Fatty acid composition and nutritional profiles of *Brycon spp.* from central Amazonia by different methods of quantification

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Abstract This study evaluated the fatty acid composition and the nutritional profile of *Brycon cephalus* and *Brycon microlepis*, fish species from the central Amazon basin, by different methods of quantification. The methods applied were: area normalization (MAN), internal standard (MIS), alternative theoretical (MAT) and alternative experimental (MAE). Significant differences were observed between the methods applied and the species studied. MAN supplied poor information about fatty acids composition and diet formulation, presenting only fatty acid profiles. MIS, MAT and MAE supplied fatty acids composition information on a mass basis. MAT and MAE overestimated results, whereas MIS presented the most accurate results. *B. cephalus* and *B. microlepis* showed high contents of approximately 65 mg g⁻¹ of n-3 fatty acids. Eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) content totaled, 104.37 mg 100 g⁻¹ and 117.89 mg 100 g⁻¹ for *B. cephalus* and *B. microlepis*, respectively. The nutritional profile of both fish species showed favorable indices for nutritional quality of the lipid fraction, indicating that both *Brycon* species are healthy dietary choices.

Keywords Amazon basin · DHA · EPA · Freshwater fish · Nutritional quality index

Introduction

The *Bryconinae* subfamily has a wide geographic distribution in South America, Central America and regions of the main Brazilian river basins, such as the Amazon and Paraná (Britski et al. 1999). *Brycon cephalus*, regionally called matrinxã, and *Brycon microlepis*, called piraputanga, are an omnivorous characids of the subfamily *Bryconinae*. These species are known to undertake periodic upriver migrations associated with reproduction (Buckup et al. 2007). In general, they feed on leaves, fruits, seeds and small fishes (Antunes et al. 2010).

The beneficial effect of fish consumption for human health has been related to, among other factors, the content of long-chain polyunsaturated fatty acids (LC-PUFAs), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3; Perini et al. 2010). These fatty acids have been shown to have benefits against several diseases, such as inflammatory diseases (Simopoulos 2008), including rheumatoid arthritis, and more recently, bowel diseases, such as Crohn's disease and ulcerative colitis (Cabr et al. 2012). LC-PUFAs also play an important role in reducing the risk of schizophrenia (Morris et al. 2005) and depression (Su et al. 2008).

Several methods are reported for quantifying fatty acids, such as the method of area normalization, the absolute quantification method with internal standard and alternative methods (Visentainer and Franco 2012). The area normalization method has been used for a long time and expresses the results as percentages derived from relative areas. However, this method has some disadvantages with error propagation due to interdependence of results (Visentainer 2012). The other methods, the absolute quantification method and the alternative methods, express their results as mass, promoting greater accuracy

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(Visentainer and Franco 2012). The alternative methods are based on the study of Exler et al. (1975), which necessitates the composition of lipid classes, triglycerides and phospholipids be determined to calculate the real content of each fatty acid in lipid fractions.

Furthermore, quantification of fatty acids, mainly the omega-3 series, is important for establishing levels presented in foods and also their nutritional profiles (Inhamuns and Franco 2008). This knowledge allows diets to be formulated more precisely and also the adaptation of technological processes of several industries, seeking to preserve the nutritional value of products and prevent the oxidation of PUFA (Almeida and Franco 2007; Moreira et al. 2001).

Due to the limited data on these aspects, this study evaluated the fatty acid composition and nutritional profile of the aforementioned fish species from the central Amazon basin by 4 different methods of quantification.

Materials and methods

Raw material

B. cephalus (matrinxã) and *B. microlepis* (Piraputanga) were collected from Teles Pires River, near Lucas do Rio Verde (13°1'59"S, 55°56'38"W), Mato Grosso, central Amazonia, Brazil. Fish samples were divided into 3 lots, each containing 10 fish per species of similar sizes. The samples were collected in the period of June–September 2015. Each sample was weighed individually and kept in polystyrene boxes with ice for transportation. Biometrics data are shown in Table 1. Fishes were beheaded and eviscerated, and fillets were obtained. Fillets of each species were homogenized, packaged and kept frozen (− 30 °C) until analysis.

Table 1 Proximate composition of 2 *Brycon* species

Species	<i>B. cephalus</i> ^A	<i>B. microlepis</i>
Moisture (%)	74.30 ± 0.02 ^a	73.89 ± 0.10 ^b
Protein (%)	18.24 ± 0.20 ^b	19.07 ± 0.15 ^a
Total lipids (%)	6.23 ± 0.10 ^b	5.34 ± 0.14 ^a
Ash (%)	1.23 ± 0.06 ^a	1.34 ± 0.09 ^a
Biometric data		
Weight (kg) ^A	1.08 ± 0.02 ^a	1.23 ± 0.06 ^b
Length (cm)	23.05 ± 0.05 ^a	27.34 ± 0.07 ^b

^AResults are expressed as mean ± standard deviation of triplicate samples. Different letters in the same line means significant difference ($P < 0.05$) by Tukey's test

Proximate composition analysis

Moisture, ash, and protein contents were determined in accordance with the AOAC (1995). Total lipids were extracted by the Bligh and Dyer (1959) method. Analyses were carried out in triplicate.

Separation of lipid classes

The procedure described by Johnston et al. (1983) was used for the separation of total lipids into lipid classes. Silica gel 60 (70–230 mesh) was used as adsorbent and the solvents chloroform, acetone and methanol for elution of neutral lipids, glycolipids and phospholipids, respectively. The percentage of each lipid classes was calculated based on the weight of the total lipids. These results were used to quantify fatty acids by the alternative methods as described by Visentainer and Franco (2012).

Fatty acid methyl esters preparation

Fatty acid methyl esters were prepared according to Hartman and Lago (1973), and modified by Maia and Rodriguez-Amaya (1993).

Fatty acid identification

Methyl esters were separated by gas chromatography using a Thermo 3300 gas chromatograph fitted with a flame ionization detector (FID) and a fused-silica CP-7420 (Select FAME) capillary column (100 m × 0.25 mm I.D. × 0.25 ml of cyanopropylpolysiloxane). Operational parameters were as follows: detector temperature, 240 °C; injection port temperature, 230 °C; column temperature, 165 °C for 18 min, programmed to increase at 4 °C min^{−1} up to 235 °C, with a final holding time of 14.5 min; carrier gas, hydrogen at 1.2 mL min^{−1}; nitrogen was used as the makeup gas at 30 mL min^{−1}; and split injection at 1:80 ratio. For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma-Aldrich, St. Louis, MO, USA). Retention times and peak area percentages were automatically computed by the software Chromquest 5.0. Analyses were carried out in triplicate.

Fatty acid quantification methods

The area normalization method (MAN) used the percentage of relative area from 1 fatty acid in relation to the sum of all fatty acids eluted, as showed in Eq. 1 (Visentainer and Franco 2012):

$$X\% = \frac{A_x \times 100}{A_t} \quad (1)$$

where, $X\%$ represents the percentage of relative area of a fatty acid (A_x) in relation to the total area for all fatty acids (A_t).

The absolute quantification method (MIS) provided a measure of a fatty acid in mg g^{-1} of total lipids compared against an internal standard of tricosanoic acid methyl ester (Sigma-Aldrich, St. Louis, MO, USA), as described by Visentainer (2012). The results were converted from mg g^{-1} of total lipids to mg 100 g^{-1} of sample. Theoretical FID correction factor values were used to calculate fatty acid concentration values in mg g^{-1} of total lipids with Eq. 2, according to Visentainer (2012):

$$FA = \frac{A_x W_{IS} CF_x}{A_{IS} W_x CF_{AE}} \quad (2)$$

where, FA is mg of fatty acids per g of total lipids, A_x is the peak area (fatty acids), A_{IS} is the peak area of internal standard methyl ester of tricosanoic acid (23:0), W_{IS} is the internal standard weight (mg) added to the sample, W_x is the sample weight (g), CF_x is the theoretical correction factor, and CF_{AE} is the conversion factor necessary to express results as mg of fatty acids rather than as methyl esters.

The alternative methods were based on the study of Exler et al. (1975), which necessitated the derivation of a reasonable factor (F) relating the total amount of fatty acids to a given quantity of total lipid. This factor was calculated easily where lipid class composition was given, and the calculation was based upon the facts that, on average, 1 g triglyceride (TG) contained 0.956 g fatty acids and 1 g phospholipids (PL) contain 0.72 g fatty acid. Therefore, the percentage of total lipid expressed as a decimal, is as follows:

$$TG \times 0.956 + PL \times 0.72 = F(\text{decimal}) \quad (3)$$

The correction factor was used to convert fatty acid methyl ester data to values suitable for food composition tables. It was assumed that, because the average fatty acid mol wt in fish lipids was relatively high, the methyl ester data could be used as a corresponding to fatty acid wt percent. Calculation then proceeds as follows in Eqs. 4 and 5:

$$F \times FA = \frac{gFA}{100gTL} \quad (4)$$

$$F \times FA \times TL(\text{decimal}) = \frac{gFA}{100gfish} \quad (5)$$

where, FA represents the respective fatty acid and TL is the total lipid content.

Theoretical values for fish were tabled by Exler et al. (1975) and were used to calculate the alternative theoretical method (MAT). To determine these factors experimentally, a lipid class separation was made as previously described. The results were applied to calculate the factors, and consequently the mass of each fatty acid, obtaining fatty acid quantification by the alternative experimental method (MAE).

Lipid nutritional quality indices

The data from fatty acid composition analyses were used to determine the nutritional profile of the lipid fraction. Nutritional quality was assessed by 3 indices: index of atherogenicity (IA), index of thrombogenicity (IT), and hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH).

IA indicated the relationship between the sum of the main saturated fatty acids (SFA) and those of the main classes of unsaturated, with the former being considered pro-atherogenic and the latter anti-atherogenic (Ulbricht and Southgate 1991). The following equation (Eq. 6) was applied to calculate IA:

$$IA = \frac{[(4 \times 14 : 0) + 16 : 0]}{MUFA + n - 6 + n - 3} \quad (6)$$

IT was defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (monounsaturated fatty acids—MUFAs, n-6 and n-3 fatty acids; Ulbricht and Southgate 1991). Equation 7 was applied:

$$IT = \frac{(14 : 0 + 16 : 0 + 18 : 0)}{\left[(0.5 \times MUFA) + (0.5 \times n - 6) + (3 \times n - 3) + \left(\frac{n - 3}{n - 6} \right) \right]} \quad (7)$$

The HH ratio was related to cholesterol metabolism and was calculated according to Eq. 8 (Santos-Silva et al. 2002):

$$HH = \frac{[(18 : 1n - 9 + 18 : 2n - 6) + 18 : 3n - 3 + 20 : 5n - 3 + 22 : 5n - 3 + 22 : 6n - 3]}{(14 : 0 + 16 : 0)} \quad (8)$$

Statistical analysis

Results of the analysis were presented as means $\pm$ standard deviations (SD). Data were submitted to one-way analysis of variance (ANOVA) and means were compared by Tukey's test. The significance level used was 5% ($P < 0.05$). Data were processed using Statistica software, version 7.0 (Statsoft 2004).

Results and discussion

Proximate composition

Table 1 shows the proximal composition of muscle tissue of *Brycon* species. Except for ash content, significant differences were observed between the species studied, at 5% of significance. The results, such as protein, ash and moisture levels were in accordance with other studies, with the same species (Moreira et al. 2001).

Moreira et al. (2001) reported minor values for total lipid content in *B. microlepis* (2.49%) collected in the Manso River, Mato Grosso, Brazil. Almeida and Franco (2007) reported levels of 4.5% for *B. cephalus* captured in the Central Amazon. Compared to these reports, the values found in this current study were higher, which could be related to differences in geographical location and season of collection. Lipid deposition in tissues is associated to many factors, such as reproductive cycle, size, age and season (Petenuci et al. 2016). *B. cephalus* and *B. microlepis* showed similar total lipid contents to those reported for *Pimelodus maculatus* (5.55%; Ramos Filho et al. 2010).

Fatty acids composition

The fatty acid compositions of *Brycon* species, as determined by different methods of quantification, are shown in Table 2. A significant difference ($P < 0.05$) was noted among the 4 methods used. The majority fatty acids were the same for both fish species, being palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9) and linoleic acid (18:2n-6). MAN, MIS, MAT and MAE all showed these same fatty acids as majority components of the total.

The area normalization method (MAN) showed no significant difference for MUFA, PUFA, n-3 and n-6 fatty acids between the 2 fish species, *B. cephalus* and *B. microlepis*. This might be due to the characteristics of the area normalization method, which did not supply the real fatty acid content in samples as reported by Visentainer and Franco (2012). According to these authors, area normalization method is just a method of fatty acid identification and does not provide the amount of fatty acids. Thus, the results obtained through this method could cause erroneous

conclusions, as showed in Table 2. The methods of fatty acids quantification, MIS, MAT and MAE, show that n-3 content was significantly different ($P < 0.05$) between the fish species, unlike MAN, which found no significant difference.

Even so, the method of area normalization has been used for a long time as a quantification method. However, the results are expressed as percentages from relative areas, which are difficult to interpret and translate to amounts for nutritional labels, informing the real amount of fatty acids in foods (Visentainer, 2012).

Comparing the other 3 methods of fatty acid quantification (MIS, MAT and MAE), a significant difference ($P < 0.05$) was noted among them for both fish species for SFA, MUFA and PUFA. SFA content ranged from 308.46 mg g⁻¹ (MIS) to 322.39 mg g⁻¹ (MAE) for *B. cephalus*, and from 281.05 mg g⁻¹ (MIS) to 306.98 mg g⁻¹ (MAT) for *B. microlepis*. PUFA content showed consistent results for both fish species (Table 2), though higher values were observed in *B. cephalus* for all methods of fatty acid quantification (MIS, MAT and MAE). These methods expressed the results as mass, promoting measures of greater accuracy, which were easier to interpret and use in diet formulations and process design. Results expressed as mass are more reliable and can be used by professionals from different areas (Visentainer 2012).

B. cephalus showed a significant difference ($P < 0.05$) in the measures for total n-6 fatty acids between the methods MIS, MAT and MAE, whereas *B. microlepis* showed similar results for MAT and MAE. In general, total n-6 fatty acids were higher than n-3 fatty acids in both species and a significant difference ($P < 0.05$) was observed between the 2 species using MIS. Carbonera et al. (2014) evaluated fatty acid composition of 21 freshwater fish species and also found that n-6 fatty acids content was higher than for n-3 fatty acids. These researchers found values ranging from 6.0 to 24.2% of n-6 fatty acids. In the current study, *B. cephalus* and *B. microlepis* presented values consistent with these results.

Linoleic acid (LA, 18:2n-6) was the predominant fatty acid in total n-6 fatty acids, representing approximately 82% in *B. cephalus* and 80% in *B. microlepis* for the 3 methods of quantification, MIS, MAT and MAE. A significant difference ($P < 0.05$) was noted among the methods used and also between the fish species. Arachidonic acid (ARA, 20:4n-6) a LC-PUFA from the n-6 series showed consistent results, with no significant difference among the methods of fatty acids quantification, MIS, MAT and MAE. *B. cephalus* and *B. microlepis* showed higher values of LA than another fish species from the *Characidae* family, *Colossoma macropomum*

Table 2 Fatty acid composition of *Brycon* species determined by different methods of quantification

Fatty Acids	<i>B. cephalus</i> ^A				<i>B. microlepis</i>			
	%		mg g ⁻¹ TL		%		mg g ⁻¹ TL	
	MAN ^B	MAE	MIS	MAT	MAN	MAE	MIS	MAT
14:0	0.62 ± 0.03 ^d	5.41 ± 0.26 ^c	5.43 ± 0.25 ^c	5.55 ± 0.26 ^c	0.96 ± 0.01 ^d	5.41 ± 0.26 ^c	8.23 ± 0.01 ^b	8.79 ± 0.03 ^a
14:1n-7	0.02 ± 0.01 ^e	0.13 ± 0.01 ^c	0.13 ± 0.01 ^c	0.14 ± 0.01 ^c	0.09 ± 0.01 ^d	0.13 ± 0.01 ^c	0.75 ± 0.02 ^b	0.81 ± 0.03 ^a
15:0	0.37 ± 0.03 ^b	3.23 ± 0.29 ^a	3.23 ± 0.29 ^a	3.33 ± 0.31 ^a	0.35 ± 0.01 ^b	3.25 ± 0.30 ^a	3.01 ± 0.07 ^a	3.25 ± 0.06 ^a
15:1n-5	0.03 ± 0.01 ^b	0.29 ± 0.06 ^a	0.29 ± 0.06 ^a	0.30 ± 0.06 ^a	0.03 ± 0.01 ^b	0.29 ± 0.06 ^a	0.25 ± 0.01 ^a	0.27 ± 0.01 ^a
16:0	22.69 ± 0.30 ^e	196.34 ± 2.87 ^b	196.34 ± 2.87 ^b	204.18 ± 2.73 ^a	20.37 ± 0.35 ^e	199.07 ± 2.66 ^{ab}	172.38 ± 2.52 ^d	187.39 ± 3.20 ^c
16:1n-9	0.38 ± 0.02 ^d	3.30 ± 0.14 ^c	3.30 ± 0.14 ^c	3.46 ± 0.15 ^{bc}	0.43 ± 0.01 ^d	3.37 ± 0.15 ^c	3.64 ± 0.05 ^b	3.99 ± 0.07 ^a
16:1n-7	0.35 ± 0.04 ^b	3.04 ± 0.39 ^b	3.04 ± 0.39 ^b	3.19 ± 0.40 ^b	1.70 ± 0.17 ^{bc}	3.11 ± 0.39 ^b	14.30 ± 1.48 ^a	15.67 ± 1.58 ^a
16:1n-5	0.09 ± 0.01 ^d	0.78 ± 0.02 ^c	0.78 ± 0.02 ^c	0.82 ± 0.02 ^c	0.11 ± 0.01 ^d	0.80 ± 0.02 ^c	0.93 ± 0.02 ^b	1.02 ± 0.02 ^a
17:0	0.31 ± 0.02 ^c	2.67 ± 0.19 ^b	2.67 ± 0.19 ^b	2.80 ± 0.20 ^b	0.36 ± 0.01 ^c	2.73 ± 0.19 ^b	2.99 ± 0.02 ^{ab}	3.27 ± 0.03 ^a
17:1n-9	0.30 ± 0.02 ^c	2.58 ± 0.16 ^a	2.58 ± 0.16 ^a	2.72 ± 0.16 ^a	0.13 ± 0.01 ^c	2.66 ± 0.16 ^a	1.08 ± 0.10 ^b	1.19 ± 0.11 ^b
18:0	10.73 ± 0.22 ^d	91.57 ± 1.75 ^{bc}	91.57 ± 1.75 ^{bc}	96.56 ± 1.98 ^a	10.51 ± 0.26 ^d	94.15 ± 1.93 ^{ab}	87.75 ± 2.41 ^c	96.73 ± 2.42 ^a
18:1n-11	0.22 ± 0.01 ^f	1.85 ± 0.02 ^c	1.85 ± 0.02 ^c	1.96 ± 0.02 ^a	0.13 ± 0.01 ^g	1.91 ± 0.02 ^b	1.10 ± 0.01 ^e	1.22 ± 0.01 ^d
18:1n-9	25.84 ± 0.43 ^e	218.95 ± 3.28 ^d	218.95 ± 3.28 ^d	232.55 ± 3.84 ^c	29.36 ± 0.05 ^e	226.73 ± 3.74 ^c	243.33 ± 0.97 ^b	270.15 ± 0.42 ^a
18:1n-7	1.55 ± 0.07 ^d	13.16 ± 0.60 ^{ab}	13.16 ± 0.60 ^{ab}	13.97 ± 0.62 ^a	1.38 ± 0.02 ^d	13.62 ± 0.60 ^{ab}	11.41 ± 0.20 ^c	12.67 ± 0.19 ^b
18:2n-6	21.93 ± 0.22 ^e	184.51 ± 1.87 ^c	184.51 ± 1.87 ^c	197.38 ± 1.97 ^a	20.70 ± 0.19 ^e	192.44 ± 1.92 ^b	170.34 ± 1.12 ^d	190.48 ± 1.72 ^b
18:3n-6	0.29 ± 0.01 ^d	2.43 ± 0.08 ^{ab}	2.43 ± 0.08 ^{ab}	2.62 ± 0.08 ^a	0.25 ± 0.02 ^d	2.55 ± 0.08 ^a	2.02 ± 0.17 ^c	2.28 ± 0.19 ^{bc}
18:3n-3	2.41 ± 0.06 ^d	20.09 ± 0.53 ^c	20.09 ± 0.53 ^c	21.65 ± 0.54 ^{ab}	2.43 ± 0.03 ^d	21.11 ± 0.53 ^b	19.89 ± 0.33 ^c	22.40 ± 0.31 ^a
20:0	0.23 ± 0.01 ^c	1.91 ± 0.01 ^a	1.91 ± 0.01 ^a	2.04 ± 0.01 ^a	0.14 ± 0.02 ^c	1.99 ± 0.01 ^a	1.14 ± 0.19 ^b	1.27 ± 0.21 ^b
18:4n-3	0.44 ± 0.02 ^d	3.61 ± 0.14 ^c	3.61 ± 0.14 ^c	3.92 ± 0.16 ^{bc}	0.50 ± 0.02 ^d	3.82 ± 0.15 ^{bc}	4.06 ± 0.13 ^b	4.61 ± 0.15 ^a
20:1n-9	0.46 ± 0.01 ^e	3.84 ± 0.02 ^{abc}	3.84 ± 0.02 ^{abc}	4.12 ± 0.02 ^a	0.40 ± 0.03 ^e	4.02 ± 0.02 ^{ab}	3.29 ± 0.26 ^d	3.70 ± 0.30 ^{bc}
20:2n-6	0.85 ± 0.02 ^e	7.08 ± 0.13 ^b	7.08 ± 0.13 ^b	7.66 ± 0.15 ^a	0.63 ± 0.01 ^e	7.46 ± 0.14 ^a	5.16 ± 0.02 ^d	5.83 ± 0.03 ^c
21:0	0.05 ± 0.01 ^h	0.42 ± 0.01 ^f	0.42 ± 0.01 ^f	0.45 ± 0.01 ^e	0.10 ± 0.01 ^g	0.44 ± 0.01 ^e	0.81 ± 0.01 ^c	0.91 ± 0.01 ^a
20:3n-6	0.73 ± 0.01 ^e	6.04 ± 0.07 ^d	6.04 ± 0.07 ^d	6.57 ± 0.07 ^c	0.96 ± 0.02 ^e	6.40 ± 0.07 ^c	7.75 ± 0.19 ^b	8.81 ± 0.19 ^a
20:4n-6	1.47 ± 0.07 ^b	12.10 ± 0.53 ^a	12.10 ± 0.53 ^a	13.25 ± 0.59 ^a	1.41 ± 0.21 ^b	12.92 ± 0.57 ^a	11.36 ± 1.73 ^a	12.99 ± 1.95 ^a
20:3n-3	0.91 ± 0.04 ^d	7.56 ± 0.31 ^c	7.56 ± 0.31 ^c	8.22 ± 0.32 ^c	1.16 ± 0.07 ^d	8.01 ± 0.31 ^c	9.39 ± 0.57 ^b	10.68 ± 0.67 ^a
20:5n-3	1.15 ± 0.02 ^e	9.43 ± 0.16 ^d	9.43 ± 0.16 ^d	10.39 ± 0.16 ^b	1.22 ± 0.04 ^e	10.13 ± 0.15 ^{bc}	9.70 ± 0.33 ^{cd}	11.18 ± 0.41 ^a
22:0	0.12 ± 0.01 ^e	1.01 ± 0.02 ^b	1.01 ± 0.02 ^b	1.09 ± 0.02 ^a	0.10 ± 0.01 ^e	1.06 ± 0.02 ^a	0.78 ± 0.02 ^d	0.88 ± 0.03 ^c
20:4n-3	0.56 ± 0.02 ^c	4.62 ± 0.14 ^a	4.62 ± 0.14 ^a	5.06 ± 0.14 ^a	0.34 ± 0.04 ^c	4.94 ± 0.14 ^a	2.77 ± 0.31 ^b	3.17 ± 0.34 ^b
20:5n-6	0.35 ± 0.01 ^c	2.83 ± 0.11 ^a	2.83 ± 0.11 ^a	3.12 ± 0.13 ^a	0.20 ± 0.03 ^c	3.04 ± 0.13 ^a	1.61 ± 0.22 ^b	1.86 ± 0.26 ^b
22:4n-6	0.14 ± 0.01 ^e	1.18 ± 0.03 ^b	1.18 ± 0.03 ^b	1.30 ± 0.04 ^a	0.11 ± 0.01 ^e	1.27 ± 0.04 ^{ab}	0.92 ± 0.06 ^d	1.06 ± 0.08 ^c
22:5n-6	0.82 ± 0.06 ^d	6.66 ± 0.43 ^{ab}	6.66 ± 0.43 ^{ab}	7.38 ± 0.57 ^a	0.63 ± 0.02 ^d	7.20 ± 0.56 ^a	5.01 ± 0.13 ^c	5.81 ± 0.17 ^{bc}
24:0	0.71 ± 0.02 ^e	5.89 ± 0.17 ^b	5.89 ± 0.17 ^b	6.39 ± 0.18 ^a	0.49 ± 0.01 ^e	6.23 ± 0.18 ^a	3.95 ± 0.05 ^d	4.48 ± 0.06 ^c
24:1n-9	0.01 ± 0.01 ^d	0.09 ± 0.01 ^a	0.09 ± 0.01 ^a	0.09 ± 0.01 ^a	0.01 ± 0.01 ^e	0.09 ± 0.01 ^a	0.05 ± 0.01 ^c	0.06 ± 0.01 ^b

Table 2 continued

Fatty Acids	<i>B. cephalus</i> ^A			<i>B. microlepis</i>		
	%			%		
	MAN ^B	MIS	MAE	MAN	MIS	MAE
22:5n-3	0.47 ± 0.03 ^d	3.82 ± 0.25 ^c	4.13 ± 0.27 ^c	0.72 ± 0.04 ^d	5.69 ± 0.31 ^b	6.53 ± 0.37 ^a
22:6n-3	2.39 ± 0.15 ^c	19.29 ± 1.23 ^a	20.99 ± 1.30 ^a	1.58 ± 0.04 ^c	12.45 ± 0.25 ^b	14.40 ± 0.33 ^b
SFA ^C	35.82 ± 0.09 ^f	308.46 ± 0.97 ^c	314.32 ± 0.78 ^b	33.37 ± 0.07 ^g	281.05 ± 0.05 ^e	304.15 ± 0.65 ^d
MUFA	29.26 ± 0.32 ^f	248.00 ± 2.37 ^c	263.33 ± 2.89 ^c	33.78 ± 0.20 ^f	280.14 ± 2.39 ^b	307.88 ± 1.85 ^a
PUFA	34.92 ± 0.39 ^f	291.26 ± 3.57 ^d	314.28 ± 3.47 ^a	32.86 ± 0.13 ^f	268.13 ± 0.42 ^e	299.49 ± 1.20 ^c
n-3	8.33 ± 0.21 ^d	68.42 ± 31.84 ^b	75.00 ± 1.89 ^a	6.94 ± 0.12 ^d	63.96 ± 0.95 ^c	63.25 ± 1.12 ^c
n-6	26.59 ± 0.30 ^e	222.84 ± 2.58 ^c	239.28 ± 2.68 ^a	25.92 ± 0.01 ^e	204.17 ± 0.53 ^d	236.24 ± 0.08 ^{ab}

^AResults are expressed as mean ± standard deviation of 3 replicates. Different letters in the same line means significant difference ($P < 0.05$) by Tukey's test

^B MAN, method of area normalization; MIS, method of internal standard; MAT, method of alternative theoretical; MAE, method of alternative experimental

^CSFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids; n-3, total n-3 fatty acids; n-6, total n-6 fatty acids

(129.67 mg g⁻¹ of total lipids), and similar values of ARA (12.70 mg g⁻¹ of total lipids; Petenuci et al. 2016).

Values of total n-3 fatty acids content in *B. microlepis* were statistically the same ($P < 0.05$) for the MIS, MAT and MAE methods (around 63 mg g⁻¹ of total lipids). However, MIS presented a lower level of n-3 (68.42 mg g⁻¹ of total lipids) than MAT and MAE (around 74 mg g⁻¹ of total lipids) in *B. cephalus*. Other researchers have reported low values for n-3 fatty acids in *B. cephalus* (3.95%; Almeida and Franco 2007) and *B. microlepis* (4.76%; Moreira et al. 2001), both evaluated by MAN. Compared to these reports, MAN in our study showed higher results for both species. *Brycon* species showed higher values than other freshwater fish species from the Amazon basin, such as *C. macropomum* (43.23 mg g⁻¹ of total lipids) and *P. nigricans* (57.28 mg g⁻¹ of total lipids; Petenuci et al. 2016).

LNA, EPA and DHA were the major fatty acids from the n-3 series in both *Brycon* species. LNA is a precursor for n-3 LC-PUFA as DHA and EPA, through elongation and desaturation processes (Tocher 2003). A significant difference ($P < 0.05$) was noted between the methods applied to each fish species (Table 2). However, both fish species showed similar LNA contents, around 20 mg g⁻¹ as noted by MIS. Lower values of LNA content were observed by Ramos Filho et al. (2008) in *P. coruscans* (15 mg g⁻¹) and *S. maxillosum* (6 mg g⁻¹).

EPA and DHA content showed a significant difference ($P < 0.05$) between the studied fish species. For EPA content, the MAT and MAE showed no significant difference. However, EPA values were statically different to MIS. For DHA content, these differences between methods were not observed. However, DHA content was higher in *B. cephalus*. According to the Agência Nacional de Vigilância Sanitária - (2012), foods containing EPA + DHA higher than 80 mg 100 g⁻¹ are considered a source for these fatty acids. Thus, *B. cephalus* and *B. microlepis* met that recommendation, with 28.72 mg g⁻¹ (approximately 104.15 mg 100 g⁻¹) and 22.15 mg g⁻¹ (around 118.44 mg 100 g⁻¹), respectively. The World Health Organization (WHO) recommends the consumption of 200–500 mg of these fatty acids per day (World Health Organization 2003). Thus, a serving of 100 g of the studied fishes supplied about 50% of the WHO daily recommended intake of EPA + DHA.

Comparing all the methods of fatty acids quantification used in this study, MAN notably supplied poorer information about fatty acids composition for diet formulation, since the results (%) of this method did not represent actual numerical quantities of fatty acids in a portion of fish. For most of evaluated parameters, MAT and MAE could be considered statistically similar. However, MAT needs further investigation, since it is an empirical method based

Table 3 Indices of nutritional quality of *Brycon cephalus* and *Brycon microlepis*

Species	Method	IA ^A	IT	HH	n-6/n-3	PUFA/SFA
<i>B. cephalus</i>	MAN ^B	0.39 ± 0.01 ^b	0.64 ± 0.02 ^b	2.32 ± 0.02 ^d	3.19 ± 0.03 ^d	0.97 ± 0.01 ^d
	MIS	0.40 ± 0.01 ^a	0.67 ± 0.01 ^a	2.26 ± 0.03 ^e	3.26 ± 0.00 ^e	0.94 ± 0.01 ^e
	MAT	0.39 ± 0.01 ^b	0.64 ± 0.01 ^b	2.33 ± 0.01 ^c	3.19 ± 0.00 ^c	0.97 ± 0.01 ^c
	MAE	0.39 ± 0.02 ^b	0.64 ± 0.02 ^b	2.33 ± 0.01 ^c	3.19 ± 0.01 ^c	0.82 ± 0.00 ^c
<i>B. microlepis</i>	MAN	0.36 ± 0.01 ^d	0.63 ± 0.01 ^c	2.63 ± 0.02 ^a	3.73 ± 0.01 ^a	0.98 ± 0.02 ^a
	MIS	0.37 ± 0.01 ^c	0.62 ± 0.01 ^d	2.55 ± 0.02 ^b	3.19 ± 0.03 ^b	0.95 ± 0.01 ^b
	MAT	0.36 ± 0.02 ^d	0.63 ± 0.01 ^c	2.63 ± 0.03 ^a	3.73 ± 0.02 ^a	0.98 ± 0.02 ^a
	MAE	0.36 ± 0.01 ^d	0.63 ± 0.01 ^c	2.63 ± 0.02 ^a	3.74 ± 0.03 ^a	0.98 ± 0.03 ^a
<i>B. microlepis</i> ^C (Moreira et al. 2001)	0.49	1.02	2.06	1.80	0.29	
<i>B. cephalus</i> (Almeida and Franco 2007)	0.59	0.79	2.63	3.17	0.47	

^AIA, index of atherogenicity; IT, index of thrombogenicity; HH, ratio of hypocholesterolemic/hypercholesterolemic; n-6/n-3, ratio between n-6 fatty acids and n-3 fatty acids; PUFA/SFA, ratio between total polyunsaturated fatty acids and total saturated fatty acids

^BDifferent letters in the same column indicate significant differences ($P < 0.05$) by Tukey's test

^CValues calculated based on the results of cited authors using the equations provided in materials and methods section

on tabulated data. MAE depends on the sampling diversity and lipid composition. The amount of phospholipids and neutral lipids can vary according to the method of lipid class separation in experimental and theoretical quantification, leading to errors in results. By contrast, MIS seemed to be the most appropriate method for the fatty acids quantification, since samples and standard run analysis occurred together. Therefore, any interference in fatty acids preparation also proportionally affected the metric for quantification.

Table 3 exhibits the nutritional profiles of lipid fractions from the *Brycon* species, represented by IA, IT, HH, n-6/n-3 and PUFA/SFA ratios. The indices, IA and thrombogenicity IT, indicated the global dietetic quality of lipids and their potential effects on the development of coronary diseases (Ulbricht and Southgate 1991). IA and IT showed a significant difference ($P < 0.05$) between the fish species. MIS showed the highest values for IA in both species. However, the results obtained were lower than the values calculated by Almeida and Franco (2007) and Moreira et al. (2001) for *Brycon* species. The values of their indices are also shown in Table 3. Lower values are desirable for both indices as this represents a better nutritional quality of fat, which is related to a decrease in cardiovascular disease risk.

HH indicates the specific effects of fatty acids on cholesterol metabolism. A significant difference ($P < 0.05$) was observed between the fish species. However, only HH values from MIS were statistically different ($P < 0.05$) from MAN, MAT and MAE. *B. cephalus* showed a lower HH ratio than reported by Moreira et al. (2001); however, similar results were reported for *B. microlepis* by Almeida and Franco (2007). Nutritionally, higher HH values are

considered more beneficial for human health. The values reported in the current study for both *Brycon* species are similar to those reported for *C. macropomum* (2.26; Petenucci et al. 2016).

Significant differences ($P < 0.05$) were observed for the n-6/n-3 ratios among the methods used and between the species studied. There was no significant difference among MAN, MAT and MAE for each species. Simopoulos (2008) associated n-6/n-3 ratios ≤ 4.0 with a 70% reduction in death caused by coronary diseases. Results shown in Table 3 suggested that both fish species constituted healthy dietary choices in this respect.

PUFA/SFA ratios also showed significant differences ($P < 0.05$) among the methods and between the fish species. Values varied from 0.88 to 0.97 for *B. cephalus* and 0.91 to 0.98 for *B. microlepis*. These results were higher than those reported by Moreira et al. (2001) and Almeida and Franco (2007) in the same species; however, they were in accordance with values reported in Brazilian wild freshwater fish by Carbonera et al. (2014).

In general terms, indices and ratios presented in Table 3 only showed differential responses when MIS was used.

Conclusion

The method of fatty acid quantification influenced the results for both *Brycon* species. In general, *B. cephalus* presented higher values of SFA, PUFA, n-3 than *B. microlepis* by all methods (MAN, MIS, MAT and MAE). MIS also quantified a high level of n-6 fatty acids in *B. cephalus*. MIS provided the most accurate results for diet formulations, whereas MAN supplied only fatty acid

profiles without numerical content of each fatty acid. The alternative methods, MAE and MAT, provided overestimated results. *B. cephalus* and *B. microlepis* showed high levels of n-3 fatty acids, mainly health-benefiting compounds, such as LNA, EPA and DHA. DHA content was higher in *B. cephalus*. Both *Brycon* species could be considered as valuable sources of EPA + DHA, since as little as 100 g could provide 50% of the recommended daily intake for these fatty acids. Except for MIS, the other methods, MAN, MAT and MAE, did not show a difference in the indices and ratios of nutritional quality for *B. cephalus* and *B. microlepis*. The quality indexes of the lipid fraction, IA, IT, HH, as well as the n-6/n-3 and PUFA/SFA ratios indicated that both fish species represent a healthy nutritional option.

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