Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*)

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Abbreviations: AA, arachidonic acid; BHT, butylated hydroxytoluene; DHA, docosahexaenoic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; FAF-BSA, fatty acid free bovine serum albumin; FO, fish oil; HBSS, Hanks balanced salt solution; HUFA, highly unsaturated fatty acids acids (carbon chain length ≥ C_{20} with ≥ 3 double bonds); PL, polar lipid; PUFA, polyunsaturated fatty acids; VO, vegetable oil; TLC, thin-layer chromatography.

Abstract

The desaturation and elongation of [1-^{14}C]18:3n-3 was investigated in hepatocytes of the tropical warm freshwater species, zebrafish (*Danio rerio*) and Nile tilapia (*Oreochromis niloticus*). The hepatocyte fatty acid desaturation/elongation pathway was assayed before and after the fish were fed two experimental diets, a control diet containing fish oil (FO) and a diet containing vegetable oil (VO; a blend of olive, linseed and high oleic acid sunflower oils) for 10 weeks. The VO diet was formulated to provide 1% each of 18:2n-6 and 18:3n-3, and so satisfy the possible EFA requirements of zebrafish and tilapia. At the end of the dietary trial, the lipid and fatty acid composition was determined in whole zebrafish, and liver, white muscle and brain of tilapia. Both zebrafish and tilapia expressed a hepatocyte fatty acid desaturation/elongation pattern consistent with them being freshwater and planktonivorous fish. The data also showed that hepatic fatty acid desaturation/elongation was nutritionally regulated with the activities being higher in fish fed the VO diet compared to fish fed the FO diet. In zebrafish, the main effect of the VO diet was increased fatty acid Δ6 desaturase activity resulting in the production of significantly more 18:4n-3 compared to fish fed the FO diet. In tilapia, all activities in the pathway were greater in fish fed the VO diet resulting in increased amounts of all fatty acids in the pathway, but primarily eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). However, the fatty acid compositional data indicated that despite increased activity, desaturation of 18:3n-3 was insufficient to maintain tissue proportions of EPA and DHA in fish fed the VO diet at the same level as in fish fed the FO diet. Practically, these results indicate that manipulation of tilapia diets in commercial culture in response to the declining global fish oil market would have important consequences for fish fatty acid composition and the health of consumers. Scientifically, zebrafish and tilapia, both the subject of active genome mapping projects, could be useful models for studies of lipid and fatty acid metabolism at a molecular biological and genetic level.

Introduction

The polyunsaturated fatty acids (PUFA), linoleate (18:2n-6) and linolenate (18:3n-3) cannot be biosynthesized *de novo* by most animals, including fish, and so they are termed essential fatty acids (EFA) (Holman 1986). The precise EFA requirements of fish varies both quantitatively and qualitatively between species (Watanabe 1982; Kanazawa 1985; Sargent et al. 1995, 1999). Thus, fish species displaying a so-called "freshwater" pattern are able to convert the C_{18} EFA, 18:3n-3 and 18:2n-6, to the longer chain, more unsaturated and physiologically important highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (20:5n-3; EPA), docosahexaenoic acid (22:6n-3; DHA) and arachidonic acid (20:4n-6; AA), via a series of fatty acid desaturation and elongations, and so only require the C_{18} PUFA (Sargent et al. 1989, 1995; Henderson and Tocher 1986). In contrast, fish species displaying a typical "marine" pattern cannot perform these conversions at an appreciable rate and so require a dietary source of the essential HUFA (Sargent et al. 1989, 1995). However, the "marine" pattern may actually be associated with adaptation to a carnivorous or, more specifically, a piscivorous, lifestyle where consumption of a predominantly fish diet, naturally rich in HUFA, has resulted in an evolutionary down-regulation of the desaturase and/or elongase enzyme activities required for the conversion of C_{18} PUFA to HUFA (Mourente and Tocher 1993, 1994; Sargent et al. 1995). These hypotheses are based on data from relatively few species including rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), pike (*Esox lucius*), turbot (*Scophthalmus maximus*) and sea bream (*Sparus aurata*) (Owen et al. 1975; Mourente and Tocher 1994; Sargent et al. 1995, 1999; Buzzi et al. 1997; Henderson et al. 1997). The fatty acid desaturation pathways in fish are currently of great interest as there is an urgent need to replace the $C_{20/22}$ HUFA-rich fish oils, derived from non-sustainable marine fisheries, with vegetable oils, rich in C_{18} PUFA, in the diets of aquacultured fish species (Sargent and Tacon 1999).

Diet is also known to directly affect desaturase enzyme activities in mammals (Brenner 1981), and we have shown that the hepatic desaturation of 18:3n-3 and 18:2n-6 was significantly greater in Atlantic salmon fed diets containing vegetable oils rich in 18:2n-6 and 18:3n-3 compared to fish fed diets containing fish oils rich in the long-chain HUFA, EPA and DHA (Bell et al. 1997; Tocher et al. 1997, 2000). Increased hepatic fatty acid desaturase activities were also induced in rainbow trout fed a diet containing olive oil compared to a diet containing fish oil (Buzzi et al. 1996) and the conversion of intraperitoneally injected ^{14}C-labelled 18:2n-6 and 18:3n-3 to HUFA was increased in Arctic charr (*Salvelinus alpinus*) fed diets containing only C_{18} PUFA compared to commercial diets containing fish

oil (Olsen and Ringo 1992). However, the effects of feeding dietary vegetable oils on hepatic fatty acid desaturation in fish has been restricted almost exclusively to these studies on salmonids.

In the present study we investigated the desaturation and elongation of [1-^{14}C]18:3n-3 in hepatocytes of two species of warm freshwater tropical fish, zebrafish (*Danio rerio*), the most important fish model species, and tilapia (*Oreochromis niloticus*), a species commercially important due to its extensive aquaculture. Fish were sampled initially immediately prior to being fed two diets, a control diet containing fish oil (FO) and a diet containing vegetable oils (VO; a blend of olive, linseed and high oleic acid sunflower oils) for 10 weeks. The VO diet was formulated to provide approximately 1% each of 18:2n-6 and 18:3n-3 and so satisfy the possible EFA requirements of zebrafish and tilapia (Sargent et al. 1995). At each sampling point, fatty acyl desaturation and elongation activities were determined in isolated hepatocytes and at the end of the trial, whole zebrafish and samples of tilapia liver, white muscle and brain were collected for analyses of lipid and fatty acid composition.

Materials and methods

Animals and diets

Zebrafish (of approximately 2 months of age) were obtained from a wholesale tropical fish supplier (?, Edinburgh, Scotland) and were maintained in duplicate 50 L aquaria (60 x 30 x 30cm) containing filtered freshwater which was maintained at a constant temperature of 26 ± 1 ^{o}C. The tanks were cleaned daily with approximately one third of the water replaced each day. Fish were fed twice a day by hand to satiety. Initially the zebrafish were fed a commercial flake diet (Tetraflake, Tetra Werke, Germany).

Tilapia were bred in the Institute of Aquaculture and were male F1s produced by natural mating of an XX female with a YY male (these parents being diploid, homozygous products of gynogenesis). Fish were 10 weeks old (approximately 4-5 g) at the initiation of the dietary trial and were fed a standard salmonid starter diet containing 54% protein and 15% oil prior to initiation of the dietary trial (Fry 02, pellet size, 1.0 – 1.5 mm; Trouw Aquaculture Ltd., Scotland). Tilapia were stocked initially at a density of 45 fish per tank in duplicate 20 L circular aquaria (0.5 x 0.5 m) supplied with recirculating, filtered (mechanical and biofilter) freshwater at a flow rate of 3 L.min^{-1} with a system replacement rate

of approximately 0.4 L.min^{-1}, representing approximately $^{1}/_{3}$ of the total system volume per day. Water temperature was maintained at a constant 27 ± 1 ºC.

The experimental diets were prepared in the Institute of Aquaculture. The diets contained 50% protein and 11% lipid and their formulation is shown in Table 1. The dry ingredients were combined and mixed before the addition of the oils and antioxidants and mixing continued for 5 minutes. Water was added to 30% of the dry weight to enable pelleting. The pellets were air dried for 48 h before storage at –20 ºC until use. Pellets of 1.0 –1.5 mm were prepared and fed directly to tilapia. For zebrafish, sieved fines of up to 0.5 mm were used. The fatty acid compositions of the diets are shown in Table 2. All diets were formulated to satisfy the nutritional requirements of freshwater fish (U.S. National Research Council 1993).

Lipid extraction and quantitation

For zebrafish, three whole fish per dietary group were analysed for lipid and fatty acid compositions at the end of the dietary trial. With tilapia, liver, brain and a fillet of white muscle were dissected from three fish per dietary group at the end of the trial. Total lipid was extracted from fish tissues and diet samples by homogenisation in ice-cold chloroform/methanol (2:1, v/v) by the method of Folch et al. (1957) and quantified gravimetrically.

Lipid class and fatty acid composition

Separation and quantification of lipid classes in fish body and tissue samples was performed by single-dimension double-development high-performance thin-layer chromatography (HPTLC) followed by scanning densitometry as described previously (Henderson and Tocher 1992). Fatty acid methyl esters (FAME) from total lipid were prepared by acid-catalyzed transmethylation (Christie 1989), and were extracted and purified by thin-layer chromatography (TLC) as described previously (Tocher and Harvie 1988). Analysis of FAME was performed by gas chromatography in a Fisons GC8000 gas chromatograph (Crawley, UK) equipped with a fused-silica capillary column (30m x 0.32 mm i.d., CP Wax 52 CB, Chrompack, UK) using hydrogen as carrier gas. Temperature programming was from 50 to 150°C at 35°C/min and to 225°C at 2.5°C/min. Individual FAME were identified by comparison with known standards and published data (Ackman 1980; Bell et al. 1983).

Preparation of isolated hepatocytes

Fish were killed by a blow to the head and the livers dissected immediately. The liver was chopped finely with scissors and incubated with 15 ml of solution A (calcium and magnesium-free Hanks balanced salt solution (HBSS) + 10 mM HEPES buffer + 1 mM EDTA) containing 0.1% (w/v) collagenase in a 25ml "Reacti-flask" in a shaking water bath at 25ºC for 30 min. The digested liver was filtered through 100 μm nylon gauze and the cells collected by centrifugation at 600 x g for 3 min. The cell pellet was washed with 20 ml of solution A containing 1% w/v fatty acid-free bovine serum albumin (FAF-BSA) and re-centrifuged as above. The hepatocytes were resuspended in 5 ml of Medium 199 containing 10 mM HEPES, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. One hundred μl of cell suspension was mixed with 400 μl of Trypan Blue and hepatocytes were counted and their viability assessed using a haemocytometer. One hundred μl of the hepatocyte cell suspension was retained for protein determination.

Assay of hepatocyte fatty acyl desaturation/elongation activities

The hepatocyte suspension (5 ml) was dispensed into a 25 cm^2 tissue culture flask. The cells were incubated with 0.25 μCi of [1-^{14}C] 18:3n-3 (final fatty acid concentration, ~ 1 μM), added as a complex with FAF-BSA in medium 199 prepared as described previously (Ghioni et al. 1997). After addition of isotope the flasks were incubated at 25 ºC for 2 h. After incubation, the cell layer was dislodged by gentle rocking, the cell suspension transferred to glass conical test tubes and the flasks washed with 1 ml of ice-cold HBSS containing 1% FAF-BSA. The cell suspensions were centrifuged at 600 g for 2 min, the supernatant discarded and the cell pellets washed with 5 ml of ice-cold HBSS/FAF-BSA. The supernatant was discarded and the tubes placed upside down on paper towels to blot for 15 sec before extraction of total lipid using ice-cold chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxy toluene (BHT) essentially as described by Folch et al. (1957) and as described in detail previously (Tocher et al. 1988).

Total lipid was transmethylated and FAME prepared as described above. The methyl esters were redissolved in 100 μl hexane containing 0.01% BHT and applied as 2.5 cm streaks to TLC plates impregnated by spraying with 2 g silver nitrate in 20 ml acetonitrile and pre-activated at 110 ºC for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v) (Wilson and Sargent 1992).

Autoradiography was performed with Kodak MR2 film for 6 days at room temperature. Areas of silica containing individual PUFA were scraped into scintillation mini-vials containing 2.5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Georgia) and radioactivity determined in a TRI-CARB 2000CA scintillation counter (United Technologies Packard, U.K.). Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Protein determination

Protein concentration in isolated hepatocyte suspensions was determined according to the method of Lowry et al. (1951) after incubation with 0.25 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60ºC.

Materials

[1-^{14}C]18:3n-3 (50-55 mCi/mmol) was obtained from NEN (DuPont (U.K.) Ltd., Stevenage, U.K.). HBSS, Medium 199, HEPES buffer, glutamine, penicillin, streptomycin, collagenase (type IV), FAF-BSA, BHT and silver nitrate were obtained from Sigma Chemical Co. (Poole, U.K.). TLC plates (20 cm x 20 cm x 0.25 mm) and HPTLC plates (10 cm x 10 cm x 0.15 mm),, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England.

Statistical analysis

Data are presented as means ± SD (n=3). The significance of dietary effects on total fatty acid desaturation in hepatocytes was determined by one-way analysis of variance (ANOVA) followed where pertinent by Tukey's multiple range test. The significance of effects on individual products of hepatocyte fatty acid desaturation and whole fish or tissue lipid and fatty acid compositions due to diet were determined by the Student's t-test. Differences were regarded as significant when $P < 0.05$ (Zar 1984).

Results

Effects of diet on hepatocyte fatty acid desaturation/elongation activities.

The total desaturation/elongation of [1-^{14}C]18:3n-3 in isolated hepatocytes was significantly higher in both zebrafish and tilapia fed the VO diet compared to fish fed the FO diet (Fig.1). Initially, the total fatty acid desaturation activity in hepatocytes from zebrafish being fed the commercial flake diet was high and not significantly different to the activity in fish fed the VO diet. In contrast, the total fatty acid desaturation activity in hepatocytes from tilapia being fed the commercial trout diet at the initiation of the dietary trial was very low and significantly lower than in fish at the end of the trial fed either the VO or FO diets (Fig.1). The main products of desaturation of [1-^{14}C]18:3n-3 in zebrafish hepatocytes at the initiation of the dietary trial were 18:4n-3 followed by 20:5n-3 (Fig.2). The primary effect of feeding the experimental diets was that the amount of 18:4n-3 was very significantly lower in fish fed the FO diet, with the amount of 20:5n-3 also significantly reduced compared to that in fish fed the VO diet (Fig.2). Summing the products of the various enzymic steps in the desaturation/elongation pathway indicated that the major difference between zebrafish fed the FO and VO diets was significantly reduced Δ6 fatty acid desaturase activity in fish fed the FO diet (Fig.2). The predominant product of desaturation of [1-^{14}C]18:3n-3 in tilapia hepatocytes at the initiation of the dietary trial was 20:5n-3 although the data are not shown due to the very low level of activity. In tilapia at the end of the dietary trial, all the individual products of desaturation were increased in fish fed the VO diet compared to fish fed the FO diet (Fig.3). The greatest increases were in the amounts of 20:5n-3 and 22:6n-3 produced, whereas the increase in 20:4n-3 was not significant (Fig.3). Summing the products of the various enzymic steps in the desaturation/elongation pathway in tilapia indicated that the products of all the steps in the pathway were significantly increased in fish fed the VO diet compared to those in fish fed the FO diet (Fig.3).

Effects of diet on lipid and fatty acid compositions of zebrafish

There was no apparent effect of diet on the lipid content at the end of the trial in zebrafish (whole fish) (Table 3). However, fish fed the FO diet had significantly higher percentages of triacylglycerol and total neutral lipid than fish fed the VO diet. Whole bodies of zebrafish fed the FO diet showed higher proportions of total n-3PUFA, due to increased percentages of 20:5n-3 and 22:6n-3, and total PUFA, whereas fish fed the VO diet had higher proportions of 18:3n-3, 20:4n-6 and total n-6PUFA (Table 4). The zebrafish fed the FO diet also showed higher proportions of 20:1 and 22:1 but lower total

monoenes due to lower 18:1n-9 than fish fed the VO diet. There were no major significant differences in whole body saturated fatty acids between zebrafish fed the FO and VO diets.

Effects of diet on lipid and fatty acid compositions of tilapia tissues

The lipid content of tilapia tissues was similar in fish fed the FO and VO diets (Table 5). In contrast to zebrafish, tissues of tilapia fed the VO diet showed increased proportions of triacylglycerol compared to fish fed the FO diet although there was some variability in tissue triacylglycerol levels as shown by the high deviations which made the increase non-significant in muscle (Table 5). However, in muscle and brain, the percentages of all the other lipid classes were generally reduced in tilapia fed the VO diet indicating that the primary effect of the VO diet was increased triacylglycerol deposition which resulted in the relative amounts of all other lipid classes decreasing. Liver was slightly different in that there were also quite large decreases in the percentages of free fatty acids and sterol esters in fish fed the VO diet and so, in consequence, the proportions of polar lipids were generally increased (Table 5).

The major differences in fatty acid compositions of tilapia fed the two experimental diets were observed in all tissues. Thus, tissues of fish fed the VO diet were characterised by having higher proportions of monoenes, due primarily to greatly increased percentages of 18:1n-9, n-9 PUFA and 18:3n-3, and greatly reduced proportions of all other n-3 PUFA, especially 20:5n-3 and 22:6n-3, compared to fish fed the FO diet (Table 6). Other dietary differences varied between tissues, with the brain being least affected. In fish fed the VO diet, liver and muscle had much lower proportions of 20:1 and 22:1, but brain showed no differences in these fatty acids. Saturated fatty acids and 18:0 were increased in livers of fish fed the VO diet, whereas in brain, saturated fatty acids and 18:0 were decreased, and muscle showed no differences in saturated fatty acids (Table 6). The percentage of 18:2n-6 was increased in muscle and decreased in liver in fish fed the VO diet, but was unaffected by diet in brain.

Discussion

The warmwater zebrafish and tilapia had all the enzymic activities necessary to produce DHA, similar to the situation in salmonids from temperate or cold waters, including Atlantic salmon (Bell at al. 1997; Tocher et al. 1997, 2000), rainbow trout (Buzzi et al 1996), and Arctic char (Olsen and Ringo 1992). Zebrafish are small and low in the food chain, and so are predominantly planktonivorous.

Tilapia are much larger fish, but they also naturally feed on zooplankton, phytoplankton, insects and bottom organisms and so are not normally piscivorous (although they can be cannibalistic under certain conditions). Consistent with their non-piscivorous feeding habits, both zebrafish and tilapia displayed hepatocyte fatty acid desaturation and elongation activities adhering to the so-called "freshwater" pattern. Previously, an *in vivo* study looking at the conversion of intraperitoneally-injected radiolabelled 18:2n-6 and 18:3n-3 had concluded that tilapia were capable of desaturating these fatty acids (Olsen et al. 1990).

Nutritional regulation of hepatocyte fatty acid desaturation was clearly observed in both tropical species, with the total desaturation activity being approximately 2-fold greater in fish fed the VO diet compared to fish fed the FO diet. The vegetable oil blend used here was olive/linseed/high oleic sunflower oils (approx. 4:1:1, by vol.) which gave an 18:3n-3/18:2n-6/n-3HUFA ratio of 9:9:1. In a previous study on salmon parr approaching smoltification, a 1:1 blend of rapeseed/linseed oil that gave a ratio of 18:3n-3/18:2n-6/n-3HUFA of 5:5:1, increased hepatocyte total desaturase activity over that obtained with a diet containing fish oil by just over 2-fold at the time of peak activity (Bell et al. 1997). In a similar trial with salmon parr, diets containing rapeseed oil (18:3n-3/18:2n-6/n-3HUFA of 2:4:1) and linseed oil (18:3n-3/18:2n-6/n-3HUFA of 8:4:1) resulted in total desaturase activities 2.4- and 1.7-fold higher, respectively, relative to that in fish fed a diet containing fish oil (Tocher et al. 2000). In another study where comparisons are possible, the total desaturase activities in hepatocytes from salmon smolts fed diets containing sunflower oil and olive oil containing predominantly 18:2n-6 as the C_{18} PUFA were approximately 2-fold greater than the activity obtained with fish fed diets containing fish oil (Tocher et al. 1997). Although not directly comparable, similar (2- to 3-fold) increases in desaturase activity were observed with trout fed olive oil (Buzzi et al. 1996) and Arctic char fed purified C_{18} PUFA (Olsen and Ringo 1992). Previously, Olsen et al. (1990) had shown that the conversion of inraperitoneally injected 18:3n-3 was increased in tilapia fed a diet containing only linoleic acid. Therefore, the effects of replacing fish oil with vegetable oil in the diets of the tropical species, tilapia and zebrafish, were similar to those observed in salmonids. The effects were also similar in all species despite differences in dietary oil contents and the type of plant oil, with, in particular, not a great deal of difference between oils rich in 18:3n-3 or 18:2n-6. The primary factor in the increased desaturase active is almost certainly the reduction of product inhibition by the considerable lowering of n-3HUFA in diets containing vegetable oil, rather than provision of more substrate PUFA for the first and, reportedly, rate-limiting enzyme in the pathway, Δ6 desaturase, although this may also contribute to the overall result.

The zebrafish prior to the trial showed high fatty acid desaturation activity due to the fact that the commercial flake diet was essentially a VO-type diet. The fatty acid composition of the diet showed that it was formulated using an 18:2n-6-rich oil (probably corn oil) and fish products which were responsible for the EPA and DHA content. However, the production of EPA and DHA from [1-^{14}C]18:3n-3 in hepatocytes was higher in fish when fed the commercial flake diet than in fish fed the VO diet, despite the fact that the percentages of EPA and DHA were higher in the commercial flake diet than in the VO diet. Even considering the slightly higher lipid content of the VO diet the absolute amounts of EPA and DHA delivered by the flake diet would be higher than the VO diet. The flake diet was characterised by very much higher 18:2n-6, and lower 18:3n-3, than the VO diet (18:2n-6/18:3n-3 ratios of 7 and 1, respectively) and it is possible that this difference in the relative amounts of the C_{18} PUFA, the direct substrates for Δ6 desaturase, were responsible for the effects observed, although the mechanism is not clear. Perhaps consistent with this was the result with salmon described above where a diet containing rapeseed oil gave a larger increase in desaturase activity than a diet containing linseed oil (18:2n-6/18:3n-3 ratios of 2 and 0.5, respectively) (Tocher et al. 2000).

In contrast, the desaturase activity in tilapia at the initiation of the experiment was very low due to the fact that the fish had been reared on trout/salmonid pellets containing a northern hemisphere fish oil (as evidenced by the high 20:1 and 22:1 contents) (Ackman 1980). The very low activity was the combined result of the high proportions of EPA and DHA, and the fact that the commercial pelleted trout diet contained 15% lipid. Therefore, the absolute amount of EPA and DHA in the diet was higher than in the experimental FO diet which was 11% lipid.

Whole zebrafish, and tilapia tissues, from fish fed the VO diet were characterised by decreased proportions of EPA, DHA, total n-3 PUFA compared to fish fed the FO diet. Fish fed the VO diet also showed generally increased proportions of 18:3n-3, 18:2n-6 and total n-6PUFA, and also increased total monoenes due to increased percentages of 18:1n-9, whereas fish fed the FO diet were characterized by high proportions of 20:1 and 22:1, monoenes characteristic of northern hemisphere fish oils. Therefore, these differences reflect the dietary fatty acid input, despite the fact that the fatty acid desaturase activities measured in hepatocytes were significantly higher in both zebrafish and tilapia fed the VO diet compared to the FO diet. In addition, the intermediates of 18:3n-3 desaturation and elongation, which are not normally in high concentration, such as 18:4n-3 and 20:4n-3, were not increased in whole zebrafish or tissues of tilapia fed the VO diet. This may indicate that desaturation of dietary 18:3n-3 was proceeding to EPA and DHA, but that the increased activity was insufficient to compensate for the lack of EPA and DHA in the VO diet and so levels of tissue EPA and DHA were

significantly reduced in VO-fed fish. This has also been observed in salmon and trout, where the increased activity of the hepatocyte fatty acid desaturation pathway did not fully compensate for the lack of EPA and DHA in the vegetable oil diets, and so levels of n-3HUFA are reduced, and those of C_{18} PUFA increased, in fish fed the diets containing vegetable oils (Buzzi et al. 1996; Bell et al. 1997; Tocher et al. 1997, 2000).

In conclusion, this study has shown that hepatic fatty acid desaturation/elongation was nutritionally regulated in wo warm water fish, zebrafish and tilapia, and that activities were higher in fish fed dietary vegetable oil compared to those in fish fed fish oil. However, the fatty acid compositional data indicated that despite increased activity, desaturation of 18:3n-3 was insufficient to maintain tissue proportions of EPA and DHA in fish fed vegetable oil at the same level as in fish fed fish oil. These results indicate that manipulation of tilapia diets in commercial culture in response to the declining global fish oil market could have important consequences for fish fatty acid composition and the health of consumers. Both zebrafish and tilapia are the subject of active genome mapping projects, and although the zebrafish genome is probably the most fully characterised of fish species, both could be useful model species in studies of lipid and fatty acid metabolism at molecular biological and genetic levels.

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Legends to Figures:

Figure 1. Effect of diet on total fatty acid desaturation activity in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*) hepatocytes. Fish were fed for 10 weeks on diets containing either 11% fish oil (FO) or 11% vegetable oil (VO) after being initially reared on either a commercial flake diet (zebrafish) or a commercial trout pellet (tilapia) as described in Materials and Methods. Columns for each species with different superscript letters are significantly different as determined by one-way ANOVA followed by Tukey's multiple range test.

Figure 2. Effect of diet on the apparent activities of the fatty acid desaturation and elongation pathway, and on the individual fatty acid products of the desaturation/elongation pathway in zebrafish (*Danio rerio*) hepatocytes. Fish were fed for 10 weeks on diets containing either 11% fish oil (FO) or 11% vegetable oil (VO) after being initially reared on a commercial flake diet as described in Materials and Methods. Differences between mean values for fish fed the VO diet compared to fish fed the FO diet were determined by the Student's t-test and are significantly different where indicated (*, $P < 0.05$).

Figure 3. Effect of diet on the apparent activities of the fatty acid desaturation and elongation pathway, and on the individual fatty acid products of the desaturation/elongation pathway in tilapia (*Oreochromis niloticus*) hepatocytes. Fish were fed for 10 weeks on diets containing either 11% fish oil (FO) or 11% vegetable oil (VO) after being initially reared on a commercial trout pellet diet containing 20% fish oil as described in Materials and Methods. Differences between mean values for fish fed the VO diet compared to fish fed the FO diet were determined by the Student's t-test and are significantly different where indicated (*, $P < 0.05$).

Fig.1

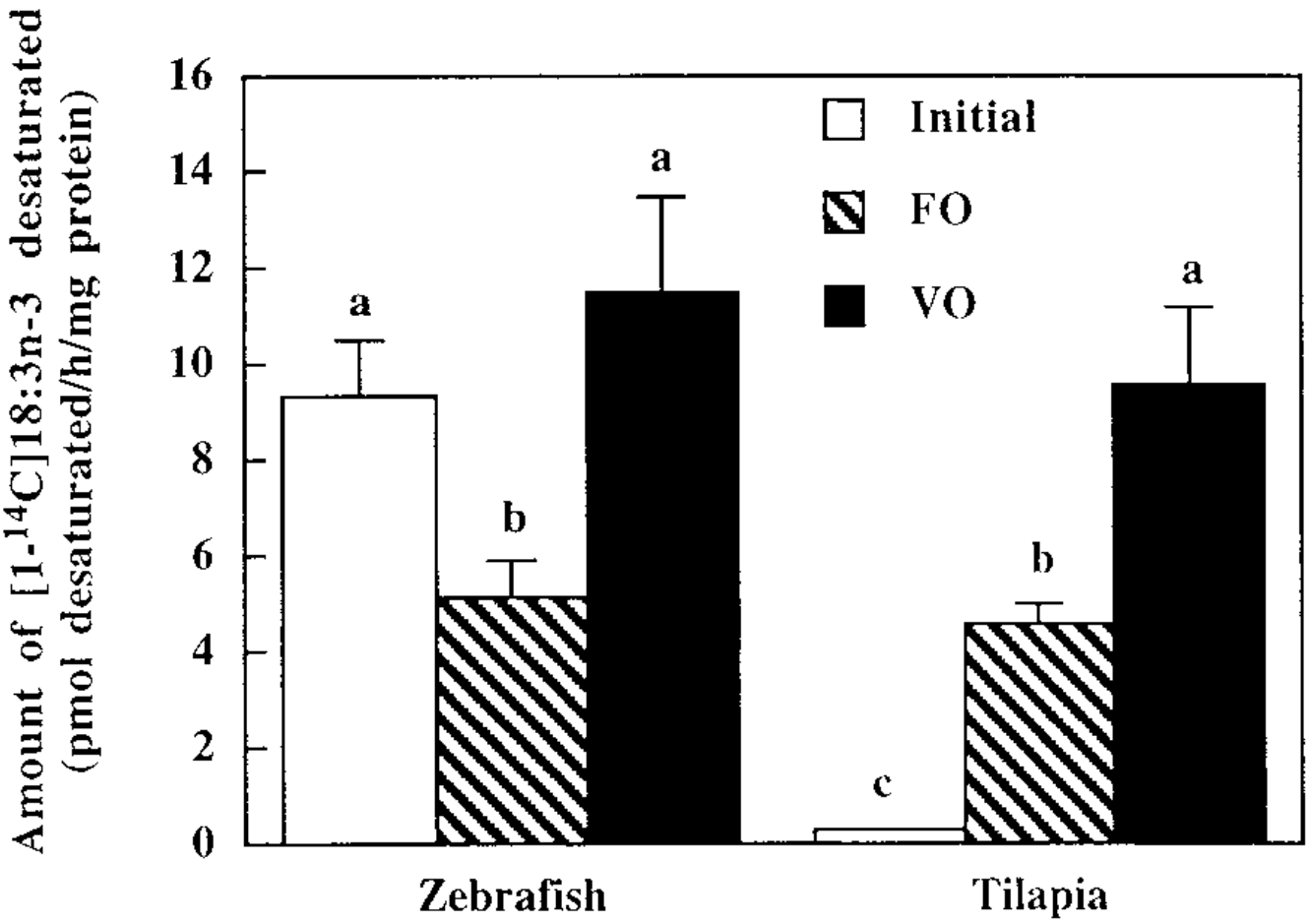
Amount of [1-14C]18:3n-3 desaturated
(pmol desaturated/h/mg protein)
16
14
12
10
8
6
4
2
0
Initial
FO
VO
a
b
a
c
b
a
Zebrafish
Tilapia

Fig.2.

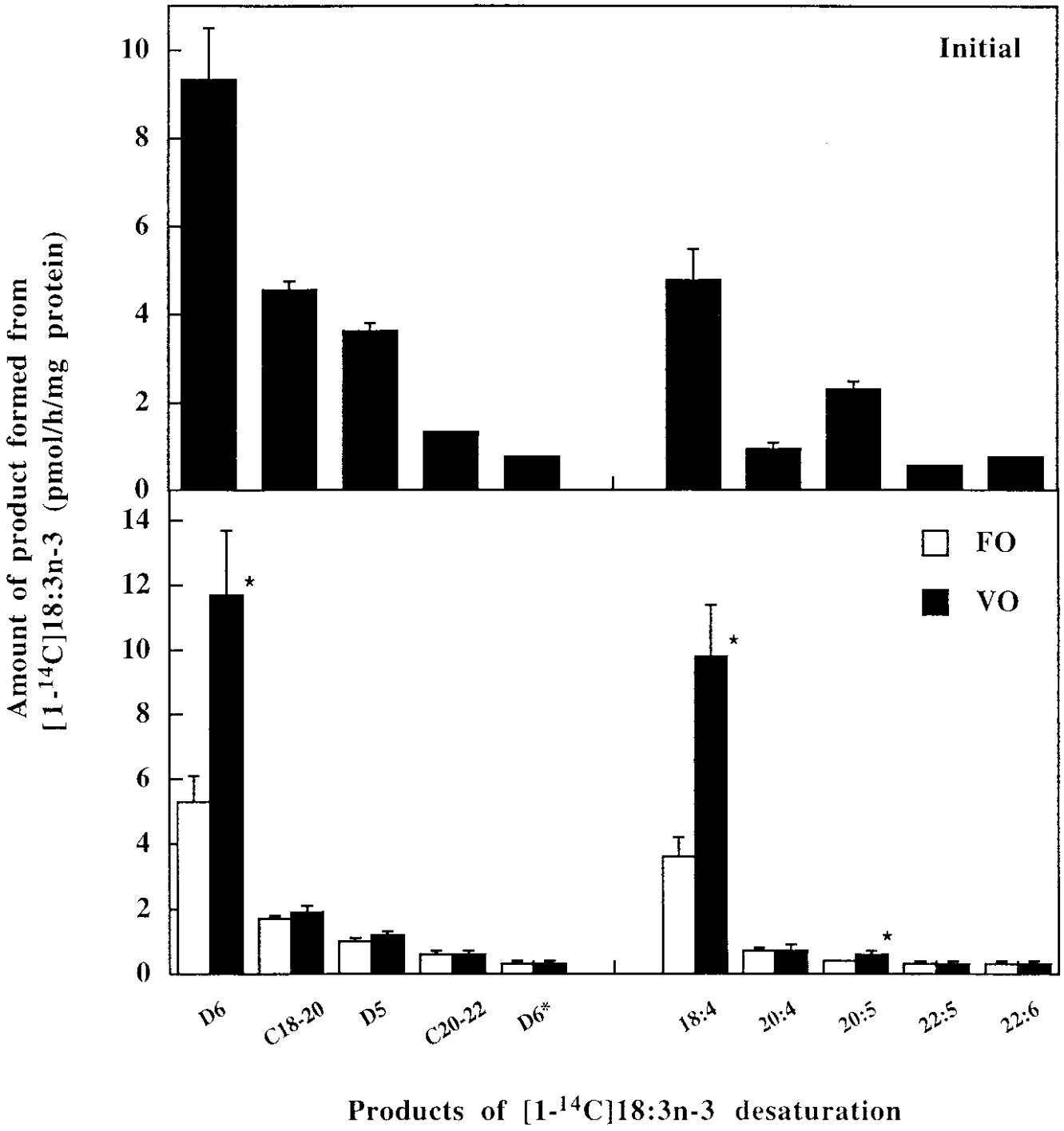
Initial
FO
VO
Amount of product formed from [1-14C]18:3n-3 (pmol/h/mg protein)
Products of [1-14C]18:3n-3 desaturation
D6
C18-20
D5
C20-22
D6*
18:4
20:4
20:5
22:5
22:6
*

Fig.3.

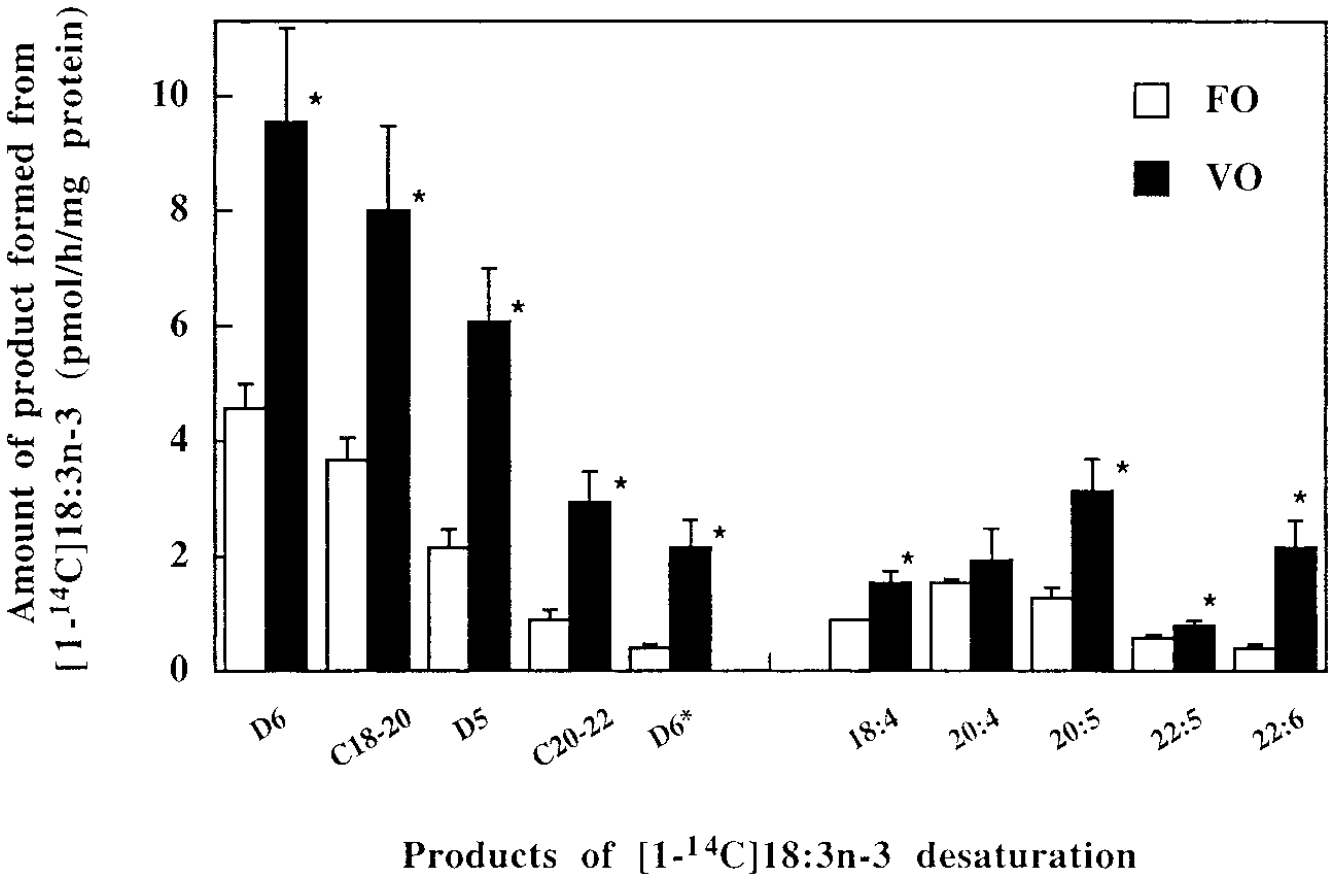
Amount of product formed from [1-14C]18:3n-3 (pmol/h/mg protein)
10
8
6
4
2
0
FO
VO
D6
C18-20
D5
C20-22
D6*
18:4
20:4
20:5
22:5
22:6
Products of [1-14C]18:3n-3 desaturation

Table 1. Components (g/Kg of dry diet) of experimental diets.

Component	Fish oil (FO) diet	Vegetable oil (VO) diet
Vitamin-free casein[1]	480	480
Potato starch[2]	150	150
Fish meal[3]	50	50
Mineral mix[4]	47	47
Vitamin mix[5]	10	10
Arginine	4	4
Leucine	4	4
Methionine	3	3
Cystine	2	2
Orange G	1	1
α-cellulose	139.6	139.6
Fish oil[6]	110	0
Olive oil[7]	0	75
Linseed oil[8]	0	17.5
High oleic sunflower oil[9]	0	17.5
Antioxidant mix[10]	0.4	0.4

[1]Vitamin-free micropulverised (ICN Biomedical Ltd., High Wycombe, U.K.).

[2]Passeli WA4 (Avebe (U.K.) Ltd., Ulceby, South Humberside, U.K.

[3]LT94, Low temperature fish meal (Ewos Ltd., Bathgate, U.K.).

[4]Supplied (per kg diet): KH_2PO_4, 22g; $FeSO_4.7H_2O$, 1.0g; $ZnSO_4.7H_2O$, 0.13g; $MnSO_4.4H_2O$, 52.8 mg; $CuSO_4.5H_2O$,12 mg; $CoSO_4.7H_2O$, 2 mg.

[5]Supplied (mg/kg diet): ascorbic acid, 1000; myo-inositol, 400; nicotinic acid, 150; calcium pantothenate, 44; all-rac-a-tocopheryl acetate, 40; riboflavin, 20; pyridoxine hydrochloride, 12; menadione, 10; thiamine hydrochloride, 10; retinyl acetate, 7.3; folic acid, 5; biotin, 1; cholecalciferol, 0.06; cyanocobalamin, 0.02.

[6]Northern hemisphere fish oil (FOSOL, Seven Seas Ltd., Hull, U.K.).

[7]Tesco, Cheshunt, U.K.

[8]ICN Biomedical Ltd., High Wycombe, U.K.

[9]Croda, Hull,U.K.

[10]Dissolved in propylene glycol and contained (g/L): butylated hydroxy anisole, 60; propyl gallate, 60; citric acid, 40.

All the other ingredients were obtained from Sigma Chemical Co. Ltd., Poole, Dorset, U.K.

Table 2. Lipid (oil) content (percentage of diet dry weight) and fatty acid composition (percentage of total fatty acids by weight) of experimental diets containing fish oil (FO) or vegetable oil (VO) and of commercial diets fed to the zebrafish (tetraflake) and tilapia (trout pellet) prior to the initiation of the dietary trials.

	Commercial diets		Experimental diets	
Fatty acid	Tetraflake	Trout pellet	FO	VO
14:0	2.5	5.6	5.3	0.4
15:0	0.6	0.5	0.7	n.d.
16:0	17.1	14.7	15.9	9.3
17:0	0.5	0.4	0.5	0.1
18:0	6.7	2.3	3.2	2.6
20:0	2.8	0.2	0.2	0.3
Total saturated	30.3	23.6	25.8	12.7
16:1n-9	0.1	0.2	0.3	n.d.
16:1n-7	3.1	4.7	5.4	0.7
18:1n-9	16.6	14.0	15.0	64.9
18:1n-7	2.5	2.5	3.2	0.4
20:1n-9	2.5	9.5	9.4	0.9
22:1n-11	2.8	13.5	12.1	0.8
22:1n-9	2.1	0.3	1.0	n.d.
24:1n-9	0.5	0.9	1.1	n.d.
Total monoenes	30.6	45.6	47.5	67.7
18:2n-6	27.2	3.2	1.5	9.1
18:3n-6	n.d.	0.2	0.2	n.d.
20:2n-6	0.1	0.4	n.d.	n.d.
20:3n-6	n.d.	0.6	n.d.	n.d.
20:4n-6	0.6	0.5	0.6	n.d.
22:5n-6	n.d.	0.2	0.2	n.d.
Total n-6 PUFA	27.9	5.0	2.5	9.1
18:3n-3	3.5	1.4	1.1	8.7
18:4n-3	0.4	3.4	2.0	0.1
20:4n-3	0.2	0.9	0.6	n.d.
20:5n-3	2.4	6.7	5.1	0.4
22:5n-3	0.5	0.9	1.0	n.d.
22:6n-3	4.2	11.3	7.9	0.7
Total n-3 PUFA	11.3	24.5	17.7	9.9
Total PUFA	39.2	30.4	20.2	19.0
Lipid content	9.2	15.0	11.0	11.0

n.d. not detected; PUFA, polyunsaturated fatty acids.

Table 3. Lipid content (percentage of wet weight) and lipid class composition (percentage of total lipid) of zebrafish (whole fish) fed diets containing fish oil (FO) or vegetable oil (VO)

Parameter	FO			VO			
Lipid content	10.2	±	0.5	10.2	±	2.7	
Lipid class							
Phosphatidylcholine	7.6	±	1.8	10.4	±	0.3	
Phosphatidylethanolamine	4.6	±	0.9	6.2	±	0.6	
Phosphatidylserine	0.8	±	0.1	1.4	±	0.3	*
Phosphatidylinositol	0.8	±	0.2	1.5	±	0.1	*
Phosphatidic acid/cardiolipin	0.8	±	0.2	0.9	±	0.2	
Shingomyelin	0.8	±	0.1	1.4	±	0.6	
Total polar lipids	15.4	±	3.0	21.9	±	1.7	*
Total neutral lipids	84.6	±	3.0	78.1	±	1.7	*
Triacylglycerol	71.1	±	5.2	60.0	±	1.5	*
Cholesterol	8.0	±	1.3	13.0	±	0.9	*
Free fatty acids	2.6	±	0.6	3.2	±	0.7	
Steryl esters	3.0	±	1.1	1.9	±	1.2	

Results are means ± SD (n = 3). Differences between mean values for fish fed the VO diet compared to fish fed the FO diet were determined by the Student's t-test and are significantly different where indicated (*, $P < 0.05$).

Table 4. Fatty acid composition (percentage of total fatty acids by weight) of carcasses of zebrafish fed the diets containing fish oil (FO) or vegetable oil (VO).

Fatty acid	FO	VO	
14:0	1.7 ± 0.2	0.7 ± 0.1	*
15:0	0.4 ± 0.0	0.3 ± 0.0	
16:0	23.1 ± 0.2	21.9 ± 1.7	
17:0	tr	tr	
18:0	3.2 ± 0.1	3.2 ± 0.1	
20:0	tr	tr	
Total saturated	28.4 ± 0.1	26.1 ± 1.7	
16:1n-9	1.4 ± 0.3	2.9 ± 0.4	*
16:1n-7	4.1 ± 0.3	3.2 ± 0.4	*
18:1n-9	37.4 ± 0.6	47.8 ± 0.5	*
18:1n-7	2.5 ± 0.2	2.6 ± 0.2	
20:1n-11	0.6 ± 0.1	0.3 ± 0.0	*
20:1n-9	2.2 ± 0.1	0.7 ± 0.1	*
20:1n-7	0.1 ± 0.0	0.0 ± 0.0	
22:1	1.9 ± 0.1	0.3 ± 0.0	*
24:1n-9	0.2 ± 0.0	0.1 ± 0.0	
Total monoenes	50.3 ± 0.5	57.8 ± 0.9	*
18:2n-6	4.5 ± 0.5	4.9 ± 0.5	
18:3n-6	0.2 ± 0.0	0.2 ± 0.0	
20:2n-6	0.5 ± 0.1	0.9 ± 0.2	*
20:3n-6	0.4 ± 0.0	0.6 ± 0.0	*
20:4n-6	1.3 ± 0.2	2.2 ± 0.4	*
22:4n-6	0.2 ± 0.0	0.4 ± 0.1	*
22:5n-6	0.2 ± 0.0	0.3 ± 0.0	*
Total n-6 PUFA	7.3 ± 0.6	9.5 ± 0.9	*
18:3n-3	1.4 ± 0.2	2.0 ± 0.2	*
18:4n-3	0.7 ± 0.1	0.2 ± 0.0	*
20:3n-3	0.1 ± 0.0	0.0 ± 0.0	
20:4n-3	0.4 ± 0.0	0.1 ± 0.0	*
20:5n-3	2.8 ± 0.2	0.4 ± 0.2	*
22:5n-3	0.9 ± 0.0	0.2 ± 0.1	*
22:6n-3	7.8 ± 0.8	3.7 ± 1.2	*
Total n-3 PUFA	14.0 ± 0.9	6.6 ± 1.8	*
Total PUFA	21.3 ± 0.4	16.1 ± 2.5	*

Results are means ± SD (n = 3). Differences between mean values for fish fed the VO diet compared to fish fed the FO diet were determined by the Student's t-test and are significantly different where indicated (*, $P < 0.05$). PUFA,polyunsaturated fatty acids; tr, trace value (< 0.05%).

Table 5. Lipid content (percentage of wet weight) and lipid class composition (percentage of total lipid by weight) of tissues from tilapia (*Oreochromis nilotica*) fed diets containing fish oil (FO) or vegetable oil (VO).

	Liver					Muscle					Brain				
	FO		VO			FO		VO			FO		VO		
Lipid content	16.3	± 3.3	15.4	± 2.0		1.3	± 0.2	1.2	± 0.3		10.0	± 2.0	12.4	± 3.8	
Lipid class composition															
PC	6.3	± 1.3	9.0	± 0.6	*	22.8	± 2.4	19.0	± 3.1		11.2	± 0.4	8.3	± 1.5	*
PE	2.7	± 0.6	4.4	± 0.3	*	11.2	± 1.6	10.5	± 2.0		13.4	± 1.3	9.7	± 1.4	*
PS	0.9	± 0.3	1.2	± 0.1		3.1	± 0.1	1.9	± 0.4	*	5.6	± 0.3	3.7	± 0.7	*
PI	1.1	± 0.3	1.4	± 0.2		4.2	± 0.2	3.3	± 0.6		2.0	± 0.2	1.2	± 0.3	*
PG/CL	0.8	± 0.2	1.3	± 0.1	*	1.7	± 0.6	1.6	± 0.5		0.5	± 0.2	0.3	± 0.1	
Sphingomyelin	1.3	± 0.3	1.5	± 0.1		3.0	± 0.3	2.1	± 0.3	*	1.4	± 0.0	0.9	± 0.2	*
Cerebrosides	n.d.		n.d.			n.d.		n.d.			5.3	± 0.4	3.4	± 0.6	*
Sulphatides	n.d.		n.d.			n.d.		n.d.			1.7	± 0.4	0.9	± 0.3	
Total polar	13.1	± 3.0	18.8	± 1.1	*	45.9	± 4.9	38.4	± 6.6		41.1	± 3.0	28.6	± 5.0	*
Total neutral	86.9	± 3.0	81.2	± 1.1	*	54.1	± 4.9	61.6	± 6.6		58.9	± 3.0	71.4	± 5.0	*
Cholesterol	8.6	± 0.6	7.7	± 0.6		13.4	± 1.0	11.6	± 1.6		17.8	± 0.9	16.5	± 0.9	
Triacylglycerol	54.3	± 3.2	64.6	± 1.8	*	35.0	± 5.8	47.3	± 8.2		37.2	± 4.2	53.7	± 6.0	*
Free fatty acids	11.6	± 1.1	1.9	± 0.8	*	2.9	± 0.6	0.5	± 0.2	*	2.0	± 0.4	0.4	± 0.2	*
Sterol esters	12.3	± 0.9	7.1	± 0.7	*	2.7	± 1.0	2.1	± 0.6		1.9	± 0.2	0.8	± 0.3	*

Results are means ± SD (n=3). Differences between mean values for fish fed the VO diet compared to fish fed the FO diet were determined by the Student's t-test and are significantly different where indicated (*, $P < 0.05$). CL, cardiolipin; n.d., not detected; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine.

Table 6. Fatty acid composition (percentage of total fatty acids by weight) of tissues from tilapia (*Oreochromis nilotica*) fed diets containing fish oil (FO) or vegetable oil (VO).

	Liver			Muscle			Brain		
	FO	VO		FO	VO		FO	VO	
14:0	3.2 ± 0.3	2.5 ± 0.3	*	2.0 ± 0.3	1.5 ± 0.3		2.0 ± 0.3	2.3 ± 0.5	
15:0	0.4 ± 0.0	0.1 ± 0.0	*	0.4 ± 0.0	0.2 ± 0.0	*	0.3 ± 0.0	0.2 ± 0.0	
16:0	15.0 ± 0.8	20.6 ± 0.3	*	18.0 ± 0.6	18.7 ± 1.3		17.6 ± 0.3	16.1 ± 1.0	
18:0	3.8 ± 0.4	6.3 ± 0.5	*	5.8 ± 0.4	6.4 ± 0.7		10.0 ± 0.9	5.6 ± 1.7	*
Total saturated	22.4 ± 1.2	29.5 ± 0.4	*	26.3 ± 0.7	26.8 ± 1.8		29.9 ± 0.8	24.2 ± 2.1	*
16:1n-9	0.3 ± 0.0	1.1 ± 0.1	*	0.3 ± 0.1	0.6 ± 0.1	*	0.4 ± 0.0	0.6 ± 0.1	
16:1n-7	4.6 ± 0.3	4.4 ± 0.6		2.7 ± 0.5	2.3 ± 0.2		3.5 ± 0.3	3.7 ± 0.6	
18:1n-9	18.2 ± 1.5	49.9 ± 1.3	*	13.1 ± 1.0	34.7 ± 2.3	*	19.6 ± 0.4	38.8 ± 2.6	*
18:1n-7	3.3 ± 0.2	2.6 ± 0.1	*	3.5 ± 0.1	3.0 ± 0.3		2.4 ± 0.1	2.5 ± 0.2	
20:1n-11	2.4 ± 0.1	0.2 ± 0.0	*	0.9 ± 0.0	0.3 ± 0.0	*	0.7 ± 0.1	0.4 ± 0.1	*
20:1n-9	8.4 ± 0.4	2.1 ± 0.1	*	5.3 ± 0.5	2.9 ± 0.3	*	3.5 ± 0.6	3.5 ± 0.7	
20:1n-7	0.5 ± 0.0	0.2 ± 0.0	*	0.2 ± 0.0	0.2 ± 0.0		0.2 ± 0.0	0.2 ± 0.0	
22:1	8.1 ± 0.6	0.5 ± 0.1	*	4.2 ± 0.6	1.3 ± 0.2	*	3.6 ± 0.6	2.5 ± 0.7	
24:1n-9	0.8 ± 0.0	0.1 ± 0.0	*	0.9 ± 0.0	0.4 ± 0.1	*	1.1 ± 0.0	0.5 ± 0.1	*
Total monoenes	46.6 ± 1.6	61.1 ± 2.1	*	31.1 ± 2.4	45.9 ± 1.9	*	34.9 ± 1.8	52.9 ± 2.9	*
18:2n-9	n.d	0.7 ± 0.1	*	n.d	0.5 ± 0.1	*	n.d	0.5 ± 0.1	*
20:2n-9	n.d	0.3 ± 0.1	*	n.d	0.2 ± 0.1	*	n.d	0.2 ± 0.0	*
20:3n-9	n.d	n.d		n.d	n.d		n.d	n.d	
Total n-9 PUFA	n.d	1.0 ± 0.2	*	n.d	0.7 ± 0.2	*	n.d	0.7 ± 0.1	*
18:2n-6	3.6 ± 0.3	2.4 ± 0.3	*	3.2 ± 0.2	4.2 ± 0.2	*	2.6 ± 0.4	3.7 ± 0.7	
18:3n-6	0.2 ± 0.0	0.2 ± 0.1		0.2 ± 0.0	0.3 ± 0.0		0.2 ± 0.0	0.3 ± 0.1	
20:2n-6	0.5 ± 0.0	0.4 ± 0.1		0.5 ± 0.0	0.9 ± 0.1	*	0.3 ± 0.0	0.4 ± 0.0	
20:3n-6	0.2 ± 0.0	0.2 ± 0.0		0.2 ± 0.0	0.6 ± 0.0	*	0.2 ± 0.0	0.2 ± 0.0	
20:4n-6	0.7 ± 0.2	0.5 ± 0.1		1.8 ± 0.2	1.6 ± 0.1		1.2 ± 0.2	0.8 ± 0.2	
22:4n-6	0.2 ± 0.0	0.1 ± 0.1		0.2 ± 0.0	0.4 ± 0.0	*	0.1 ± 0.1	0.2 ± 0.0	
22:5n-6	0.2 ± 0.0	0.2 ± 0.1		0.5 ± 0.0	0.6 ± 0.1		0.2 ± 0.0	0.2 ± 0.0	
Total n-6 PUFA	5.7 ± 0.5	4.1 ± 0.7	*	6.6 ± 0.2	8.6 ± 0.2	*	4.7 ± 0.4	5.7 ± 0.7	
18:3n-3	0.9 ± 0.1	1.2 ± 0.3		0.6 ± 0.1	1.8 ± 0.2	*	0.6 ± 0.1	1.9 ± 0.3	*
18:4n-3	0.6 ± 0.0	0.1 ± 0.0	*	0.6 ± 0.1	0.3 ± 0.0	*	0.7 ± 0.1	0.5 ± 0.2	
20:3n-3	0.3 ± 0.0	0.2 ± 0.0		0.2 ± 0.0	0.1 ± 0.0		0.1 ± 0.1	0.2 ± 0.1	
20:4n-3	0.9 ± 0.1	0.1 ± 0.0	*	0.6 ± 0.0	0.2 ± 0.0	*	0.5 ± 0.1	0.3 ± 0.1	
20:5n-3	1.8 ± 0.2	0.1 ± 0.0	*	4.2 ± 0.4	1.1 ± 0.1	*	2.2 ± 0.2	1.1 ± 0.4	*
22:4n-3	0.2 ± 0.0	0.0 ± 0.0	*	0.1 ± 0.1	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0	
22:5n-3	6.0 ± 1.0	0.4 ± 0.1	*	5.7 ± 0.1	2.2 ± 0.2	*	3.2 ± 0.3	1.9 ± 0.7	*
22:6n-3	14.6 ± 1.7	2.2 ± 0.6	*	24.0 ± 1.6	11.0 ± 1.3	*	23.2 ± 2.3	8.7 ± 2.1	*
Total n-3 PUFA	25.3 ± 2.3	4.3 ± 1.1	*	36.0 ± 1.9	16.7 ± 1.2	*	30.5 ± 1.7	14.7 ± 2.7	*
Total PUFA	31.0 ± 2.3	9.4 ± 1.9	*	42.7 ± 1.8	26.1 ± 1.1	*	35.2 ± 1.6	21.1 ± 3.2	*
Total DMA	n.d	n.d		n.d	1.3 ± 1.1		n.d	1.7 ± 1.6	

Results are means ± SD (n = 3). Differences between mean values for fish fed the VO diet compared to fish fed the FO diet were determined by the Student's t-test and are significantly different where indicated (*, $P < 0.05$). DMA, dimethylacetals; n.d., not detected; PUFA, polyunsaturated fatty acids.