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Quantification of Essential Fatty Acids and Assessment of the Nutritional Quality Indexes of Lipids in Tilapia Alevins and Juvenile Tilapia Fish (*Oreochromis niloticus*)

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Abstract

To determine the nutritional quality of the lipid segment in tilapia (*Orechromis niloticus*) during different periods of development (alevins and juveniles), the total lipids (TL), linolenic (LNA), eicosapentaenoic (EPA), docosapentaenoic (DHA), linoleic (LA) and arachidonic (AA) acids were quantified , and the lipid nutritional quality indexes were calculated for the tilapia. The lipid profile showed that the species present high indexes of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in both development phases. The indexes of nutritional quality of lipids, atherogenic index (AI) and thrombogenic index (TI) present low values and represent beneficial health effects, both in the alevin and juvenile fish. The quantifications of LNA, EPA, DHA, LA and AA show the greatest values (mg/g of total lipids) for the juveniles. However, regardless of the development phase in which the tilapia is, the fish may be considered a source of essential fatty acids a kind of potential and nutritional food, reflecting beneficial effects for consumer's health.

Keywords: lipid profile, tilapia, nutritional quality, atherogenic index, thrombogenic index

1. Introduction

To achieve a healthy diet, consumers all over the world are looking for food that provides energy and offers other nutritional benefits.

The tilapia cultivated in approximately 100 countries (Tonial, Bravo, de Souza, Matsushita, Furuya, & Visentainer, 2011) belong to the second group of major fish in the world aquaculture (Pantoja, Dias, Marinho, Montagner, & Tavares-Dias, 2012; Tonial, Stevanato, Matsushita, de Souza, Furuya, & Visentainer, 2009; Borghetti, Ostrensky, & Borghetti, 2003). The tilapia species include the Nile tilapia (*Oreochromis niloticus*), which is considered to be the most resistant to high temperatures, low dissolved oxygen concentration, high concentration of ammonia in water and adverse environmental conditions (Tuker, 2011; Sardella & Brauner, 2008).

In addition, the Nile tilapia provides excellent sensorial and nutritional characteristics, such as tasty meat, low amounts of fat and calories, absence of Y-shaped fishbone and good indexes for being converted into food and have its fillet used as food, which optimizes the industrialization of this species (Furtado, Resende, & Freitas 2010; Chukwu, 2009; Sklan, Prag, & Lupatsch, 2004).

In general, fish meat is considered to be a positive factor in the human diet because it is a good source of proteins, vitamins and lipids of a good quality, such as polyunsaturated fatty acids, especially Omega-3 and 6 fatty acids (Ponzoni, Hamzah, Tanan, & Kamaruzzaman, al., 2005; Köprücü & Ozdemir, 2005; Gutiérrez-Espinosa, 2011).

The linoleic (LA-18:2n-6) and alpha-linolenic (LNA, 18:3n-3) acids are known as essential fatty acids (Martin, Almeida, Ruiz, Visentainer, Matshushita, de Souza, & Visentainer, 2006) because they are precursors of other

fatty acids of this series. LA is the precursor of arachidonic acid (AA, 20:4n-6), one of the precursors of the biosynthesis of eicosanoids (prostaglandin, thromboxanes and leukotrienes), which perform important functions in the human body (Visentainer, Carvalho, Ikegaki, & Park, 2000; Aguiar, Morais, Santos, Stevanato, Visentainer, de Souza, & Visentainer, 2007). Alpha-linolenic acid (LNA) is the precursor of eicosapentaenoic acid (EPA, 20:5n-3), which is associated with decreased cardiac diseases (Nestel, 2000), and docosaheaenoic acid (DHA – 22:6n-3), which is considered to be essential in the formation of nervous tissues and vision (Crawford, Bloom, Broadhurst, Schmidt, Cunnane, Galli, Gehbremeskel, Linseisen, Lloyd-Smith, & Parkington, 1999).

Polyunsaturated fatty acids (PUFA/n-3) are also directly connected to preventing of uterine and colon cancer and immunologic diseases (Chen, Lochmann, Goodwin, Praveen, Dabrowski, & Lee, 2004) as well as decreasing chronically neurodegenerative diseases (Hunter & Roberts, 2000), rheumatoid arthritis, osteoporosis (Albertazzi & Coupland, 2002) and kidney disease (Donadio, Berstralh, & Offord, 1994).

The aim of this study was to assess the nutritional quality of *Oreochromis niloticus* alevins and juveniles by quantifying the fatty acids n-3 and n-6 and nutritional quality indexes of lipids.

2. Material and Methods

2.1 Sampling

Alevin and juvenile tilapia samples with an estimated age of approximately 40 or 60 days, respectively, were acquired from a recreational fishing site in the city of Maringá, Paraná State. The juvenile tilapia (20 individuals) were eviscerated, filleted and ground in a food processor to obtain a homogeneous consistency. The alevins (40 individuals) were also ground in a food processor, discarding only the viscera. The samples were stored in a nitrogen atmosphere and frozen at -18°C until the time of analysis. All of the chemical analyses were performed in triplicate.

2.2 Physical and Chemical Characterization

Moisture, ash, and protein contents were determined according to AOAC (1997). Total lipids were extracted by the Bligh and Dyer (1959) method.

2.3 Fatty Acids Composition

Fatty acid methyl esters (FAME) were prepared as described by Joseph and Ackman (1992) and analyzed using gas chromatograph 14-A (Shimadzu, Japan) with a flame ionization detector and a capillary column made of melted silica CP Sil-88, a capillary column (100 m, 0.25 mm and 0.25 mm). The temperature of the column was programmed at 2 °C/min from 180 °C to 240 °C, and the temperature of the injector was kept at 220 °C and 245 °C. The gas flux was 1.4 $mL.min^{-1}$ for the carrier gas (H_2), 30 $mL.min^{-1}$ for the make-up gas (N_2) and 30 $mL.min^{-1}$ and 300 $mL.min^{-1}$ for the H_2 and for the flame synthetic air. The ratio for the sample partition (split) was 1:100. Peak areas were determined using the CG-300 computing integrator, and the fatty acid composition was expressed as the weight percentage of each fatty acid relative to the total fatty acids (%). FAME were identified by comparison with the retention times of standards from Sigma (St. Louis, MO, USA) and equivalent chain-length values (ECL).

2.4 Quantification of Acids LNA, EPA, DHA, AA, LA

The fatty acids LNA, EPA, DHA, AA and LA were quantified in mg/g of total lipids through their internal standardization by means of those methyl esters standards for fatty acids. Because it was not present in the analyzed samples, tricosanoic acid methyl ester (23:0) was used as the internal standard, and calculations were performed using the following equation (Visentainer, 2012):

LNA, EPA, DHA, AA or LA (mg/g) = [(Ax x Mp x Fcx) / (Ap x MA x Fc)],

where

Ax = LNA, EPA, DHA, AA or LA area;

Ap = internal standard area;

Fcx = theoretical factor for correction of the acids LNA, EPA, DHA, AA or LA;

Mp = mass of the internal standard added to the to the sample in milligrams;

MA = mass of the total lipids sample in grams; and

Fc = conversion Factor to present the results in mg of total fatty acids starting from the LNA, EPA, DHA, AA and LA methyl esters.

2.5 Nutritional Quality Index (NQI) of Lipids

The nutritional quality of the lipid portion in the tilapia (*Oreochromis niloticus*) samples in two different phases of development, the alevin and the juvenile phases, was determined through the fatty acids composition. The nutritional quality of the lipids was assessed by considering three indexes: atherogenicity (AI), thrombogenicity (TI) and the ratio between the hypocholesteronic and hypercholesteronic. The following calculations were used to determine these indexes:

a) Atherogenicity Index (Ulbricht & Southgate, 1991)

(AI) = [(C12:0 + 4xC14:0+C16:0)]/ΣAGMI+ Σn-6+Σn-3

b) Thrombogenicity Index (Ulbricht; Southgate, 1991)

(TI) = (C14:0 + C16:0 +C18:0)/[(0,5xΣAGMI)+(0,5xΣn-6)+(3xΣn-3)+(Σn-3/n-6)]

c) Ratio between hypocholesterolemic and hypercholesterolemic fatty acids (Santos-Silva, Bessa, & Santos-Silva, 2002).

(HH) = (C18:1n-9+C18:2n-6+C20:4n-6+C18:3n-3-+C20:5n-3+C22:5n-3+C22:6n-6)/(C14:0+C16:0)

2.6 Statistical Analysis

The values for the proximate composition and the fatty acid composition were subjected to the statistics variance analysis (ANOVA). The Tukey's test was applied to the unequal variances among the average sample values, keeping the significance level in 5% in all analyses through the software Statistica, version 7.0 (2004).

3. Results

In The mean values of the proximate composition for samples of tilapia alevins and juveniles tilapia are shown in Table 1.

Table 1. Proximate composition of tilapia alevins and juvenile tilapia

Constituents	Tilapia alevin (%)	Juvenile tilapia (%)
Moisture	78.82±0.26[a]	75.36±0.27[b]
Ash	1.65±0.13[a]	1.52±0.09[b]
Protein	16.85±0.33[a]	16.94±0.30[b]
Total Lipids	2.65±0.24[a]	6.13±0.37[b]

The results are averages in triplicates with the respective estimates for the standard deviation. Values in the same line that are followed by the same letter do not differ between them ($p > 0.05$).

Table 2 presents the fatty acids composition obtained in samples of tilapia alevins and juvenile tilapias.

Table 2. Fatty acid composition from the total lipids of tilapia alevin and juvenile tilapia samples

Fatty Acids	Tilapia Alevin (%± d.p)	Juvenile Tilapia (%± d.p)	Fatty Acids	Tilapia Alevin (%± d.p)	Juvenile Tilapia (%± d.p)
14:0	1.74 ±0.17	1.97±0.01	20:0	0.23±0.02	0.23±0.01
16:0	27.17 ±0.46	28.12±0.19	18:3n-3	0.58±0.01[a]	0.71±0.01[b]
16:1n-9	0.60 ±0.04	0.60±0.02	20:1n-9	0.82±0.02[a]	0.74±0.01[b]
16:1n-7	4.95 ±0.11	5.31±0.13	21:0	0.2 ±0.01	0.21±0.01
17:0	0.39 ±0.01	0.41±0.01	20:2n-6	0.39±0.01	0.34±0.01
17:1n-7	0.24 ±0.01	0.26±0.01	20:3n-6	0.40±0.01[a]	0.32±0.01[b]
18:0	7.80 ±0.05	7.79 ±0.03	22:0	0.68±0.03	0.70±0.01
18:1t-9	0.25 ±0.03	0.27 ±0.01	20:4n-6	3.23±0.11[a]	3.26±0.01[b]
18:1n-9	29.95 ±0.42	29.41 ±0.04	20:5n-3	0.17±0.01[a]	0.21±0.01[b]
18:1n-7	3.62±0.14	3.66±0.07	22:4n-6	0.87±0.05[a]	0.61±0.01[b]
18:2t-6	0.43±0.01[a]	0.47±0.00[b]	22:5n-6	1.50±0.06[a]	1.04±0.03[b]
18:2n-6	10.57 ±0.15	10.84±0.04	22:5n-3	0.58±0.02[a]	0.48±0.01[b]
18:3n-6	0.65 ±0.01[a]	0.70±0.01[b]	22:6n-3	1.95 ±0.05	1.98±0.04

The values given in the table above are standard deviation means ± SD of triplicate analysis. Different letters in the same line indicate differences ($p < 0.05$) by the Tukey's test. The individual fatty acids were expressed as a percentage of total fatty acids.

The sum of SFA, MUFA, PUFA, n-6, n-3, the ratios of PUFA/SFA and n-6/n-3 and the nutritional quality index of lipid fraction in alevins and juvenile tilapia are shown in Table 3.

Table 4 shows the concentration in mg/g of total lipids of the fatty acids, LNA, EPA, DHA, LA and AA on the samples of alevins and Juvenile tilapia.

Table 3. Sum and ratios of fatty acids and nutritional quality index of lipid fraction in tilapia alevin (*Orechromis niloticus*) and juvenile tilapia (*Orechromis niloticus*)

Sum and ratio	Tilapia alevin (%± d.p)	juvenile tilapia (%± d.p)
PUFA	21.33 ±0.42[a]	20.04 ±0.13[a]
MUFA	40.44±0.12[a]	40.37 ±0.24[a]
SFA	38.23±0.01[a]	39.59 ±0.12[b]
n-6	18.00±0.35[a]	16.64 ±0.06[b]
n-3	3.27 ±0.08[a]	3.34 ±0.06[a]
PUFA/SFA	0.56 ±0.02[a]	0.51 ±0.01[a]
n-6/n-3	5.50 ±0.02[a]	4.98 ±0.08[b]
AI	0.55	0.60
TI	0.82	0.87
HH	1.63	1.56

Results are the means of three repetitions with the respective standard deviation estimates. The results are expressed as the percentage of fatty acids. Different letters in the same line indicate significant differences ($p < 0.05$) by Tukey's test. (SFA) saturated fatty acids, (MUFA) monounsaturated fatty acids, (PUFA) polyunsaturated fatty acids, (PUFA/SFA) polyunsaturated fatty acid/saturated fatty acid ratio, (n-6/n-3) omega-6/omega-3 fatty acid ratio. AI: atherogenec index - [(C12:0+4xC14:0+C16:0)]/ΣAMUFA+Σn-6+Σn-3. TI: thrombogenic index - (C14:0 + C16:0 +C18:0)/[(0,5xΣMUFA)+(0,5xΣn-6)+(3xΣn-3)+(Σn-3/n-6)]. HH: ratios

between hypocholesterolemic and hypercholesterolemic fatty acids - (C18:1n-9+C18:2n-6+C20:4n-6+C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3)/(C14:0+C16:0).

Table 4. Absolute quantification of fatty acids (mg/g of total lipids) LNA, EPA, DHA, AA and LA

Fatty acids	Tilapia alevin	Juvenile tilapia
	Average ±dp	Average ±dp
LNA (mg/g)	4.75±0.61[a]	5.82±0.50[b]
EPA (mg/g)	1.16±0.55[a]	1.43±0.38[b]
DHA (mg/g)	14.97±0.19[a]	15.20±0.78[b]
AA (mg/g)	25.40±0.19[a]	25.63±0.68[b]
LA (mg/g)	89.01±0.85[a]	91.28±0.89[b]

Each value is the triplicate average of fatty acids LNA (Linolenic acid), EPA (Eicosapentaenoic acid), DHA (Docosahexanoic acid), AA (Arachidonic acid) and LA (Linoleic acid) expressed in mg/g of total lipids with respective estimates of standard deviations. Averages on the same line followed by different letters are meaningfully different at level 0.5%.

4. Discussion

The proximate composition of a certain type of food provides information about its nutritional potential. The knowledge of the chemical composition of fish presents a fundamental importance to the standardization of food products on the basis of nutritional criteria when it provides assistance for dietary decisions (Castagnolli, 1992).

The samples of tilapia alevins have higher percentages of moisture (78.82%) and ash (1.65%) compared to the samples of juvenile tilapia, which presented higher percentages of proteins (16.94%) and lipids (6.13%). This fact may be due to the alevins samples were in a development stage at the time of the collection, thus, the energy generated from feeding did not accumulate as fat. According to Souza and Maranhão (1998), young fish have more moisture and less fat in their meat than adult fish because they are in a developing phase and have less fat available for storage.

Ribeiro, Logato, Paula, Costa, Murgas and Freitas (2008) found moisture contents in tilapia fillets of approximately 78%, which are similar to those found in this study. Moreover, the same authors found a higher percentage of ashes (5.30%) compared to those observed in this study (1.52 to 1.65%). To Omolara and Omotayo (2008), the high moisture content is a disadvantage because it increases susceptibility to microbial spoilage and degradation of polyunsaturated fatty acids and consequently decreases the preservation time of the fishes.

The lipid content obtained by us for both alevins (2.65%) and for juveniles(6.13%) corroborates with the assessment by Beirão, Teixeira, Batista, Santo, Damian and Meinert (2004), which reported that tilapia can be considered to be a lean fish because their average fat content is below 7.0%. Furthermore, the data obtained for moisture also corroborate the values (79.16%) found by Beirão, Teixeira, Batista, Santo, Damian & Meinert (2004), who note that the moisture content of fish in general varies from 60.0 to 80.0% and that lean fish have higher moisture in their muscle structure. According to Olagunju Muhammad, Mada, Mohammed, Mohammed and Mahmoud (2012), considering their fat content, fish can be grouped into four general categories: lean fish (< 2%), low fat (2 to 4%), medium fat (4 to 8%), and high fat (> 8%). Therefore, the alevines and juveniles analyzed in this study can be considered to have a low fat and medium fat, respectively, this study was similar. The proximate composition values of the tilapia samples analyzed in this study are similar to the values found by Simões, Ribeiro, Ribeiro, Park and Murr (2007): 77.13% of moisture, 2.60% of lipids, 19.36% of protein and 1.09% of ash in tilapia fillets.

Abimbola, Kolade, Ibrahim, Oramadike and Ozor (2010) found results of approximately 18.65% protein, 0.55% lipid, 1.30% ash and 79.50% moisture in tilapia *guineensis*, whereas for tilapia *melanotheron,* the values were 18.74%, 0.70%, 1.06% and 79.50% for protein, lipid, ash and moisture, respectively. According to Furtado et al (2010), although the fish are from the same species, differences in the physical-chemical composition of the fish meat are expected because of several factors; age can be considered the main factor.

From a total of 26 fatty acids found in the tilapia samples, seven were saturated, seven were monounsaturated and twelve were polyunsaturated. The predominant fatty acids in the lipid fraction were palmitic acids (16:0), with 27.17 and 28.12%; stearic acid (18:0), with 7.80 and 7.79%; oleic acid (18:1n-9), with 29.95 and 29.41%; and linoleic acid (18:2n-6), with 10.57 and 10.84%, for the tilapia alevins and the juveniles, respectively. The same fatty acids were also prevalent in studies of tilapia conducted by Visentainer, Carvalho, Ikegaki & Park (2000), which presented an average percentage of 22.47% (16:0), 12.82% (18:0), 24.19% (18:1n-9) and 17.96% (18:2n-6). Moreover, Furtado et al (2010) found an average of 26.15% (16:0), 6.93% (18:0), 39.67% (18:1n-9) and 10.38% (18:2n-6).

According to Henderson (1996), the saturated and monounsaturated fatty acids in fish may be the result of a lipidic diet. In addition to the diet, the saturated fatty acids may be generated through non-lipidic carbon sources and then transformed into monounsaturated fatty acids.

Ramos Filho, Ramos, Hiane and Souza (2008) and Souza et al. (2007), found that the acids 16:0, 18:0, 18:1 n-9 and 18:2n-6 seem to be present in higher concentrations in freshwater species. However, Bentes, Souza, Mendonça and Simões (2009) did not observe significant differences in the saturated fatty acid content of the Gurijuba (marine fish) in relation to the Piramutaba and Dourada (freshwater fish).

For the juvenile samples, the fatty acids 18:3n-3, 20:5n-3, 18:2t-6 and 18:3n-6 showed a significantly higher percentage ($p < 0.05$) in the alevin samples. The fatty acids 20:1n-9, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-3 were significantly higher ($p < 0.05$) in in the alevin samples.

The sums of PUFA and MUFA (Table 3) were not significantly different ($p > 0.05$) between the alevin and juvenile samples but are higher to the values (PUFA = 16.7%; MUFA = 28.2%) found by Inhamuns and Franco (2001) in a study performed with Marapá muscles and the values (PUFA=0.67%; MUFA=2.67%) found by Furtado et al. (2010) in a study performed on tilapia filets.

Concerning the SFA sum, the contents presented a difference ($p < 0.05$) of 38.23% in alevins and 39.59% in juveniles; such values are lower to the values found by Inhamuns and Franco (2001) in Mapará samples (50.4%).

The ratios n-6/n-3 were (5.50) and (4.98) for tilapia alevins and juvenile tilapia, respectively. Steffens (1997) found inferior values in non-cultivated freshwater fish (0.25-1).

According to Martin, Almeida, Ruiz, Visentainer, Matshushita, de Souza and Visentainer (2006), diets based on a ratio of n-6/n-3 lower than 1:1 are not recommended because they inhibit transformation of linoleic acid into long chain polyunsaturated fatty acids (PUFA-VLC). However, the results obtained in this study corroborate those of Simopoulos (1999), who recommends a ratio of n-6/n-3 between 5 and 10.

Food with polyunsaturated and saturated fatty acids ratio (PUFA/SFA) below 0.45 have been considered as undesirable by the Department of Health and Social Security (1984); this ratio coincide with recommended by Enser et al (1998) based on information from the English Health Department: foods with ratio under 0.45 have the potential to raise blood cholesterol levels. In this study, the ratio of PUFA/SFA was 0.56 and 0.51 for alevins and juveniles, respectively; such values are higher than the value (0.45) However, the index of PUFA/SFA, which was evaluated separately, has received restrictions as does not consider the metabolic effects of monounsaturated fatty acids (Menezes, Miranda, Pinheiro, Cintra, Freire, & Cabral Júnior, 2009).

The evaluation of the nutritional quality of the lipid fraction of tilapia in two different developmental phases, alevin and juvenile phases, presented similar values to the those of the atherogenic index (IA) and thrombogenic (IT), 0.60 and 0.87, respectively, which were higher for tilapia in the juvenile phase,. However, the index related to ratio between hypocholesterolemic and hypercholesterolemic fatty acids (HH) was higher in the alevins (1.63). Testi, Bonaldo, Gatta and Badiani (2006) found superior values of HH to fish varying from 2.03 to 2.46.

Low values of IA and IT are beneficial to health according to Assunção (2007), who suggested that fatty acids are more favorable to health because they prevent the emergence of coronary diseases according to Turan, Sonmez and Kaya (2007). Moreover, the higher the ratio between hypocholesterolemic and hypercholesterolemic fatty acids, the more adequate that oil or fat is for the nutrition (Santos-Silva, Bessa & Santos-Silva, 2002; Sousa Bentes, Souza, Simões, & Mendonça, 2009). The values found in this study are similar to those found by Tonial, Bravo, de Souza, Matsushita, Furuya & Visentainer (2011) in tilapia from Nile feed that were fed rations supplemented with soybean oil for a period of 90 days; these values varied from 0,41 - 067 (IA); 0,90 - 1,09 (IT) and 1,39 - 2,10 (HH).

The polyunsaturated fatty acids LNA, EPA, DHA, AA, and LA are highly important to nutrition, especially LNA, because it is a precursor of n-3 fatty acids, and LA, because it is a precursor of n-6 fatty acids.

The fatty acids LNA, EPA, DHA, AA, and LA levels were significantly higher ($p < 0.05$) in the juvenile tilapia samples (5.82, 1.43, 15.20, 25.63, and 91.28 mg/g of total lipids, respectively) than in the alevin samples. According to the study by Visentainer (2003), the average values found for these fatty acids in tilapia samples submitted to different treatments with linseed oil were between (5.52-59.29 mg/g), (0.15-2.54 mg/g) and (9.93-26.13 mg/g) for LNA, EPA and DHA, respectively.

Tonial, Bravo, de Souza, Matsushita, Furuya and Visentainer (2011) found values that varied from 2.26 - 4.44 mg/g (LNA), 0.53 - 0.74 mg/g (EPA), 1.72 - 6.67 mg/g (DHA), 73.98 - 102.14 mg/g (LA), and 15.90 - 17.58 mg/g (AA) in samples of tilapia fed with rations supplemented with soybean oil for a period of 90 days.

The results of this study show that despite that the analyzed tilapia were not of market size, they presented a satisfactory amount of essential fatty acids for consumer health.

5. Conclusion

Although tilapia were analyzed below the weight recommended for commercialization, we conclude that the percentage of PUFA and MUFA in alevin and juvenile tilapia were high, suggesting benefits to human health.

The indexes of nutritional quality of lipids IA and IT presented low values, representing beneficial health effects from both alevins and juveniles. Despite tilapia alevins presenting lower quantities (mg/g of TL) of LNA, EPA, DHA, LA, and AA compared to juveniles, the lipid fraction of tilapias evaluated in the two different development phases allows us to consider tilapia as a rich source of essential fatty acids; therefore, as these fish have good potential as a nutritional food with beneficial effects for the consumer's health.

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