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Effect of flaxseed oil in diet on fatty acid composition in the liver of Nile Tilapia (*Oreochromis niloticus*)

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SUMMARY. This study analyzed the effects of different concentrations of flaxseed oil (FO) on the proximate composition and the contents of alpha-linolenic acid (LNA, 18:3n-3), eicosapentaenoic (EPA, 20:5n-3), and docosahexaenoic (DHA, 22:6n-3) fatty acids in the liver of cultured Nile tilapia (*Oreochromis niloticus*). During the five-month culture period, tilapias were given diets with incremental concentrations of FO (0.00%; 1.25%; 2.50%; 3.75%, and 5.00%) as a replacement of sunflower oil (control). There was no significant difference in moisture and ash content in the liver between treatments. Protein values ranged from 12.1% (treatment II) to 13.9% (treatment V) and total lipids ranged from 5.6% (treatment V) to 7.2% (treatment II). There was no significant difference between most treatments. Fatty acid methyl esters (FAMEs) were quantitatively analyzed by capillary gas chromatography against a $C_{23:0}$ internal standard. Variations in concentrations (in mg g^{-1} of total lipids) of fatty acids between treatment I and treatment V ranged from 4.2 to 51.2 (LNA), from 0.2 to 2.3 (EPA), and from 10.6 to 56.2 (DHA), respectively. This experiment demonstrated that increasing amounts of LNA in feed may markedly increase the amounts of LNA, EPA, and DHA in the liver of Nile tilapia.
Key words: Fatty acids, flaxseed oil, composition, liver, Nile tilapia.

RESUMEN. Efecto de la dieta com aceite de linaza sobre la composición de ácidos grasos en el hígado del tilapia de Nilo (*Oreochromis niloticus*). Este estudio analiza los efectos de diversas concentraciones del aceite de linaza (AL) en la composición centesimal y el contenido del ácido alfa-linolenico (LNA, 18:3 n-3), eicosapentaenoic (EPA, 20:5 n-3), y (DHA, 22:6 n-3), los ácidos grasos docosahexaenoic en el hígado del tilapia del Nilo (*Oreochromis niloticus*) criada em cautiverio. Durante un período de cinco meses, a las tilapias le fueron administradas dietas con concentraciones crecientes de AL (0,00%; 1,25%; 2,50%; 3,75%, y 5,00%) como reemplazo del aceite de girasol (control). No se encontró diferencia significativa en el contenido de humedad y de ceniza en el hígado entre los tratamientos. Los valores de la proteina variaron entre 12,1% (tratamiento II) y (al tratamiento 13,9% V) y los lípidos totales entre el 5,6% (tratamiento V) y 7,2% (tratamiento II). No se encontró diferencia significativa entre la mayoría de los tratamientos. Los ésteres metílicos de los ácidos grasos (FAMEs) fueron analizados cuantitativamente por cromatografía de gas capilar contra la CA. 23:0 estándares internos. Las variaciones en concentraciones (en mg g^{-1} de lípidos totales) de ácidos grasos entre el tratamiento I y el tratamiento V variaron entre 4,2 y 51,2 (LNA), 0,2 y 2,3 (EPA), y 10,6 a 56,2 (DHA), respectivamente. Esto experimento demostró que cantidades crecientes de LNA en la alimentación puede aumentar significativamente las cantidades de LNA, de EPA, y de DHA en el hígado de la tilapia del Nilo.
Palabras clave: Acidos grasos, aceite de la linaza, composición, hígado, tilapia del Nilo.

INTRODUCTION

A freshwater fish, Nile tilapia is widely distributed and is one of the most commercially cultured species, being the sixth most cultured finfish species in the world. It is well known for its fast growth, ability to grow in a wide range of culturing conditions, and its high consumer acceptability (1).

Recent studies have indicated that some parts of fish not used as food are appropriate for human nutrition (2) and may be used in oil extraction. Research has shown the existence of significant concentrations of n-3 polyunsaturated fatty acids (n-3 PUFA) in viscera (3), heads (4), and liver (5).

The importance of fish as a source of omega-3 polyunsaturated fatty acids (n-3 PUFA) in human nutrition is widely recognized (6, 7). Among these acids, mainly eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), as well as its precursor, alpha linolenic acid (LNA, 18:3n-3) stand out. These acids are associated to numberless benefits to human health. DHA plays an important role in the formation, development, and working of the brain and retina (8). EPA has anti-inflammatory properties (7), and in general contributes to the prevention of heart diseases and to the reduction of biochemical factors associated to cancer (9).

Flaxseed oil is one of the word's most important vegetable sources of LNA (10), a precursor of the n-3 PUFA series in freshwater fish (11). In a recent study, n-3 PUFA composition

INTRODUCTION

of feeds at supply directly reflected in the fatty acid composition in Nile tilapia given flaxseed oil, presenting the highest content of n-3 PUFA in muscle tissue (12).

Considering the few studies related with fatty acid manipulation in fish liver and alternative sources of n-3 PUFA, this work investigated the incremental addition of flaxseed oil in substitution of sunflower oil in feed and its influence on the concentrations of LNA, EPA, and DHA (in mg/g of total lipids) in liver of Nile tilapia (*Oreochromis niloticus*) maintained in captivity for five months.

MATERIALS AND METHODS

Animal and diets

The experiments were carried out in the Aquaculture Laboratory of the Biology Department of Universidade Estadual de Maringá, Brazil. It utilized 125 Nile 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 the 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 slaughtered and the liver 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.

Proximate composition

Proximate composition of liver and feeds were determined as described by Cunniff (13). Total lipids (TL) were determined by Bligh & Dyer (14).

Fatty acid composition

Fatty acid methyl esters (FAME) were prepared by methylation of total lipids by Joseph & Ackman (15). Methyl esters were separated by gas chromatography using a Varian 3300 (USA) gas chromatographer fitted with a flame ionization detector (FID) and a fused-silica DB-WAX capillary column (30 m x 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 at 2°C/min up to 210°C with final holding time of 25 min; carrier gas, hydrogen at 0.8 mL/min with linear velocity of 38 cm/s and oxygen filter coupled to the feed line; make-up gas, nitrogen at 30 mL/min; 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 (ECL) were used (16, 17), 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.

Quantification of LNA, EPA and DHA

The concentration of n-3 series fatty acids, LNA, EPA, and DHA in mg g^{-1} of total lipids, in liver was measured against tricosanoic acid methyl ester (23:0) from Sigma (USA) as an internal standard as described by (15). Theoretical FID (flame ionization detector) correction factor values (15, 18, 19) were used to obtain fatty acid concentration values. The following formula was used to calculate the concentrations:

$$\text{Fatty acid (mg g}^{-1}\text{ TL)} = \frac{(A_X)\,(W_{IS})\,(CF_X)}{(A_{IS})\,(W_X)\,(1.04)} \times 1000$$

where LT = total lipid, A_X is the peak area (LNA, EPA, and DHA), A_{IS} is the peak area of the internal standard (IS) tricosanoic acid methyl ester (23:0), W_{IS} is the weight (mg) of IS added to the sample (in mg), W_X is the sample weight (in mg), CF_X is the theoretical correction factor, and 1.04 is conversion factor necessary to express results as mg of fatty acids rather than as methyl esters.

Statistics

The values of the means were statistically compared by Tukey test at 5% with one-way ANOVA. Data were processed using the Statistica software (20).

RESULTS

The experimental FID correction factor values for the LNA (0.97 ± 0.02), EPA (0.99 ± 0.01), and DHA (0.98 ± 0.02) were determined experimentally. As the experimental values were close to the calculated ones, the theoretical corrections factors (CF_X) of LNA (1.01), EPA (0.99), and DHA (0.97) were used to determine concentrations as recommended by (15, 18).

According (Table 1), there were no significant differences ($P > 0.05$) in either total lipids or moisture contents of feeds among the treatments. The increase in the concentration of LNA acid was well established; values ranged between 13.6 and 272.4 mg/100 g of feed with a significant difference ($P < 0.05$) between treatments with flaxseed oil. EPA and DHA were not detected in feeds. Fatty acids profiles of commercial feeds used in treatment of cultured species in Brazil presented low values of LNA (3.3%) and high values of LA -18:2n-6 (38.8%) (21).

TABLE 1
Composition of experimental feeds

Ingredients (g/100g)	Treatments[A] I	II	III	IV	V
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
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	0.02	0.02	0.02	0.02	0.02
NaCl	0.50	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50	0.50
Composition[B]					
Total lipids (g/100g)	7.6±0.3a	7.7±0.4a	8.0±0.6a	8.0± 0.7a	7.8±0.3 a
Moisture (g/100g)	9.7±1.4a	9.6±1.4 a	9.6±1.6a	9.4± 1.7a	9.9± 2.0a
LNA (mg/100g feed)	13.6±2.0a	79.4±3.8b	140.2±130c	202.1±11.6d	272.4±8.6e
EPA (mg/100g feed)	nd	nd	nd	nd	nd
DHA (mg/100g feed)	nd	nd	nd	nd	nd

[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]Data are presented as mean (n=9) ± SD. Different letters in the same line are significantly different (P<0.05) by Tukey test. Abbreviations: LNA = alpha linolenic acid. EPA = eicosapentaenoic acid. DHA = docosahexaenoic acid. BHT = butylated hydroxytoluene. Premix = mineral and vitamin supplement. nd = not detected.

The liver of Nile tilapia (Table 2) did not present any significant difference between treatments I, III, IV, and V for total lipids and protein contents. However, treatment II (1.25% flaxseed oil) presented the largest lipid content (7.2%) and the lowest protein content (12.1%), differing significantly from other treatments. The values of total lipids and protein in this experiment were lower than those found by researchers (22), who studied the liver of juvenile Nile tilapia, with mean values of 10.2% (total lipids) and 14.3% (protein), while total lipid content was higher than those found in the liver of Atlantic salmon (23). Moisture and ash contents did not vary significantly (P > 0.05) between treatments, with mean values of 72.5% (moisture) and 1.1% (ash). These values are close to those determined by (5) in Nile tilapia, moisture (72.7%) and ash (1.0%).

TABLE 2
Proximate composition (g/100g) and LNA, EPA, and DHA concentrations (mg g^{-1}LT) in liver

Composition	Treatments[A] I	II	III	IV	V
Total lipids	6.1±0.2a	7.2±0.2b	5.8±0.1a	5.7±0.1a	5.6±0.1a
Protein	13.8±0.4a	12.1±0.4b	13.0± 0.3a	13.4±0.4a	13.9±0.3a
Moisture	73.2±0.8a	72.3±0.5a	72.2±0.7a	72.8±0.6a	72.1±0.5a
Ash	1.1±0.1a	1.1±0.1a	1.2±0.1a	1.1±0.1a	1.2±0.1a
Fatty acids (mg g^{-1}LT)					
18:3n-3 (LNA)	4.2±1.2a	17.0±4.9b	23.8± 5.4c	34.0±6.2d	51.2±10.6e
20:5n-3 (EPA)	0.2±0.1a	0.5± 0.2b	0.9±0.4c	1.5± 0.3d	2.3±1.0e
22:6n-3 (DHA)	10.6±1.6a	16.1± 2.6b	19.3±3.4c	25.3±5.3d	56.2±9.5e

[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. Data are presented as mean (n = 30) ± SD. Different letters in the same line are significantly different ($P < 0.05$) by Tukey test. Abbreviations: LT = total lipids. LNA = alpha linolenic acid. EPA = eicosapentaenoic acid. DHA = docosahexaenoic acid.

DISCUSSION

It was observed differences in the protein concentration and the total lipids between treatments I and II, with an increase in the lipid concentration and a decrease in the protein concentration. After these treatments, the concentrations were stabilized.Total lipids (Table 2) average of 7.8% was larger than 1.1% in fillets (24) and protein average of 13.2% was smaller than 18.0% (25) in fillets of Nile tilapia.

The fatty acid LNA is a precursor of the n-3 PUFA series and only LNA was present and in increasing amounts in the different feeds of this experiment (Table 1). In the elongation and desaturation conversions of the series, EPA and DHA in the liver of Nile tilapia (Table 2) and some LNA were rather stored in the liver rather than converted. Similar results were obtained in muscular tissue (26) and visceras (27) of Nile tilapias.

Increases in the concentrations of LNA, EPA, and DHA (in mg g^{-1} total lipids) were well established in liver, with a significant difference ($P<0.05$) between all the treatments with the incremental substitution of sunflower oil by flaxseed oil. Therefore, increasing the amounts of LNA in feed can markedly increase the amounts of EPA and DHA in Nile tilapia liver.

CONCLUSION

There was no significant alteration in the proximate composition, except in treatments I and II. The increase in the concentration of alpha-linolenic acid (LNA) in feeds resulted in a gradual increase in fatty acids alpha-linolenic (LNA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) in all treatments. The largest concentration of these fatty acids in Nile tilapia liver was obtained with treatment V (largest level of flaxseed oil).

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