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Influence of diets enriched with flaxseed oil on the α-linolenic, eicosapentaenoic and docosahexaenoic fatty acid in Nile tilapia (*Oreochromis niloticus*)

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Abstract

This study examined the effects of different levels of flaxseed oil in increasing the α-linolenic (LNA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids contents in tilapias raised in captivity. Nile tilapia were raised in captivity for a period of five months, receiving increasing levels (0%; 1.25%; 2.50%; 3.75% and 5.00%) of flaxseed oil in substitution for sunflower oil (control). No significant differences ($P > 0.05$) of moisture or total lipids contents were found among fillets from tilapia fed the different diets. Analyses of the fatty acid methyl esters (FAMEs) were quantitatively measured by capillary gas chromatography against a $C_{23:0}$ internal standard. Increases of the concentration of LNA, EPA and DHA (in mg/g of total lipids), were well established in the fillets, with a significant difference ($P < 0.05$) among all the treatments, as the replacement of the sunflower oil by flaxseed oil was increased.

Keywords: Flaxseed oil; Nile tilapia; LNA; EPA; DHA

1. Introduction

In Brazil, in recent years, there has been an increase in the production and consumption of freshwater fish reared in aquaculture systems, mainly the Nile tilapia (*Oreochromis niloticus*). However, studies have shown that these freshwater fish, including the tilapia, have low contents of α-linolenic (LNA, 18:3$n-3$), eicosapentaenoic (EPA, 20:5$n-3$) and docosahexaenoic (DHA, 20:5$n-3$) fatty acids, when compared to those from the sea or from natural habitats (Andrade, Rubira, Matsushita, & Souza, 1995; Andrade, Visentainer, Matsushita, & Souza, 1996; Justi, Hayashi, Visentainer, Souza, & Matsushita, 2003; Luzia, Sampaio, Castellucci, & Torres, 2003). It is well established, in humans, that an increase in the ingestion of long-chain polyunsaturated fatty acids (LC-PUFA), especially EPA and DHA, in diet reduces the risk of heart disease (Steffens, 1997) and rheumatoid arthritis (Kromann & Green, 1980). Consumption of LC-PUFA and fish oils is also asserted to reduce the biochemical factors associated with cancer (Kimura, Takaku, Nakajima, & Okuda, 2001), psoriasis (Mayser et al., 1997), and human infertility (Conquer, Martin, Tummon, Watson, & Tekpetey, 2000). Researchers in Brazil have demonstrated that commercial feeds supply low levels of $n-3$ fatty acids and high levels of $n-6$ fatty acids (Maia, 1992; Moreira, Visentainer, Souza, & Matsushita, 2001). The diet of the fish has a great influence on their general chemical composition, and particularly on their fatty acid composition (Henderson & Tocher, 1987). Flaxseed oil is one of the world's most important vegetable sources of LNA (Wanasundara & Shahidi, 1994), a precursor of the LC-PUFA $n-3$ polyunsaturated fatty acid series

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(Hendersom, 1996). In recent study, the fatty acid compositions of the feeds at their time of supply were reflected directly in the Nile tilapia composition. The fish that received, during 30 days, a diet with addition of flaxseed oil presented the highest content of $n-3$ polyunsaturated fatty acids ($n-3$ PUFA) and a smaller $n-6/n-3$ ($n-6$ PUFA/$n-3$ PUFA) ratio (Justi et al., 2003). The purpose of this work was to investigate incremented addition of flaxseed oil in substitution for the sunflower oil in the feeds and their influences on the amounts of the LNA, EPA and DHA (in mg/g of total lipids) of Nile Tilapia (*Oreochromis niloticus*) maintained in captivity for five months.

2. Materials and methods

2.1. Sampling

The experiments were carried out in the Aquaculture Laboratory of the Biology Department of State University Maringá. It utilized 125 tilapia (*Oreochromis niloticus*) with initial mean individual weights of 88 ± 6 g, distributed in 25 ponds (1000 l water capacity) in five treatments and five duplications. The treatments consisted of addition of flaxseed oil (0%; 1.25%; 2.50%; 3.75% and 5.00%) in substitution for sunflower oil (control) in feeds (Table 1). After five months, the fish were killed, filletted and held in polyethylene packing (in N_2 atmosphere) at −18 °C. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature, and homogenized.

2.2. Analysis

The moisture content in fillets and feeds was determined as described by Cunniff (1998), and the total lipids (TL) were determined by the Bligh and Dyer (1959) method. The fatty acid methyl esters (FAME) were prepared by methylation of the tracylglycerols, as described by method of Joseph and Ackman (1992). Methyl esters was separated by gas chromatography using a Varian 3300 (USA) gas chromatograph fitted with a flame ionization detector (FID) and a fused-silica DB-WAX capillary column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA). The operation parameters were as follows: detector temperature, 280 °C; injection port temperature, 250 °C; column temperature, 170 °C for 16 min, programmed to increase at 2 °C/min up to 210 °C, with final holding time of 25 min; carrier gas, hydrogen at 0.8 ml/min, linear velocity of 38 cm/s, with an oxygen filter coupled to the feed line; nitrogen was used as the makeup gas at 30 ml/min; split injection at 1:50 ratio. For identification of the LNA, EPA and DHA acids, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma, St. Louis, MO). Equivalent chain-length values (ECL) were used (Stràsky, Jursík, & Vitek, 1997; Thompson, 1996), as

Table 1
Composition of experimental feeds[A]

Ingredients[a] (%)	Treatments				
	A	B	C	D	E
Corn	16.93	16.93	16.93	16.93	16.93
Soybean meal	51.62	51.62	51.62	51.62	51.62
Wheat meal	20.00	20.00	20.00	20.00	20.00
Sugarcane silage	1.28	1.28	1.28	1.28	1.28
Calcareous	1.74	1.74	1.74	1.74	1.74
Bicalcium phosphate	2.41	2.41	2.41	2.41	2.41
Flaxseed oil	0.00	1.25	2.50	3.75	5.00
Sunflower oil	5.00	3.75	2.50	1.25	0.00
BHT[B]	0.02	0.02	0.02	0.02	0.02
NaCl	0.50	0.50	0.50	0.50	0.50
Premix[C]	0.50	0.50	0.50	0.50	0.50
Composition[D]					
Total lipids (%)	7.6 ± 0.3a	7.7 ± 0.4a	8.0 ± 0.6a	8.0 ± 0.7a	7.8 ± 0.3a
Moisture (%)	9.7 ± 1.4a	9.6 ± 1.4a	9.6 ± 1.6a	9.4 ± 1.70a	9.9 ± 2.0a
LNA (mg/100 g)	13.6 ± 2.0a	79.4 ± 3.8b	140.2 ± 13.0c	202.1 ± 11.6d	272.4 ± 8.6e
EPA	nd[E]	nd	nd	nd	nd
DHA	nd	nd	nd	nd	nd

[a,b,c,d,e] Different letters in the same line are significantly different ($P < 0.05$) by Tukey's test.
[A] Data provided by Aquaculture Laboratory – Department of Biology of the State University of Maringá.
[B] Butylated hydroxytoluene (antioxidant).
[C] Mineral and vitamin supplement.
[D] Results expressed as averages of the three replicates.
[E] Not detected.

Table 2
Total lipids, moisture and LNA, EPA and DHA contents of the tilapia fillets[A,B]

Composition	Treatments[A]				
	A	B	C	D	E
Total lipids (g/100 g)	1.1 ± 0.2a	1.1 ± 0.2a	1.2 ± 0.3a	1.2 ± 0.2a	1.1 ± 0.2a
Moisture (g/100)	77.4 ± 0.7a	76.8 ± 0.4a	77.2 ± 0.7a	77.3 ± 1.2a	76.9 ± 0.4a
LNA[C] (mg/g)	6.5 ± 1.8a	18.8 ± 3.0b	34.2 ± 3.3c	55.3 ± 7.3d	59.3 ± 7.5e
EPA[D] (mg/g)	0.1 ± 0.0a	0.8 ± 0.1b	1.4 ± 0.1c	2.0 ± 0.2d	2.5 ± 0.4e
DHA[E] (mg/g)	9.9 ± 2.6a	16.8 ± 2.2b	22.7 ± 2.7c	25.9 ± 2.6d	26.1 ± 2.0e

[A] Treatments: A (0.00%); B (1.25%); C (2.50%); D (3.75%) e E (5.00%) of flaxseed oil completed up to 5.00% with sunflower oil.
[B] Results expressed as an average of 30 replicates. Averages followed by different letters in the same line are significantly different ($P < 0.05$) by Tukey's test.
[C] LNA: α-linolenic acid.
[D] EPA: eicosapentaenoic acid.
[E] DHA: docosahexaenoic acid.

well as a coupled system of a gas chromatograph–mass spectrometer Shimadzu QP 5000 and fragmentation by electron impact, 70 eV. Retention times and peaks area percentages were automatically computed by a Varian 4290 integrator. Quantification of the LNA, EPA and DHA (in mg/g of total lipids), were made against a $C_{23:0}$ internal standard from Sigma (USA), as described by Joseph and Ackman (1992).

2.3. Statististcs

The values of the means were statistically compared by Tukey's test at 5% with one-way ANOVA. Data were processed using the Statistica soft-ware (StatSoft, USA, 1996).

3. Results and discussion

There were no significant differences ($P > 0.05$) in total lipids and moisture contents of feeds (Table 1) among the treatments.

The increase of the concentration of LNA acid was well established; values between 13.6 and 272 mg/100 g of the total lipids were found in the feeds, with a significant difference ($P < 0.05$) among the treatments with additional flaxseed oil. EPA and DHA were not detected in the feeds (Table 1). Fatty acid profiles of commercial feeds used in the treatment of species grown in fish farms in Brazil, presented low values of LNA (3.3%) and a high value of LA-18:2$n-6$ (38.8%) (Moreira et al., 2001).

The values of the experimental FID detector correction factor were determined experimentally for the LNA, EPA and DHA, to be 0.97 ± 0.02, 0.99 ± 0.02 and 0.98 ± 0.02, respectively. The theoretical values, correction, factors are 1.01 for LNA, 0.99 for EPA and 0.97 for DHA (Craske & Bannon, 1988); these values are close to (LNA and DHA) or the same (EPA) as the experimental values. Thus, in the determination of the concentration of the fatty acids, the theoretical FID factor could be used as recommended by Bannon, Craske, and Hilliker (1986).

Total lipid contents ranged from 1.1 to 1.2 g/100 g of tilapia fillet, classified as lean fish (<2% of TL) by Ackman (1989). According to Ogawa and Koike (1987), the moisture in fish ranged from 70% to 85% on average, and in this experiment the values obtained ranged from 76.8% to 77.4%. Total lipids and moisture contents did not show any significant differences ($P > 0.05$) among the treatments.

The same enzymes serve to elongate both $n-3$ and $n-6$ fatty acids. There is therefore a competitive effect in that an excess of ALA could interfere with conversion of 18:2$n-6$ to 20:4$n-6$, a highly desirable fatty acid (Ackman, 1996). In this study, the value of the 20:4$n-6$ ranged from 3.96% to 2.62%.

LNA is a precursor of the $n-3$ fatty acid series and only LNA was present in the different feeds for this experiment, but with increasing amounts (Table 1). In conversions by elongation and desaturation of the series, EPA and DHA in the fillets of the tilapia (Table 2), and some of the LNA, were not converted but stored in the fillets. Increases of the concentrations of LNA, EPA and DHA (in mg/g of total lipids), were well established in the fillets, with a significant difference ($P < 0.05$) among all the treatments, as the substitution of the sunflower oil by flaxseed oil was increased. The LNA, EPA and DHA concentrations in the tilapia fillet increased significantly in the A to D treatments. While the contents of LNA and DHA stabilized with increase of the concentration of LNA in the feeds, after the D treatment, the EPA concentration still tended to increase. Therefore, increasing the amounts of LNA in the feed can markedly increase the amounts of EPA in the tilapia fillets relative to DHA.

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