Fish Physiol Biochem (2007) 33:269–280
DOI 10.1007/s10695-007-9139-4

Does dietary tocopherol level affect fatty acid metabolism in fish?

Gabriel Mourente · J. Gordon Bell · Douglas R. Tocher

Received: 15 February 2007 / Accepted: 2 April 2007 / Published online: 7 July 2007

Abstract Fish are a rich source of the n-3 polyunsaturated fatty acids (PUFA), particularly the highly unsaturated fatty acids (HUFA) eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids, which are vital constituents for cell membrane structure and function, but which are also highly susceptible to attack by oxygen and other organic radicals. Resultant damage to PUFA in membrane phospholipids can have serious consequences for cell membrane structure and function, with potential pathological effects on cells and tissues. Physiological antioxidant protection involves both endogenous components, such as free-radical-scavenging enzymes, and exogenous dietary micronutrients including tocopherols and tocotrienols, the vitamin E-type compounds widely regarded as the primary lipid-soluble antioxidants. The antioxidant activities of tocopherols are imparted by their ability to donate their phenolic hydrogen atoms to lipid (fatty acid) free radicals, resulting in the stabilization of the latter and the termination of the lipid peroxidation chain reaction. However, tocopherols can also prevent PUFA peroxidation by acting as quenchers of singlet oxygen. Recent studies on marine fish have shown correlations between dietary and tissue PUFA/tocopherol ratios and incidence of lipid peroxidation, as indicated by the levels of thiobarbituric-acid reactive substances (TBARS) and isoprostanes. These studies also showed that feeding diets containing oxidized oil significantly affected the activities of liver antioxidant defence enzymes and that dietary tocopherol partially attenuated these effects. However, there is evidence that dietary tocopherols can affect fatty acid metabolism in other ways. An increase in membrane PUFA was observed in rats deficient in vitamin E. This was suggested to be due to overproduction of PUFA arising from increased activity of the desaturation/elongation mechanisms responsible for the synthesis of PUFA. Consistent with this, increased desaturation of 18:3n-3 and 20:5n-3 in hepatocytes from salmon fed diets deficient in tocopherol and/or astaxanthin has been observed. Although the mechanism is unclear, tocopherols may influence the biosynthesis of n-3PUFA through alteration of cellular oxidation potential or peroxide tone.

Keywords Vitamin E · α-tocopherol · Antioxidant defence enzymes · Lipid peroxidation

G. Mourente (✉)
Departamento de Biología Animal, Biología Vegetal y Ecología, Facultad de Ciencias del Mar, Universidad de Cádiz, 11510 Puerto Real (Cádiz), Andalucía, Spain
e-mail: gabriel.mourente@uca.es

J. G. Bell · D. R. Tocher
Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

Introduction

Vitamin E is a minor component present among the lipid constituents of cell membranes and lipoproteins. The vitamin E-type compounds, tocopherols and tocotrienols, are not synthesized by animals and must be obtained in the diet, ultimately from higher plant and algal sources (Hess 1993). Tocopherols are widely regarded as the primary lipid-soluble exogenous antioxidant nutrients (Buettner 1993; Kamal-Eldin and Appelqvist 1996; Wang and Quinn 1999). Their antioxidant effects are imparted partly by their ability to donate their phenolic hydrogen atoms to lipid-free radicals, resulting in the termination of the lipid peroxidation chain reaction (Burton and Ingold 1989) and partly by acting as quenchers of singlet oxygen free radicals to prevent damage to tissues and specifically to unsaturated lipids (Gorman et al. 1984; Wang and Quinn 1999). The relative antioxidant efficacies of the tocopherols in vivo have been established as $\alpha > \beta > \gamma > \delta$ (Burton and Traber 1990; Wang and Quinn 1999) with α-tocopherol identified as the major naturally occurring tocopherol in the lipids of marine fish and salmon (Ackman and Cormier 1978; Parazo et al. 1998). Moreover, vitamin E has been found to possess functions that are independent of its antioxidant radical-scavenging capacity such as effects on protein kinase, gene expression, cell proliferation and disease (Azzi and Stocker 2000) and enhanced immune responses of fish (Ortuño et al. 2000). However, the effects of tocopherols (vitamin E) on cellular oxidation potential or peroxide tone may affect lipid metabolism in other ways, including alteration of fatty acid desaturation and/or elongation. This article reviews the mechanisms of oxidative stress and lipid peroxidation, and the effects of vitamin E in fish, focusing on both its role in an integrated antioxidant defence mechanism and its effects on the biosynthesis of highly unsaturated fatty acids (HUFA).

Biochemistry of oxidative stress

Reactive oxygen species (ROS)

Molecular oxygen (O_2) is essential for aerobic organisms, with its dominant role in eukaryotes being that of terminal electron acceptance in mitochondrial respiration, where it is ultimately reduced to water during the process of oxidative phosphorylation, the major source of ATP in aerobes. The reduction of O_2 to water requires four electrons, and this reduction proceeds sequentially through one-, two-, and three-electron products. These univalent reductions of O_2 to water occur as presented in the following equations:

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$$

$$H_2O_2 + e^- + H^+ \rightarrow \cdot OH + H_2O$$

$$\cdot OH + e^- \rightarrow H_2O$$

The sum result of these four reactions being:

$$O_2 + 4e^- \rightarrow 2\ H_2O$$

The products of the sequential reductions depicted in the first three equations are the superoxide radical anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (·OH), respectively. These activated or ROS, particularly ·OH, are very reactive and potentially deleterious to biological systems. Both $O_2^{\bullet -}$ and ·OH are oxygen-based free radicals (oxy-radicals). Although not a free radical, that is, one possessing an unshared electron, H_2O_2 is also reactive and serves as an important precursor of ·OH by reacting with $O_2^{\bullet -}$ through the Haber–Weiss reaction below.

$$O_2^{\bullet -} + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$$

This reaction, although thermodynamically favourable, is kinetically slow but is catalyzed by transition metals such as iron and copper. Thus, metal-catalyzed (i.e., chelated iron) Haber–Weiss reactions are an important source of ·OH in biological systems. Other important species of activated oxygen include singlet oxygen ($^1O_2$), and alkoxy radicals (RO·), and peroxy radicals (ROO·) formed by oxidation of organic molecules and, in particular, lipids and fatty acids (see below). In addition to mitochondrial

electron transport, other sources of endogenous ROS production include the electron transport chains of microsomes (Winston and Cederbaum 1983) and chloroplasts (Asada et al. 1974), the respiratory burst associated with phagocytosis by leukocytes (Chung and Secombes 1988) and the activities of enzymes such as xanthine oxidase, tryptophan dioxygenase, diamine oxidase, and prostaglandin synthase (Fridovich 1978; Halliwell 1978).

Oxidative damage

Reactive oxygen species and free radicals can react with a wide variety of biomolecules and in a rather nonspecific manner, particularly in the case of highly reactive radicals such as ·OH. Lesions associated with ROS include oxidation of membrane lipids, proteins, and nucleic acids and altered cellular redox status, resulting in the tissue pathologies often associated with redox-active contaminants, and possibly chemical carcinogenesis and aging (Ames 1989). The role of ROS and free-radical intermediates in DNA alterations, including adduct formation, is a topic of intense research interest with the most specific genotoxic effect being the oxidation of DNA resulting in oxidized bases such as thymine glycols and 8-hydroxyguanine (Dizdaroglu and Bergtolt 1986). In erythrocytes, haemoglobin can also be a target for ROS attack, resulting in the formation of methaemoglobin (MetHb) in which the iron centers of the haeme moieties are oxidized (Fe^{3+}), preventing the molecules from functioning normally in O_2 binding and transport (Stern 1985). In fish, excess build-up of nitrite due to incomplete ammonia oxidation is a relatively common problem in aquaculture that results in methaemoglobinemia, or brown blood disease, named after the dark colour of MetHb (Bowser et al. 1983). The examples described above demonstrate the consequences of ROS attack on nucleic acids and proteins, however this article is primarily concerned with oxidative damage to another major group of biomolecules, the lipids, and this is described below.

Lipid peroxidation

Lipid peroxidation can be defined as the oxidative deterioration of polyunsaturated fatty acids (PUFA), and is an important consequence of oxidative stress. Lipid peroxidation proceeds by a chain reaction whereby a single radical species has the ability to propagate a number of deleterious biochemical reactions (Fig. 1). Lipid peroxidation can be initiated by the ROS-mediated, particularly ·OH, abstraction of a hydrogen atom from a methylene group of a PUFA, yielding a lipid radical. This organic radical can be stabilized by molecular rearrangement to a conjugated diene radical, which readily reacts with O_2 to produce the peroxy radical. The peroxy radical can readily abstract a hydrogen from another methylene group of PUFA to yield a lipid hydroperoxide, and a new lipid radical, which can continue propagating additional lipid hydroperoxide and lipid radicals in a chain reaction. Lipid hydroperoxides are relatively stable in isolation, but can react with transition-metal complexes to yield alkoxyl radicals. The lipid peroxidation chain reaction can be terminated by two lipid radicals reacting to form a non-radical product or by quenching by a radical scavenger such as tocopherol (or similar antioxidant molecule) (Fig. 1). In general, the overall effects of lipid peroxidation are to decrease membrane fluidity, increase the permeability of the membrane to normally impermeable substances, and inactivate membrane-bound enzymes. For example, lipid peroxidation of erythrocyte membranes alters their ability to change shape and pass through capillaries and eventually leads to haemolysis (Halliwell and Gutteridge 1996). Continued fragmentation of fatty acid side-chains to produce potentially toxic compounds such as aldehydes and hydrocarbons will eventually lead to a complete loss of membrane integrity (Fig. 1). Peroxidation-induced damage to the lysosome membrane can result in hydrolytic enzymes escaping into the cell cytoplasm, further damaging the cell.

Antioxidant defence mechanisms

An array of antioxidant defence mechanisms to detoxify ROS has evolved to counter the potentially deleterious effects of activated oxygen (Yu 1994). Antioxidant compounds can be classified as water-soluble reductants such as glutathione, uric acid, and ascorbic acid (vitamin C), or as lipid-soluble radical scavengers, chief amongst which are the subject of this volume, the tocopherols (vitamin E), but which

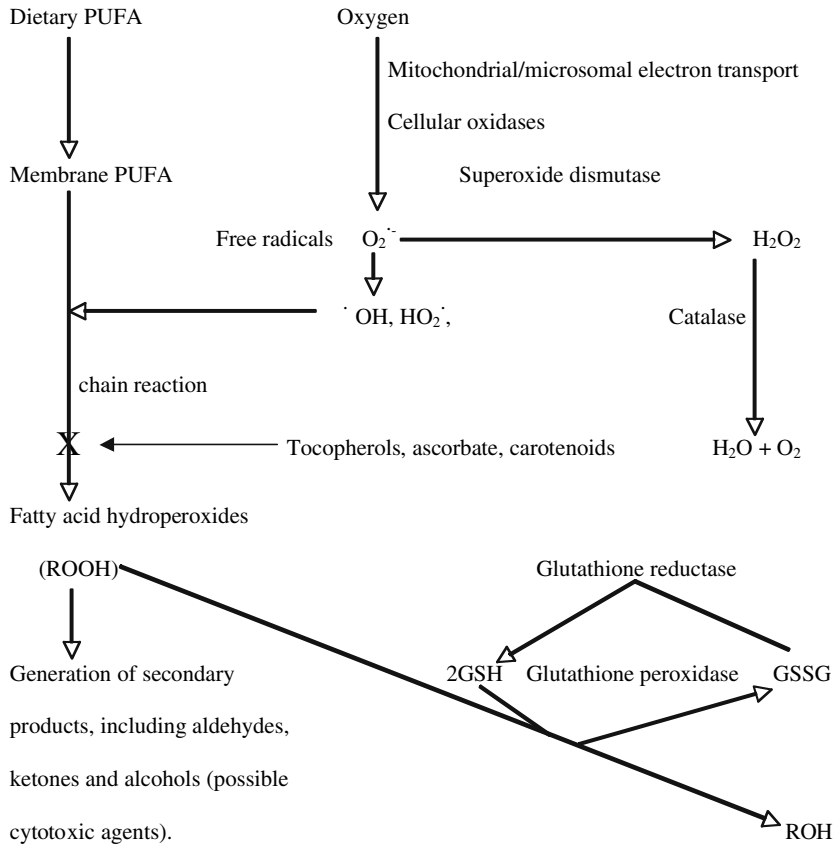

Fig. 1 Mechanisms of lipid peroxidation and antioxidant protection. Adapted from Sargent et al. (2002)

also include retinol (vitamin A), carotenoids and various xanthophylls. Antioxidant enzymes include radical-scavenging enzymes such as superoxide dismutase (SOD) and catalase, peroxidases such as glutathione peroxidase (GPX), and glutathione reductase (GR) (Fig. 1). Thus, components of both endogenous and exogenous origins contribute protection (Jacob 1995), and interactions between components and synergistic effects have been described. For instance, it is well documented that ascorbate can provide protection synergistically with α-tocopherol (Leung et al. 1981).

The antioxidant enzymes comprise a series of enzyme scavengers of oxyradicals and other free radicals. SOD is a group of metalloenzymes that converts $O_2^{-}\cdot$ to H_2O_2 (Fridovich 1986). SOD plays a pivotal antioxidant role, catalyzing the dismutation of $O_2^{\bullet\,-}\cdot$ at rates approximating diffusion limits, making it among the most active enzymes described. Numerous studies have indicated induction of SOD in many organisms by factors associated with increased oxyradical production, such as elevated O_2 and exposure to redox-active contaminants. The product of SOD activity, H_2O_2, can be removed by the activities of catalase or peroxidases such as GPX (Fig. 1). Catalase is associated primarily with peroxisomes, where it detoxifies H_2O_2 arising as a by-product of fatty acid oxidation (Fahimi and Sies 1987). GPX is a cytosolic and mitochondrial enzyme, and in addition to reducing H_2O_2, it can reduce lipid peroxides (ROOH) to their corresponding alcohols (ROH), an important reaction for quenching lipid-peroxidizing chain reactions (Reed 1990). GR plays an important antioxidant role by catalyzing the reduction of oxidised glutathione (GSSG) to glutathione (GSH) at the expense of Reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Reed 1990) and, thereby, recycling antioxidant cofactor molecules.

Like all aerobic organisms, fish are susceptible to attack by ROS and have developed antioxidant

defences including low-molecular-weight antioxidants together with adapted antioxidant enzymes. Therefore, to prevent oxidative damage, effective antioxidant defences are, in part, dependent on the adequate dietary supply of essential antioxidants, including vitamin E. Some recent reviews in the literature have dealt with information about oxidative stress and antioxidant defences in fish in relation to specific situations including pollution and aquaculture (Livingstone 2003), environment and temperature (Abele and Puntarulo 2004), egg and larval quality (Palace and Werner 2006), and a range of biotic and abiotic factors (Martínez-Alvarez et al. 2005).

Tocopherols, oxidative stress and lipid peroxidation in fish

Oxidative stress and lipid peroxidation

The fundamental aspects of oxyradical production, antioxidant defences, and biochemical manifestations of oxidative injury are shared among biological systems including fish. Deleterious cellular effects associated with oxidative stress, such as lipid peroxidation, methaemoglobinemia, and DNA oxidation, have also been investigated in fish. In this regard, lipid peroxidation has received the greatest attention. Due to their poikilothermic nature, fish lipids are more highly unsaturated than those from homeotherms, particularly those adapted to cold-water environments (Abele and Puntarulo 2004), which would seem to predispose them to lipid peroxidation. Indeed, in vivo lipid peroxidation caused by oxygen radicals is a principal cause of several diseases in fish such as jaundice (Sakai et al. 1989, 1998), nutritional muscular dystrophy (Watanabe et al. 1970; Murai and Andrews 1974; Cowey et al. 1984; Bell et al. 1985; Frischknecht et al. 1994), haemolysis (Kawatsu 1969), liver degeneration, anaemia, depletion of antioxidant vitamins, blood pathologies and myopathy of skeletal muscle (Cowey et al. 1984; Bell et al. 1985; Tacon 1992; Frischknecht et al. 1994; Sargent et al. 2002), and the development of skeletal abnormalities (Lewis-McCrea and Lall 2007). Clearly, the presence of oxidized lipids can have toxic consequences for fish, whether they arise from dietary input of toxicants or by deficiencies in essential antioxidant nutrients. However, evidence suggests that pathological symptoms can be controlled or eliminated by supplying sufficient dietary antioxidant, particularly tocopherol, to prevent the production of excessive levels of free-radical-generated toxic compounds (Cowey et al. 1984; Baudin-Laurencin et al. 1989; Baker and Davies 1997a; Livingstone 2003; Abele and Puntarulo 2004; Martínez-Alvarez et al. 2005).

Role of tocopherol in preventing lipid peroxidation in fish

The vitamin E requirements of many fish species have been established and generally fall in the range of 20–50 mg/kg dry feed (NRC 1993). Specific deficiency symptoms include muscular dystrophy, exudative diathesis, anaemia, impaired erythropoiesis, erythrocyte fragility, skin discolouration, and ceroid pigment deposition.

Recent research has focused more on the relationship of tocopherol with increased dietary PUFA, temperature and interaction with other antioxidants. Thus, in fish, increased levels of dietary and tissue PUFA require increased dietary supplementation with tocopherol to prevent the occurrence of oxidative damage (Watanabe et al. 1981; Cowey et al. 1981). A correlation between increased dietary PUFA and tocopherol requirement was found in blue tilapia (Roem et al. 1990), turbot (Stephan et al. 1995), carp (Runge et al. 1992) and Atlantic salmon (Waagbø et al. 1991). When fish are fed tocopherol-deficient diets, there is a rapid loss of tocopherol from liver and muscle but selective retention in the neural tissues of the brain and eye. In a study with Atlantic salmon, feeding a tocopherol-deficient diet for a period of 22 weeks resulted in liver tocopherol levels falling to 3% of their original value, whereas levels in brain and eye were only reduced to 35 and 40% of their original values (Bell et al. 2000). These results suggest selective conservation of tocopherol in tissues with a high n-3 HUFA content and probably reflect the functionality of n-3 HUFA-rich biomembranes in neural tissues. In general, levels of tocopherol are higher in fish tissues than in mammals and this probably reflects the higher degree of antioxidant protection required in n-3 PUFA-rich organisms (Hamre and Lie 1995; Martínez-Alvarez et al. 2005). In juvenile African

catfish, elevated dietary tocopherol resulted in decreased levels of thiobarbituric-acid reactive substances (TBARS), common indicators of lipid peroxidation (Baker and Davies 1996a, 1997b), although doses of tocopherol above the requirement only marginally improved the protection against peroxidation. In contrast, Olsen et al. (1999) found that α-tocopherol did not influence the tissue TBARS content in juvenile Arctic char (*Salvelinus alpinus* L.), while high dietary PUFA increased the content of TBARS in liver and in muscle. Moreover, there is no evidence that dietary vitamin E above a minimum requirement does not significantly improve the antioxidant defences and health of the fish (Olsen et al. 1999; Kiron et al. 2004). Nevertheless, vitamin E requirements depend not only on dietary lipids, but also on vitamin C (Olsen et al. 1999) since ascorbic acid, by donating electrons to the α-tocopheroxyl radical, reduces it back to functional α-tocopherol (Tappel 1962).

Several studies have suggested that carotenoids, including *β*-carotene, astaxanthin and canthaxanthin, are potent antioxidants in *in vitro* membrane models and that they operate synergistically with tocopherol (Krinsky 1993; Nishigaki et al. 1994; Fukuzawa et al. 1998). Thus, synergism between tocopherol and astaxanthin was recently observed in Atlantic salmon (Bell et al. 2000), and antioxidant synergism has also been observed between tocopherol and selenium in trout and salmon (Bell et al. 1985; Poston et al. 1976). Other potential synergistic effects include regeneration of α-tocopherol from its radical by glutathione (Wefers and Sies 1988) or dihydro-lipoic acid (Freiselben and Packer 1993). In addition, phospholipids having a primary amine group, e.g., phosphatidylethanolamine or phosphatidylserine, can function as peroxyl radical scavengers and thereby have a sparing effect on tocopherol (Lambelet et al. 1984), whereas phosphatidylinositol and other acidic phosphatides can act synergistically with tocopherols due to their metal-chelating activity (Pokorny 1987; Ishihara 1996). Phospholipids have been shown to enhance the antioxidant efficacy of tocopherols in oils by forming reverse micelles or microemulsions, such that tocopherols were positioned in the micelles with their active phenolic group adjacent to the polar region where peroxy radicals are concentrated (Kago and Terao 1995).

Tocopherol and the antioxidant defence enzymes

Few studies have investigated the activities of antioxidant enzymes and concentrations of oxyradical scavengers, including tocopherol, in aquatic animals (Winston and Di Giulio 1991; Abele and Puntarulo 2004; Martínez-Alvarez et al. 2005), and studies with fish have often failed to reveal consistent antioxidant responses (Di Giulio et al. 1995; Abele and Puntarulo 2004; Martínez-Alvarez et al. 2005). The activities of the antioxidant enzymes have been measured in marine fish including dab (*Limanda limanda*) (Livingstone et al. 1992), sardine (*Sardina pilchardus*) (Peters et al. 1994), turbot larvae (Peters and Livingstone 1996) and Senegal sole larvae (Solé et al. 2004), and freshwater fish such as rainbow trout and black bullhead (*Ameiurus melas*) (Aceto et al. 1994; Otto and Moon 1996). These studies focussed on the role of the enzymes in pollutant detoxification (Peters et al. 1994) or developmental aspects (Aceto et al. 1994; Otto and Moon 1996; Peters and Livingstone 1996) rather than the effects of dietary nutrients. In mammals, the effects of dietary PUFA and tocopherol on the activity of the antioxidant enzymes in liver are contradictory. However, supplementation of PUFA, including EPA, to Swiss 3T3 cells resulted in increased levels of PUFA in phospholipids and the activities of SOD, GPX and GST increased with the degree of unsaturation of the phospholipids (Benito et al. 1997).

Previously, we have shown that SOD and GST activities decreased as the PUFA/tocopherol ratio decreased during early development in unfed common dentex (*Dentex dentex*) larvae (Mourente et al. 1999a). Conversely, the activities of catalase and, to a lesser degree, GPX actually increased with decreasing PUFA/tocopherol ratio in that study. In a subsequent study on larval dentex, there were no significant effects on the antioxidant enzyme activities of increasing dietary HUFA, but in this study the level of dietary tocopherol increased in parallel with dietary HUFA (Mourente et al. 1999b). Similarily, no interactions were observed between dietary tocopherol and antioxidant enzyme activities in Atlantic salmon (Lygren et al. 2000).

Recently, we showed that the level of dietary tocopherol had significant effects on the activities of the enzymes of the liver antioxidant defence system in juvenile marine fish (Mourente et al. 2000; Tocher

et al. 2002). In the earlier trial, a clear relationship between liver PUFA/tocopherol ratio and the activities of the liver antioxidant enzymes was not observed in juvenile turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippoglossus*) and gilthead sea bream (*Sparus aurata*) (Mourente et al. 2000). This was possibly due to the level of dietary HUFA being insufficient to exert a significantly high oxidative load, and/or the level of tocopherol in the deficient diets was not sufficiently low. In a subsequent trial, the dietary HUFA load was increased and some significant effects on antioxidant enzyme activities were obtained that could have been predicted based on current knowledge. Thus, higher activity of GPX in turbot fed a tocopherol-deficient diet, and lower levels of catalase and GPX in halibut, and of catalase in sea bream fed a diet supplemented with high levels of tocopherol, were consistent with the expected pattern (Tocher et al. 2002).

These studies suggested that feeding high-HUFA diets and/or decreased tocopherol 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. 2002). In a further trial with juvenile turbot, halibut and sea bream, the level of dietary HUFA was increased by using higher levels of dietary oil and by using an oil with a much higher n-3 HUFA content (Mourente et al. 2002; Tocher et al. 2003). In order to increase the potential peroxidative stress to an even higher level, oxidized oil was also used with peroxidation induced by controlled heating (50°C) in an oxygen-rich atmosphere, with the extent of peroxidation monitored regularly by sampling and determination of peroxide value (PV). Therefore, the dietary trial had a factorial two design [oxidized (X) versus unoxidised oil and ± tocopherol] giving four diets, HO, HE, HXO and HXE. The effects of dietary oxidized oil with or without supplementary dietary tocopherol on the activities of the liver antioxidant defence enzymes were characterized. Finally, the levels of liver and whole-body lipid peroxidation products, including malondialdehyde, determined as TBARS, and isoprostanes, were measured.

Dietary oxidized oil significantly reduced growth in turbot and especially in halibut, but not in sea bream. Tocopherol improved growth in sea bream fed oxidised oil but not in turbot or halibut, although it improved survival in all three species. In sea bream and turbot, liver catalase and SOD activities were increased by feeding oxidized oil and reduced by dietary tocopherol (Figs. 2, 3). Conversely, in halibut, the liver enzyme activities were not increased by feeding oxidized oil, but SOD was reduced by feeding tocopherol (Figs. 2, 3). Consistent with these data, feeding oxidized oil increased lipid peroxidation products in halibut, but generally not in turbot (Figs. 4, 5). Furthermore, lipid peroxidation products were generally reduced by dietary tocopherol in turbot, but not in halibut (Fig. 4). Similar attenuating effects of dietary tocopherol in fish fed oxidized oil were obtained in previous studies in which sea bream (Obach et al. 1993) and African catfish were fed oxidized oil (Baker and Davies 1997a, b).

Therefore, halibut liver antioxidant defence enzymes did not respond to dietary oxidized oil or tocopherol as occurred in turbot and sea bream. This resulted in increased levels of lipid peroxides in halibut compared to turbot in fish given dietary oxidized oil. In addition, supplemental tocopherol did not reduce lipid peroxides in halibut as it did in turbot and sea bream. Therefore, the increased peroxidation stress in halibut may have been responsible for their

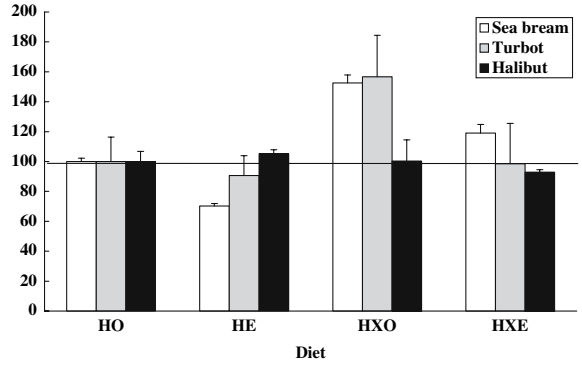

Fig. 2 Effects of dietary tocopherol and oxidized oil on liver catalase activity in turbot, sea bream and halibut. Diets were fed to juvenile fish for 3 months. HO, unoxidized oil (PV = 5) without tocopherol; HE, unoxidized oil plus tocopherol (200 ppm); HXO, oxidized oil (PV = 45) without tocopherol; HXE, oxidized oil plus tocopherol. Activities are presented relative to diet HO (=1) and are means ± SD ($n = 3$). Significant effects due to tocopherol (toc) and dietary oil (oil) supplementation, and interaction (int) as determined by two-way analysis of variations (ANOVA) are indicated below. Sea bream (toc, oil), turbot (toc) and halibut (none). Data taken from Tocher et al. (2003)

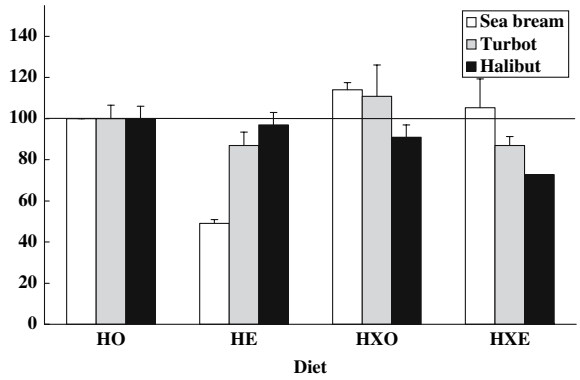

Fig. 3 Effects of dietary tocopherol and oxidised oil on liver superoxide dismutase activity in turbot, sea bream and halibut. Diets were fed to juvenile fish for three months. HO, unoxidized oil (PV = 5) without tocopherol; HE, unoxidized oil plus tocopherol (200 ppm); HXO, oxidized oil (PV = 45) without tocopherol; HXE, oxidized oil plus tocopherol. Activities are presented relative to diet HO (=1) and are means ± SD (n = 3). Significant effects due to tocopherol (toc) and dietary oil (oil) supplementation, and interaction (int) as determined by two-way ANOVA are indicated below. Sea bream (toc, oil, int), turbot (toc) and halibut (toc, oil, int). Data taken from Tocher et al. (2003)

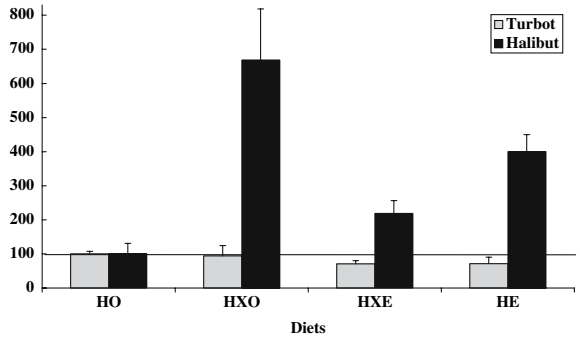

Fig. 4 Effects of dietary tocopherol and oxidized oil on thiobarbituric acid-reactive substances (TBARs) in turbot and halibut. Diets were fed to juvenile fish for three months. HO, unoxidized oil (PV = 5) without tocopherol; HE, unoxidized oil plus tocopherol (200 ppm); HXO, oxidized oil (PV = 45) without tocopherol; HXE, oxidized oil plus tocopherol. Activities are presented relative to diet HO (=1) and are means ± SD (n = 3). Significant effects due to tocopherol (toc) and dietary oil (oil) supplementation, and interaction (int) as determined by two-way ANOVA are indicated below. Turbot (toc) and halibut (oil, int). Data taken from Tocher et al. (2003)

poorer growth and survival in comparison to turbot and sea bream. It was speculated that this effect in halibut may, in part, be due to the temperature of culture (14°C) compared to turbot and sea bream that

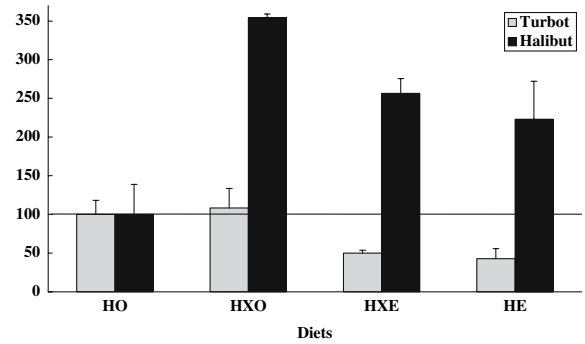

Fig. 5 Effects of dietary tocopherol and oxidized oil on isoprostanes in turbot and halibut. Diets were fed to juvenile fish for three months. HO, unoxidized oil (PV = 5) without tocopherol; HE, unoxidized oil plus tocopherol (200 ppm); HXO, oxidized oil (PV = 45) without tocopherol; HXE, oxidized oil plus tocopherol. Activities are presented relative to diet HO (=1) and are means ± SD (n = 3). Significant effects due to tocopherol (toc) and dietary oil (oil) supplementation, and interaction (int) as determined by two-way ANOVA are indicated below. Turbot (toc) and halibut (oil, int). Data taken from Tocher et al. (2003)

were cultured at higher temperatures (19°C). Previous studies have indicated that development of lipid peroxidation pathologies is increased at lower water temperatures (Cowey et al. 1984). These data from juvenile marine fish provide some of the best evidence that dietary tocopherol interacts with endogenous antioxidants including liver enzymes in an integrated antioxidant defence system in fish.

In a recent study, the physiological response of rainbow trout to oxidative stress induced by feeding large amounts of unsaturated fatty acids depended on vitamin E levels in the diet and upregulation of antioxidant enzyme activities corresponded to mechanisms combating the elevation of free radicals under oxidative stress (Puangkaew et al. 2005). It seems evident that the role of vitamin E as an effective antioxidant depends on the extent of the oxidative stress of the fish.

Tocopherol and fatty acid desaturation and elongation pathway

Various effects on tissue fatty acid compositions have been reported in response to diets deficient in tocopherol. Very often no major effects have been observed, but in other trials both decreased and

increased levels of PUFA have been observed. Decreased levels of PUFA have been easily and logically explained by increased levels of lipid peroxidation leading to loss of membrane PUFA and consequently reduced levels of tissue PUFA. However, in rats deficient in both tocopherol and selenium, Buttriss and Diplock (1988) observed an increase in the HUFA, 22:6n-3 and 20:4n-6 in mitochondrial and microsomal membranes. They theorized that this increase was due to an overproduction of these HUFA arising from increased activity of the desaturation and elongation mechanisms responsible for the synthesis of HUFA. A similar effect has also been found in African catfish fed oxidized oil (Baker and Davies 1996b). In a more recent study, the ability of isolated salmon hepatocytes to desaturate and elongate [1-^{14}C]18:3n-3 and [1-^{14}C]20:5n-3 was studied in fish fed diets deficient in tocopherol, astaxanthin or both (Bell et al. 2000). The results showed that fatty acid desaturation and elongation was increased in fish fed diets deficient in either tocopherol or astaxanthin and, especially, in the fish deficient in both (Table 1). There are very few similar studies in which fatty acid desaturation and elongation have been determined and related to dietary tocopherol. Despret et al. (1992) investigated the relationship between tocopherol levels and fatty acid desaturase activities in rat and found it to be tissue dependent. Thus, in rat, increased vitamin E was associated with increased fatty acyl Δ6 desaturase activity in brain, but a similar increased level of tocopherol was associated with decreased Δ6 desaturase activity in liver. The mechanism of these effects of dietary tocopherol on fatty acid desaturation is unclear. In an early meta-analysis study, Infante (1986) reassessed a range of early data and hypothesized that vitamin E (tocopherol) and selenium may have direct roles in fatty acid desaturation. Specifically, he suggested that tocopherol (and selenium) might affect fatty acid desaturation by actually participating in the microsomal electron transport chain via the involvement of a terminal vitamin E-containing electron donor (Infante 1986). However, no data from subsequent studies have supported this hypothesis and few researchers in the field would see a direct role for tocopherol in this way. It appears likely, therefore, that the effect of tocopherol on fatty acid desaturation and elongation occurs through a more indirect mechanism. It may be mediated by a membrane effect, either through affecting membrane fatty acid composition or, more indirectly, by affecting fluidity, as there is evidence that tocopherol decreases membrane fluidity and/or acts as a membrane stabilizer (Wang and Quinn 1999). Alternatively, and possibly most plausibly, it is possible that tocopherol may affect the desaturation reaction even more indirectly through affecting general cellular peroxide tone/antioxidant status. This is supported by the fact that an increase in peroxide tone, whether achieved by restricted dietary intake of one or more antioxidants and/or by inclusion of dietary pro-oxidants in the form of oxidized triacylglycerol oils or other lipid classes, appears to result in the activation of fatty acyl desaturation and elongation.

Table 1 Effect of diets deficient in either tocopherol (E) or astaxanthin (Ax) on the desaturation of [1-^{14}C]18:3n-3 and [1-^{14}C]20:5n-3 by hepatocytes from Atlantic salmon (pmol/h/mg protein; means ± SD, $n = 3$)

	Diet			
	+E + Ax	−E + Ax	+E − Ax	−E − Ax
[1-^{14}C]18:3n-3				
Total products	10.0 ± 1.7^{b}	16.7 ± 1.6^{ab}	17.6 ± 2.8^{ab}	24.2 ± 4.9^{a}
Tetraenes	7.7 ± 1.2^{b}	12.2 ± 1.0^{b}	11.9 ± 1.1^{b}	17.2 ± 3.0^{a}
Pentaenes	1.9 ± 0.6^{b}	3.5 ± 0.5^{ab}	4.0 ± 0.9^{ab}	5.4 ± 1.4^{a}
22:6n-3	0.4 ± 0.1	1.0 ± 0.5	1.7 ± 1.2	1.5 ± 0.4
[1-^{14}C]20:5n-3				
22:6n-3	7.0 ± 0.8^{c}	7.7 ± 1.4^{bc}	12.7 ± 2.3^{ab}	14.8 ± 3.3^{a}

Values within a row with different superscript letters are significantly different ($P < 0.05$) as determined by one-way ANOVA followed, where appropriate, by Tukeys multiple comparison test (JG Bell et al. unpublished data)

Conclusions

Dietary tocopherols can affect fatty acid metabolism in at least two ways. They are the primary lipid-soluble antioxidants and can prevent PUFA peroxidation both by acting as quenchers of singlet oxygen and by stabilizing fatty acid free radicals and terminating the lipid peroxidation chain reaction. The role of tocopherol as an effective antioxidant depends on the extent of the oxidative stress in the fish, and is thus related to the quantity and quality, in terms of degrees of unsaturation and peroxidation, of dietary fatty acids. The role of dietary tocopherol as a lipid antioxidant is well researched and established. Less well understood is the role of tocopherol in fatty acid desaturation and elongation. Meta-analysis of early studies in mammals suggested that dietary tocopherol levels could affect fatty acid desaturation, and led to the hypothesis that tocopherol may have a direct role participating in microsomal electron transport. This has not been supported by subsequent studies and, in fish, the most common observation has been increased membrane PUFA in vitamin E deficiency. Consistent with this, we have measured increased desaturation of 18:3n-3 and 20:5n-3 in hepatocytes from salmon fed diets deficient in tocopherol. Although the mechanism of the effects of dietary tocopherol on fatty acid desaturation and elongation is unclear, it appears likely that tocopherol has its effect through an indirect mechanism possibly involving alteration of cellular oxidation potential or"peroxide tone" that also affects cellular synthesis of long-chain PUFA.

References

Abele D, Puntarulo S (2004) Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrate and fish. Comp Biochem Physiol 138A:405–415

Aceto A, Amicarelli F, Sacchetta P, Dragani B, Bucciarelli T, Masciocco L, Miranda M, Di Ilio C (1994) Developmental aspects of detoxifying enzymes in fish (*Salmo iridaeus*). Free Radic Res 21:285–294

Ackman RG, Cormier MG (1978) α-Tocopherol in some Atlantic fish and shellfish with particular reference to live holding without food. J Fish Res Bd Can 24:357–373

Ames BN (1989) Endogenous oxidative DNA damage, aging and cancer. Free Radic Res Commun 7:121–128

Asada K, Kiso K, Yoshikawa K (1974) Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. J Biol Chem 249:2175–2181

Azzi A, Stocker A (2000) Vitamin E: non-antioxidant roles. Prog Lipid Res 39:231–255

Baker RTM, Davies SJ (1996a) Changes in tissue α-tocopherol status and degree of lipid peroxidation with varying α-tocopheryl-acetate inclusion in diets for African catfish. Aquac Nutr 2:71–79

Baker RTM, Davies SJ (1996b) Increased production of docosahexaenoic acid (22:6 n-3, DHA) in catfish nutritionally stressed by the feeding of oxidized oils and the modulatory effect of dietary α-tocopheryl acetate. J Fish Biol 49:748–752

Baker RTM, Davies SJ (1997a) Muscle and hepatic fatty acid profiles and α-tocopherol status in African catfish (*Clarius gariepinus*) given diets varying in oxidative state and vitamin E inclusion. Anim Sci 64:187–195

Baker RTM, Davies SJ (1997b) Modulation of tissue α-tocopherol in African catfish, *Clarias gariepinus* (Burchell), fed oxidized oils, and the compensatory effect of supplemental dietary vitamin E. Aquac Nutr 3:91–97

Baudin-Laurencin F, Messager JL, Stephan G (1989) Two examples of nutritional pathology related to vitamin E and vitamin C deficiencies. Adv Trop Aquac Tahiti Actes Colloq Ifremer 9:171–181

Bell JG, Cowey CB, Adron JW, Shanks AM (1985) Some effects of vitamin E and selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in rainbow trout (*Salmo gairdnei*). Br J Nutr 53:149–157

Bell JG, McEvoy J, Tocher DR, Sargent JR (2000) Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. J Nutr 130:1800–1808

Benito S, Fernandez Y, Mitjavila S, Moussa M, Anglade F, Periquet A (1997) Phospholipid fatty acid composition affects enzymatic antioxidant defenses in cultured Swiss 3T3 fibroblasts. Redox Rep 3:281–286

Bowser PR, Falls WW, Van Zandt J, Collier N, Philips JD (1983) Methemoglobinemia in channel catfish: methods of prevention. Prog Fish Cult 45:154–158

Buettner GR (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, α- tocophenol, and ascorbate. Arch Biochem Biophys 300:535–543

Burton GW, Ingold KU (1989) Vitamin E as in vitro and in vivo antioxidant. Ann N Y Acad Sci 570:7–22

Burton GW, Traber MG (1990) Vitamin E: antioxidant activity, biokinetics and bioavailability. Ann Rev Nutr 10:357–382

Buttriss JL, Diplock AT (1988) The α-tocopherol and phospholipid fatty acid content of rat liver subcellular membranes in vitamin E and selenium deficiency. Biochim Biophys Acta 963:61–69

Chung S, Secombes CJ (1988) Analysis of events occurring within teleost macrophages during the respiratory burst. Comp Biochem Physiol 89B:539–544

Cowey CB, Adron JW, Walton MJ, Murray J, Youngson A, Knox D (1981) Tissue distribution, uptake, and requirement for a-tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. J Nutr 3:1556–1567

Cowey CB, Degener E, Tacon AGJ, Youngson A, Bell JG (1984) The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) grown at natural, varying water temperatures. Br J Nutr 51:443–451

Despret S, Dinh L, Clement M, Bourre JM (1992) Alteration of vitamin E in rat brain and liver. Neurosci Lett 145:19–27

Di Giulio RT, Benson WH, Sanders BM, Van Veld PA (1995) Biochemical mechanisms: metabolism, adaptation and toxicity. In: Rand GM (ed) Fundamentals of aquatic toxicology: effects, environment fate, and risk assessment. Taylor and Francis, London, pp 523–562

Dizdaroglu M, Bergtolt DS (1986) Characterization of free radical induced base damage in DNA at biologically relevant levels. Anal Biochem 156:182–188

Fahimi HD, Sies H (eds) (1987) Peroxisomes in biology and medicine. Springer Verlag, New York

Freisleben H-J, Packer L (1993) Free-radical scavenging activities, interactions and recycling of antioxidants. Biochem Soc Trans 21:325–330

Fridovich I (1978) The biology of oxygen radicals. Science 201:875–880

Fridovich I (1986) Superoxide dismutases. Adv Enzymol 58:61–97

Frischknecht R, Wahli T, Meier W (1994) Comparison of pathological changes due to deficiency of vitamin C, vitamin E and combinations of vitamins C and E in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 17:31–45

Fukuzawa K, Inokami Y, Tokumura A, Terao J, Suzuki A (1998) Rate constants for quenching singlet oxygen and activities for inhibiting lipid peroxidation of carotenoids and tocopherol in liposomes. Lipids 33:751–756

Gorman AA, Gould IR, Hamblett I, Standen MC (1984) Reversible exciplex formation between singlet oxygen ($^1\Delta_g$) and vitamin E: solvent and temperature effects. J Am Chem Soc 106:6956–6959

Halliwell B (1978) Superoxide dependent formation of hydroxyl radicals in the presence of iron chelates. FEBS Lett 92:321–326

Halliwell B, Gutteridge JMC (1996) Lipid peroxidation: a radical chain reaction. In: Halliwell B, Gutteridge JMC (eds) Free radicals in biology and medicine. Clarendon Press, Oxford, pp 188–266

Hamre K, Lie O (1995) Alpha-tocopherol levels in different organs of Atlantic salmon (*Salmo salar* L.)—effect of smoltification, dietary levels of n-3 polyunsaturated fatty acids and vitamin E. Comp Biochem Physiol 111A: 547–554

Hess JL (1993) Vitamin E: α-Tocopherol. In: Alscher RG, Hess JL (eds) Antioxidants in higher plants. CRC Press, Boca Raton, pp 111–134

Infante JP (1986) Vitamin E and selenium participate in fatty acid desaturation. A proposal for an enzyme function of these nutrients. Mol Cell Biochem 69:93–108

Ishihara K (1996) Antioxidant mechanisms of phospholipid. Bull Nat Res Inst Fish Sci 8:139–146

Jacob RA (1995) The integrated antioxidant system. Nutr Res 15:755–776

Kago T, Terao J (1995) Phospholipids increase radical scavenging activity of vitamin E in a bulk oil model system. J Agric Fd Chem 43:1450–1454

Kamal-Eldin A, Appelqvist L-A (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671–701

Kawatsu H (1969) Studies on the anemia of fish-III. An example of macrcytic anemia found in brook trout, *Salvelinus fontinalis*. Bull Freshw Res Lab 19:161–167

Kiron V, Puangkaew J, Ishizaka K, Satoh S, Watanabe T (2004) Antioxidant status and non-specific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with three lipid sources. Aquaculture 234:361–379

Krinsky NI (1993) Actions of carotenoids in biological systems. Annu Rev Nutr 13:561–587

Lambelet P, Saucy F, Loliger J (1984) Radical exchange reactions between vitamin E, vitamin C and phosphatides in autoxidising polyunsaturated lipids. Free Radic Res 20:1–10

Leung HW, Vang MJ, Mavis RD (1981) The cooperative interactions between vitamin E and vitamin C in suppression of peroxidation of membrane phospholipids. Biochem Biophys Acta 664:266–272

Lewis-McCrea LM, Lall SP (2007) Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnormalities in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 262:142–155

Livingstone DR (2003) Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Revue Med Vet 154:427–430

Livingstone DR, Archibald S, Chipman JK, Marsh JW (1992) Antioxidant enzymes in liver of dab *Limanda limanda* from the North Sea. Mar Ecol Prog Ser 91:97–104

Lygren B, Hamre K, Waagbo R (2000) Effect of induced hyperoxia on the antioxidant status of Atlantic salmon *Salmo salar* L. fed three different levels of dietary vitamin E. Aquac Res 31:401–407

Martínez-Alvarez RM, Morales AE, Sanz A (2005) Antioxidant defenses in fish: biotic and abiotic factors. Rev Fish Biol Fish 15:75–88

Mourente G, Tocher DR, Díaz E, Grau A, Pastor E (1999a) Relationships between antioxidant enzyme activities and lipid peroxidation products during early development in *Dentex dentex* eggs and larvae. Aquaculture 179:309–324

Mourente G, Tocher DR, Díaz-Salvago E, Grau A, Pastor E (1999b) Study of the n-3 highly unsaturated fatty acids requirement and antioxidant status of *Dentex dentex* at *Artemia* feeding stage. Aquaculture 179:291–307

Mourente G, Díaz-Salvago E, Tocher DR, Bell JG (2000) Effects of dietary polyunsaturated fatty acid/vitamin E (PUFA/tocopherol) ratio on antioxidant defence mechanisms of juvenile gilthead sea bream (*Sparus aurata* L., Osteichthyes, Sparidae). Fish Physiol Biochem 23:337–351

Mourente G, Díaz-Salvago E, Bell JG, Tocher DR (2002) Increased activities of hepatic antioxidant defence enzymes in juvenile gilthead sea bream (*Sparus aurata* L., Osteichthyes, Sparidae) fed dietary oxidised oil: attenuation by dietary vitamin E. Aquaculture 214:343–361

Murai T, Andrews JW (1974) Interaction of dietary α-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish (*Ictalurus punctatus*). J Nutr 104: 1416–1431

National Research Council (1993) Nutrient requirements of fish. National Academy Press, Washington DC

Nishigaki I, Dmitrovski AA, Miki W, Yagi K (1994) Suppressive effect of astaxanthin on lipid peroxidation induced in rats. J Clin Biochem Nutr 16:161–166

Obach A, Quentel C, Laurencin FB (1993) Effects of alpha-tocopherol and dietary oxidized fish oil on the immune response of sea bass *Dicentrarchus labrax*. Dis Aquat Org 15:175–185

Olsen RE, Lovaas E, Lie O (1999) The influence of temperature, dietary poly-unsaturated fatty acids, α-tocopherol and spermine on fatty acid composition and indices of oxidative stress in juvenile Arctic char, *Salvinus alpinus* (L.). Fish Physiol Biochem 20:13–29

Ortuño J, Esteban MA, Meseguer J (2000) High dietary intake of α-tocopherol acetate enhances the non-specific immune response of gilthead sea bream (*Sparus aurata* L.). Fish Shellfish Immunol 10:293–307

Otto DME, Moon TW (1996) Endogenous antioxidant systems of two teleost fish, the rainbow trout and the black bullhead, and the effect of age. Fish Physiol Biochem 15: 349–358

Palace VP, Werner J (2006) Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. Sci Mar 70S2:41–57

Parazo MPM, Lall SP, Castell JD, Ackman RG (1998) Distribution of α- and γ-tocopherols in Atlantic salmon (*Salmo salar*) tissues. Lipids 33:697–704

Peters LD, Livingstone DR (1996) Antioxidant enzyme activities in embryologic and early larval stages of turbot. J Fish Biol 49:986–997

Peters LD, Porte C, Albaiges J, Livingstone DR (1994) 7-Ethoxyrosorufin O-deethylase (EROD) and antioxidant enzyme activities in larvae of sardine (*Sardina pilchardus*) from the North coast of Spain. Mar Pollut Bull 28:299–304

Pokorny J (1987) Major factors affecting the autoxidation of lipids In: Chan HWS (ed) Autoxidation of unsaturated lipids. Academic Press, London, pp 141–206

Poston HA, Combs GF, Leibovitz L (1976) Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): gross, histological and biochemical deficiency signs. J Nutr 106:892–904

Puangkaew J, Kiron V, Satoh S, Watanabe T (2005) Antioxidant defense of rainbow trout (*Oncorhynchus mykiss*) in relation to dietary n-3 highly unsaturated fatty acids and vitamin E contents. Comp Biochem Physiol 140C:187–196

Reed DJ (1990) Glutathione: toxicological implications. Annu Rev Pharmacol Toxicol 30:603–631

Roem AJ, Kohler CC, Stickney RR (1990) Vitamin E requirement of the blue tilapia, *Oreochromis aureus* (Steindachner), in relation to dietary lipid level. Aquaculture 87:155–164

Runge G, Steinhart H, Schwarz FJ, Kirchgessner M (1992) Influence of type of fats and α-tocopherol acetate additions to the feed rations on the tocopherol and tocotrienol composition of carp (*Cyprinus carpio* L.). J Anim Physiol Anim Nutr 67:16–24

Sakai T, Murata H, Endo M, Yamauchi K, Tabata N, Fukudome M (1989) 2-Thiobarbituric acid values and contents of α-tocopherol and bile pigments in the liver and muscle of jaundiced yellowtail, *Seriola aquiqueradiata*. Agric Biol Chem 53:1739–1740

Sakai T, Murata H, Endo M, Shimomura T, Yamauchi K, Ito T, Yamaguchi T, Nakajima H, Fukudome M (1998) Severe oxidative stress is thought to be a principal cause of jaundice of yellowtail *Seriola quinqueradiata*. Aquaculture 160:205–214

Sargent JR, Tocher DR, Bell JG (2002) The lipids. In: Halver JE, Hardy RW (eds) Fish nutrition, 3rd edn. Academic Press, San Diego, pp 182–246

Solé M, Potrykus J, Fernández-Díaz C, Blasco J (2004) Variation on stress defences and metallothionein levels in the Senegal sole, *Solea senegalensis*, during early larval stages. Fish Physiol Biochem 30:57–66

Stéphan G, Guillaume J, Lamour F (1995) Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary n-6 or n-3 polyunsaturated fatty acids. Aquaculture 130:251–268

Stern A (1985) Red cell oxidative damage. In: Sies H (ed) Oxidative stress. Academic Press, London, pp 331–349

Tacon, AGJ (1992) Nutritional fish pathology: morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Fisheries Technical Paper no 330, Rome

Tappel AL (1962) Vitamin E as the biological lipid antioxidant. Vitam Horm 20:493–510

Tocher DR, Mourente G, Van der Eeken A, Evjemo JO, Diaz E, Bell JG, Geurden I, Lavens P, Olsen Y (2002) Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquac Nutr 8:195–207

Tocher DR, Mourente G, Van Der Eeken A, Evjemo JO, Diaz E, Wille M, Bell JG, Olsen Y (2003) Comparative study of antioxidant defence mechanisms in marine fish fed variable levels of oxidised oil and vitamin E. Aquac Internat 11:195–216

Waagbø R, Sandnes K, Sandevin A, Lie O (1991) Feeding three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E to Atlantic salmon (*Salmo salar*): growth and chemical composition. Fiskeridir Skr (Ernaering) 4:51–63

Wang X, Quinn PJ (1999) Vitamin E and its function in membranes. Prog Lipid Res 38:309–336

Watanabe T, Takashima F, Ogino C, Hibiya T (1970) Effect of α-tocopherol on carp. Nippon Suisan Gakkaishi 36: 623–630

Watanabe T, Takeuchi T, Wada M, Uchara R (1981) The relationship between dietary lipid levels and a-tocopherol requirement of rainbow trout. Bull Jap Sci Fish 47: 1463–1471

Wefers H, Sies H (1988) The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. Eur J Biochem 174:353–357

Winston GW, Cederbaum AI (1983) Oxyradical production by purified components of the liver microsomal mixed-function oxidase system. I oxidation of hydroxyl radical scavenging agents. J Biol Chem 258:1508–1513

Winston GW, Di Giulio RT (1991) Pro-oxidant and antioxidant mechanisms in aquatic organisms. Aquat Toxicol 19: 137–161

Yu BP (1994) Cellular defenses against damage from reactive oxygen species. Physiol Rev 74:139–162