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The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids

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Abstract

The purpose of the study was to examine the fatty acid profiles of Nile tilapia (*Oreochromis niloticus*) submitted to different feeding times (0, 10, 20 and 30 days) on a diet enriched with n-3 fatty acids, by addition of flaxseed oil in substitution for sunflower oil. The main fatty acids detected were palmitic (C16:0), stearic (C18:0), oleic (C18:1n9), linoleic (C18:2n6) and α-linolenic (C18:3n3) in all the treatments. The 30 day-fed fish presented the best values for total n-3 fatty acids, with a prominence of α-linolenic acids, showing that the flaxseed oil as well as the feed supply time influenced the fatty acid profiles.

Keywords: Nile tilapia (*Oreochromis niloticus*); Fatty acid; Time of supply; Flaxseed oil

1. Introduction

Marine fish have been recognized as important sources of high quality protein in the human diet. In recent years, the lipids also have been recognized as having high nutritional value, due their protective effect against the development of cardiovascular diseases and rheumatoid arthritis (Puwastien, Judprasong, Kettwan, Vasanachitt, Nakngamanong, & Bhattacharjee, 1999). Reasons for the great interest in the fatty acid effects include studies with populations, such as Eskimos, who regularly consume fish, rich in n-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic (EPA, C20:5n3) and docosahexaenoic (DHA, C22:6n3) acids, and have a low incidence of inflammatory and cardiovascular disorders (Andrade, Rubira, Matsushita, & Souza, 1995; Archer, Green, Chamberlain, Dyer, & Liu, 1998; Hunter & Roberts, 2000; Peterson et al., 1998; Schacky, 2000; Visentainer, Carvalho, Ikegaki, & Park, 2000).

The consumption of fish, either marine or freshwater, has consequently been stimulated (Puwastien et al., 1999). The fatty acid (FA) compositions of fish muscle is clearly influenced by their diet . The lipids of freshwater feeds are characterized by linoleic (C18:2n6), α-linolenic (C18:3n3) and EPA (Henderson & Tocher, 1987). Hence, the fatty acid composition of freshwater fish is characterized by high contents of n-6 PUFA and mainly of linoleic and arachidonic (AA, C20:4n6) acids (Steffens, 1997). On the other hand, the plankton of marine feeds presents low levels of n-6 PUFA, of which EPA and DHA are the predominants acids. The basic difference between freshwater and marine fishes is due to differences in the composition of their diets (Henderson & Tocher, 1987).

Fish is an important source of EPA and AA, precursors for biosynthesis of eicosanoids (prostaglandins, thromboxanes and leukotrienes) which exercise important functions in the human body (Archer et al. 1998; Ascherio, Rimm, Stampfer, Giovannucci, & Willett, 1995; Hunter & Roberts, 2000; Peterson et al., 1998; Schacky, 2000; Visentainer et al., 2000).

There is a nutritional deficit of n-3 PUFA in the human diet; therefore, a higher consumption of food containing these acids is recommended. Feed modification (increasing n-3 PUFA) of animals, such as fish, that form part of our diet, is an another alternative for

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increasing the consumption of these fatty acids in our food (Valenzuela & Uauy, 1999).

The purpose of this study was to evaluate the fatty acid profiles in Nile tilapia (*Oreochromis niloticus*) submitted to different times of feeding (0, 10, 20 and 30 days) with diets containing flaxseed oil.

2. Materials and methods

2.1. Sampling

The experiments were carried in the Aquaculture Laboratory of the Biology Departament of State University of Maringá. One hundred fish were utilized with initial mean individual weights of 41.66 ± 0.56 g, distributed in 20 ponds (1000 l water capacity) in four treatments and five repetitions. The treatments consisted of the supply of feed (Table 1) with addition of flaxseed oil (in substitution of sunflower oil) for different times: 0, 10, 20 and 30 days. In the first 30 days, fish received the same basic feed (without addition of the flaxseed oil) for adaptation. After 60 days 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, triturated and homogenized.

Table 1
Compositions of experimental feeds[a]

	Without flaxseed oil	With flaxseed oil
Ingredients (%)		
Maize	16.93	16.93
Soybean bran	51.62	51.62
Wheat bran	20.00	20.00
Cane bagasse	1.28	1.28
Calcareous	1.74	1.74
Bicalcium phosphate	2.41	2.41
Sunflower oil	5.00	1.25
Flaxseed oil	0.00	3.75
Salt	0.50	0.50
Premix[b]	0.50	0.50
Antioxidant	0.02	0.02
Nutrients		
Available energy (kcal/kg)	3100.00	3100.00
Total phosphorus (%)	1.05	1.05
Calcium (%)	1.40	1.40
Crude fibre (%)	6.00	6.00
Fat (%)	6.77	6.77
Methionine + cystine (%)	0.96	0.96
Lysine (%)	1.64	1.64
Crude protein (%)	28.00	28.00

[a] Data provided by Aquaculture Laboratory—Department of Biology of the State University of Maringá.
[b] Mineral and vitamin supplement.

3. Analysis

Moisture and ash were determined gravimetrically by desiccation at 105 °C and by incineration in an oven at 600 °C, respectively, and the crude protein was obtained by the Kjeldahl method (Cunniff, 1998).

Total lipids were extracted by the Bligh and Dyer method (1959), and the fatty acid methyl esters (FAME) were prepared by methylation of the triacylglycerols, as described by method 5509 of ISO (1978).

The FAME were analyzed by a Shimadzu 14A (Japan) gas chromatograph, equipped with a flame ionization detector (FID) and fitted with a fused silica capillary column (50 m, 0.25 mm i. d. and 0.20 μm of Carbowax 20M). Column temperature was programmed at 2 °C/min from 150 to 240 °C. Injector and detector temperatures were 220 and 245 °C, respectively. Carrier gas was hydrogen (1.2 ml/min) and the make-up gas was nitrogen (30 ml/min). The split used was 1/100. Peaks areas were determined using the CG-300 computing integrator and FAME identification was made by comparison with the retention times of the known standards from Sigma (USA).

4. Statistics

The results were submitted to analysis of variance (ANOVA), at 5% significance level, by Statistica software (StatSoft, USA, 1996), version 5.0. The mean values were compared by Tukey's test.

5. Results and discussion

The proximate analyses of the *Oreochromis niloticus* fillets are presented in Table 2. For the same fish species, Puwastien et al. (1999) found similar values for moisture (78.1%), crude protein (19.8%), lipids (1.8%) and ash (1.0%), for *Oreochromis* sp., Izquierdo, Ferrari, Martínez, Salas, and Cagnasso (2000) obtained different results for moisture (72.4%), crude protein (23.3%), lipids (2.26%) and ash (1.94%).

The fatty acid composition of feeds are listed in Table 3. The feed containing the flaxseed oil presented the highest values for α-linolenic and docosahexaenoic acids; therefore this feed presented the highest total value for n-3 fatty acids.

From Table 4 it can be seen that the length of the feeding time is directly related to the incorporation of n-3 PUFA into *Oreochromis niloticus* fillet, mainly as α-linolenic acid, and consequently the n-3 fatty acids total. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and n-6 FA total showed similar results and no differences ($P > 0.05$) were observed between the treat-

Table 2
Proximate analyses of Nile tilapia (*Oreochromis niloticus*) fillet submitted to different times of supply[a]

Parameters	Time[b] (days)			
	30	20	10	0
Moisture (%)	79.12a ± 0.61	78.98a ± 0.13	77.91b ± 0.23	79.00a ± 0.58
Ash (%)	1.25ab ± 0.03	1.15a ± 0.04	1.26ab ± 0.09	1.36b ± 0.02
Protein (%)	17.2a ± 1.22	18.8a ± 0.28	18.6a ± 0.25	18.2a ± 0.80
Lipids (%)	1.15a ± 0.17	1.02a ± 0.12	1.07a ± 0.16	1.09a ± 0.13

[a] Results expressed as averages of three replicates. Different letters in the same line are significantly different ($P < 0.05$) by Tukey's test.
[b] Time of supply of feed containing flaxseed oil.

Table 3
Fatty acid (FA) compositions of feeds utilized in the experiments[a]

FA	Without flaxseed oil	With flaxseed oil
C14:0	0.24a ± 0.01	0.23a ± 0.03
C16:0	9.94a ± 0.15	9.26a ± 0.08
C16:1n7	0.30a ± 0.01	0.31a ± 0.01
C17:0	0.09a ± 0.01	0.10a ± 0.00
C18:0	4.06a ± 0.10	5.61b ± 0.07
C18:1n9	27.2a ± 0.19	24.6b ± 0.47
C18:1n7	0.96a ± 0.03	1.61b ± 0.10
C18:2n6	53.8[a] ± 0.21	32.3b ± 0.40
C18:3n3	1.58a ± 0.03	24.2b ± 0.20
C20:1n11	0.42a ± 0.00	0.32b ± 0.02
C20:1n9	0.26a ± 0.04	0.38a ± 0.01
C20:5n3	0.07a ± 0.02	0.11a ± 0.04
C22:1n11	0.66a ± 0.04	0.32b ± 0.05
C22:1n9	0.15a ± 0.01	0.40b ± 0.03
C22:6n3	0.12a ± 0.02	0.28b ± 0.03
C24:1n9	0.10 ± 0.01	Nd
PUFA[b]	55.6a ± 0.21	57.2a ± 0.45
MUFA[c]	30a ± 0.20	27.6b ± 0.49
SFA[d]	14.3a ± 0.18	15.2b ± 0.11
n6	53.8a ± 0.21	32.6b ± 0.40
n3	1.78a ± 0.04	24.6b ± 0.20
PUFA/SFA	3.88a ± 0.05	3.76a ± 0.04
n6/n3	30.3a ± 0.78	1.32b ± 0.02

[a] Results expressed as a percentage of the total fatty acids. Averages followed by different letters in the same line are significantly different ($P < 0.05$) by Tukey's test.
[b] PUFA = total of polyunsaturated FA.
[c] MUFA = total of monounsaturated FA.
[d] SFA = total of saturated FA; n3 = total of n-3 FA; n6 = total of n-6 FA.

ments. The PUFA and SFA values were different from those found by Moreira, Visentainer, Souza, and Matsushita (2001), i.e. 41.9% (SFA) and 7.19% (PUFA) for "piraputanga" (*Brycon microlepis*) and 35.6% (SFA) and 12.0% (PUFA) for "piracanjuba" (*Brycon orbignyanus*). In relation to MUFA, Méndez, González, Inocente, Grudice, and Grompone (1996) observed different values for white corvine (*Micropogonias furnieri*) and for palometa (*Parona signata*) i.e. 39.0 and 41.9%, respectively.

The treatment with flaxseed oil presents the best result for the n6/n3 ratio: 4.34. The Department of Health of the UK (HMSO, 1994) recommends a maximum value of 4.0 for this ratio. Analyzing the PUFA/SFA ratio verifies that the values were higher than the minimum recommended of 0.45 (HMSO, 1994) and no differences ($P > 0.05$) were observed between the treatments.

The n-3 PUFA total value found by Andrade et al. (1995), 4.81% for tilapia (*Oreochromis niloticus*), was lower than the results observed for the treatments of 20 and 30 days, similar to the result of 10 days and higher than treatment without addition of the flaxseed oil. Our results for EPA and DHA were lower than those obtained by Izquierdo et al. (2000), i.e. EPA (5.4%) and

Table 4
Fatty acid (FA) compositions of Nile tilapia (*Oreochromis niloticus*) fillet submitted to different times of supply[a]

FA	Time[b] (days)			
	0	10	20	30
C14:0	0.70a±0.13	0.72a±0.07	0.73a±0.24	0.60a±0.06
iC16:0	0.19a±0.01	0.18a±0.01	0.17a±0.02	0.16a±0.01
C16:0	16.6a±0.18	16.5a±0.92	17.5a±1.05	16.0a±0.50
C16:1n9	0.45a±0.02	0.46a±0.02	0.43a±0.04	0.36b±0.03
C16:1n7	1.33a±0.26	1.25a±0.13	1.32a±0.30	1.12a±0.13
iC17:0	0.19a±0.05	0.19a±0.04	0.18a±0.03	0.25a±0.05
aiC17:0	0.14±0.01	Nd	Nd	Nd
C17:0	0.32a±0.02	0.33a±0.05	0.32a±0.04	0.31a±0.02
C18:0	6.98a±0.46	6.76a±0.50	7.52a±0.76	7.83a±0.83
C18:1n9	23.2a±0.63	23.7a±0.96	22.7a±0.84	24.4a±1.11
C18:1n7	2.34ab±0.11	2.23b±0.25	2.66a±0.21	2.38ab±0.12
C18:2n6	30.8a±1.24	30.9a±2.62	27.2ab±1.62	26.6b±1.62
C18:3n6	0.95a±0.11	0.92a±0.09	0.77ab±0.06	0.71b±0.08
C19:1n11	Nd	Nd	Nd	0.16±0.03
C18:3n3	1.04a±0.22	2.11a±0.70	2.51a±0.58	4.06b±1.15
C20:1n11	0.30a±0.02	0.30a±0.03	0.30a±0.03	0.36b±0.02
C20:1n9	1.02ab±0.05	0.99ab±0.04	0.99b±0.06	1.12a±0.09
C20:2n6	Nd	Nd	Nd	0.12±0.01
C20:3n9	2.46a±0.21	2.33a±0.09	2.21a±0.18	2.08a±0.21
C20:3n6	0.18a±0.01	0.14ab±0.03	0.15ab±0.02	0.13b±0.02
C21:0	1.25a±0.15	1.20a±0.09	1.19a±0.06	1.23a±0.19
C20:4n6	3.02a±0.49	2.61a±0.68	3.24a±0.69	2.66a±0.61
C20:3n3	0.27a±0.05	0.45ab±0.13	0.55b±0.08	0.90c±0.17
C20:4n3	0.08a±0.02	0.09a±0.03	0.10a±0.02	0.10a±0.01
C20:5n3	0.12a±0.01	0.12a±0.02	0.17a±0.05	0.16a±0.01
C22:1n11	0.19a±0.01	0.17a±0.02	0.16a±0.01	0.19a±0.01
C22:1n9	0.11a±0.01	0.10a±0.01	0.11a±0.02	0.12a±0.03
C21:5n3	0.17a±0.03	0.15a±0.01	0.17a±0.07	0.16a±0.01
C22:4n6	1.12a±0.08	1.04a±0.22	1.18a±0.16	1.15a±0.26
C22:5n6	2.55a±0.39	2.22a±0.56	2.71a±0.88	2.01a±0.57
C22:5n3	0.57a±0.09	0.52a±0.05	0.76a±0.07	0.79a±0.14
C22:6n3	1.39a±0.17	1.30a±0.31	1.90a±0.24	1.60a±0.44
C24:1n9	Nd	Nd	0.12a±0.02	0.16a±0.05
PUFA[c]	44.7a±1.45	44.9a±2.88	43.7a±2.09	43.2a±2.24
MUFA[d]	28.9a±0.69	29.2a±1.00	28.8a±0.92	30.3a±1.13
SFA[e]	26.3a±0.53	25.9a±1.05	27.6a±1.32	26.4a±0.99
n6	38.7a±1.40	37.81a±2.77	35.3a±1.98	33.4a±1.85
n3	3.64a±0.30	4.74ab±0.78	6.15b±0.64	7.77c±1.25
PUFA/SFA	1.70a±0.06	1.74a±0.13	1.58a±0.11	1.64a±0.10
n6/n3	10.9a±0.97	8.26ab±1.49	5.81bc±0.69	4.34c±0.74

[a] Results expressed as a percentage of the total fatty acids. Averages followed by different letters in the same line are significantly different ($P < 0.05$) by Tukey's test.
[b] Time of supply of feed containing flaxseed oil.
[c] PUFA = total of polyunsaturated FA.
[d] MUFA = total of monounsaturated FA.
[e] SFA = total of saturated FA; n3 = total of n-3 FA; n6 = total of n-6 FA.

DHA (5.0%) for tilapia (*Oreochromis sp.*); Andrade et al. (1995) found a higher value for EPA (1.98%) and lower value for DHA (0.94%).

The fatty acid compositions of the feeds and as their times of supply were reflected directly in the Nile tilapia composition. The fishes that received, during 30 days, a diet with the addition of flaxseed oil presented the highest index for n-3 PUFA and the best n6/n3 ratio.

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