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Fatty acid profile of fillet, liver and mesenteric fat in tilapia (*Oreochromis niloticus*) fed vegetable oil supplementation in the finishing period of fattening

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Abstract

Tilapia (*Oreochromis niloticus*) previously reared on a commercial feed were shifted to 3 experimental diets with added 5 % of soybean, linseed oil or fish oils, for 42 days as a finishing diet, according to literature recommendations. Fillet, liver and mesenteric fat total lipid fatty acid composition was determined and evaluated taking health and dietary recommendations into consideration. It was found that dietary vegetable oil fatty acids are effectively incorporated into tilapia hepatic and muscular total lipids, but have no pronounced effect on further fatty acid metabolism, in particular on the n-3 fatty acids. Liver was found to sensitively indicate elevated dietary lipid intake, as proven by its higher, most probably endogenous palmitate synthesis. Based on our results the application of vegetable oils to partially substitute fish oil for tilapia can be recommended in relation to the most important dietary lipid quality indicators.

Keywords: tilapia, fatty acid, soybean oil, linseed oil

Introduction

Before the agricultural revolution about 10 000 years ago humans ingested about equal amounts of n-6 and n-3 essential fatty acids. Over the past 150 years this balance has been upset. Current estimates in Western cultures suggest a ratio of n-6 to n-3 fatty acids of 10-20:1 optimal for human health instead of generally recommended 1-4:1 (Simopoulos 1999). However, in the past 30 years, intakes of animal fats have declined and those of soybean, sunflower, and rapeseed oils have increased in northern Europe. Sunflower oil, with its dominant linoleic acid proportion is now used widely, albeit soybean and rapeseed oils are currently the most plentiful liquid vegetable oils and both have desirable ratios of n-6 to n-3 fatty acids (Sanders 2000).

After all, fish oil is still the main dietary source of long chain n-3 polyunsaturated fatty acids (PUFA) in the human diet. N-3 PUFA family consists of α-linolenic acid (ALA, C18:3n-3) and its longer-chain metabolites: eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic (C22:5n-3) and docosahexaenoic (DHA, C22:6n-3) acids. N-3 PUFA may be beneficial factors in the prevention and treatment of many diseases, i.e. cardiovascular diseases (CVD), certain types of cancer and diseases with an immuno-inflammatory component, and they also play

a suggested role in the cerebral development and function (Kolanowski & Laufenberg 2006). Despite recommendations from organizations to increase fish consumption in general, Weaver *et al.* (2008) demonstrated that not all fish are »created« equal. Whereas farmed Atlantic salmon and farmed trout have some of the highest levels of n-3 fatty acids, coupled with low levels of arachidonic acid (AA C20:4n-6), farmed tilapia and catfish have low levels n-3 fatty acids along with levels of AA, so high they can be considered less advantageous. Since tilapia grows rapidly on formulated feeds with lower protein levels and tolerates higher carbohydrate levels than many carnivorous farmed species, it is ideal for intensive cost-effective recirculation systems. Karapanagiotidis *et al.* (2006) reported that in case of tilapia, the wild fish and fish reared under the most extensive conditions had a more favourable fatty acid profile for human consumption as they contained higher proportions of ALA, EPA, and DHA, higher n-3 to n-6 PUFA ratios, and lower proportions of linoleic acid (LA, 18:2n-6). Muscle tissue of intensively cultured fish was characterized by increased fat deposition, consisting mainly of saturated and monounsaturated fatty acids and LA.

Despite increases in the total global consumption of fish oil by the aquaculture sector, the average dietary fish oil inclusion levels within compound aquafeeds have been steadily declining. The main reason for this decrease is a combination of a decreasing market availability of fish oil from capture fisheries, increasing market cost and increased global use of cheaper plant and animal alternative lipid sources (Tacon & Metian 2008). Several publications (Ng *et al.* 2001, Visentainer *et al.* 2005, De Souza *et al.* 2007, Karapanagiotidis *et al.* 2007, Tonial *et al.* 2009, Szabó *et al.* 2009) demonstrated that the use of different vegetable oils (palm oil, linseed oil, sunflower oil) could substitute a significant amount of dietary fish oil without compromising fish growth and feed utilisation efficiency. However, apart from economically acceptable growth the post-harvest quality of farmed fish is an important aspect that should be taken into consideration when evaluating the suitability of vegetable oils as possible dietary fish oil alternatives (Ng & Bahurmiz 2009). In case of the fillet fatty acid composition Tocher *et al.* (2002) and Karapanagiotidis *et al.* (2007) reported that tilapia has a limited hepatic capacity to elongate and desaturate 20:5n-3 and 22:6n-3 from dietary ALA precursor. By feeding vegetable oil containing diets, the desaturation and elongation of ALA is generally insufficient to compensate for the lack of EPA and DHA in the vegetable oil source, leading ultimately to compromised fatty acid composition. This consequently produces an aquaculture product of lower lipid nutritional value for the consumer.

In marine fish, fatty acid incorporation experiments are highly successful in the accurate prediction of the fillet fatty acid composition (Jobling 2004), as well in the pre-defined modification of the fillet fatty acid profile, e.g. for the production of cardioprotective human diets (Torstensen *et al.* 2004). In case of tilapia Justi *et al.* (2003) found that the length of the feeding time (in a period of 30 days) is directly related to the incorporation of n-3 PUFA into fillet, mainly for α-linolenic acid. Tonial *et al.* (2009) demonstrated that 45 days is the shortest time period required for the inclusion of linseed oil in tilapia feeds to raise the nutritional value (n-6 to n-3 ratio of muscle tissue) of adult Nile tilapia.

The purpose of the present study was to evaluate the fatty acid profiles of intensively reared Nile tilapia (*Oreochromis niloticus*) shifted to feeds containing soybean oil and linseed oil compared to fish oil in the last 42 days of the fattening period, i.e. in the finishing phase.

Material and methods

Experimental fish, feeding and culture facilities

Tilapia originated from the »Tuka« fish farm of the Szavasfish Ltd. (Tuka, Hungary) and were fed the basal diet *ad libitum* (Table 1) during the fattening period. The experimental stock (585 fish, average weight: 175.3±7.8 g; mean±SD) was transferred to nine, aerated tanks (1 m^3 volume) working in a recirculation system at the Fish Laboratory of the Kaposvár University, Hungary. The stocking density was 11.4 kg/m^3. The average temperature was 27.95±1.07 °C (means±SD, n=42) and the pH changed between 7.7-7.9 during the 42 days experiment. The O_2, NO_2-N, NO_3-N, TAN and PO_4-P content of the water in the rearing system were 8.1±0.67 mg/l, 0.08±0.02 mg/l, 5.39±1.63 mg/l, 0.22±0.45 mg/l and 2.38±0.65 mg/l (n=6), respectively. Rearing conditions were determined to correspond the culture parameters in intensive indoor recirculating aquaculture systems (RAS) described by Muir *et al.* (2000) and Watanabe *et al.* (2002).

During the experimental period, three experimental diets (soybean oil, linseed oil, fish oil complementation) were fed in three replications (65 fish per replication for each treatment groups), of which the chemical and fatty acid composition is given in Table 1. The basal diet containing 60 g/kg fat (originated from the ingredients; mainly fish oil) was complemented with the following vegetable oils: soybean oil, linseed oil and fish oil, resulting approximately 110 g ether extract/kg feed. The average digestible energy accounted for 16.5 MJ/kg and the size of pellets was 5.0 mm. The complete feed (daily dose of 1.61±0.06 % of the fish biomass) was administered manually three times a day until satiation.

On the 43rd day of the experiment 6 male fish from each treatment were selected (the mean body mass was 250.8 g) over-anaesthetised with clove oil (dose 0.025 ml/l, 2 min) and processed to gain different samples for chemical analysis. On the first day of the experiment 6 fish were also sampled (as initial value). The left fillet, liver, mesenteric fat obtained after fish dissection were washed in ice-cold physiological saline, wiped dry and stored frozen (–70 °C) until analysis.

Fatty acid analysis: extraction and gas liquid chromatography

Tissue samples were extracted with the method of Folch *et al.* (1957). All solvents used were ultrapure-grade by Sigma-Aldrich (Schnelldorf, Germany), and 100 mg L^{-1} butylated hydroxitoluene was added to the extraction mixture (chloroform/methanol 2/1 vol/vol) as antioxidant.

Gas liquid chromatography was performed on a Shimadzu 2100 apparatus (Shimadzu, Kyoto, Japan), equipped with a SP-2380 (Supelco, Bellefonte, USA) type capillary column (30 m × 0.25 mm internal diameter, 0.20 µm film) and flame ionisation detector. Characteristic operating conditions were: injector temperature: 270 °C, detector temperature: 300 °C, helium flow: 28 cm sec^{-1}. The oven temperature was graded: from 80 to 205 °C: 2.5 °C min^{-1}, 5 min at 205 °C, from 205 to 250 °C 10 °C min^{-1} and 5 min at 250 °C. To identify individual fatty acid in the chromatogram, a fatty acid standard mixture (Me100; Larodan Fine Chemicals, Malmö, Sweden) was used. Results were expressed as weight % of the total fatty acid methyl esters.

Table 1
Ingredients and fatty acid composition of the experimental diets

Ingredient	Basal diet	Soybean oil	Flaxseed oil	Fish oil
Soybean oil	-	5.0	-	-
Fax seed oil	-	-	5.0	-
Fish oil	-	-	-	5.0
Others[a]	100.0	95.0	95.0	95.0
Fatty acid composition[b]				
C12:0	0.11	0.07	0.04	0.07
C14:0	0.86	3.65	0.88	4.22
C14:1n-5	0.13	0.10	0.03	0.12
C15:0	0.18	0.37	0.13	0.42
C16:0	21.2	15.05	11.52	15.20
C16:1n-7	4.79	3.27	1.24	3.70
C17:0	0.37	0.41	0.12	0.48
C17:1n-7	0.19	0.56	0.18	0.61
C18:0	5.75	2.91	3.67	2.67
C18:1n-9	27.3	16.03	17.61	14.9
C18:1n-7	ND	1.93	1.39	1.96
C18:2n-6t	ND	0.33	0.08	0.38
C18:2n-6c	33.2	23.10	30.91	19.1
C18:3n-6	0.06	0.07	0.02	0.08
C18:3n-3	1.73	4.16	24.5	3.72
C20:0	0.12	0.25	0.20	0.23
C20:1n-9	0.63	5.67	1.20	7.11
C20:2n-6	0.2	0.30	0.18	0.31
C20:3n-3	0.07	0.06	0.03	0.06
C20:3n-6	ND	0.12	0.08	0.14
C20:4n-6	0.43	0.33	0.16	0.36
C20:5n-3	0.7	4.64	1.37	5.20
C22:1n-9	ND	7.05	1.11	8.49
C22:5n-3	0.16	0.75	0.34	0.81
C22:6n-3	1.66	8.43	2.95	9.27
C24:0	ND	0.06	0.02	0.04
C24:1n-9	0.1	0.33	0.09	0.35
Σ SFA	28.7	22.7	16.6	23.3
Σ UFA	71.3	77.3	83.4	76.7
Σ MUFA	33.2	35.0	22.8	37.3
Σ PUFA	38.1	42.3	60.6	39.4
Σ n-3 PUFA	4.3	18.0	29.1	19.1
Σ n-6 PUFA	33.9	24.3	31.4	20.4
Σ n-6 / Σ n-3	7.8	1.3	1.1	1.1

[a]The basal diet was formulated from wheat meal, fish meal, soybean, feed yeast, fish premix, monocalcium phosphate and methionine. The proximate chemical composition was the following: protein 39.0 %, carbo-hydrates 35.5 %, ether extract 6.0 %, ash 4.9 % and fibre 2.6 %, total phosphorous 0.86 %, vitamin A 10 000 IU/kg, vitamin D3 1 000 IU/kg, vitamin E 50 mg/kg. The fatty acids of wheat meal dominated in the fat of basal diet. [b] ND: non detectable (<0.01 g/100 g fatty acids)

Atherogenic and thrombogenicity indices

Atherogenic (IA) and thrombogenicity (IT) indices were calculated according to Ulbricht and Southgate (1991), as follows:

$$IA = [12:0 + (4 \times 14:0) + 16:0] / [(PUFA\ n\text{-}6 + n\text{-}3) + 18:1 + other\ MUFA] \quad (1)$$

$$IT = [14:0 + 16:0 + 18:0] / [0.5 \times 18:1 + 0.5 \times other\ MUFA + 0.5 \times n\text{-}6\ PUFA + 3 \times n\text{-}3\ PUFA + (n\text{-}3\ PUFA / n\text{-}6\ PUFA)] \quad (2)$$

Statistical analysis

Differences between mean values were computed using a one-way analysis of variance (ANOVA) with the Tukey »post hoc« test. All calculations were performed with the SPSS 10 (SPSS Inc., Chicago, IL, USA) software.

Results

In the experimental period the feed was changed to three diets with a chemical and fatty acid composition given in Table 1. The diets were produced as a result of the different oil supplementations (5 % of soybean oil [SO], linseed oil [LO] and fish oil [FO]) of the basal diet. Data referring to the fatty acid composition of the different oil supplementations show that the proportion of total unsaturated fatty acids was higher in the experimental diets, as compared to the basal diet due to the higher PUFA level. This was especially pronounced in the LO diet, where the 1.5 times higher PUFA proportion was accompanied with lower MUFA proportion. The greatest differences in the individual fatty acid proportions were observed for the lower level of oleic acid (C18:1n-9) and arachidonic acid (AA, 20:4n-6) and higher proportion of EPA, and DHA by the experimental diets, as compared to the basal diet. In case of LA the SO and FO groups contained lower levels, as compared to the basal diet, but in the LO group LA and also ALA proportion was found to be rather high.

The fat content of the different organs showed an increasing tendency as effect of feeding the experimental feeds. In the fillet the difference was significant only between the FO group and the initial value (1.16±0.09 %; 1.31±0.16 %; 1.42±0.06 %; 0.83±0.23 %, in the groups SO, LO, FO and initial, respectively), and but in the liver the changes were not significant (6.31±1.44 %; 6.19±0.65 %; 7.35±0.55 %; 5.08±0.48 %). The quantity of mesenteric fat was found to be lower in the SO group (3.81±1.31g; 7.25±5.58g; 8.24±4.64g, in the groups SO, LO and FO, respectively), but the difference was not significant due to the high individual variance.

The fatty acid composition of the tissue total lipids is presented in Tables 2, 3, and 4.

In the fillet almost all of the fatty acid proportions were significantly affected by the different treatments (Table 2). In the SO group the proportion of C18:0, C18:2n-6, C20:2n-6, C20:3n-3, C20:3n-6, C20:4n-6, C22:0, C24:0 increased. Similar changes were observed in the LO group where the proportion of C20:2n-6, C20:3n-3, C20:3n-6, C22:0, C24:0 and also C18:3n-3, increased but the difference in C18:0, C18:2n-6 and C20:4n-6 was not significant. The effect of vegetable oil complementation resulted decreasing proportion of C17:1n-7, C20:1n-9, C22:1n-9 in both vegetable oil groups (SO and LO). The proportion of C14:0, C22:5n-3

and C24:1n-9 decreased only in LO group. In the main fatty acid groups, the total n-6 PUFA increased significantly in both vegetable oil groups, the SO group exceeding the LO group. This resulted in a higher n-6 to n-3 ratio in the former, since in the LO and FO groups this ratio showed no significant differences in the fillet.

Table 2
Fatty acid composition of the fillet of tilapia fed different vegetable oil diets (% of total fatty acids, mean ±SD)

Fatty acid	Fillet		
	Soybean oil	Linseed oil	Fish oil
12:0	0.04±0.00	0.04±0.01	0.05±0.01
C14:0	2.21±0.15[a]	2.44±0.13[ab]	3.09±0.26[b]
C14:1n-5c	0.09±0.00	0.12±0.01	0.12±0.03
C15:0	0.19±0.05	0.19±0.01	0.25±0.02
C16:0	22.52±1.21	22.03±0.32	22.36±0.40
C16:1n-7c	3.17±0.26	4.07±0.48	4.75±0.87
C17:0	0.37±0.03	0.39±0.01	0.37±0.12
C17:1n-7c	0.20±0.00[a]	0.22±0.03[a]	0.35±0.01[b]
C18:0	8.58±0.07[b]	7.29±0.84[ab]	6.43±0.16[a]
C18:1n-9c	21.56±1.69	22.25±2.14	23.08±2.71
C18:2n-6c	15.87±0.29[b]	13.76±1.00[ab]	11.47±0.14[a]
C18:3n-6c	0.63±0.16	0.57±0.12	0.45±0.11
C18:3n-3c	1.07±0.10[a]	4.51±0.03[b]	1.14±0.10[a]
C20:0	0.23±0.01	0.23±0.02	0.20±0.01
C20:1n-9c	2.37±0.14[a]	2.08±0.15[a]	3.92±0.09[b]
C20:2n-6c	1.20±0.04[c]	0.84±0.07[b]	0.63±0.02[a]
C20:3n-3c	1.37±0.02[c]	0.98±0.07[b]	0.76±0.04[a]
C20:3n-6c	0.28±0.01[b]	0.84±0.01[c]	0.19±0.01[a]
C20:4n-6c	3.55±0.11[b]	2.71±0.35[ab]	1.89±0.03[a]
C20:5n-3c	0.35±0.03	0.44±0.01	0.94±0.25
C22:0	0.08±0.00[b]	0.07±0.00[b]	0.06±0.00[a]
C22:1n-9c	0.11±0.00[a]	0.12±0.01[a]	0.24±0.02[b]
C22:5n-3c	1.75±0.12[a]	2.14±0.00[ab]	2.89±0.38[b]
C22:6n-3c	11.97±0.91	11.39±0.12	14.10±3.13
C24:0	0.09±0.02[b]	0.10±0.01[b]	0.04±0.01[a]
C24:1n-9c	0.15±0.00[a]	0.16±0.01[ab]	0.20±0.01[b]
∑SFA	34.32±1.53	32.78±1.35	32.87±0.06
∑MUFA	27.64±2.10	29.03±2.83	32.67±3.48
∑n-3 PUFA	16.50±1.15	19.47±0.06	19.83±3.83
∑n-6 PUFA	21.54±0.59[b]	18.72±1.54[b]	14.63±0.29[a]
∑PUFA	38.04±0.57	38.19±1.48	34.46±3.54
n-6/n-3	1.31±0.12[b]	0.96±0.08[ab]	0.75±0.16[a]
IA	0.48±0.04	0.47±0.02	0.52±0.01
IT	0.44±0.01	0.38±0.02	0.38±0.04

[a]Different lower case superscript in the same row represents significant ($P<0.05$) differences.

Liver lipids (Table 3) of all treated groups were characterized with higher palmitic acid (C16:0) proportions compared to the levels of other tissues. In the vegetable oil groups the average of LA proportion increased approximately to double value, but differences between groups were not significant due to the high individual variances. The proportion of ALA was significantly higher in the LO group and this treatment increased also the level of C20:3n-6

in the liver. The proportion of n-3 PUFA showed a decreasing tendency in SO group, and both vegetable oil groups resulted 1.5 -2 times higher average in the n-6 PUFA level, total PUFA, and n-6 to n-3 ratios but none of them showed significant differences due to the high individual variances.

Table 3
Fatty acid composition of the liver of tilapia fed different vegetable oil diets (% of total fatty acids, mean ±SD)

Fatty acid	Liver		
	Soybean oil	Linseed oil	Fish oil
12:0	0.06±0.00	0.06±0.02	0.06±0.01
C14:0	5.11±0.59	4.88±0.26	5.32±1.15
C14:1n-5c	0.14±0.02	0.19±0.02	0.18±0.01
C15:0	0.14±0.01	0.14±0.05	0.15±0.03
C16:0	28.91±4.72	29.73±1.14	31.13±2.57
C16:1n-7c	5.05±0.41	6.40±1.33	6.83±0.03
C17:0	0.34±0.06	0.33±0.05	0.48±0.07
C17:1n-7c	0.22±0.07	0.21±0.03	0.30±0.04
C18:0	10.39±1.30	8.82±0.26	9.51±0.18
C18:1n-9c	30.11±2.52	27.78±4.31	29.31±0.13
C18:2n-6c	9.47±4.99	8.49±3.44	4.31±1.91
C18:3n-6c	0.50±0.24	0.43±0.14	0.25±0.08
C18:3n-3c	0.58±0.26[a]	2.54±0.68[b]	0.40±0.22[a]
C20:0	0.19±0.01	0.19±0.06	0.16±0.00
C20:1n-9c	1.73±0.12	1.51±0.42	2.69±0.50
C20:2n-6c	0.57±0.19	0.41±0.17	0.24±0.07
C20:3n-3c	0.58±0.04	0.49±0.17	0.35±0.06
C20:3n-6c	0.12±0.05[ab]	0.43±0.12[b]	0.07±0.03[a]
C20:4n-6c	1.38±0.38	1.14±0.12	0.85±0.07
C20:5n-3c	0.08±0.01	0.19±0.07	0.25±0.10
C22:0	0.04±0.00	0.05±0.02	0.03±0.01
C22:1n-9c	0.06±0.02	0.09±0.03	0.17±0.04
C22:5n-3c	0.41±0.04	0.87±0.32	0.81±0.32
C22:6n-3c	3.73±1.09	4.53±0.35	5.99±0.49
C24:0	0.01±0.02	0.04±0.00	0.00±0.00
C24:1n-9c	0.06±0.01	0.10±0.04	0.15±0.02
∑SFA	45.20±6.50	44.22±0.41	46.85±3.78
∑MUFA	37.39±2.30	36.27±5.15	39.63±0.43
∑n-3 PUFA	5.38±0.90	8.62±1.57	7.79±1.19
∑n-6 PUFA	12.03±5.10	10.89±3.99	5.72±2.16
∑PUFA	17.41±4.20	19.51±5.56	13.52±3.35
n-6/n-3	2.34±1.33	1.24±0.24	0.72±0.16
IA	0.92±0.24	0.88±0.01	1.00±0.21
IT	1.08±0.18	0.87±0.08	0.98±0.18

[a]Different lower case superscript in the same row represents significant ($P<0.05$) differences.

Table 4
Fatty acid composition of the mesenteric fat of tilapia fed different vegetable oil diets (% of total fatty acids, mean±SD)

Fatty acid	Mesenteric fat		
	Soybean oil	Linseed oil	Fish oil
12:0	0.06±0.00	0.05±0.01	0.06±0.00
C14:0	3.22±0.34	3.04±0.34	3.67±0.94
C14:1n-5c	0.15±0.00	0.17±0.02	0.16±0.01
C15:0	0.20±0.02	0.26±0.02	0.29±0.05
C16:0	22.83±1.14	21.56±0.88	22.56±0.19
C16:1n-7c	5.09±0.38	5.80±0.53	6.10±0.60
C17:0	0.35±0.05	0.43±0.01	0.52±0.08
C17:1n-7c	0.27±0.01^{a}	0.32±0.02^{ab}	0.42±0.06^{b}
C18:0	6.57±0.04^{b}	5.42±0.33^{a}	5.86±0.03^{ab}
C18:1n-9c	30.17±1.83	28.95±2.63	30.77±2.48
C18:2n-6c	19.80±0.54^{b}	17.04±1.16^{b}	13.50±0.09^{a}
C18:3n-6c	0.83±0.21	0.71±0.20	0.50±0.17
C18:3n-3c	1.48±0.17^{a}	5.13±0.03^{b}	1.22±0.25^{a}
C20:0	0.25±0.02	0.26±0.05	0.20±0.01
C20:1n-9c	2.83±0.07^{a}	3.03±0.12^{a}	4.42±0.44^{b}
C20:2n-6c	0.96±0.04^{b}	0.75±0.03^{ab}	0.58±0.11^{a}
C20:3n-3c	0.81±0.09	0.62±0.11	0.49±0.13
C20:3n-6c	0.28±0.04^{a}	0.78±0.03^{b}	0.19±0.01^{a}
C20:4n-6c	0.63±0.06	0.58±0.09	0.49±0.11
C20:5n-3c	0.18±0.00^{a}	0.33±0.04^{ab}	0.61±0.16^{b}
C22:0	0.09±0.01	0.09±0.01	0.07±0.00
C22:1n-9c	0.14±0.00	0.20±0.02	0.27±0.05
C22:5n-3c	0.78±0.07^{a}	1.32±0.07^{ab}	1.91±0.41^{b}
C22:6n-3c	1.84±0.21	2.89±0.32	4.84±1.20
C24:0	0.03±0.01	0.03±0.01	0.01±0.02
C24:1n-9c	0.16±0.01	0.21±0.02	0.29±0.06
∑SFA	33.61±1.55	31.14±1.65	33.24±0.88
∑MUFA	38.80±2.26	38.69±3.03	42.42±2.49
∑n-3 PUFA	5.09±0.36^{a}	10.30±0.07^{b}	9.07±1.89^{ab}
∑n-6 PUFA	22.50±0.35^{b}	19.87±1.45^{b}	15.26±0.29^{a}
∑PUFA	27.59±0.71^{ab}	30.17±1.38^{b}	24.33±1.60^{a}
n-6/n-3	4.42±0.24^{b}	1.93±0.15^{a}	1.72±0.39^{a}
IA	0.54±0.05	0.49±0.04	0.56±0.06
IT	0.71±0.03^{b}	0.50±0.03^{a}	0.57±0.03^{a}

^{a}Different lower case superscript in the same row represents significant ($P<0.05$) differences.

In the mesenteric fat the characteristic dietary fatty acids significantly affected their tissue proportions, indicating a likewise direct incorporation. In the SO group the proportion of LA and C20:2n-6 increased, while the proportion of C17:1n-7, C20:1n-9, EPA and DHA decreased significantly as compared to the FO group. In the LO group the level of LA, ALA and C20:3n-6 increased, while C20:1n-9 decreased. The higher LA proportion in the SO and LO group showed a higher total n-6 PUFA proportion in both treatments, while the higher ALA levels in the LO group resulted higher n-3 PUFA and total PUFA levels, too. Comparing the different vegetable oil treatments to each other significant difference was found between SO and FO groups in the C18:0 proportions. LO group differed significantly from the other groups in the

ALA proportion. In the proportions of C20:3n-6 the vegetable oil complementation led to a higher tissue level, with significant difference in the LO group. LO group showed higher n-3 PUFA level, n-6 to n-3 ratio and IT value compared to the SO group.

Discussion

Coronary heart disease (CHD) occurs in most instances due to obstruction of coronary vessels by atherosclerosis or thrombosis, singly or in combination. Ulbricht & Southgate (1991) reported seven dietary factors that are implicated in these processes. Two are promoters (atherogenic and thrombogenic SFAs) and five are protective (PUFA of the n-6 series, PUFA of the n-3 series, MUFA, dietary fibre, and antioxidants).

Karapanagiotidis *et al.* (2006) reported on the elevated level of SFA and MUFA in the fillet of intensively farmed tilapia due to the increased fat deposition characterised mainly by SFA, MUFA and LA. They recommend the substitution of vegetable oils rich in LA with oils abundant in oleic acid and ALA. In our study the dietary fatty acid incorporation was confirmed in all organs. However, the elevation of SFA in the different treatments did not occur, and neither MUFA levels were affected by the vegetable oil supplementations.

Analysing the three, functionally divergent organs (fillet, liver, mesenteric fat) the three diets led to basically similar alterations in their fatty acid profiles. Liver was the only organ where the palmitate proportion was altered by all three diets. The reason of this may be not merely the diet, as all experimental diets contained largely similar palmitate proportions (Table 1). Instead of this in *Perciformes* liver is one of the main sites of lipid storage and palmitate is generally acting as an oxidisable energy source (Stubhaug *et al.* 2005). The dominant palmitate accretion as a result of hepatic lipogenesis is otherwise characteristic for increased energy uptake.

In all three organs investigated the LO complementation was merely effective in increasing of the tissue ALA proportions. However; it is very interesting that this increase failed to mirror the effect of the large C18:3n-3 provision by the LO diet, as further elongated and desaturated products (EPA, DPA, and DHA) were not affected by the precursor fatty acid feeding. The vegetable oil feeding (especially SO diet) led ultimately to a reduction of the fillet DPA proportion, and was found not to be effective in either enriching or maintaining the fillet EPA and DHA proportions. Opposite results were described by SHAPIRA *et al.* (2009), where farmed mango tilapia (*Sarotherodon galilaeus galilaeus*) fed increased n-3 PUFA in the form of linseed showed moderate increases in the n-3 long chain PUFA proportion, supporting the capacity for n-3 PUFA transformation and accretion. Our result confirms the report of Karapanagiotidis *et al.* (2007), that tilapia (*Oreochromis niloticus*) has a limited capacity to synthesize EPA and DHA from dietary ALA precursor. Agaba *et al.* (2005) reported that freshwater fish were found to effectively metabolize C18 PUFA to highly unsaturated fatty acid, but the pattern of activity shown by the elongases from tilapia was found to be slightly unusual in that the activity towards C20:5n-3 was equal to that towards C18:4n-3 and it had the highest activity towards C20:4n-6. In that study the warm water species (zebrafish, catfish, tilapia and sea bream) all displayed higher activities towards the n-6 fatty acids than the colder water species (salmon, turbot and cod), most probably because their original environments basically lacking these fatty acids. In accordance with this assumption, an increase was experienced for arachidonic acid (AA) in vegetable oil groups, which was the

most expressed by the SO diet. This can be regarded as a less favourable tendency, as AA is the precursor of the most effective inflammation mediators, thromboxanes, prostaglandins and leukotrienes (Allayee *et al.* 2009). The ratio of arachidonic acid to long-chain n-3 PUFAs (EPA and DHA) in human diets is also an important factor. Weaver *et al.* (2008) reported that the average ratio of AA to EPA in farmed tilapia varied around the extremely high value of 11:1, which is considered to be detrimental. Our data demonstrated low ratios (2.02, 1.26, and 0.65 in the groups of SO, LO and FO, respectively) and the fact that the different supplementations especially FO used as a finishing diet could effectively reduce this values. Although, the issue whether dietary AA is harmful or not is unequivocal since AA has both pro- and antithrombotic and inflammatory effects, as well as important functions in cell signalling (Netleton 2008).

In the publication of Weaver *et al.* (2008) about the fatty acid composition of commonly consumed farmed fish species collected in supermarkets in the United States tilapia (fillet) was characterized with high (>2) n-6 to n-3 fatty acid ratio since both farm raised Atlantic salmon and trout have ratios below 1 due to the high n-3 proportions. Tonial *et al.* (2009) demonstrated that LO feeding for 45 days gives a better n-6 to n-3 ratio (1.1) of muscle tissue due to the reduction of n-6 and an increase in the proportion of n-3 FAs. Our data partially confirmed this; LO feeding resulted asimilar n-6 to n-3 ratio as in the FO group, indicating that by LO supplementation n-3 PUFA proportion corresponds to effect of FO on the fatty acid composition better than SO. This was however mostly attributed to the direct increment of the ALA and not to its further elongated and desaturated metabolites.

The ratios of PUFA to SFA or n-6 to n-3 are often handled as indicators of the dietary lipid quality. Characterization of diets in terms of their total fat content, their saturated fatty acid ratio, their P/S ratio, the proportion of energy from fat, or their PUFA n-3 or n-6 proportion alone can lead to misleadingly naive statements about diets and to simplistic dietary advice. Atherogenic index (IA) and thrombogenicity index (IT) developed by Ulbricht & Southgate (1991) indicate the global dietetic quality of lipids and their potential effect on the development of coronary disease. Concerning fish, Jankowska *et al.* (2010) found that the wild and reared perch (*Perca fluviuatilis L.*) were found not to differ in the values of these indices except for IA in the liver and omental fat lipids. Thus, it may be stated that relationships between pro-atherogenic and anti-atherogenic fatty acids of perch muscles were not determined by its origin. No differences between the groups were either observed in comparing dependencies between pro- and anti-thrombogenic fatty acids of all body parts of both wild and reared perch. Our data showed similar tendencies, while vegetable oil supplementation does not affect the value of the two indices in the fillet and the liver, and in mesenteric fat only IT was negatively affected by the soybean oil treatment.

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References

Agaba MK, Tocher DR, Zheng XZ, Dickson CA, Dick JR, Teale AJ (2005) Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish. Comp Biochem Phys 142 B, 342-352

Allayee H, Roth N, Hodis HN (2009) Polyunsaturated fatty acids and cardiovascular disease: implications for nutrigenetics. J Nutrigenet Nutrigenomics 3, 140-148

de Souza NE, Matsushita M, de Oliveira CC, Franco MR B, Visentainer JV (2007) Manipulation of fatty acid composition of Nile tilapia (*Oreochromis niloticus*) fillets with flaxseed oil. J Sci Food Agr 87, 1677-1681

Folch J, Less M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues

Jankowska B, Zakes Z, Zmijewski T, Szczepkowski M (2010) Fatty acid profile of muscles, liver and mesenteric fat in wild and reared perch (*Perca fluviatilis L.*). Food Chem 118, 764-768

Jobling M (2004) »Finishing« feeds for carnivorous fish and the fatty acid dilution model. Aquac Res 35, 706-709

Justi KC, Hayashi C, Visentainer JV, de Souza NE, Matsushita M (2003) 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. Food Chem 80, 489-493

Karapanagiotidis IT, Bell MV, Little DC, Yakupitiyage A, Rakshit SK (2006) Polyunsaturated fatty acid content of wild and farmed tilapias in Thailand: Effect of aquaculture practices and implications for human nutrition. J Agr Food Chem 54, 4304-4310

Karapanagiotidis IT, Bell MV, Little DC, Yakupitiyage A (2007) Replacement of dietary fish oils by alpha-linolenic acid-rich oils lowers omega 3 content in tilapia flesh. Lipids 42, 547-559

Kolanowski W, Laufenberg G (2006) Enrichment of food products with polyunsaturated fatty acids by fish oil addition. Eur Food Res Technol 222, 472-477

Muir JF, van Rijn J, Hargreaves J (2000) Production in intensive and recycle systems. In: Beveridge MCM, McAndrew BJ (eds.) Tilapias: Biology and Exploitation. Kluwer Academic Publishers, Dordrecht, The Netherlands, 405-446

Netleton JA (2008) Concerning PUFA in fish. J Am Diet Assoc 108, 1830-1831

Ng WK, Lim PK, Sidek H (2001) The influence of a dietary lipid source on growth, muscle fatty acid composition and erythrocyte osmotic fragility of hybrid tilapia. Fish Physiol Biochem 25, 301-310

Ng WK, Bahurmiz OM (2009) The impact of dietary oil source and frozen storage on the physical, chemical and sensorial quality of fillets from market-size red hybrid tilapia, *Oreochromis* sp. Food Chem 113, 1041-1048.

Sanders TAB (2000) Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 71, S176-S178

Shapira N, Weill P, Sharon O, Loewenbach R, Berzak O (2009) n-3 PUFA fortification of high n-6 PUFA farmed tilapia with linseed could significantly increase dietary contribution and support nutritional expectations of fish. J Agr Food Chem 57, 2249-2254

Simopoulos AP (1999) Essential fatty acids in health and chronic disease. Am J Clin Nutr 70, S560-S569

Stubhaug I, Tocher DR, Bell JG, Dick JR, Torstensen BE (2005) Fatty acid metabolism of Atlantic salmon (*Salmo salar L.*) hepatocytes and influence of dietary vegetable oil. Biochem Biophys Acta 1734, 277-288

Szabó A, Romvári R, Szathmári L, Molnár T, Locsmándi L, Bázár G, Molnár E, Horn P, Hancz C (2009) Effects of dietary vegetable oil supplementation on fillet quality traits, chemical and fatty acid composition of African catfish (*Clarias gariepinus*). Arch Tierz 52, 321-333

Tacon AGJ, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285, 146-158

Tocher DR, Agaba M, Hastings N, Bell JG, Dick JR, Teale AJ (2002) Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). Fish Physiol Biochem 24, 309-320

Tonial I.B., Stevanato F.B., Matsushita M., De Souza N.E., Furuya W.M., Visentainer J.V. (2009): Optimization of flaxseed oil feeding time length in adult Nile tilapia (*Oreochromis niloticus*) as a function of muscle omega-3 fatty acids composition. Aquacult Nutr 15, 564-568

Torstensen BE, Froyland L, Ornsrud R Lie O (2004) Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (Salmo salar) fed vegetable oils. Food Chem 87, 567-580

Ulbricht TLV, Southgate DAT (1991) Coronary heart-disease – 7 dietary factors. Lancet 338, 985-992

Visentainer JV, de Souza NE, Makoto M, Hayashi C, Franco MRB (2005) Influence of diets enriched with flaxseed oil on the a-linolenic, eicosapentaenoic and docosahexaenoic fatty acid in Nile tilapia (*Oreochromis niloticus*). Food Chem 90, 557-560

Watanabe WO, Losordo TM, Fitzsimmons K, Hanley F (2002) Tilapia production systems in the Americas: Technological advances, trends, and challenges. Rev. Fisheries Sci. 10, 465-498

Weaver KL, Ivester P, Chilton JA, Wilson MD, Pandey P, Chilton FH (2008) The content of favorable and unfavorable polyunsaturated fatty acids found in commonly eaten fish. J Am Diet Assoc 108, 1178-1185

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