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Incorporation and fatty acid composition in liver of Nile tilapia fed with flaxseed oil

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ABSTRACT. One of the most consumed freshwater fish in South America is Nile tilapia. The present study examined the effects of flaxseed oil (FO), source of alfa-linolenic acid (LNA), on the total lipid composition and polyunsaturated fatty acid, n-6 and n-3 PUFA, contents on Nile tilapia (*Oreochromis niloticus*) liver. Tilapias were given diets with increasing levels 0.00, 1.2, 2.50, 3.75 and 5.00% (w w^{-1}) of FO as a replacement of sunflower oil for five months. Fatty acids analysis of methyl esters revealed 45 fatty acids common to all treatments. The increase of flaxseed oil resulted in a decrease in total n-6 PUFA (35.1 to 21.1%) and an increase in n-3 PUFA (3.3 to 18.5%). The diet with LNA underwent sequential desaturation and elongation in liver, leading to an increase in all n-3 PUFA and a decrease in n-6/n-3 ratios (10.7 to 1.1). The manipulation of fatty acids with FO may be used to increase n-3 PUFA and to help balance n-6/n-3 PUFA in dietary supplements, thus, the liver tilapia becomes one product with major nutritional value.

Keywords: fatty acids manipulation, lipids, Omega-3.

RESUMO. Incorporação e composição de ácidos graxos no fígado de tilápia-do-Nilo alimentada com óleo de linhaça. A tilápia-do-Nilo é um dos peixes de água doce mais consumido na América do Sul. No presente experimento foram avaliados os efeitos do óleo de linhaça, fonte do ácido alfa-linolênico (LNA) sobre a composição de lipídios totais e dos ácidos graxos poli-insaturados (AGPI) das séries n-6 e n-3, contidos no fígado de tilápia-do-Nilo (*Oreochromis niloticus*). As tilápias receberam dietas com níveis de óleo de linhaça de 0,00; 1,25; 2,50; 3,75 e 5,00% (massa $massa^{-1}$), em substituição ao óleo de girassol, por cinco meses. Nas análises dos ésteres metílicos de ácidos graxos foram detectados 45 ácidos graxos comuns em todos os tratamentos. O aumento na ingestão de óleo de linhaça resultou na diminuição do total de AGPI n-6 de 35,1 para 21,1% e um aumento de AGPI n-3 de 3,3 para 18,5% no fígado. O LNA, no fígado da tilápia, sofreu sequencial dessaturação e elongação, levando a um aumento de todos os AGPI n-3 e diminuição da razão n-6/n-3 de 10,7 para 1,1. A manipulação de ácidos graxos com óleo de linhaça pode ser utilizada para aumentar a concentração de ácidos graxos AGPI n-3 e balancear a razão n-6/n-3 no fígado da tilápia, tornando-se um subproduto da pesca com maior potencial nutritivo.

Palavras-chave: manipulação de ácidos graxos, quantificação de lipídios, Omega-3.

Introduction

Tilapia is one of the most cultured freshwater fish worldwide (YASMIN et al., 2004). Recent studies indicate that some fish parts not used as food are appropriate for human nutrition (ARRUDA, 2004) and may be used in fish oil extraction. Research has shown the existence of significant concentrations of n-3 polyunsaturated fatty acids (n-3 PUFA) in visceras (SOUZA et al., 2005) and head (MOREIRA et al., 2003).

The importance of fish as a source of polyunsaturated fatty acids (PUFA), particularly n-3 fatty acids in human nutrition is widely recognized (SIMOPOULOS, 2004). These fatty acids (n-3 PUFA) have been associated to numberless benefits to human health. In general, they contribute to the prevention of heart diseases (LEE; LIP, 2003; LEIGH-FIRBANK et al., 2002; KRIS-ETHERTON et al., 2002).

In freshwater fish, linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3) are metabolized by the same sequential desaturation and elongation enzyme systems, which results in the production of long-chain PUFA n-3 and n-6 series (HENDERSON; TOCHER, 1996; STUBHAUG et al., 2005).

Flaxseed oil is one of the world's most important vegetable sources of ALA (WANASUNDARA; SHAHIDI, 1994), a precursor of the long chain n-3 PUFA series, in freshwater fish (HENDERSON; TOCHER, 1996).

This work investigated the influence of the incremental addition of flaxseed oil as a substitute of sunflower oil in feed on the concentrations of fatty acids, particularly n-3 and n-6 in the liver of Nile tilapias (*Oreochromis niloticus*) maintained in captivity for five months.

Material and methods

The experiments were carried out in the Aquaculture Laboratory of the Biology Department of Universidade Estadual de Maringa, Brazil. Five treatments in five duplications were used with 125 Nile tilapias (*Oreochromis niloticus*) with initial mean individual weight of 88 ± 6 g distributed in 25 ponds (1000 L each). The treatments consisted of the addition of flaxseed oil (0.00; 1.25; 2.50; 3.75 and 5.00%) as a substitute of sunflower oil (control) in feeds (Table 1) (AGUIAR et al., 2007). After five months, the liver of slaughtered fish was removed and kept 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.

Total lipids were determined by Bligh; Dyer (1959). FAME was prepared by methylation of total lipids by Joseph and Ackman (1992) method. FAME was separated by gas chromatography using a Varian 3300 (USA) gas chromatographer fitted with a flame ionization detector and a fused-silica DB-WAX capillary column (30 m x 0.25 mm i.d.) (J and 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. at 2°C $min.^{-1}$ up to 210°C with final holding time of 25 min.; carrier gas, hydrogen at 0.8 mL $min.^{-1}$ with linear velocity of 38 cm s^{-1} and oxygen filter coupled to the feed line; make-up gas, nitrogen at 30 mL $min.^{-1}$; split injection, 1:50 ratio (injection in duplicate). For the identification of fatty acids, fatty acid retention times were compared to those of standard methyl esters (Sigma, St. Louis, MO). Equivalent chain-length values were used (STRANSKY et al., 1997; THOMPSON, 1996), as well as coupled system gas chromatograph-mass spectrometer Shimadzu QP 5000 and electron impact fragmentation at 70 eV. Retention times and peak area percentages were automatically computed in a Varian 4290 integrator.

Mean values were statistically compared by Tukey test at 5% with one-way ANOVA. The data were processed using the Statsoft (1996).

Results and discussion

There were no significant difference for total lipids in the diets of the treatments I, II, III, IV and V, with values of 7.6, 7.7, 8.0, 8.0 and 7.8 respectively. The composition of experimental feeds used in treatments is showed in Table 1.

Table 1. Composition (%) of experimental feeds.

Ingredients (g 100 g^{-1})	Treatments[a]				
	I	II	III	IV	V
Flaxseed oil	0.00	1.25	2.50	3.75	5.00
Sunflower oil	5.00	3.75	2.50	1.25	0.00
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
Calcium(carbonate)	1.74	1.74	1.74	1.74	1.74
Dicalcium phosphate	2.41	2.41	2.41	2.41	2.41
Premix[‡]	0.50	0.50	0.50	0.50	0.50
NaCl	0.50	0.50	0.50	0.50	0.50

[a]Treatments: I (0.00%), II (1.25%), III (2.50%), IV (3.75%), and V (5.00%) of flaxseed oil completed up to 5.00% with sunflower oil. [‡]Premix (mineral and vitamin supplement).

A total of 24 fatty acids were found in feeds. The only fatty acids of the n-3 and n-6 series found in feeds were ALA and LA. The increase in feed ALA content (Table 2) from treatment I (1.8%) to treatment V (31.9%) was due to the added flaxseed oil (an ALA source), while the decrease in LA content from treatment I (54.3%) to treatment V (27.4%) was due to the substitution of sunflower oil (an LA source). Of the 24 fatty acids present in feeds in this experiment, 19 were detected in tilapia liver, while the other five (8:0, 13:0, 18:2n-4, 20:2n-9, and 20:3n-9) were not verified.

Table 2. Fatty acid composition (%) of experimental feeds.

Fatty acids[b]	Treatments[a]				
	I	II	III	IV	V
16:0	9.7 ± 0.1a	9.6 ± 0.1b	9.6 ± 0.2ab	9.5 ± 0.1c	9.6 ± 0.2ab
18:0	3.7 ± 0.1a	3.7 ± 0.1b	3.9 ± 0.19c	4.1 ± 0.3d	4.1 ± 0.1d
18:1n-9	26.6 ± 0.3a	25.9 ± 0.3b	25.1 ± 0.4c	24.4 ± 0.3d	23.6 ± 0.3e
18:1n-7	0.8 ± 0.0a	0.9 ± 0.0b	1.0 ± 0.0c	1.1 ±0.0d	1.1 ± 0.0e
18:2n-6 (LA)	54.3 ± 1.0a	48.0 ± 1.1b	40.4 ± 1.5c	33.8 ± 1.1d	27.4 ± 1.1e
18:2n-3 (ALA)	1.8 ± 0.8a	9.1 ± 0.9b	17.5 ± 1.6c	24.8 ± 1.0d	31.9 ± 1.1e
22:0	0.7 ± 0.1a	0.6 ± 0.0b	0.4 ± 0.1c	0.4 ± 0.1d	0.3 ± 0.1e

[a]Treatments: I (0.00%), II (1.25%), III (2.50%), IV (3.75%), and V (5.00%) of flaxseed oil completed up to 5.00% with sunflower oil. Premix (mineral and vitamin supplement); [b]Fatty acids present at < 0.2% were 8:0, 13:0, 14:0, 17:0, 16:1n-9, 16:1n-7, 18:2n-4, t,t-18:2n-6, 17:1n-9, 20:0, 20:1n-11, 20:1n-9, 20:2n-9, 20:3n-9, 22:1n-11, 22:1n-9 and 24:0; Values are mean ± standard deviation of six fatty acids Different letters in the same line are significantly different ($p < 0.05$) by Tukey test.

Nile tilapia liver did not present any significant difference in total lipid contents for treatments I (6.1%), III (5.8%), IV (5.7%), and V (5.6%). However, it presented major contents (7.2%) for treatment II. The values of total lipids found in this experiment are lower than those found by VISENTAINER et al. (2003a), who studied the liver of juvenile Nile tilapias, and lower values were found by VISENTAINER et al. (2003b) in tilapia head.

A total of 45 fatty acids and 2 dimethylacetals (DMA) (16:0 and 18:0) were identified in liver total lipids (Table 2). The major fatty acids were oleic acid (18:1n-9), linoleic acid (18:2n-6), and palmitic acid (16:0).

Saturated (SFA) and monounsaturated (MUFA) fatty acid compositions in liver were little affected by the different diets. Nevertheless, it was observed a decrease in total n-6 PUFA in liver from 35.1 (treatment I) to 21.1 (treatment V) and an increase in total n-3 PUFA from 3.3 (treatment I) to 18.5 (treatment V) with the increase in flaxseed oil in feeds. The values of n-3 PUFA in liver for all flaxseed oil treatments were higher than those in Nile tilapia head (1.71) (VISENTAINER et al., 2003b). The increase in n-3 PUFA in liver was significantly different for ALA and DHA fatty acids for all treatments (Table 3).

Table 3. Fatty acids composition in liver of Nile tilapia subjected to different treatments.

Fatty acids	Treatments[a]				
	I	II	III	IV	V
SFA					
14:0	1.6 ± 0.3a	1.6 ± 0.3a	1.9 ± 0.3b	1.4 ± 0.3a	1.4 ± 0.3a
i15:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
15:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
i16:0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
16:0	15.3 ± 0.6	15.6 ± 0.7	15.7 ± 0.8	15.2 ± 0.6	15.5 ± 0.6
i17:0	0.8 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
ai17:0	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
17:0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
18:0	7.1 ± 0.6	6.7 ± 0.5	7.0 ± 0.6	7.7 ± 0.7	7.3 ± 0.6
19:0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
22:0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
24:0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Sum	25.6 ± 1.4	25.2 ± 1.3	25.7 ± 1.0	25.7 ± 1.1	25.9 ± 1.2
MUFA					
14:1n-7	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
15:1n-7	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
16:1n-9	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
16:1n-7	2.3 ± 0.2	2.4 ± 0.3	2.4 ± 0.2	2.0 ± 0.3	2.0 ± 0.2
16:1n-5	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
17:1n-9	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
18:1n-11	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
18:1n-9	26.2 ± 1.7a	26.8 ± 1.8a	26.1 ± 1.9a	24.5 ± 1.6b	24.3 ± 1.5b
18:1n-7	2.3 ± 0.2	2.4 ± 0.3	2.3 ± 0.2	2.4 ± 0.3	2.5 ± 0.3
18:1n-5	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
20:1n-11	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
20:1n-9	0.9 ± 0.2a	1.2 ± 0.1b	1.0 ± 0 2a	0.9 ± 0.2a	0.9 ± 0.2a
22:1n-11	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ±0.1
22:1n-9	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Sum	34.0 ± 1.1a	35.2 ± 1.2b	34.5 ± 0.9b	32.0 ± 0.9c	32.1 ± 0.8c
n-6 PUFA					
18:2n-6	23.8 ± 1.4a	22.8 ± 1.3b	22.8 ± 1.3b	17.3 ± 1.7d	15.5 ± 1.6e
18:3n-6	1.1 ± 0.1a	0.9 ± 0.1b	0.9 ± 0.1b	0.4 ± 0.1d	0.2 ± 0.1e
20:2n-6	1.8 ± 0.2a	1.7 ± 0.2a	1.7 ± 0.2a	1.1 ± 0.1c	0.9 ± 0.1d
20:3n-6	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
20:4n-6	3.5 ± 0.3a	3.0 ± 0.2b	3.0 ± 0.2b	2.7 ± 0.2c	2.3 ± 0.2e
22:2n-6	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:3n-6	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:4n-6	0.8 ± 0.1a	0.7 ± 0.1a	0.7 ± 0.1a	0.5 ± 0.1b	0.4 ± 0.1b
22:5n-6	3.0 ± 0.2[a]	2.0 ± 0.1b	2.0 ± 0.1b	1.0 ± 0.1d	1.0 ± 0.1d
Sum	35.1 ± 1.8a	31.9 ± 2.0b	31.9 ± 2.0b	23.7 ± 1.9d	21.1 ± 1.6e
n-3 PUFA					
18:3n-3 (LNA)	0.7 ± 0.2a	2.0 ± 0.1b	4.7 ± 0.3c	6.4 ± 0.2d	6.9 ± 0.3e
18:4n-3	0.1 ± 0.1a	0.1 ± 0.1a	0.4 ± 0.1b	0.5 ± 0.1b	0.8 ± 0.1c
20:3n-3	0.2 ± 0.1a	0.5 ± 0.1b	0.8 ± 0.1c	1.2 ± 0.1d	1.2 ± 0.1d
20:4n-3	0.1 ± 0.1a	0.1 ± 0.1a	0.2 ± 0.1a	0.4 ± 0.1b	0.5 ± 0.1b
20:5n-3 (EPA)	0.1 ± 0.1a	0.1 ± 0.1a	0.1 ± 0.1a	0.6 ± 0.1b	0.8 ± 0.1c
22:4n-3	0.1 ± 0.1a	0.1 ± 0.1a	0.3 ± 0.1b	0.3 ± 0.1b	0.4 ± 0.1b
22:5n-3	0.3 ± 0.1a	0.3 ± 0.1a	0.4 ± 0.1a	0.8 ± 0.1b	0.9 ± 0.1b
22:6n-3 (DHA)	1.7 ± 0.2a	2.4 ± 0.3b	3.9 ± 0.4c	6.1 ± 0.4d	6.9 ± 0.4e
Sum	3.3 ± 05a	5.6 ± 0.7b	10.8 ± 0.8c	16.3 ± 0.9d	18.5 ± 0.9e
DMA					
16:0DMA	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
18:1DMA	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
n-6/n-3 ratio	10.7 ± 1.1a	5.7 ± 0.8b	2.5 ± 0.4c	1.5 ± 0.3d	1.1 ± 0.2e

[a]Treatments: I (0.00%), II (1.25%), III (2.50%), IV (3.75%) and V (5.00%) of flaxseed oil completed up to 5.00% with sunflower oil; [b]Values are mean ± standard deviation of six replicates. Different letters in the same line are significantly different ($p < 0.05$) by Tukey test.

Significant differences between treatments (Table 3) were observed in n-6/n-3 ratios. They decreased from 10.7 (treatment I) to 1.1 (treatment V). This decrease was also observed in fillets of adult tilapia submitted to flaxseed oil treatment (SOUZA et al., 2008), ranging from 7.6 to 1.1 according to TONIAL et al. (2008), and 10.9 to 4.34 according to JUSTI et al. (2003). The n-6/n-3 ratios for treatment II (5.7) were lower than those in cultured *Brycon* head (mean value of 6.9) (MOREIRA et al., 2003) and in the muscle tissue of pacu (9.8) and pintado (7.3) cultured in captivity (TANAMATI et al., 2009). The desaturation and elongation of fatty acids in freshwater fish hepatocytes has been well established by several researchers, including by TOCHER et al. (2002).

Conclusion

This study confirmed the increase in n-3 PUFA and the reduction in n-6 PUFA by the sequential desaturation and elongation (enzyme systems) of fatty acids ALA and LA in Nile tilapias. Thus, the fatty acid composition may be manipulated and fish liver may be commercialized to produce dietary supplements that may help balance n-6/n-3 PUFA in diet. In the future, it may also be used as a food and pharmaceutical industry raw material and in the study of the liver fatty acids in other fish species.

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