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Quality of polyunsaturated fatty acids in Nile tilapias (*Oreochromis niloticus*) fed with vitamin E supplementation

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ABSTRACT

Freshwater fish are an important source of protein, but they also contain other highly nutritive components such as fats. Polyunsaturated fatty acids (PUFAs) are essential for normal growth, development and reproduction of vertebrates. The antioxidant role of vitamin E in cell membranes prevents fatty acid and cholesterol oxidation, thereby promoting PUFA and subcellular particle stabilization. The effects of vitamin E supplementation on the quality of Nile tilapia (*Oreochromis niloticus*) carcass were investigated. The experiments were carried out in an experimental laboratory over 106 d. After sex reversal, 400 early juvenile *O. niloticus* were tested in a completely randomized experiment with 5 treatments (4 repetitions each), consisting of vitamin E monophosphate supplementation at 0, 50, 100, 150 or 200 mg/kg of a base diet. Treatment diets contained equal amounts of protein and energy. Tilapias supplemented with vitamin E contained arachidonic acid (20:4 ω-6; AA) which participates in inflammatory response. Nile tilapia carcasses that received vitamin E at 100 and 150 mg/kg diet had improved carcass quality by increasing the PUFA:SFA ratio and had the highest levels of polyunsaturated fatty acids from the omega-3 (linolenic acid; 18:3 ω-3) and omega-6 (linoleic acid; 18:2 ω-6) series.

1. Introduction

Freshwater fish are an important source of protein, but they also contain other highly nutritive components such as fats. Lipids are fundamental to the health, survival and success of fish populations (Adams, 1998). The functions these molecules have in the growth of the fish are well defined, namely: energy, structural, hormonal and biochemical precursors of eicosanoids, among others (Haliloglu, Abdulkadir, Sirkecioglu, Aras, & Atamnalp, 2004). Within lipids, polyunsaturated fatty acids (PUFAs) are required for normal growth and development, especially by maintaining structural and functional integrity of membranes (Sargent & La Mcevoy, 1997; Navarro, Navarro, Seixas Filho, & Ribeiro Filho, 2010a). In addition, the prevention of coronary heart disease, cardiovascular disease, rheumatoid arthritis, depression, postpartum depression, cancers, diabetes, anti-inflammatory action, among others are some of the benefits of PUFA to human health (Puwastien, Nakngamanong, & Bhattacharjee, 1999; Sanderson et al. 2002).

Vitamin E is important for many physiological processes in animals. Its antioxidant role in cell membranes prevents fatty acid and cholesterol oxidation (Guerra, Evans, & Maxwell, 2004; Navarro, Ribeiro Filho, Ferreira, & Pereira, 2009), thereby promoting PUFA and subcellular particle stabilization. Consequently, vitamin E prevents the formation of toxic lipid peroxides that can damage biological membranes, blood vessels, change capillary permeability and produce a number of pathologies in vertebrates (Fogaça & Sant'Ana, 2007).

Tocher et al. (2002) showed that diet supplementation with vitamin E increases the growth of juvenile sea bream and decreases the levels of lipid peroxidation products in both sea bream and turbot (*Psetta maxima*) tissues. It is believed that vitamin E and PUFA content in tissues is closely related (Izquierdo & Fernández-Palacios, 1997). Both nutrients have a synergetic effect on nonspecific immune responses and resistance against diseases in the bastard halibut (*Paralychthis olivaceous*) (Wang et al., 2006). Bai and Lee (1998) found increased levels of linoleic (18:2, n-6), γ-linolenic

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(18:3, n-6) and α-linolenic acid (18:3, n-3) associated to high vitamin E levels, as well as an increase in arachidonic acid (20:4, n-6) levels associated to an elevated vitamin E levels to 120 mg/kg diet. Therefore, PUFA content must combine with vitamin E levels to protect against physiological oxidation (Sargent & La Mcevoy, 1997).

Given that fishery products are important ingredients for improving the nutritional status of consumers, studies that assess fatty acid and antioxidant content in fish diet are crucial to increasing fish meat quality. However, information on fatty acid profile and vitamin E in fish carcass is scarce. Accordingly, the present study evaluates the effects of vitamin E supplementation on the fatty acid profile of Nile tilapia carcasses.

2. Material and methods

The experiments were carried out in an experimental laboratory at the Department of Animal Biology – UFV over 106 d (9 Jan to 25 Apr, 2005). The 400 sex-reversed, early juvenile tilapias (*Oreochromis niloticus*), weighing 1.40 ± 0.88 g and measuring 4.77 ± 0.37 cm, were obtained from a reputable producer. They were distributed among twenty 1000-l tanks (Souza, Castagnolli, & Kronka, 1998), renewal with water at a constant rate of 7.5 mL/min and 12 light/12 dark photoperiod. To assess fish performance, a completely randomized design was established, with five treatments (4 repetitions each) consisting of the addition of vitamin E monophosphate at 0, 50, 100, 150 and 200 mg/kg of a base diet composed of 36% crude protein and 3600 kcal of digestible energy/kg. Treatments were initiated after a 5-day adaptation period to the base diet.

The diets were composed of 21.5% soybean meal, 30% corn gluten, 28.50% corn, 9% of fish meal, 7.60% soybean oil, 1.37% phosphate dicalcium, 0.51% L-methionine, 0.60% NaCl, 0.60% vitamin premix and mineral-free vitamin E, 0.15% lysine and 0.02% BHT. The percentage of vitamin E was added to the experimental diet at levels of 0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg and 200 mg/kg.

Diets were pelleted and portions corresponding to 5 percent of body weight were offered three times a day (8:00, 13:00 and 18:00 h). Portion size was adjusted every 15 d to accompany fish growth. Fifteen percent of the fish were collected in 3 cm-mesh nets and measured with a caliper and precision scale. A 12:12 h light/dark cycle was adopted. Temperature was measured twice a day (7:00 and 17:00 h) and pH, dissolved oxygen and ammonia every 7 d.

After the 106-day experiment and a 24-h fast, the fish were anesthetized with benzocaine and sacrificed. Carcasses were weighed on a precision scale (0.001 g) to determine initial carcass composition. For chemical analyses, carcasses were dried in a forced ventilation oven at 55 °C for 48 h. The dried carcasses were then ground in a ball mill until the particle sizes were homogenous.

Analyses of crude protein was determined by the micro Kjeldahl method (titration with 0.05 N sulphuric acid), the ether extract was determined by extraction with ethyl ether for 30 h, the mineral content was determined after incineration in muffle at 550 °C for 4 h, and crude fibre was determined by digestion with sulphuric acid 1.25 N and sodium hydroxide 1.25 N. The analysis of the ingredients used in the diets and fish samples, were performed at the Laboratory of Animal Nutrition Department of Animal Science (LNA / DZO), University Federal of Minas Gerais – UFMG. The procedures are in accordance with AOAC (1995).

The fatty acids present in fish carcass were extracted by the technique recommended by Folch, Lees, and Stanley (1957) and identified fatty acid composition were determined by gas chromatography second Firestone (1998). The samples were transmethylated based on the methodology of Hartmann and Lago (1973), which consists of saponification and conversion of fatty acid methyl esters.

Three repetitions of each fatty acid were used and fatty acid profile was determined in a Varian CP-338 GC gas chromatograph fitted with a DB-WAX 25 m × 0.25 mm × 0.25 μm column (J&W Scientific), at the Chemistry Department of UFMG. Hydrogen was used as the carrier gas at a velocity of 40 cm/s. The initial column temperature of 50 °C was maintained for 2 min, increased at a rate of 4 °C/min until reaching 220 °C and kept at this temperature for more than 25 min. The temperature was 260 °C in both injection port (split of 1/50) and detector. The assays used 2 μl of sample and Sigma 189–19 as the standard fatty acid mixture.

The identification of fatty acids was by comparison of retention times of methyl esters of standards with the sample and the measurement made by standardization.

Statistical analysis was performed using the SAS program version 6 software package and the means were compared by SNK test at 5% significance level.

The results were analysed with the SAS version 6 software package (SAS Institute INC, North Caroline, USA, 1997). The means were compared by SNK test at 5% significance level.

3. Results and discussion

Mean temperature in the tanks was 28.23 ± 0.63 °C, pH was 7.25 ± 0.58 and dissolved oxygen was 5.23 ± 0.85 mg l^{-1}. These values meet the optimal conditions for tilapia growth according to Navarro et al. (2010b).

The fatty acid profile in Nile tilapia carcasses was different among treatments with different vitamin E supplementation. Fish receiving 100 or 150 mg vitamin E/kg diet had the highest levels of omega-3 and omega-6 PUFAs, as indicated by the higher levels of linoleic (18:2, ω-6) and linolenic (18:3, ω-3) acid in the carcass (Table 1). Maintaining high levels of PUFA is associated to the presence of vitamin E, which is added to diets not only to improve nutritional properties, but also to combat and neutralise free radicals before they oxidise these fats in cell membranes (Pita, Piber Neto, Nakaoka, & Mendonça Junior, 2004). The vitamin E to promote the protection of PUFAs in fish meat, contributing to product quality and preservation during processing (Gonçalves et al., 2010).

Nile tilapias receiving supplementation of 100 and 150 mg of vitamin E/kg diet had higher PUFA levels compared to saturated fatty acids (SFA), but this was not observed with other supplementation levels (Table 1). Due to this fatty acid balance, carcasses had lower SFA deposition and higher meat quality. A number of studies have shown a direct association between SFA consumption and blood cholesterol levels (HMSO Nutritional aspects of cardiovascular disease. Report on health & Department of, 1994). Palmitic acid (16:0) was the main SFA in the Nile tilapia carcasses tested (Table 1). Similar results were found by Moreira, Visentainer, Souza, and Matsushita (2001) in three species of Brazilian freshwater fish and by Rahman, Huah, Hassan, and Daud (1995) and Justi, Hayashi, Visentainer, Souza, and Matsushita (2003) in Nile tilapias.

Tilapias supplemented with vitamin E contained arachidonic acid (20:4 ω-6; AA) (Table 2). However, it was not detected in non-supplemented fish. Vitamin E may therefore be involved in the activation of elongase and desaturase enzymes, which participate in the transformation of linoleic acid (18:2 ω-6) into AA, as reported by Mourente, Good, and Bell (2005). Tocher et al. (2002) found no effects of vitamin E supplementation on liver fatty acid composition in *Scophthalmus maximus* and *Hippoglossus hippoglossus*. However, they found that a supplementation level of 1000 mg of vitamin E/kg in the diet increased AA levels in *Sparus aurata*. Despite these results, treatment with the highest vitamin E

Table 1
Fatty acid profile in the carcass of Nile tilapia fed diets supplemented with vitamin E.

Vitamin E mg/kg						
Fatty acid[a]	0	50	100	150	200	C.V.
8:0	0.14 ± 0.07	0.12 ± 0.07	ND	0.05 ± 0.01	0.08 ± 0.04	24
12:0	ND	0.11 ± 0.02	ND	0.07 ± 0.01	0.14 ± 0.03	20.6
13:0	ND	0.18 ± 0.02	ND	0.06 ± 0.04	0.26 ± 0.01	14.4
14:0	2.60 ± 0.05	2.10 ± 0.02	1.95 ± 0.02	1.96 ± 0.16	2.73 ± 0.04	5.8
15:0	0.46 ± 0.02	1.29 ± 0.03	0.25 ± 0.03	0.38 ± 0.04	1.75 ± 0.02	79
16:0	24.64 ± 1.2	23.55 ± 0.2	19.65 ± 0.1	19.85 ± 0.0	25.39 ± 0.0	2.48
17:0	0.58 ± 0.07	0.30 ± 0.0	0.43 ± 0.0	0.41 ± 0.0	0.14 ± 0.0	25.7
18:0	6.16 ± 0.22	6.35 ± 0.23	5.29 ± 0.10	9.20 ± 0.8	6.12 ± 1.0	9.18
20:0	1.13 ± 0.17	1.10 ± 0.34	0.40 ± 0.03	0.43 ± 0.0	0.79 ± 0.0	22.0
21:0	0.24 ± 0.05	0.26 ± 0.02	0.85 ± 0.02	0.65 ± 0.01	0.25 ± 0.0	10.2
22:0	1.17 ± 0.24	0.87 ± 0.37	0.52 ± 0.23	0.67 ± 0.0	1.10 ± 0.35	27.3
23:0	0.15 ± 0.02	0.18 ± 0.02	0.47 ± 0.03	0.36 ± 0.01	0.18 ± 0.03	9.3
14:1	0.63 ± 0.08	0.56 ± 0.14	0.09 ± 0.01	0.10 ± 0.0	0.49 ± 0.04	19.3
15:1	ND	0.16 ± 0.02	ND	ND	ND	51.0
16:1	4.34 ± 0.18	3.73 ± 0.12	3.68 ± 0.05	3.79 ± 0.0	4.42 ± 0.13	3.0
17:1	0.32 ± 0.02	0.35 ± 0.0	0.33 ± 0.0	0.30 ± 0.0	0.35 ± 0.0	4.2
18:1	34.09 ± 1.33	32.52 ± 0.92	27.57 ± 0.12	25.32 ± 0.7	31.03 ± 0.93	3.0
20:1ω9	1.63 ± 0.4	1.11 ± 0.08	1.23 ± 0.03	1.44 ± 0.0	1.15 ± 0.04	16.0
22:1 ω9	0.21 ± 0.05	0.35 ± 0.02	0.12 ± 0.02	0.17 ± 0.01	0.42 ± 0.0	7.2
18:2 ω6c	13.30 ± 0.62	14.98 ± 1.55	27.73 ± 0.05	25.66 ± 0.0	14.01 ± 0.32	3.9
20:2	1.29 ± 0.7	1.48 ± 0.03	1.30 ± 0.0	1.13 ± 0.01	0.72 ± 0.17	31.6
22:2	0.90 ± 0.02	0.75 ± 0.02	0.23 ± 0.01	0.38 ± 0.02	0.48±.01	10.6
18:3 ω3	0.60 ± 0.1	0.80 ± 0.08	2.04 ± 0.0	1.81 ± 0.01	0.73 ± 0.01	5.5
18:3 ω6	0.27 ± 0.02	0.37 ± 0.04	1.10 ± 0.0	0.91 ± 0.0	0.37 ± 0.01	3.9
20:3 ω3	0.18 ± 0.00	0.24 ± 0.05	0.78 ± 0.02	0.62 ± 0.02	0.29 ± 0.35	7.86
20:4 ω6	ND	0.25 ± 0.02	0.30 ± 0.05	0.30 ± 0.05	0.17 ± 0.05	22.1
20:5n-3	0.51 ± 0.1	0.29 ± 0.01	0.20 ± 0.03	0.27 ± 0.04	0.29 ± 0.01	17.8
NI	4.36 ± 0.0	6.65 ± 0.04	3.49 ± 0.01	3.71 ± 0.02	6.15 ± 0.02	0.54

Values correspond to mean ± sd; CV, coefficient of variation; ND, not detected; NI, detected but not identified.
[a] The FAMES were calculated so as follows % FAMES/10 mg the fat.

Table 2
SFA, MUFA, PUFA, EPA, omega-3, omega-6 levels and the omega-3:omega-6 ratio.

Vitamin E mg/kg	0	50	100	150	200	C.V.
AA (20:4 ω6)	ND	0.25 ± 0.0	0.30 ± 0.05	0.30 ± 0.05	0.17 ± 0.03	22.2
EPA (20:5 ω3)	0.51 ± 0.1	0.29 ± 0.01	0.20 ± 0.03	0.27 ± 0.04	0.29 ± 0.01	17.8
ω-3	1.29 ± 0.0b	1.33 0.2 ± b	3.02 ± 0.0a	2.7 ± 0.1a	1.31 ± 0.0 b	6.7
ω-6	13.57 ± b	15.6 ± b	29.13 ± a	26.87 ± a	14.55 ± b	5.91
AGS	37.27 ± ab	36.41 ± ab	29.81 ± c	34.09 ± b	38.93 ± a	2.54
AGMI	41.22 ± a	38.78 ± ab	33.02 ± dc	31.12 ± d	37.86 ± bc	2.71
AGPI	17.05 ± b	19.16 ± b	33.68 ± a	31.08 ± a	17.06 ± b	5.12
NI	4.36 ± 0.02b	6.65 ± 0.03a	3.49 ± 0.04c	3.71 ± 0.02c	6.15 ± 0.03a	0.56
ω-3/ω-6	0.10 ± 0.01b	0.09 ± 0.02b	0.10 ± 0.03a	0.10 ± 0.02ab	0.09 ± 0.02b	2.98
AGPI/AGS	0.45 ± 0.04 c	0.49 ± 0.03 c	1.12 ± 0.08 a	0.89 ± 0.11 b	0.45 ± 0.02 c	4.04

Values correspond to mean ± se and were determined from duplicates.
ND, not detected; AGS, saturated fatty acids; AGMI, monounsaturated fatty acids. AGPI, polyunsaturated fatty acids NI, detected but not identified.

supplementation (200 mg/kg diet) did not produce carcasses with high AA content. AA is a prostaglandin and thromboxane biosynthesis precursor, indirectly affecting processes such as blood coagulation and endothelial healing in humans (Memon, Talpur, Bhanger, & Balouch, 2011).

Docosahexaenoic acid (22:6 ω-3; DHA) and eicosapentaenoic acid (20:5 ω-3; EPA) are long-chain fatty acids that prevent and attenuate inflammatory processes and heart diseases. The present study did not detect DHA (22:6, ω-3) in the Nile tilapia carcasses evaluated and only a small fraction of EPA (20:5, ω-3) (Table 2). This result is expected because DHA derives from EPA which, in turn, derives from linolenic acid (18:3, ω-3), which was detected at low levels in the carcasses. Probably, the activity of desaturase and elongase enzymes, involved in the synthesis of omega-3 PUFA series are also low. Although Nile tilapias do not need PUFA addition to their diet (Kanazawa, Teshima, & Sakamoto, 1980; Takeuchi & Watanabe, 1983), tilapia meat with higher PUFA content is more popular with consumers (Huang, Huang, & Lee, 1998). This is because the human body has little ability to convert into EPA and DHA PUFAs, occurring with low efficiency, about 10 to 15% (Emken, Adlof, & Gulley, 1994) and due to the health benefits of these acids (Visentainer, Carvalho, Ikegaki, & Park, 2000). EPA and DHA are known to protect against heart diseases (Guler, Aktumsek, Citil, Arslan, & Torlak, 2008). Monounsturated fatty acids also protect humans against heart diseases, but less efficiently than PUFA (Visentainer et al., 2000).

The omega-3:omega-6 ratio was higher in Nile tilapia carcasses receiving 100 and 150 mg of vitamin E/kg diet than in fish using other treatments (Table 2). These values are under those of 0.5 to 3.8 reported by Henderson and Tocher (1987), but similar to that found by Maia, Rodriguez-Amaya, and Fraco (1992) for tambaqui (*Colossoma macropomum*) meat.

With respect to the PUFA:SFA ratio, the overall values were above 0.45, the minimum value recommended by the Health Department (HMSO, 1994). The PUFA:SFA ratio in Nile tilapias treated with 100 and 150 mg of vitamin E/kg diet was much higher

than 0.45 and that detected in the other treatments. This result is likely associated to the increase in PUFA levels in these fish. SFA levels were significantly lower in fish receiving supplementation of 100 mg of vitamin E/kg diet, but not in fish supplemented with 150 mg/kg (Table 2). This improved PUFA:SFA ratio produces animals with lower saturated fat deposition in the body.

In the present study, the levels of omega 3, omega 6, PUFA and SFA as well as the PUFA:SFA ratio in Nile tilapias supplemented with 200 mg of vitamin E/kg diet was similar to those of non-supplemented fish. This effect is likely dose-dependent since vitamin E is liposoluble and can be toxic at excessive levels, compromising its antioxidant activity.

4. Conclusion

Of the treatments tested, supplementation of Nile tilapia diets with vitamin E at 100 and 150 mg of vitamin E/kg diet improves carcass quality by increasing the PUFA:SFA ratio and omega 3 and omega 6 levels.

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