Research paper

Comparative study of antioxidant defence mechanisms in marine fish fed variable levels of oxidised oil and vitamin E.

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Running head: Vitamin E and antioxidant defences in marine fish

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Abbreviations: AA, all-cis-5,8,11,14-eicosatetraenoic acid (arachidonic acid, 20:4n-6); CDNB, chlorodinitrobenzene; DHA, all-cis-4,7,10,13,16,19-docosahexaenoic acid (22:6n-3); EPA, all-cis-5,8,11,14,17-eicosapentaenoic acid (20:5n-3); GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSH, oxidized glutathione; GST, glutathione-S-transferase; HUFA, highly unsaturated fatty acids ($\geq C_{20}$ with ≥ 3 double bonds); isoprostane, 8-iso-prostaglandin $F_{2\alpha}$; PUFA, polyunsaturated fatty acid; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

Abstract

The aim of the study was to compare the antioxidant systems in juvenile marine fish of commercial importance in European aquaculture, namely turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippoglossus*) and gilthead sea bream (*Sparus aurata*). The present dietary trial was specifically designed to investigate the antioxidant effects of vitamin E under moderate oxidising conditions, including high dietary levels of highly unsaturated fatty acids and the feeding of oxidised oils. The objective was to induce a stressful pro-oxidant status to enable characterisation of the biochemical responses to peroxidative stress without causing unnecessary suffering to the experimental animals or high mortalities during the trials. Both sea bream and turbot showed excellent growth, whereas growth was poorer in halibut. Dietary oxidised oil significantly reduced growth in turbot and especially in halibut, but not in sea bream. Vitamin E improved growth in sea bream fed oxidised oil but not in turbot or halibut. However, vitamin E supplementation appeared to improve survival in all three species. In sea bream and turbot, liver antioxidant defence enzyme activities were generally increased by feeding peroxidised oil and reduced by vitamin E. Conversely, in halibut, the liver antioxidant defence enzyme activities were not increased by feeding peroxidised oil and only superoxide dismutase was reduced by feeding vitamin E. Consistent with these data, feeding oxidised oil increased lipid peroxidation products in halibut, but generally not in sea bream or turbot. Furthermore, lipid peroxidation products were generally reduced by dietary vitamin E in both sea bream and turbot, but not in halibut. Therefore, halibut liver antioxidant defence enzymes did not respond to dietary oxidised oil or vitamin E as occurred in turbot and, especially sea bream. This resulted in increased levels of lipid peroxides in halibut compared to turbot and sea bream in fish given dietary oxidised oil. In addition, supplemental vitamin E did not reduce lipid peroxides in halibut as it did in turbot and sea bream. The increased peroxidation stress in halibut may account for their poorer growth and survival in comparison to turbot and especially sea bream. Halibut were reared at a lower temperature, although relatively high for halibut, than either turbot or sea bream but they were also slightly younger/smaller fish and possibly, therefore, more developmentally immature, and either or all of these factors may be important in the lack of response of the liver enzymes in halibut.

INTRODUCTION

Lipid and polyunsaturated fatty acid (PUFA) peroxidation is highly deleterious, resulting in damage to biomembranes and it is implicated in several pathological conditions in fish (Kawatsu, 1969; Watanabe *et al*., 1970; Murai and Andrews, 1974; Sakai *et al*., 1989). Physiological antioxidant protection involves both endogenous components such as free radical scavenging enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) (Winston and Di Giulio, 1991; Halliwell and Gutteridge, 1996), and exogenous dietary micronutrients such as vitamin E. Tissue lipid and polyunsaturated fatty acid (PUFA) contents and compositions are critical factors in lipid peroxidation. As fish tissues and fish diets contain large quantities of n-3 highly unsaturated fatty acids (HUFA) they are potentially more at risk from peroxidative attack than those of mammals (Sargent *et al*., 1999). However, information in relation to *in vivo* lipid peroxidation and antioxidant defences either in wild or cultured marine fish species is quite limited (De Silva and Anderson, 1995; Stéphan *et al*., 1995; Murata *et al*., 1996; Peters and Livingstone, 1996; Mourente *et al*., 1999a,b). The principal objectives of this project were 1) To demonstrate the significance of oxidative stress, and the role of the antioxidant systems under controlled experimental conditions, in weaned juvenile marine fish of commercial importance in European aquaculture, namely turbot (*Scophthalmus maximus*), halibut (*Scophthalmus maximus*) and gilthead sea bream (*Sparus aurata*) and 2) to characterise and understand the antioxidant systems in marine fish during early development in order to enhance the growth and quality of early life stages by avoiding oxidation problems that may cause pathologies and disease.

Our previous work suggested that feeding high HUFA diets resulted in signs of increased peroxidative stress in juvenile marine fish, as evidenced by increased levels of tissue lipid peroxidation products, but only moderate effects on liver antioxidant defence enzyme activities were observed (Tocher *et al*., 2001). Therefore, in the present trial, the level of dietary HUFA was increased by using higher levels of oil in the diet (19%) and, especially, by using an oil with a much higher n-3HUFA content (a high quality anchovy/sardine oil concentrate). In order to increase the potential peroxidative stress to an even higher level, oxidised oil was also used with peroxidation induced by controlled heating (50 ^{o}C) in an oxygen-rich atmosphere, with the extent of peroxidation monitored regularly by sampling and determination of peroxide value (P.V.). Therefore, the dietary trial had a factorial two design (oxidised (X) v. unoxidised oil and ± vitamin E) giving four diets, HO,

HE, HXO and HXE. The effects of dietary oxidised oil with or without supplementary dietary vitamin E on survival and growth parameters of the fish were determined. Dietary effects on tissue lipid and fatty acid contents and compositions were determined, and the effects of the dietary oxidised oil and vitamin E on the activities of the liver antioxidant defence enzymes were characterised. Finally, the levels of liver and whole body lipid peroxidation products, including malondialdehyde, determined as thiobarbituric acid reactive substances (TBARS), and isoprostanes, were measured.

MATERIALS AND METHODS

Experimental diets

The experimental diets were based on a modified commercial extruded formulation utilising fishmeal as protein source (Table 1). Mineral and vitamin premixes, vitamin E-stripped anchovy/sardine oil concentrate (both oxidised and unoxidised) and vitamin E (tocopheryl acetate) were prepared and supplied by the Lipid Nutrition Group, Institute of Aquaculture, University of Stirling. The diets were manufactured by a commercial feed producer (Ewos Ltd., Livingston, Scotland). Vitamin E was removed from the oil by charcoal absorption and half of the oil was oxidised by heating at 50 ºC with vigorous aeration for 24 h, with the degree of oxidation monitored by determination of the peroxide value at 8 h intervals. Four diets were produced at two pellet sizes (500 and 1500 μm). The PUFA contents, vitamin E levels, PUFA/vitamin E ratios, unsaturation index, peroxide values and TBARS contents of the diets are shown in Table 2, and the fatty acid compositions are shown in Table 3.

Experimental fish and dietary trial conditions

The turbot experiment was performed in the facilities of the Laboratory of Aquaculture & Artemia Reference Center (Ghent University, Belgium). Juvenile turbot were obtained from a commercial hatchery (France Turbot, Noirmoutier, France) and were maintained in a 1000 l tank for acclimatization to laboratory conditions during which time they were fed a standard ICES weaning diet (INVE Aquaculture N.V., Belgium). The dietary trial was conducted in 3 separate recirculating water units with each unit consisting of four 30-liter rectangular tanks with separate biofilter. The 75 day-old fish were stocked in the experimental system at an initial weight of 0.95 ± 0.22 g, at a density of 120 individuals per tank. Each diet was fed to

triplicate groups of fish (one tank in each unit) with the feed supplied by automatic feeders for 600 degree.days at a water temperature of 19 ± 1ºC. Water quality parameters in each unit were monitored daily (temperature, NH_3, NO_2^-, salinity; ammonia never exceeded 0.3 ppm). Excess feed was siphoned off and any dead fish were removed. The light regime was 12 h light:12h dark with feeding during the light period only.

The halibut experiment was performed in the facilities of the Norwegian University of Science and Technology (NTNU) in Trondheim. Normally pigmented halibut fry were obtained from a commercial fish farm (Norwegian Halibut AS, Rørvik, Norway). At NTNU, 67-day old halibut fry of initial weight 0.31 ± 0.11g were divided randomly into groups of 100 –120 individuals in polyethylene tanks (1x1 m, 40 cm depth) with a water flow of 6.3 - 7.5 l min^{-1}. The dietary trial was performed at a temperature of 14 ± 1°C for a period of 600 degree.days. All groups of fish were fed continuously to satiation throughout the experimental period with the ration adjusted weekly as fish grew. The tanks were rinsed every day and there were always food particles in excess at the bottom of each unit.

The sea bream experiment was performed in the facilities of the University of Cadiz, Cadiz, Spain. Sea bream from the same batch, completely weaned, with a functional swimbladder, were obtained from CUPIMAR S. A. (Cadiz, Andalucia, Spain). After acclimatization to the experimental diet and conditions for two weeks, the 80 days post-hatch fish with a live mass of 1.52 ± 0.21 g were stocked randomly at an initial density of 5 fish/l into rectangular tanks of 100 l each. Each diet was fed to triplicate groups of fish. The ration varied from 4% to 3% of the biomass/day between the beginning and end of the experiment and was offered to fish 6 times during the daylight hours (natural photoperiod) by hand. The length of the experiment was established at 600 degree.days. The tanks were in an open system continuously supplied with running borehole water of 39 ppt salinity at a temperature of 19.4 ± 0.2ºC. The water was treated with biological filters to eliminate ammonia, by nitrification processes, to sea water quality criteria (1 μg/l NH_3-N maximum). Oxygen was supplied by aeration with the minimum level observed during trials being 5.6 mg/l or 77.8% saturation. Water renewal was set at 10 times total volume per day (0.7 l.min^{-1}).

Sampling

In all trials, survival was determined by counting the number of fish remaining at the end of the experiment and was expressed as a percentage of the initial number. Fish were sampled

and dissected on ice after a 24 h starvation period to avoid interference of gut contents in the analysis. For morphometric measurements, a minimum of 25 specimens were used from each treatment. The head-tail length and live mass were determined for whole fish, while dry mass was determined for both whole fish and liver. Live masses were determined by blotting fish and liver on filter paper before weighing, and dry masses were determined after heating in an oven at 60 ºC for 24 h. After removal, the liver was carefully cleaned of adhering tissue before weighing and the hepato-somatic index (HSI) calculated (HSI = 100* [liver weight/body weight]). Growth was assessed by determining the specific growth rate (SGR) as % weight gain per day (100* [ln (final weight) – ln (initial weight)]/days) (Wootten, 1990). Samples of diets, whole fish and dissected livers for lipid and fatty acid analysis, vitamin E content, lipid peroxidation products (TBARS and isoprostane) and hepatic antioxidant defence enzyme activities were immediately frozen in liquid nitrogen and stored under nitrogen at –80 ºC prior to analysis. All liver samples were triplicates of pooled livers, the number of livers dependent upon the size of the fish and weight of individual livers, varying between three and six livers per sample.

Lipid extraction and lipid class composition

Total lipid content was determined gravimetrically after extraction by homogenisation in chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch *et al*., (1957). Separation of lipid classes was performed by high-performance thin-layer chromatography (HPTLC). Approximately 10 μg of lipid extract was loaded as a 2 mm streak and the plate developed to two-thirds distance with methyl acetate/isopropanol/chloroform/methanol/0.25 % aqueous KCl (25:25:25:10:9, by vol.). After desiccation, the plate was fully developed with hexane/diethyl ether/acetic acid (85:15:1, by vol.). The classes were quantified by charring at 160 ºC for 15 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid, followed by calibrated densitometry using a Shimadzu CS-9000 dual-wavelength flying spot scanner and DR-13 recorder (Henderson and Tocher, 1992).

Total lipid fatty acid analyses

Fatty acid methyl esters (FAME) from total lipids were prepared by acid-catalysed transmethylation for 16 h at 50 ºC, using heptadecanoic acid (17:0) as internal standard

(Christie, 1989). FAME were extracted and purified as described previously (Tocher and Harvie, 1988) and were separated in a Fisons GC8000 gas chromatograph equipped with a chemically bonded CP Wax 52CB fused silica wall coated capillary column (30 m x 0.32 mm i. d., Chrompack U.K. Ltd., London), on-column injection system and flame ionisation detection. Hydrogen was used as the carrier gas with an oven thermal gradient from an initial 50 ºC to 150 ºC at 40 ºC/min and then to a final temperature of 230 ºC at 2 ºC/min. Individual FAME were identified by comparison with known standards, a well-characterised fish oil and by reference to published data as described previously (Tocher and Harvie, 1988) and quantified using a PC with Chromcard for Windows software (Thermo-Quest Italia S.P.A., Milan, Italy).

Measurements of thiobarbituric acid reactive substances (TBARS)

The measurement of TBARS was carried out using a method adapted from that of Burk *et al*., (1980). Between 20-30 mg of tissue per sample was homogenised in 1.5 ml of 20 % (w/v) trichloroacetic acid containing 0.05 ml of 1% BHT in ethanol. To this was added 2.95 ml of freshly prepared 50 mM thiobarbituric acid solution. The reagents were mixed in a stoppered test tube and heated at 100 ºC for 10 min. After cooling the tubes and removing protein precipitates by centrifugation at 12000 x g, the supernatant was read in a spectrophotometer at 532 nm. The absorbance was recorded against a blank at the same wavelength. The concentration of TBARS, expressed as mmol TBARS.mg^{-1} of tissue protein, was calculated using the extinction coefficient 0.156 μM^{-1}.cm^{-1}.

Determination of 8-isoprostane levels

The levels of 8-isoprostane, a novel lipid peroxidation product formed non-enzymatically, and thus a potentially good indicator of lipid peroxidation in tissues, were determined by enzyme immunoassay (EIA). Isoprostanes were determined in the same homogenates of liver and whole fish that were prepared for TBARS analyses. Samples should be assayed immediately after collection or, as in this case, stored at –80 ºC, as they can also appear in samples as an artifact of prolonged storage at temperatures above –80 ºC. Most 8-isoprostanes are found esterified in lipids, and so an extraction and hydrolysis was performed in order to determine total amounts of 8-isoprostane. Briefly, 2 ml ethanol was added to 1.5 ml of homogenate, mixed, and allowed to stand for 5 minutes at 4 ºC before precipitated

protein was removed by centrifugation. The supernatant was decanted into a clean test tube and 3.5 ml 15 % KOH added and incubated for 60 min at 40 ºC. The solution was diluted to 10 ml with ultrapure water and the pH lowered to below 4.0 with 2 ml concentrated formic acid. Isoprostanes were purified by applying the solution to a C_{18} reverse-phase mini-column ("Sep-Pak", Millipore UK, Watford, UK) after activating the column with 5 ml methanol followed by 5 ml ultrapure water. The columns were then washed with 5 ml ultrapure water and 5 ml HPLC grade isohexane before eluting isoprostanes with 5 ml ethyl acetate containing 1 % methanol. The solvent was evaporated under a stream of nitrogen and 1 ml EIA kit buffer added. Total isoprostane was quantified using an EIA kit and 8-isoprostane standard as per manufacturers instructions (Cayman Chemical Co., Ann Arbor, USA).

Determination of vitamin E contents

Vitamin E (α-tocopherol) was determined by HPLC with fluorescence detection, as described in Huo *et al.,* (1996). Samples were homogenized in 2 ml of methanol containing 1mg/ml BHT, and tocol added as an internal standard, using a Potter Elvehjem tube. The samples were then centrifuged for 2 min at 1500 x g and the supernatant transferred to a polypropylene tube. The solid residue was homogenized in 2 ml methanol/BHT and the extract combined with the first one, and with 1 ml methanol/BHT used to rinse the Potter tube. The combined extracts were centrifuged for 10 min at 12000 x g and an aliquot of 100 μl was injected. Column and elution details were as in Huo *et al*., (1996). Quantitation was based on peak height ratios (analyte versus the internal standard tocol).

Determination of enzyme activities in liver homogenates

Samples of liver were homogenised in 9 volumes of 20 mM phosphate buffer pH 7.4, 1 mM EDTA and 0.1 % Triton X-100, the homogenates were centrifuged at 600 x g to remove debris, and the resultant supernatants used directly for enzyme assays. Catalase activity was measured by following the reduction of hydrogen peroxide at 240 nm using the extinction coefficient 0.04 $mM^{-1}.cm^{-1}$ (Beers and Sizer, 1952). Immediately before assay, 50 ml of 67 mM potassium phosphate buffer pH 7.0 was mixed with 80 μl of 30% (v/v) hydrogen peroxide. The assay cuvette (quartz) contained 3.0 ml of above buffered hydrogen peroxide solution plus 25 μl of sample.

Total superoxide dismutase (SOD) activity was assayed by measuring the inhibition of

the oxygen-dependent oxidation of adrenaline (epinephrine) to adenochrome by xanthine oxidase plus xanthine (Panchenko *et al*., 1975). Plastic semi-micro-cuvettes containing 0.5 ml of 100 mM potassium phosphate buffer pH 7.8 / 0.1 mM EDTA, 200 μl adrenaline, 200 μl xanthine and 50 μl distilled water (uninhibited control) or 50 μl sample were prepared and the reaction initiated by the addition of 10 μl xanthine oxidase (Sigma X4875). The reaction was followed at 480 nm and 1 unit of superoxide dismutase activity is described as the amount of the enzyme which inhibits the rate of adenochrome production by 50 %.

Glutathione peroxidase (GPX) was assayed by following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase (Bell *et al*., 1985). Plastic semi-micro-cuvettes containing 0.75 ml of 60 mM potassium phosphate buffer pH 7.4/1 mM EDTA/2 mM sodium azide, 50 μl reduced glutathione, 100 μl NADPH and 5 μl glutathione reductase (Sigma G4751) were prepared. The basal reaction was initiated by the addition of 50 μl hydrogen peroxide solution and the non-enzymic rate without sample added was measured for later subtraction. Sample (50 μl) was then added and the assay continued by measuring absorbance at 340 nm with specific activity determined using the extinction coefficient of 6.22 mM^{-1} .cm^{-1}.

Glutathione-S transferase (GST) activity was determined by following the formation of glutathione-chlorodinitrobenzene (CDNB) adduct at 340 nm. Standard plastic cuvettes containing 2.5 ml of 120 mM potassium phosphate buffer pH 6.5, 100 μl GSH and 100 μl CDNB were prepared and the reaction initiated by the addition of 50 μl sample. Specific activities were determined using an extinction coefficient of 9.6 $mM^{-1}.cm^{-1}$ (Habig *et al*., 1974).

Glutathione reductase (GR) activity was assayed as described by Racker (1955) by measuring the oxidation of NADPH at 340 nm using the extinction coefficient 6.22 $mM^{-1}.cm^{-1}$. Plastic semi-micro-cuvettes containing 0.6 ml of 0.2 M potassium phosphate buffer pH 7.0 /2 mM EDTA, 200 μl oxidised glutathione and 100 μl NADPH were prepared and the reaction initiated by the addition of 100 μl of sample.

Protein content in the homogenate supernatants was determined by the Folin-phenol reagent method, according to Lowry *et al*., (1951) following digestion for 1 h at 60 ºC in 1M NaOH/0.25% sodium dodecyl sulphate.

Statistical analysis

Results are presented as means ± SD (n = 3 or as otherwise stated). The data were checked

for homogeneity of the variances by the Bartlett test and, where necessary, the data were arc-sin transformed before further statistical analysis. Differences between mean values were analysed by one- or two-way analysis of variance (ANOVA) followed, when pertinent, by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P < 0.05$ (Zar, 1984).

RESULTS

The diets had high unsaturation indices of around 345 as a result of the the high PUFA content (~ 80 $g.kg^{-1}$ of diet) (Table 2), primarily due to very high levels of n-3HUFA (Table 3). Peroxide values were significantly higher (up to 9-fold) in the diets containing the oxidised oil although the TBARS contents were all similar at around 6 $\mu mol.mg^{-1}$ dry mass of diet. The vitamin E contents of the supplemented diets, at between 200 and 260 $mg.kg^{-1}$ diet, were around 7- fold higher than in the diets without supplemental vitamin E and, as a result, the PUFA/vitamin E molar ratios were 5- to 8-fold lower in the supplemented diets (Table 2).

Both the sea bream and turbot showed excellent growth, with specific growth rates (SGRs) varying between 2.9 and 3.7, whereas growth was poorer in halibut and an SGR of 3 was only observed with fish fed diet HE (Table 4). Dietary oxidised oil significantly reduced growth in turbot, and especially in halibut, but not in sea bream where dietary oxidised oil had a slight stimulatory effect on growth. Vitamin E supplementation improved growth in sea bream fed oxidised oil but not in turbot or halibut. However, vitamin E supplementation improved survival in all three species. Dietary oxidised oil reduced survival in both turbot and, especially halibut, with the greatest mortalities recorded in turbot and halibut fed the diet containing oxidised oil without vitamin E supplementation, diet HXO (Table 4).

Dietary oxidised oil increased the proportions of triacylglycerol and neutral lipids in livers of sea bream (Table 5) but had no significant effects on lipid class compositions in turbot or halibut (Tables 6 and 7). Similarly, the experimental diets had few significant effects on liver fatty acid compositions other than dietary oxidised oil decreased the percentages of 14:0 and 18:3n-3 in sea bream (Table 5) and supplementary vitamin E reduced the proportions of total saturated fatty acids in halibut (Table 7).

In sea bream, the activities of the primary radical scavenging enzymes, catalase and SOD, were increased by feeding the diets containing peroxidised oil and reduced by supplementary dietary vitamin E. In turbot, GST and GR activities were increased by feeding

oxidised oil and catalase, SOD and GST activities were reduced by dietary vitamin E (Table 8). Dietary oxidised oil tended to increase both catalase and SOD activities in turbot although the data were just short of statistical significance. By contrast, in halibut only GST was increased by feeding diets containing oxidised oil and only SOD was reduced by feeding vitamin E. Interestingly, GR was generally increased by dietary vitamin E in all three species (Table 8). Feeding diets containing oxidised oil increased lipid peroxidation products in halibut, but generally less so in sea bream and turbot (Table 9). The increased lipid peroxidation products in response to feeding dietary oxidised oil were generally more pronounced in whole fish than in liver. The lipid peroxidation products were generally reduced by dietary vitamin E in both sea bream and turbot, but generally not in halibut (Table 9).

DISCUSSION

Our previous study demonstrated relationships between dietary vitamin E levels, liver vitamin E levels, the activities of the liver antioxidant enzymes and the levels of liver lipid peroxidation products (Tocher *et al*., 2001). The overall balance of the data showed that these relationships were logical in that decreased dietary vitamin E levels and increased dietary PUFA/vitamin E ratios led to decreased levels of vitamin E and increased PUFA/vitamin E ratios in liver, and generally higher activities of the liver antioxidant enzymes and higher levels of lipid peroxides. The present study was specifically designed to further investigate the antioxidant effects of vitamin E under more severe peroxidising conditions, including increased dietary HUFA (more than doubling the previous level in Tocher *et al*., 2001) and the feeding of oxidised oils. The aim was to induce a more stressful pro-oxidant status to enable further characterisation of the biochemical indicators of peroxidative stress without causing unnecessary suffering to the experimental animals or high mortalities during the trials. The results showed that the dietary formulations were significantly more severe than in our previous trials and, as hoped, this resulted in clearer antioxidant defence responses in the fish. However, in addition, the present study showed clear species differences such that a gradient of deleterious effects was observed from very few in sea bream to considerable stress in halibut with turbot intermediate. The extent of peroxidative stress and deleterious effects appeared inversely proportional to the responses of the hepatic antioxidant defence enzyme activities.

In sea bream, none of the diets had any deleterious effects on the overall health and well-

being of the fish, which showed excellent growth with very low levels of mortality. In complete contrast, halibut did not appear able to respond to the diets in a same way as the sea bream. Although lower survival rates would be expected for halibut, the mortalities in this trial were, at best, double and, at worst, approximately four-fold greater than could have been expected (Tocher *et al*., 2001). That, coupled with the very much lower growth rate compared to sea bream and turbot, showed that the diets were clearly inducing high levels of stress. As could have been predicted, halibut fed the HXO diet were the most affected whereas fish fed the HE diet were the least affected. The response in turbot was somewhere between the two extremes described above for sea bream and halibut. Growth and survival were still fairly good. However, growth was slightly reduced by feeding oxidised oil and survival was substantially lower in fish fed diet HXO. In several previous studies, dietary vitamin E had no significant effects on growth in turbot (Stephan *et al*., 1995), Atlantic salmon (Lygren *et al*., 2000), African (*Clarius gariepinus*) and channel catfish (*Ictalurus punctatus*) (Baker and Davies, 1996; Bai and Gatlin, 1993). However, dietary vitamin E deficiency reduced growth in amago salmon (*Oncorhynchus rhodurus*) (Taveekijakarn *et al*., 1996) and juvenile Korean rockfish (*Sebastes schlegeli*) (Bai and Lee, 1998).

Both dietary oxidised oil and supplemental dietary vitamin E gave significant effects on liver antioxidant defence enzyme activities and lipid peroxidation products in sea bream and turbot. In sea bream, the activities of the primary radical scavenging enzymes, catalase and SOD were increased by feeding dietary oxidised oil and reduced by dietary vitamin E. Similarly in turbot, the activities of catalase, SOD, GST and GR were increased by dietary oxidised oil, whereas vitamin E supplementation significantly reduced the activities of catalase, SOD and GST. These effects are entirely consistent with the commonly perceived biochemical mechanisms of these enzyme systems (Winston and Di Giulio, 1991; Miller *et al*., 1993; Halliwell and Gutteridge, 1996). In contrast, in halibut, there was only a very moderate response by the liver antioxidant defence enzymes to the added peroxidation stress of oxidised oil and the protective effect of vitamin E was much less apparent. Studies in which these enzyme activities have been measured in fish have tended to focus on their role in pollutant detoxification (Peters *et al*., 1994) or developmental aspects (Aceto *et al*., 1994; Otto and Moon, 1996; Peters and Livingstone, 1996). No interactions were observed between dietary vitamin E and antioxidant enzyme activities in Atlantic salmon (Lygren *et al*. 2000) and no clear relationship between dietary or tissue PUFA/vitamin E ratios and liver antioxidant enzyme activities were observed in gilthead sea bream (Mourente *et al*., 2000). Recently, we specifically showed that variation in dietary and, consequently, tissue

PUFA/vitamin E ratios could significantly affect peroxidation status, as determined by the levels of lipid peroxidation products, in juvenile marine fish with few physiologically significant affects on liver antioxidant enzyme activities (Tocher *et al*., 2001). The present study has perhaps indicated that a more direct peroxidative stress, such as that induced by feeding oxidised oil is required before clear effects on the liver antioxidant enzyme activities are observed.

Therefore, and perhaps as a result of the responses in enzymic activities detailed above, feeding oxidised oil did not generally increase lipid peroxidation products in liver of sea bream and turbot but they were generally reduced by dietary vitamin E. In a similar way, the general lack of response of the liver antioxidant defence enzymes, and the fact that dietary vitamin E appeared to have no moderating effect, were probably important contributing factors responsible for the higher levels of lipid peroxidation products observed in halibut fed diets containing oxidised oil. The fact that the increased levels of lipid peroxidation products in response to feeding dietary oxidised oil were generally more pronounced in whole fish rather than in liver was not unexpected as vitamin E is concentrated in the liver (Stéphan *et al*., 1995; Lygren *et al*., 2000; Tocher *et al*., 2001). Decreasing PUFA/vitamin E ratios as a result of increasing dietary vitamin E levels reduced the levels of lipid peroxidation products in juvenile marine fish (Tocher *et al*., 2001) and increased dietary vitamin E levels resulted in decreased levels of TBARS in juvenile African catfish (Baker and Davies, 1996; 1997).

CONCLUSIONS

Overall therefore, the present study was successful in further characterising peroxidative stress responses in juvenile marine fish. Both sea bream and turbot generally responded to both dietary oxidised oil and dietary vitamin E in a logical and predictable manner whereas halibut appeared to be less able to respond adequately to the increased peroxidative stress the diets imposed. Thus, halibut liver antioxidant defence enzymes did not respond to dietary oxidised oil or vitamin E as occurred in turbot and, especially sea bream. This resulted in increased levels of lipid peroxides in halibut compared to turbot and sea bream given dietary oxidised oil. In addition, supplemental vitamin E did not reduce lipid peroxides in halibut as it did in turbot and sea bream. The increased peroxidation stress in halibut would account for their poorer growth and survival in comparison with turbot and, especially, sea bream. The halibut were reared at a lower temperature than either turbot or sea bream but they were also slightly younger/smaller fish and possibly, therefore, more

developmentally immature, and either or all of these factors may be important in the general lack of response of the liver enzymes in halibut. These data suggested that the capability of the antioxidant system may be species dependent, and perhaps related to developmental stage. In addition, halibut may have a higher requirement for vitamin E compared to the other two species perhaps related to culture temperature and/or HUFA requirement. These apparent differences between species warrant further investigation.

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TABLE 1. Formulation of experimental diets showing composition of the base pellet and oil coatings

Component	HO	HXO	HXE	HE
Pellet				
Fishmeal[1]	72	72	72	72
Starch[2]	10	10	10	10
Finnstim/Betafin	0.5	0.5	0.5	0.5
Mineral premix M2[3]	2.4	2.4	2.4	2.4
Vitamin E-free premix[4]	0.5	0.5	0.5	0.5
Vitamin E-stripped fish oil[5]	2	2	2	2
Coating				
Vitamin E-stripped fish oil[5]	12.6	-		-
Oxidised oil[6]	-	12.6	-	-
Oxidised oil + Vitamin E[7]	-	-	12.6	-
Stripped fish oil[5] + Vitamin E[7]	-	-	-	12.6

[1] LT94, Low temperature fish meal (Ewos Ltd., Livingston, U.K.).
[2] Paselli WA4 (Avebe Ltd., Ulceby, U.K).
[3] 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; $CuSO_4.5H2O$, 12 mg; $CoSO_4.7H_2O$, 2 mg; KI, 2 mg.
[4] Supplied (mg/kg diet): ascorbic acid, 1000; myo-inositol, 400; nicotinic acid, 150; calcium pantothenate, 44; 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.
[5] Tuna orbital oil concentrate (Croda Universal Ltd., Hull, U.K.), stripped by activated charcoal (Sigma Chemical Co. Ltd.) adsorption in hexane.
[6] Vitamin E-stripped anchovy/sardine oil concentrate oxidised by heating at 50 °C in an oxygen-rich atmosphere for 24 h.
[7] Tocopherol acetate added to oils at 2000 mg/Kg.

TABLE 2. Unsaturation index, peroxide value, TBARS content (μmol.mg^{-1} dry mass), tocopherol content (mg.kg^{-1} diet), PUFA content (g.kg^{-1} diet), and ratio of PUFA/Vitamin E (mol/mol) of the experimental diets.

	HO		HXO		HXE		HE	
Unsaturation index	343.1 ± 0.1		346.1 ± 1.7		342.5 ± 2.3		346.3 ± 3.0	
Peroxide value	9.2 ± 0.5	b	42.0 ± 1.6	a	42.0 ± 1.6	a	4.5 ± 0.4	b
TBARS content	6.2 ± 0.1		6.2 ± 0.4		6.0 ± 0.2		6.2 ± 0.1	
Tocopherol (vitamin E) content	37.9 ± 3.0	b	35.3 ± 7.3	b	260 ± 38	a	197 ± 5	a
PUFA content	86.2 ± 3.8		82.9 ± 2.5		76.5 ± 0.3		78.3 ± 2.7	
Molar ratio PUFA/Vitamin E	3063 ± 609	a	3795 ± 926	a	467 ± 64	b	628 ± 11	b

Data are mean ± SD (n=3). SD = 0 implies an SD < 0.05. Values within a row with different superscript letters are significantly different ($p < 0.05$) as determined by one-way analysis of variance followed, where appropriate, by Tukey's multiple range test. PUFA, polyunsaturated fatty acid; TBARS, thiobarbituric acid-reactive substances; unsaturation index, no. of double bonds x content (percentage).

TABLE 3. Fatty acid composition (percentage of total fatty acids) of the experimental diets.

Diet name	HO		HXO		HXE		HE	
size (mm)	0.5	1.5	0.5	1.5	0.5	1.5	0.5	1.5
14:0	2.5	2.5	2.4	2.4	2.4	2.6	2.4	2.4
16:0	7.8	7.7	7.4	7.6	7.7	7.9	7.4	7.8
18:0	1.5	1.5	1.4	1.5	1.5	1.5	1.4	1.5
Total saturated[1]	12.2	12.2	11.3	11.9	12.0	12.4	11.7	12.1
16:1n-7	2.9	2.9	2.8	2.9	2.8	2.9	2.8	2.8
18:1n-9	7.8	7.7	7.5	7.7	7.8	7.9	7.5	7.8
18:1n-7	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
20:1[1]	4.7	4.7	4.6	4.7	4.6	4.7	4.5	4.6
22:1[2]	6.5	6.5	6.3	6.5	6.4	6.5	6.3	6.4
24:1n-9	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.7
Total monoenes[4]	24.6	24.5	23.9	24.5	24.3	24.8	23.9	24.4
18:2n-6	2.0	1.9	1.9	1.9	1.9	1.8	1.8	1.9
20:4n-6	1.6	1.7	1.7	1.7	1.6	1.6	1.7	1.6
Total n-6PUFA[5]	4.9	4.9	5.0	4.9	4.8	4.7	4.8	4.8
18:3n-3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
18:4n-3	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
20:4n-3	1.8	1.8	1.9	1.8	1.8	1.8	1.8	1.8
20:5n-3	27.4	27.8	28.5	28.0	28.2	27.7	28.5	27.8
22:5n-3	3.2	3.1	3.2	3.2	3.2	3.1	3.2	3.1
22:6n-3	20.9	20.7	20.6	20.7	20.6	20.4	21.0	20.9
Total n-3PUFA[6]	57.5	57.6	58.7	58.0	58.1	57.4	58.9	58.0
Total PUFA[7]	63.2	63.3	64.5	63.6	63.7	62.8	64.4	63.5

[1]Includes 15:0 present at < 0.05%; [2]predominantly n-9 isomer; [3]predominantly n-11 isomer; [4]includes 16:1n-9, present at < 0.5%; [5]includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 and 22:5n-6, present at < 0.5%; [6]includes 20:3n-3 and 22:4n-3, present at < 0.5%; [7]includes C_{16} PUFA; PUFA, polyunsaturated fatty acids.

TABLE 4. Effects of dietary oxidized oil and vitamin E on growth and survival of sea bream (80 days old), turbot (75 days old), and halibut (67 days old).

		HO	HXO	HXE	HE	Significance		
						ox. oil	Vit. E	inter.
Sea bream	Initial weight (g)	1.52±0.21	1.52±0.21	1.52±0.21	1.52±0.21			
	Final weight (g)	4.12±0.5	4.20±0.34	4.55±0.04	4.05±0.58	Y	Y	Y
	SGR (%.day^{-1})	3.30±0.44	3.38±0.27	3.66±0.03	3.24±0.50	Y	Y	Y
	Survival (%)	99.0±0.9	99.2±1.0	99.5±0.5	99.5±0.9	N	Y	N
Turbot	Initial weight (g)	0.95±0.22	0.95±0.22	0.95±0.22	0.95±0.22			
	Final weight (g)	3.02±0.65	2.61±0.65	2.52±0.69	2.74±0.59	Y	Y	N
	SGR (%.day^{-1})	3.47±0.68	3.04±0.72	2.92±0.78	3.20±0.63	Y	Y	N
	Survival (%)	90	75	99	91			
Halibut	Initial weight (g)	0.31±0.11	0.31±0.11	0.31±0.11	0.31±0.11			
	Final weight (g)	0.66±0.24	0.58±0.33	0.51±0.31	0.89±0.41	Y	N	Y
	SGR (%.day^{-1})	2.2±0.3	1.8±0.2	1.4±0.2	3.0±0.3	Y	Y	Y
	Survival (%)	57	29	41	59			

Data are presented as means ± SD (n = 3). Significant effects due to dietary oil or vitamin E supplementation as determined by two-way analysis of variance are indicated (Y). N, not significant. SGR, specific growth rate.

TABLE 5. Effects of dietary oxidised oil and vitamin E on lipid content (percentage of wet weight), proportions of polar lipid, neutral lipid and triacylglycerol (percentages of total lipid), and fatty acid composition (percentage of total fatty acids by weight) of total lipid of liver from sea bream (*Sparus aurata*)

	HO	HXO	HXE	HE
Total lipid	10.9 ± 1.9	16.0 ± 3.0	14.1 ± 3.9	16.3 ± 1.4
Total polars	27.0 ± 2.9	19.5 ± 3.6	18.7 ± 3.0	20.6 ± 4.4
Total neutrals	73.0 ± 2.9	80.5 ± 3.6	81.1 ± 3.2	79.4 ± 4.4
Triacylglycerol	64.5 ± 3.2	72.6 ± 4.0	72.8 ± 3.0	70.1 ± 4.4
Total saturated	13.6 ± 1.2	12.2 ± 1.1	13.4 ± 1.1	12.7 ± 0.6
Total monoenes	23.8 ± 0.8	22.6 ± 1.4	24.0 ± 0.7	21.4 ± 0.2
18:2n-6	1.8 ± 0.0	1.8 ± 0.0	1.8 ± 0.1	1.9 ± 0.0
18:3n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:2n-6	0.5 ± 0.1	0.3 ± 0.2	0.3 ± 0.0	0.3 ± 0.1
20:3n-6	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
20:4n-6	1.9 ± 0.0	1.9 ± 0.2	1.8 ± 0.1	1.8 ± 0.0
22:4n-6	0.4 ± 0.0	0.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.0
22:5n-6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Total n-6PUFA	5.7 ± 0.1	5.6 ± 0.2	5.5 ± 0.0	5.5 ± 0.1
18:3n-3	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.9 ± 0.0
18:4n-3	2.1 ± 0.2	1.9 ± 0.2	1.9 ± 0.1	2.0 ± 0.1
20:3n-3	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:4n-3	2.1 ± 0.3	2.4 ± 0.2	2.2 ± 0.1	2.1 ± 0.1
20:5n-3	20.3 ± 1.2	19.3 ± 2.0	19.5 ± 1.6	21.2 ± 0.9
22:5n-3	5.6 ± 0.9	8.3 ± 2.2	6.7 ± 0.6	6.3 ± 0.6
22:6n-3	21.6 ± 0.9	22.6 ± 1.8	20.7 ± 1.0	23.4 ± 0.8
Total n-3PUFA	54.2 ± 1.7	57.1 ± 1.9	53.4 ± 1.6	57.5 ± 0.8
Total PUFA	61.1 ± 1.8	63.9 ± 1.6	60.2 ± 1.6	64.2 ± 0.8

Results are means ± SD (n = 3). An SD of 0.0 implies an SD of < 0.05%. Significant effects due to dietary oil or vitamin E supplementation were determined by two-way analysis of variance. The only significant differences were that dietary oxidised oil increased the proportions of total neutral lipid and triacylglycerol and decreased the percentages of 14:0 and 18:3n-3. PUFA, polyunsaturated fatty acids.

TABLE 6. Effects of dietary oxidised oil and vitamin E on lipid content (percentage of wet weight), proportions of polar lipid, neutral lipid and triacylglycerol (percentages of total lipid), and fatty acid composition (percentage of total fatty acids by weight) of total lipid of liver from turbot (*Scophthalmus maximus*)

	HO	HXO	HXE	HE
Lipid content	7.9 ± 1.5	8.9 ± 1.6	8.9 ± 1.2	8.8 ± 2.0
Polar lipid	26.5 ± 2.8	27.0 ± 3.2	24.2 ± 2.5	26.7 ± 1.3
Neutral lipid	73.5 ± 2.8	73.0 ± 3.2	75.8 ± 2.5	73.3 ± 1.3
Triacylglycerol	56.7 ± 4.0	55.7 ± 3.7	59.9 ± 1.5	60.5 ± 3.1
Total saturated	15.2 ± 1.1	15.2 ± 1.2	15.0 ± 0.3	14.8 ± 0.4
Total monoenes	19.4 ± 0.6	19.3 ± 0.8	20.4 ± 0.8	19.3 ± 0.2
18:2n-6	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.0	2.0 ± 0.0
18:3n-6	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
20:2n-6	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
20:3n-6	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
20:4n-6	2.3 ± 0.2	2.4 ± 0.1	1.9 ± 0.7	2.3 ± 0.1
22:4n-6	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
22:5n-6	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.1
Total n-6 PUFA	6.1 ± 0.2	6.3 ± 0.2	5.7 ± 0.7	6.0 ± 0.2
18:3n-3	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.0	0.8 ± 0.1
18:4n-3	2.5 ± 0.1	2.4 ± 0.3	2.6 ± 0.2	2.3 ± 0.2
20:3n-3	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
20:4n-3	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.0
20:5n-3	21.7 ± 0.9	20.3 ± 1.6	21.0 ± 0.4	20.8 ± 0.5
22:5n-3	5.1 ± 0.1	5.1 ± 0.2	5.6 ± 0.4	5.4 ± 0.7
22:6n-3	27.1 ± 0.5	28.5 ± 1.6	26.7 ± 0.8	28.4 ± 0.9
Total n-3 PUFA	59.2 ± 1.1	59.1 ± 0.5	58.9 ± 0.6	59.9 ± 0.5
Total PUFA	65.3 ± 1.0	65.4 ± 0.4	64.6 ± 0.5	65.9 ± 0.6

Results are means ± SD (n=3). There were no significant effects due to either dietary oil or vitamin E supplementation as determined by two-way analysis of variance. PUFA. polyunsaturated fatty acids.

TABLE 7. Effects of dietary oxidised oil and vitamin E on lipid content (percentage of wet weight), proportions of polar lipid, neutral lipid and triacylglycerol (percentages of total lipid), and fatty acid composition (percentage of total fatty acids by weight) of total lipid of liver from halibut (*Hippoglossus hippoglossus*)

	HO	HXO	HXE	HE
Total lipid	7.8 ± 1.0	7.5 ± 1.2	6.1 ± 1.1	7.4 ± 1.0
Total polars	29.2 ± 1.0	26.6 ± 3.1	27.2 ± 2.1	26.8 ± 2.1
Total neutrals	70.8 ± 1.0	73.4 ± 3.1	72.8 ± 2.1	73.2 ± 2.1
Triacylglycerol	55.0 ± 1.7	54.5 ± 5.6	53.7 ± 3.3	54.6 ± 2.5
Total lipid	10.9 ± 1.9	16.0 ± 3.0	14.1 ± 3.9	16.3 ± 1.4
Total saturated	22.8 ± 0.3	22.2 ± 0.8	21.9 ± 0.4	20.5 ± 0.2
Total monoenes	41.7 ± 0.9	43.0 ± 2.5	43.5 ± 1.6	42.8 ± 1.4
18:2n-6	5.5 ± 0.1	5.6 ± 0.2	5.6 ± 0.2	5.9 ± 0.2
18:3n-6	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
20:2n-6	0.9 ± 0.1	0.8 ± 0.3	0.9 ± 0.2	0.8 ± 0.1
20:3n-6	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.0
20:4n-6	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.5 ± 0.1
22:5n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
Total n-6 PUFA	8.2 ± 0.1	8.2 ± 0.5	8.1 ± 0.3	8.5 ± 0.3
18:3n-3	1.4 ± 0.1	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.1
18:4n-3	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
20:3n-3	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.0
20:4n-3	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
20:5n-3	7.5 ± 0.3	7.4 ± 0.2	7.2 ± 0.2	8.0 ± 0.2
22:5n-3	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.0
22:6n-3	14.7 ± 0.4	13.8 ± 1.4	13.9 ± 1.1	14.9 ± 1.4
Total n-3 PUFA	27.3 ± 0.5	26.6 ± 1.5	26.4 ± 1.1	28.2 ± 1.4
Total PUFA	35.5 ± 0.7	34.8 ± 1.7	34.5 ± 1.3	36.7 ± 1.6

Results are means ± SD (n=3). Effects due to dietary oil or vitamin E supplementation were determined by two-way analysis of variance. The only significant effect was that vitamin E supplementation reduced the proportions of total saturated fatty acids. PUFA, polyunsaturated fatty acids.

TABLE 8. Effects of dietary oxidized oil and vitamin E on the activities of liver antioxidant enzymes

		HO	HXO	HXE	HE	Significance		
						ox. oil	vit E	inter.
Sea bream	Catalase	437±10	667±23	520±25	307±7	Y	Y	N
	SOD	5.7±0.0	6.5±0.2	6.0±0.8	2.8±0.1	Y	Y	Y
	GPX	101.6±5.9	84.7±3.7	89.8±5.6	85.7±1.5	Y	N	Y
	GST	895±80	802±100	944±61	833±15	N	N	Y
	GR	26.3±1.6	59.2±2.7	71.3±7.2	77.8±28.9	N	Y	N
Turbot	Catalase	129±21	202±36	127±35	117±17	N	Y	N
	SOD	4.6±0.3	5.1±0.7	4.0±0.2	4.0±0.3	N	Y	N
	GPX	0.93±0.19	0.81±0.09	0.83±0.08	0.79±0.07	N	N	N
	GST	104±3	117±5	102±8	86±9	Y	Y	N
	GR	8.5±1.5	13.1±0.9	11.5±1.4	10.2±0.6	Y	N	Y
Halibut	Catalase	170.1±11.6	170.5±24.3	158.0±2.8	178.8±4.6	N	N	N
	SOD	3.3±0.2	3.0±0.2	2.4±0.0	3.2±0.2	Y	Y	Y
	GPX	38.7±17.3	n.d.	36.6±0.0	14.9±6.8	N	N	Y
	GST	154.0±5.9	158.0±5.1	190.0±1.4	155.0±6.1	Y	Y	Y
	GR	9.1±3.0	6.2±0.9	9.6±0.4	14.2±0.8	Y	Y	N

Data are presented as means ± SD (n = 3). Significant effects due to dietary oil or vitamin E supplementation as determined by two-way analysis of variance are indicated (Y). N, not significant. Catalase, (μmol.min^{-1}.mg protein^{-1}); GPX (glutathione peroxidase), GR (glutathione reductase) and GST (glutathione-S-transferase) are all nmol.min^{-1}.mg protein^{-1}; SOD, superoxide dismutase (Units.min^{-1}.mg protein^{-1}).

TABLE 9. Effects of dietary oxidized oil and vitamin E on lipid peroxidation products in liver and whole fish

		HO	HXO	HXE	HE	Significance		
						ox. oil	vit E	inter.
Sea bream	TBARS							
	liver	5.4±0.5	5.0±0.5	4.2±0.3	3.3±0.1	N	Y	Y
	Isoprostane							
	liver	110±6	188±3	125±22	177±3	N	N	Y
Turbot	TBARS							
	liver	0.66±0.18	1.20±0.14	0.54±0.18	0.60±0.20	Y	Y	Y
	fish	10.2±0.8	9.6±3.1	7.2±1.0	7.3±1.9	N	Y	N
	Isoprostane							
	liver	79.1±1.0	64.3±5.0	64.0±14.5	77.9±3.2	Y	N	N
	fish	42.4±7.7	46.0±10.6	21.2±1.6	18.1±5.6	N	Y	N
Halibut	TBARS							
	liver	0.18±0.02	0.22±0.01	0.21±0.01	0.19±0.01	Y	N	N
	fish	1.6±0.5	10.7±2.4	3.5±0.6	6.4±0.8	Y	N	Y
	Isoprostane							
	liver	58.3±4.3	57.1±6.7	83.9±5.8	60.8±6.5	Y	Y	Y
	fish	14.7±5.7	52.1±0.7	37.7±2.8	32.8±7.2	Y	N	Y

Data are presented as means ± SD (n = 3). Significant effects due to dietary oil or vitamin E supplementation as determined by two-way analysis of variance are indicated (Y).

N, not significant. 8-isoprostane ($pg.mg^{-1}$ protein); TBARS, thiobarbituric acid-reactive substances ($\mu mols.mg^{-1}$ protein).