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Chemometrics applied to the incorporation of omega-3 in tilapia fillet feed flaxseed flour

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Abstract

This study evaluated the effect of adding flaxseed flour to the diet of Nile tilapia on the fatty acid composition of fillets using chemometrics. A traditional and an experimental diet containing flaxseed flour were used to feed the fish for 60 days. An increase of 18:3 n-3 and 22:6 n-3 and a decrease of 18:2 n-6 were observed in the tilapia fillets fed the experimental diet. There was a reduction in the n-6:n-3 ratio. A period of 45 days of incorporation caused a significant change in tilapia chemical composition. Principal Component Analysis showed that the time periods of 45 and 60 days positively contributed to the total content of n-3, LNA, and DHA, highlighting the effect of omega-3 incorporation in the treatment containing flaxseed flour.

Keywords: Nile tilapia; essential fatty acids; flaxseed flour; principal component analysis.

1 Introduction

The intake of fish meat and fish oil has attracted the interest of researchers due to their beneficial effects on human health (Givens & Gibbs, 2006; Kirkhus et al., 2012). The lipid fraction in fish is composed of polyunsaturated fatty acids; the omega-6 and omega-3 have gained particular attention because they have important metabolic functions, including pro- and anti-inflammatory effects, respectively. The recommended n-6:n-3 ratio ranges from 1:1 to 4:1 in order to avoid adverse effects on metabolism. However, the Western diets provide ratios of between 10:1 and 20:1, and there are reports of 50:1 (Ratnayake & Galli, 2009).

Some fish species have an enzymatic system (elongases and desaturases) that enables the conversion of linoleic acid (LA, 18:2 n-6) into arachidonic acid (AA, 20:4 n-6) and alpha-linolenic acid (ALA, 18:3 n-3) into eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic acids (DHA, 22:6 n-3). These compounds are considered essential fatty acids because they cannot be synthesized by the human body and must be taken in through the diet (Simopoulos, 2011). EPA and DHA are associated with metabolic and biochemical processes involved in the prevention of cardiovascular disease, psoriasis, arthritis, atherosclerosis, eczema, cancer, reducing depression risk, diabetes, rheumatoid arthritis, osteoporosis, cognitive decline, and neurologic dysfunctions such as Alzheimer and schizophrenia (Mateos et al., 2012; Ottestad et al., 2012).

Marine fish are considered rich in omega-3 fatty acids due to ingestion of plankton (a source of LNA). The levels of EPA and DHA in tilapia fillets in captivity are dependent on the lipid

profile of the feed offered, and their fatty acid composition can be improved through the fish farming system (Zanqui et al., 2013).

The use of vegetable oil from soybean, rapeseed, sunflower, and flaxseed has been evaluated in diets of fish species of temperate climates, and it has shown no effects on growth performance; however, some differences in the lipid composition of the muscle tissue according to the oil used have been observed (Bahurmiz & Ng, 2007).

Flaxseed has 51.8-60.4% ALA and is considered an excellent omega-3 source (Ryan et al., 2007). The use of flaxseed to enrich foods without causing radical changes in eating habits is promising (Pagamunici et al., 2014a, 2014b; Souza et al., 2014). Multivariate analysis enables extracting more information when compared to univariate analysis (Luciano & Naes, 2009), which is a chemometric tool allows for pattern recognition, the gathering of information, reduction of data dimensionality and the organization of data in different groups according to similarities in an easier to understand structure (Matos et al., 2003). Principal component analysis (PCA) is based on performing linear comparisons of the original variables. The principal components (PC) are mutually orthogonal and explain the variance decreases with an increase in the PC number (Correia & Ferreira, 2007). Therefore, this study evaluated the effect of the addition of flaxseed flour to the diet of Nile tilapia on the fatty acid composition of its muscle tissue as a function of the feeding time using chemometrics.

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2 Materials and methods

The experiment lasted 60 days and was performed in the Crow River, in the city of Diamante do Norte, Parana, Brazil (22°39'S; 052°46'O). A total of 400 tilapias with an initial live weight of 245 g were distributed into eight cages with a unit volume of 1.2 m³ (1.0 × 1.0 × 1.2 m high) and at density of 50 fishes per cage in a completely randomized design with two treatments and four replications; each tank was considered an experimental unit.

Two feeds were prepared in this experiment: a control feed, using soybean oil, and an experimental feed, where soybean oil was substituted for flaxseed flour (50 g kg⁻¹), as shown in Table 1. The experimental diets were formulated according to the nutritional requirements of the species (National Research Council, 1993). The fish were fed twice daily, at 9am and 3pm, at 2% of total biomass.

Three individuals were collected per experimental unit starting at time zero (before starting treatment) and every 15 days up to 60 days. After every collection, the fish were killed by an overdose of xylocaine (10 g L⁻¹), weighed, gutted, and filleted; the fillets were ground in a multiprocessor, homogenized, and packed in polyethylene bags. The samples were stored in a freezer (-24 °C) under a nitrogen atmosphere until analyses.

2.1 Analysis

Moisture, crude protein, and ash were determined according to Cunniff (1998), and 6.25 was used as the conversion factor of total nitrogen to crude protein. Total lipids were extracted according to the method proposed by Bligh & Dyer (1959).

To determine their fatty acid composition, the lipids were converted into fatty acid methyl esters (FAME) and methylated according to Hartman & Lago (1973). The FAME were separated in a gas chromatograph CP-3380 (Varian, USA) fitted with a flame ionization detector following the conditions used by Fuchs et al. (2013).

The retention times were compared to those of standard methyl esters (Sigma, USA). The fatty acids were identified using tricosanoic acid methyl esters (Sigma, USA) as internal standards, as described by Joseph & Ackman (1992). The peak areas were determined using the Star 5.0 software (Varian, USA), and the fatty acid concentrations were expressed in mg FA per g of total lipids.

2.2 Ethical aspects and biosecurity

This study was approved by the Ethics Committee on Animal Use in Trials of Maringá State University, File No. 011/2011, 036/2011.

2.3 Statistical and multivariate analysis

The Fatty acid composition and physicochemical analyses were carried out in triplicate. The results were submitted to variance analysis (ANOVA), and the means were compared using Tukey's post hoc and the Student "t" tests by comparing

the control and flaxseed treatment as a function of feeding time. Multivariate analysis was performed applying the Principal Component Analysis (PCA). The average of the contents of LA, AA, LNA, EPA, DHA, SFA, MUFA, PUFA, n-3, n-6, n-6:n-3; PUFA:SFA, and proximal composition that were obtained in three individual batches was used. The averages were auto-scaled using the NIPALS algorithm. Regression analyses were performed to verify the incorporation of LA, LNA, and DHA

Table 1. Control and flaxseed feed formulations.

Ingredients (g kg ⁻¹)	Feeds	
	Control	Flaxseed
Corn	389.6	408.6
Soybean meal	320.0	320.0
Fish meal	120.0	120.0
Wheat bran	80.0	80.0
Rice flour	50.0	0.0
Flaxseed whole meal	0.0	50.0
Soybean oil	19.0	0.0
Dicalcium phosphate	6.0	6.0
Salt	5.0	5.0
Vitamin and mineral supplement ^a	5.0	5.0
Lysine	1.2	1.2
Propionic acid	1.0	1.0
Vitamin C	0.8	0.8
Methionine	1.0	1.0
Threonine	1.0	1.0
Tryptophan	0.2	0.2
BHT ^b	0.2	0.2
Proximal composition ^c (g kg ⁻¹)		
Moisture	526.9±6.5	525.4±5.8
Crude protein	301.3±4.7	280.7±5.1
Ash	61.1±0.7	78.5±0.3
Total lipid	104.5±0.3	53.7±2.4
Fatty acid ^d (mg g ⁻¹ total lipid)		
14:0	7.83±0.28	5.13±0.12
16:0	155.54±5.44	124.38±2.45
18:0	15.37±0.49	10.16±0.17
16:1n-7	37.14±1.31	41.34±0.75
18:1n-9	295.69±9.51	268.62±6.22
18:2n-6 (LA)	407.96±13.29	296.12±7.27
18:3n-3 (LNA)	30.67±1.00	177.40±5.16
Parameters		
SFA	178.74±5.47	139.66±2.46
MUFA	332.83±9.60	309.96±6.27
PUFA	438.63±13.33	473.52±8.92
PUFA:SFA	2.45±0.04	3.39±0.03
n-6:n-3	13.30±0.05	1.67±0.04

SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids. ^aAssurance levels per kilogram of product: Vitamin A (24,000 UI); Vitamin K (30 mg); Vitamin B1 (40 mg); Vitamin B6 (35 mg); Vitamin (80 mg); Folic acid (12 mg); Pantothenate Calcium (100 mg); Vitamin C (600 mg); Biotin (2 mg); Choline (1000 mg); iron (200 mg); Copper (35 mg); Manganese (100 mg); Zinc (240 mg); Iodine (1.6 mg); Cobalt (0.8 mg). ^bButil Hidroxy Toluene. ^cMean and standard deviation. ^dOther fatty acids identified: 14:1n-9, 14:1n-7, 15:0, 16:1n-9, 16:1n-5, 17:0, 17:1n-11, 17:1n-9, 17:1n-7, 18:1n-7, 18:1n-11, 18:1n-5, 20:0, 20:1n-9, 20:1n-7, 21:0, 20:2n-6, 20:3n-6, 22:0, 22:4n-6, 22:5n-6, 24:0, 24:1n-9.

fatty acids into the muscle tissue of fish fed with flaxseed. The software Statistica (StatSoft, 2007), version 8.0, was used with a 5% ($p < 0.05$) significance level for rejection of the null hypothesis.

3 Results and discussion

The proximal and fatty acid composition of the diets used in the experiment are shown in Table 1. The diets had different fatty acid compositions. The diet containing flaxseed flour had a higher content of LNA because of the fatty acid composition of this ingredient.

Table 2 shows the proximal composition of muscle tissues analyzed in each treatment. Moisture and ash content did not differ ($p < 0.05$) during the study period for both treatments. In the control diet there was a significant difference ($p < 0.05$) in protein content between the experimental times, but there was no significant difference ($p < 0.05$) between the diets according to the t test. In the present study, total lipids ranged from 2.5 to 3.5% without significant difference ($p < 0.05$) between the experimental times and the treatments. Simões et al. (2007) obtained a proximal composition similar to that of the present study.

Table 3 and Figure 1 show the principal component analysis (PCA). The NIPALS algorithm enabled us to select PC1 and PC2, which were significant ($p < 0.05$) and explained 54.50 and 38.99% of the data variance, respectively. As can be seen in Figure 1, PC1 allowed to set apart the times 0, 15, and 30 days for both treatments and 45 days for the flaxseed treatment due to the positive contributions of the contents of ash and moisture. Analyzing PC2 (Figure 1 and Table 3), the time periods of 45 and 60 days indicate that both diets positively contributed to increase the crude protein and moisture content.

As for the fatty acids, an increase in n-3 PUFA was observed over time in the treatment supplemented with flaxseed, particularly LNA, (Table 3). The incorporation can be evidenced by linear regression analysis using the equation: $Y = 9.2880 + 0.2274 * X$, with $R^2 = 0.9087$ and high Pearson correlation coefficient $r = 0.9532$. This may demonstrate the influence of LNA from the flaxseed on the lipid composition, with an

increase of 52% after 60 days. The studies of Justi et al. (2003), Matsushita et al. (2006) and Aguiar et al. (2011), which included the use of flaxseed aiming at the incorporation of n-3 into muscle tissue, also described satisfactory effects; however, these authors used flaxseed oil. Analyzing the time periods of 45 and 60 days, it can be seen that there was no significant difference ($p < 0.05$) in the incorporation of LNA. This effect was described by Tonial et al. (2009), when assessing the addition of flaxseed oil as a source of n-3 in the diet adult tilapia. These authors found an increase in the levels of LNA in the muscle tissue up to 45 days, which remained constant until the treatment period of 90 days was completed.

As can be seen in Figure 2, PC1 allowed to distinguish the control treatment because of the positive contributions of LA, AA, SFA, MUFA, n-6 and n-6:n-3. Analyzing PC1 and PC2 (Figure 2 and Table 3), the time periods of 15 and 60 days of the flaxseed treatment showed positive contributions to the

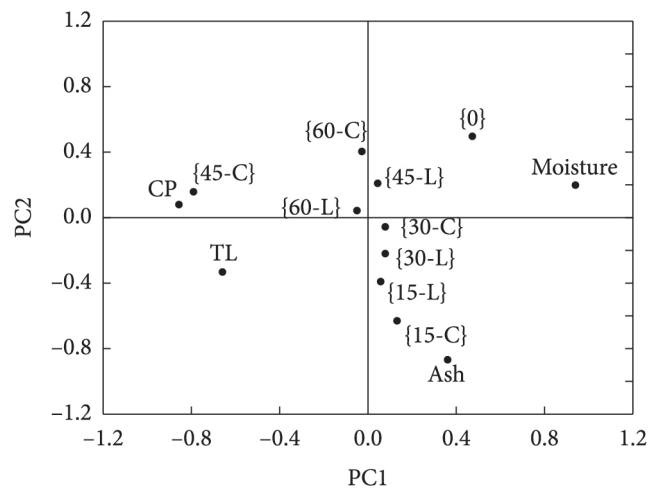


Figure 1. Principal component analysis of proximal composition of the treatments. PC: Principal Component. CP: crude protein. TL: Total lipid. {0}: initial time of the experiments of the control and linseed treatments; {15-C}, {30-C}, {45-C} and {60-C}: experimental times of the control treatment; {15-L}, {30-L}, {45-L} and {60-L}: experimental times of the linseed treatment.

Table 2. Proximal composition of Tilapia fillets.

Parameters	Time (days)				
	0	15	30	45	60
Control					
Moisture	76.17 ^{Aa} ±0.46	74.35 ^{Ac} ±0.67	74.61 ^{Ac} ±0.09	72.34 ^{Bb} ±0.82	74.26 ^{Ac} ±1.09
Ash	1.04 ^{Ac} ±0.01	1.26 ^{Aa} ±0.03	1.14 ^{Ab} ±0.07	1.00 ^{Ac} ±0.02	1.00 ^{Bc} ±0.06
Total lipid	2.53 ^{Aa} ±0.23	3.12 ^{Aa} ±0.70	2.80 ^{Aa} ±0.35	3.55 ^{Aa} ±0.75	2.96 ^{Aa} ±0.43
Crude protein	20.22 ^{Ab} ±0.77	20.59 ^{Ab} ±0.37	21.11 ^{Aa} ±0.16	22.60 ^{Aa} ±0.83	20.67 ^{Ab} ±1.12
Linseed					
Moisture	76.17 ^{Aa} ±0.46	74.09 ^{Ab} ±0.92	74.66 ^{Aab} ±0.51	74.33 ^{Ab} ±0.83	73.96 ^{Ab} ±0.82
Ash	1.04 ^{Aa} ±0.01	1.18 ^{Bb} ±0.02	1.16 ^{Ab} ±0.06	1.08 ^{Aab} ±0.10	1.10 ^{Aab} ±0.03
Total lipid	2.53 ^{Aa} ±0.23	3.20 ^{Aa} ±0.30	3.11 ^{Aa} ±0.22	2.58 ^{Aa} ±0.46	2.78 ^{Aa} ±0.27
Crude protein	20.22 ^{Aa} ±0.77	20.40 ^{Aa} ±0.39	20.81 ^{Aa} ±0.27	21.17 ^{Ba} ±0.78	21.20 ^{Aa} ±0.87

Means obtained from analysis performed in triplicates followed by standard deviation. Same uppercase letters in the same column for the same parameter analyzed do not show significant difference ($p < 0.05$) between the treatments. Same lowercase letters in the same line do not show significant difference ($p < 0.05$) over time.

Table 3. Eigen analysis of the correlation matrix loadings of the significant principal component (PC) of the fatty acids and proximal composition of the treatments.

	Proximal composition	
	PC1	PC2
Eigenvalues	3.0805351	1.9881894
% Total variance	23.696424	15.293765
Moisture	0.942311	0.197904
Ash	0.359125	-0.870550
Total Lipid	-0.658702	-0.337443
Protein	-0.857940	0.084077
	Fatty acid	
	PC1	PC2
Eigenvalues	8.3980760	3.0477510
% Total variance	39.990838	14.5131
18:2n-6 (LA)	0.951105	0.103293
18:3n-3 (LNA)	-0.946017	0.107835
20:4n-6 (AA)	0.949895	-0.030956
20:5n-3 (EPA)	-0.351285	-0.283959
22:6n-3 (DHA)	-0.874725	0.106105
SFA	0.579596	-0.726977
MUFA	0.260461	-0.543966
PUFA	0.655780	0.528182
n-6	0.942007	0.255775
n-3	-0.972420	0.102177
n-6:n-3	0.988349	-0.095847
PUFA:SFA	0.224829	0.899655

PC: Principal component. SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids.

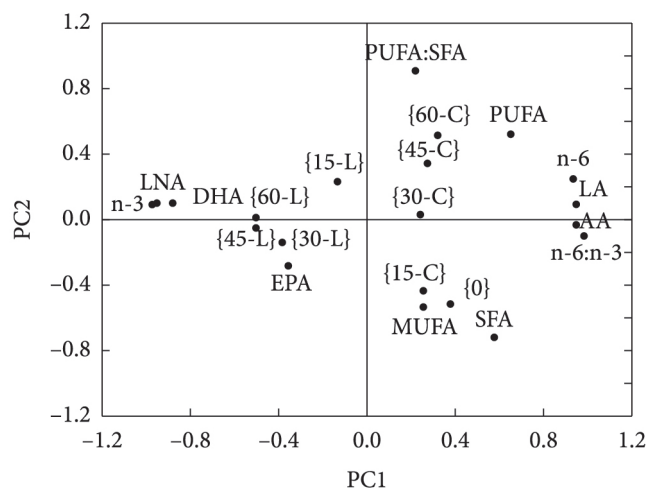


Figure 2. Principal component analysis of essential fatty acids and the relationship between them in both treatments. AA: arachidonic acid; LNA: alpha-linolenic acid; EPA: eicosapentanoic acid; DHA: docsaheaxanoic acid; LA: linolenic acid; SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-6: total omega-6 fatty acids and n-3: total omega-3 fatty acids. {0}: initial time of the experiments of the control and linseed treatments; {15-C}, {30-C}, {45-C} and {60-C}: experimental times of the control treatment; {15-L}, {30-L}, {45-L} and {60-L}: experimental times of the linseed treatment.

total content of n-3, LNA and DHA, highlighting the effect of incorporation of omega 3 fatty acids through supplementation with flaxseed flour.

As can be seen in Table 3, the EPA content (20:5 n-3) of fillets from fish fed with flaxseed did not differ significantly through the time. The fishes have two enzymes, elongase and desaturase, which act in the bioconversion of LNA into EPA. They also convert EPA into DPA (docosapentaenoic acid) and DPA into DHA. Therefore, EPA and DPA are considered secondary metabolites and are converted into DHA via metabolic pathway. Due this conversion, some variations in the contents of EPA and DPA may be observed (Perini et al., 2010). Table 4 also shows that feed supplemented with flaxseed flour significantly increased ($p < 0.05$) the level of DHA (22:6 n-3) in the fillets. This increase was around 73% (Table 4). The incorporation of DHA could be verified by linear regression analysis using the equation: $Y = 7.9040 + 0.0908 \cdot X$, with $R^2 = 0.9098$ and high Pearson correlation coefficient $r = 0.9538$. These data confirm the fatty acid conversion of in the fish metabolism during farming.

In the control treatment, there were significant variations ($p < 0.05$) in the levels of EPA, but DHA remained constant during the experimental period. According to Visentainer et al. (2003), Nile tilapia has low levels of LNA, EPA, DHA, and other n-3 fatty acids in their muscle tissue when compared to those of marine fish, according to Luzia et al. (2003). Blanchet et al. (2005) found 0.322 g kg of DHA in wild trout and tilapia fed with flaxseed flour.

The level of LA (18:2 n-6) in the control treatment varied throughout the experiment (Table 4); however, there was no significant difference between T0 and T60. In the flaxseed treatment, there was a significant decrease of 15% ($p < 0.05$) (Table 4). This was probably due to the absence of soybean oil (as a source of n-6) in the feed. Using regression analysis, a decrease in LA was verified by the equation: $Y = 128.3360 - 0.3540 \cdot X$, with $R^2 = 0.8355$ and Pearson correlation coefficient $r = 0.9140$. During the experiment, the concentration of AA decreased in the flaxseed treatment. This fact may be justified by the competition for $\Delta 6$ desaturase enzyme, which catalyze the conversion of LA and LNA into AA and DHA, respectively. The n-6:n-3 ratio of the flaxseed diet was close to 1:1, but the fish metabolism has a preference for LNA fatty acid resulting in a lower concentration of AA in the tilapia fillets (Perini et al., 2010).

The changes in LA and LNA fatty acids in the treatments led to a decrease in the n-6:n-3 ratio. According to Simopoulos (2011), the scientific literature suggests that there has been an improvement in human diet; it used to have n-6:n-3 ratio of 1:1, but today the (mainly Western diets) ratios range from 10:1 to 25:1). These data indicate that current diets have low levels of n-3 fatty acids, which can causing many problems in the human body because they are important components of cell membranes. The n-6:n-3 balance is essential for the maintenance of normal development and homeostasis throughout the life cycle. Visentainer et al. (2005) reports that high values of the n-6:n-3 ratio generate an imbalance of fatty acids in the human body and probably contribute to the development of inflammatory processes, immune system disorders, hypertension, and neurological disorders. During

Table 4. Absolute quantification of fatty acids in the fillets.

Fatty acids* (mg g ⁻¹)	Time (days)																			
	0				15				30				45				60			
	Control	Linseed	Control	Linseed	Control	Linseed	Control	Linseed	Control	Linseed	Control	Linseed	Control	Linseed	Control	Linseed	Control	Linseed		
14:0	23.14 ^{Abb} ±0.02	23.14 ^{Ab} ±0.02	20.41 ^{Ac} ±0.88	19.44 ^{Abb} ±0.88	23.96 ^{Abc} ±1.06	20.80 ^{Bbb} ±1.59	20.96 ^{Abc} ±1.26	21.84 ^{Abb} ±1.59	21.27 ^{Abc} ±1.39	21.10 ^{Abb} ±1.94										
16:0	248.82 ^{Aa} ±5.02	248.82 ^{Ab} ±5.02	239.64 ^{Bbb} ±4.22	224.57 ^{Abb} ±4.05	240.19 ^{Abb} ±1.76	221.35 ^{Bbb} ±3.72	236.69 ^{Abb} ±5.27	222.97 ^{Bbb} ±3.10	233.52 ^{Abb} ±1.67	219.43 ^{Bbb} ±6.07										
16:1n-7	45.37 ^{Abb} ±0.22	45.37 ^{Ab} ±0.22	44.90 ^{Abb} ±0.78	43.96 ^{Abb} ±1.63	46.18 ^{Abb} ±2.91	43.42 ^{Ab} ±2.27	47.40 ^{Aa} ±1.61	44.19 ^{Abb} ±2.29	42.92 ^{Ab} ±2.00	43.64 ^{Ab} ±3.53										
18:0	66.38 ^{Aa} ±0.20	66.38 ^{Ab} ±0.20	61.56 ^{Abb} ±1.79	57.18 ^{Bbb} ±1.80	60.13 ^{Ab} ±3.28	59.27 ^{Ab} ±2.18	58.60 ^{Abb} ±1.87	58.85 ^{Abb} ±4.01	59.66 ^{Ab} ±0.81	58.85 ^{Ab} ±2.81										
18:1n-9	304.57 ^{Aa} ±0.50	304.57 ^{Ab} ±0.50	287.90 ^{Abb} ±5.76	284.90 ^{Aa} ±13.44	283.31 ^{Ab} ±13.88	295.87 ^{Aa} ±3.02	285.86 ^{Abb} ±4.74	289.30 ^{Aa} ±9.25	288.33 ^{Abb} ±9.30	290.24 ^{Aa} ±11.45										
18:2n-6	130.87 ^{Aa} ±1.41	130.87 ^{Ab} ±1.41	128.46 ^{Abb} ±2.17	123.92 ^{Bb} ±2.30	128.81 ^{Aab} ±0.44	112.12 ^{Bb} ±3.00	126.98 ^{Ab} ±0.24	110.78 ^{Bb} ±2.25	129.34 ^{Abb} ±0.24	110.89 ^{Bb} ±6.90										
18:3n-3	7.51 ^{Aa} ±0.13	7.51 ^{Ab} ±0.13	7.60 ^{Ba} ±0.32	13.82 ^{Ab} ±2.55	9.70 ^{Bb} ±0.26	17.25 ^{Abc} ±1.49	9.60 ^{Bb} ±0.13	20.99 ^{Ac} ±4.37	9.60 ^{Bb} ±0.30	20.98 ^{Ac} ±3.39										
20:4n-6	225.39 ^{Ab} ±0.35	225.39 ^{Ab} ±0.35	15.62 ^{Abb} ±0.69	0.47 ^{Bb} ±0.08	13.63 ^{Aa} ±0.41	0.40 ^{Bb} ±0.06	15.04 ^{Ab} ±0.17	0.32 ^{Bb} ±0.07	16.28 ^{Ab} ±1.09	0.38 ^{Bb} ±0.07										
20:5n-3	0.51 ^{Aa} ±0.05	0.51 ^{Ab} ±0.05	0.39 ^{Abc} ±0.02	0.34 ^{Ab} ±0.05	0.44 ^{Abb} ±0.05	0.43 ^{Abb} ±0.02	0.35 ^{Ac} ±0.02	0.47 ^{Abb} ±0.16	0.32 ^{Bc} ±0.04	0.54 ^{Ab} ±0.06										
22:6n-3	7.24 ^{Aa} ±0.73	7.24 ^{Ab} ±0.73	6.51 ^{Ba} ±0.58	9.71 ^{Aab} ±2.33	6.24 ^{Ba} ±0.30	10.94 ^{Ab} ±1.11	6.70 ^{Bb} ±0.29	12.69 ^{Ab} ±1.25	6.99 ^{Bb} ±0.70	12.56 ^{Ab} ±2.27										
sums and ratios																				
SFA	362.32 ^{Aa} ±0.36	362.32 ^{Ab} ±0.36	350.44 ^{Abb} ±5.38	312.29 ^{Bb} ±5.38	338.26 ^{Ac} ±2.68	311.59 ^{Bb} ±4.35	327.91 ^{Ad} ±4.46	312.19 ^{Ab} ±4.49	326.76 ^{Ad} ±1.90	309.19 ^{Bb} ±8.03										
MUFA	408.74 ^{Aa} ±0.84	408.74 ^{Ab} ±0.84	385.81 ^{Ab} ±6.34	375.11 ^{Ab} ±13.26	384.22 ^{Ab} ±14.12	385.41 ^{Aab} ±6.23	389.69 ^{Ab} ±5.16	380.43 ^{Ab} ±6.79	388.38 ^{Ab} ±10.49	381.89 ^{Bb} ±12.05										
PUFA	202.08 ^{Abc} ±1.72	202.08 ^{Ab} ±1.72	186.78 ^{Aa} ±2.81	188.15 ^{Aa} ±9.72	197.23 ^{Ac} ±1.44	178.06 ^{Bb} ±5.98	201.77 ^{Abc} ±3.73	179.76 ^{Ba} ±10.10	206.31 ^{Ab} ±4.00	181.90 ^{Ba} ±12.50										
n-6	185.98 ^{Ac} ±1.55	185.98 ^{Ab} ±1.55	171.42 ^{Ab} ±2.40	163.85 ^{Ab} ±7.24	179.95 ^{Ab} ±1.28	148.94 ^{Bbc} ±5.06	184.21 ^{Abc} ±3.42	145.22 ^{Bc} ±6.36	188.47 ^{Ac} ±3.35	147.41 ^{Bbc} ±13.23										
n-3	16.1 ^{Aa} ±0.74	16.1 ^{Ab} ±0.74	15.36 ^{Ba} ±0.84	27.33 ^{Ab} ±3.45	17.28 ^{Bbc} ±3.07	32.73 ^{Abc} ±2.44	17.56 ^{Bbc} ±0.41	38.85 ^{Ac} ±4.69	17.84 ^{Bb} ±1.01	38.94 ^{Ac} ±1.56										
n-6:n-3	11.57 ^{Ab} ±0.04	11.57 ^{Ab} ±0.04	11.18 ^{Abb} ±0.60	6.05 ^{Bb} ±0.59	10.42 ^{Ab} ±0.32	4.57 ^{Bc} ±0.31	10.49 ^{Ab} ±0.18	3.77 ^{Bc} ±0.37	10.59 ^{Ab} ±0.52	3.79 ^{Bc} ±0.38										
PUFA:SFA	0.56 ^{Abc} ±0.01	0.56 ^{Ab} ±0.01	0.53 ^{Bc} ±0.01	0.60 ^{Aa} ±0.04	0.58 ^{±0.01}	0.57 ^{Ba} ±0.02	0.62 ^{Aa} ±0.02	0.58 ^{Aa} ±0.04	0.63 ^{±0.01}	0.59 ^{Ba} ±0.05										

Means obtained in the analysis performed in triplicates followed by standard deviation. Same uppercase letters in the same line and time do not show significant difference between the treatments. Same lowercase letters in the same line for the same treatment do not show significant difference over time. *Concentration (mg g⁻¹ total lipids) of fatty acids in tilapia fillets for the control and flaxseed treatments.

this experiment, the diet with flaxseed showed an n-6:n-3 ratio ranging from 11.6:1 to 3.8:1; the latter was observed after 45 days of cultivation. Ramos Filho et al. (2008) found the n-6:n-3 ratio of 3.65 for other freshwater fish such as *Piaractus mesopotamicus*.

The PUFA: SFA ratio remained constant for the fish fed the flaxseed diet. According to Enser et al. (1998), the expected value for PUFA:SFA must be greater than 0.45 since according to these authors, lower ratios indicate unhealthy meat, especially in terms of cardiovascular disease risk. Accordingly, foods with higher PUFA:SFA indicate higher nutritional value.

4 Conclusion

The addition of flaxseed flour in the diet of tilapia provided incorporation of satisfactory levels of n-3 fatty acids. Increased levels of omega 3 fatty acids in the fish muscle tissue were observed during the time the fish were fed the experimental flaxseed diet.

A time period of 45 days was enough to produce a distinctive fish. There were increases in the levels of LNA, EPA, and DHA in the fish fed flaxseed flour, but a lower n6:n3 ratio was found. Therefore, the dietary supplementation with flaxseed and the period of time it was given contributed to increase the nutritional quality of the fatty acids in tilapia muscle tissue. Analyzing PC1 and PC2, the time periods of 15 and 60 days of the flaxseed treatment showed positive contributions to the total content of n-3, LNA and DHA, highlighting the effect of incorporation of omega 3 fatty acids through supplementation with flaxseed flour.

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