Fish oil replacement in finfish nutrition

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Abstract

Unsustainable fishing practices have placed a heavy emphasis on aquaculture to meet the global shortfalls in the supply of fish and seafood, which are commonly accepted as the primary source of health-promoting essential omega-3 (n-3 highly unsaturated fatty acids). However, dietary fish oil is required for the production of omega-3-rich farmed fish and this commodity, in a vicious circle, is at present derived solely from wild fisheries. Decreasing global availability coupled with the highly variable price of this resource has forced the aquaculture industry to investigate the possibilities of alternative dietary lipid sources. This review attempts to compile all principal information available regarding the effects of fish oil replacement for the diets of farmed finfish, analysing the findings using a comparative approach among different cultured fish species. The review initially focuses on the present situation with regard to the production, availability and main nutritional characteristics of fish oil and the principal alternative lipid sources (such as vegetable oils and animal fats). Following this, the effects of fish oil replacement in finfish nutrition on feed quality, fish performance, feed efficiency, fish lipid metabolism, final eating quality and related economic aspects are presented and discussed.

Key words: animal fat, aquaculture, canola oil, fatty acid, fish oil, fish quality, linseed oil, lipid, lipid catabolism, lipid metabolism, lipogenesis, palm oil, phytosterols, plant oil, rapeseed oil, seafood quality, soybean oil, vegetable oil.

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1 Introduction

Lipids are hydrophobic compounds that are soluble in organic solvents. They include triacylglycerols, wax esters, sterols and phospholipids. Lipids are required by fish as a source of available energy, as structural components of biomembranes, carriers of fat-soluble vitamins, precursors to eicosanoids, hormones and vitamin D, and as enzyme co-factors (Higgs & Dong 2000). In fish, dietary lipids are an important source of essential fatty acids for regular growth, health, reproduction and bodily functions. All vertebrate species, including fish, have an absolute dietary requirement for both n-6 and n-3 polyunsaturated fatty acids (PUFA). The biologically active forms of essential fatty acids are generally the C_{20} and C_{22} metabolites of linoleic acid (LA; 18:2 n-6) and α-linolenic acid (ALA; 18:3 n-3). The roles of lipids in fish nutrition have become more important in recent years given the production and implementation of high-lipid, energy-dense diets. Improvements in growth, feed utilization efficiency and nutrient retention in fish fed such energy-dense diets (Sargent *et al.* 2002) benefit not only the fish farmer, by giving a shorter grow-out period, but also the environment. Lipid levels as high as 40% are currently used in commercial salmon feeds. Marine fish oils (FO) have traditionally been used as the sole dietary lipid source in commercial fish feeds given their ready availability, competitive price and the abundance of essential fatty acids contained within this product.

The aquaculture industry is currently the fastest growing food production sector in the world, expanding at an average annual rate of 8.8% from 1950 to 2004 (FAO 2007a). World aquaculture produces approximately 60 million tons of seafood, which is worth more than US$70 billion annually. Farmed fish accounts for approximately 50% of all fish consumed globally and this percentage is expected to continue to increase as a result of dwindling catches from capture fisheries and ever-increasing total and *per capita* seafood consumption. Capture fisheries are not expected to increase production and aquaculture has been challenged to meet the seafood demand of a growing human population. Based on current exploitation trends, some scientists have predicted a collapse of all species of wild seafood that are currently fished by the year 2050 (Worm *et al.* 2006). The rapid expansion of the global aquaculture industry results, in part, from the increasing intensification of culture systems and the subsequent use of manufactured aquafeeds. According to estimates, aquafeeds currently use approximately 87% of the global supply of FO as a lipid source (Tacon *et al.* 2006). In 2003, salmonid fish, such as Atlantic salmon (*Salmo salar*, Linnaeus, 1758) and rainbow

trout (*Oncorhynchus mykiss*, Walbaum, 1792), accounted for 66.4% of the total FO used in aquaculture followed by marine fish (13.8%), marine shrimp (7.3%), carp (5.5%), tilapia (2.0%), freshwater prawns (1.7%), eels (1.4%) and milkfish (0.6%) (Tacon *et al.* 2006). The global use and demand for FO by various aquaculture species up to 2012 had been estimated by Tacon *et al.* (2006), who predicted that the future consumption of FO will hover at approximately 88% of global supplies. Others, however, predict that aquaculture will consume up to 98% of the global FO supply by the year 2010 (Pike & Barlow 2003). What remains undisputed is the fact that for the past 25 years, annual FO production has not increased beyond 1.5 million tons per annum (Table 1) and the rapidly growing aquaculture industry cannot continue to rely on finite stocks of marine pelagic fish as a supply of FO. The International Fishmeal and Fish Oil Organization (IFFO) predicted a further 12% decline in FO production in 2006 (Mittaine 2005), further exacerbating the dramatic situation of available FO stocks (Table 1).

In the North-Western European market, the price of FO increased from US$314 per ton in 1999 to a record high of US$812 per ton in 2006 within a short 7 year span (Table 2). The price of marine FO is expected to continue to increase in the near future. Despite the current high costs of FO, aquafeed millers continue to use this product in their fish feed formulations because of their familiarity with the product and the perception that

it is the best lipid source for the culture of fish, particularly marine carnivorous species. The major characteristic of FO is the high level of n-3 highly unsaturated fatty acids (HUFA), which are known to be essential for the optimal growth and health of farmed fish (Table 3). However, this scenario is not expected to persist because of a combination of factors. In addition to the economic factor of rising global FO prices and limited supplies, the aquaculture industry is under intense criticism from both scientists and environmental groups with regard to the long-term ecological sustainability of these finite fishery resources (Naylor *et al.* 2000; Worm *et al.* 2006). The ethics of using fishery resources with food-grade potential (pelagic fish, such as mackerel, hake, whiting, pilchards, sardines and capelin) for animal feeding rather than for direct human consumption is also an issue of heated global debate (FAO 2007a), and potential remedial policies have recently been developed (FAO 2007b).

Therefore, there is currently great urgency within the aquafeed industry to find and implement sustainable alternatives to FO. The challenge for fish production is to maintain, if not improve, the recognised benefits of fish for human health while simultaneously seeking to maximize sustainability, fish health and economic benefits. In recent years, intensive research activities have been conducted globally to evaluate alternative lipid sources. The main results of this research are reported and discussed in the present review.

Table 1 World production (thousand metric tonnes) of fish oil, vegetable oils and animal fats from 1980 to 2006†

Oils/fats	1980	1985	1990	1995	2000	2005	2006
Fish oil	1217	1481	1412	1379	1307	1054	988
Vegetable oils							
Palm oil	4543	6832	11 020	20 322	25 594	33 733	36 733
Soybean oil	13 382	13 974	16 097	15 119	21 743	33 575	35 187
Canola/rapeseed oil‡	3478	6066	8160	10 936	14 496	16 205	18 340
Sunflower oil	5024	6564	7869	7003	9808	9661	11 094
Cottonseed oil	2992	3942	3782	3312	3815	4989	4917
Groundnut oil	2864	3575	3897	4325	4382	4523	4497
Palm kernel oil	571	868	1450	1877	2620	3975	4308
Coconut oil	2716	2627	3387	3253	3147	3257	3166
Olive oil	1701	1796	1855	1863	2513	2916	2746
Corn oil	–§	–	–	1855	1966	2099	2252
Sesame oil	–	–	–	589	705	823	871
Linseed oil¶	–	–	–	701	705	607	710
Animal fats							
Tallow and greese	6283	6518	6813	7013	8071	8211	8446
Lard	4691	4989	5509	5141	6580	7568	7877
Butter fat	5746	6315	6500	4834	5829	6665	6790

†Data compiled from Basiron (2007), Tacon *et al.* (2006) and Malaysian Palm Oil Board (2005).
‡Canola or rapeseed oil.
§Data not available.
¶Linseed or flaxseed oil.

Table 2 Average prices of fish oil, tallow and major vegetable oils in the North-Western European market ($US/metric tonne)†

Oil/fat	1999	2000	2001	2002	2003	2004	2005	2006
Fish oil (FO)	314	262	451	586	563	683	719	812
Crude palm oil (CPO)	436	310	286	390	443	471	422	478
Soybean oil (SBO)	427	338	354	454	554	616	545	599
Canola/rapeseed oil (C/RO)	423	347	402	485	600	685	669	794
Sunflower oil (SFO)	507	392	484	594	593	684	677	658
Tallow (TAL)	361	290	324	360	461	463	449	451
FO/CPO price ratio‡	0.72	0.85	1.58	1.50	1.27	1.45	1.70	1.70
FO/SBO price ratio	0.74	0.78	1.27	1.29	1.02	1.11	1.32	1.36
FO/(C/RO) price ratio	0.74	0.76	1.12	1.21	0.94	1.00	1.07	1.02
FO/SFO price ratio	0.62	0.67	0.93	0.99	0.95	1.00	1.06	1.23
FO/TAL price ratio	0.87	0.90	1.39	1.63	1.22	1.48	1.60	1.80

†Compiled from Malaysian Palm Oil Board (2005) and Globefish (2006).
‡Fish oil price ratios calculated from average commodity prices.

Table 3 Typical iodine value and fatty acid composition (% total fatty acids) of fish oils, vegetable oils and animal fats used in fish feed formulations†

Oils/fats	Iodine value‡	SFA	MUFA	LA	AA	ALA	EPA	DHA	n-6 PUFA	n-3 PUFA	n-3/n-6 ratio
Fish oils											
Anchovy oil	180–200	28.8	24.9	1.2	0.1	0.8	17.0	8.8	1.3	31.2	24.0
Capelin oil	95–160	20.0	61.7	1.7	0.1	0.4	4.6	3.0	1.8	12.2	6.8
Menhaden oil	150–200	30.5	24.8	1.3	0.2	0.3	11.0	9.1	1.5	25.1	16.7
Herring oil	115–160	20.0	56.4	1.1	0.3	0.6	8.4	4.9	1.4	17.8	12.7
Cod liver oil	n.a.§	19.4	46.0	1.4	1.6	0.6	11.2	12.6	3.0	27.0	9.0
Vegetable oils											
Crude palm oil	44–58	48.8	37.0	9.1	–¶	0.2	–	–	9.1	0.2	0.0
Soybean oil	120–141	14.2	23.2	51.0	–	6.8	–	–	51.0	6.8	0.1
Canola/rapeseed oil	110–126	4.6	62.3	20.2	–	12.0	–	–	20.2	12.0	0.6
Sunflower oil	110–143	10.4	19.5	65.7	–	–	–	–	65.7	0.0	0.0
Cottonseed oil	99–113	45.3	17.8	51.5	–	0.2	–	–	51.5	0.2	0.0
Groundnut oil	n.a.	11.8	46.2	32.0	–	–	–	–	32.0	0.0	0.0
Corn oil	103–128	12.7	24.2	58.0	–	0.7	–	–	58.0	0.7	0.0
Linseed oil	177	9.4	20.2	12.7	–	53.3	–	–	12.7	53.3	4.2
Animal fats											
Beef tallow	41–52	47.5	40.5	3.1	0.4	0.6	–	–	3.1	0.6	0.2
Pork lard	52–74	38.6	44.0	10.2	–	1.0	–	–	10.2	1.0	0.1
Poultry fat	80–85	28.5	43.1	19.5	–	1.0	–	–	19.5	1.0	0.0

†Data compiled from National Research Council (1993), Gunstone et al. (1994) and Hertrampf and Piedad-Pascual (2000).
‡Expressed as grams of iodine absorbed per 1000 g of fat/oil, which is a measure of the chemical saturation of the oil/fat.
§Data not available.
¶Not detectable.
AA, arachidonic acid, 20:4 n-6; ALA, α-linolenic acid, 18:3 n-3; DHA, docosahexaenoic acid, 22:6 n-3; EPA, eicosapentaenoic acid, 20:5 n-3; LA, linoleic acid, 18:2 n-6; MUFA, monounsaturated fatty acids; n-3 PUFA, polyunsaturated fatty acids with the first double bond at the 3rd carbon atom; n-6 PUFA, polyunsaturated fatty acids with the first double bond at the 6th carbon atom; SFA, saturated fatty acids.

1.1 Vegetable oils

Unlike the production of FO, which has essentially remained static over the past three decades, the production of vegetable oils (VO) has increased considerably (Table 1). The most rapid increase in production has been observed in crude palm oil (CPO), which overtook soybean oil (SBO) in 2005 as the most produced oil in the world. In Malaysia, approximately 3.7 tons of palm oil (PO) is produced annually per hectare, making palm oil the most prolific oil-producing plant (note on terminology: PO is the refined form of CPO). As a perennial species, palm crops produce continuously for approximately 25 years, guaranteeing the reliability of PO supply

and lowering production costs compared with other major oilseed plants, which are mostly annuals. Oil productivities of soybean, canola (also referred to as rapeseed) and sunflower are 0.36, 0.59 and 0.42 tons ha^{-1} year^{-1}, respectively (Basiron 2007). The total global production of VO amounted to more than 115 million tons in 2005 (Malaysian Palm Oil Board 2005). The availability of the major vegetables oils continues to increase yearly with CPO, SBO, canola/rapeseed oil (C/RO) and sunflower oil (SFO) registering more than 1 million tons of ending stocks in 2006 (Table 1).

With the exception of SFO, the prices of the three major VO (CPO, SBO and C/RO) have historically been lower than the price of FO (Table 2). In the North-Western Europe market in 2005, CPO was priced at US$422 per ton compared with US$545 and US$669 per ton for SBO and C/RO, respectively. In the same year, marine FO cost US$719 per ton. In 2006, FO prices were approximately US$800 per ton, exceeding those of the major VO by up to 40% (Globefish 2006). According to industry estimates, it is predicted that this price differential will widen further in the near future, with FO experiencing heavy demand from the aquafeed industry and the decline in global FO production. Oceanic phenomena, such as the re-occurring El Niño, can greatly affect pelagic fisheries causing further FO price hikes. This makes VO increasingly attractive from an economic standpoint to fish feed manufacturers who are looking for cheaper, reliable alternatives to FO. Current research seems to indicate that similar to FO, VO are readily catabolised by fish as an energy source for growth (Bell *et al.* 2001; Regost *et al.* 2003a; Ng *et al.* 2007a; Stubhaug *et al.* 2007). Consequently, much of the FO used in current aquafeeds might be wasteful and could be replaced with VO, which are more available, sustainable and cost effective. However, the chemical characteristics of VO, specifically the fatty acid composition, might pose a problem and currently limits the sole use of these alternative lipid sources.

Most VO are relatively poor sources of n-3 fatty acids in comparison to marine FO. As previously mentioned, n-3 HUFA are essential for the growth of healthy fish and these fatty acids are non-existent in all VO (Table 3). Vegetable oils are rich sources of n-6 and n-9 fatty acids, mainly LA and oleic acid (OA; 18:1 n-9), with the exception of linseed oil (LO), which is rich in ALA. Vegetable oils have been evaluated as FO substitutes either singly or as a blend of VO formulated to replicate the fatty acid composition found in FO in terms of relative total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA ratios (Torstensen *et al.* 2005; Francis *et al.* 2007a). However, such VO blends are still lacking in the HUFA that are abundantly present in FO. Depending on the species, fish have varying abilities to bioconvert (elongate and desaturate) 18:3 n-3 up to n-3 HUFA (Sargent *et al.* 2002), but in most cases, particularly for marine carnivorous species, the quantity of n-3 HUFA produced *in vivo* is insufficient to support the optimal growth and health of the fish (Mourente *et al.* 2005a). The effects of various VO on fish performance and fish tissue chemical composition will be addressed in further detail in the following sections of this review. In general, research has reported that VO can replace substantial amounts of FO in the diets of many fish species without affecting growth or feed efficiency, provided that adequate amounts of specific essential fatty acids (EFA) are supplied in the diet.

Henderson and Sargent (1985) have reported the preferential use of SFA (i.e. 16:0; abundant in CPO) and MUFA (i.e. 18:1 n-9; abundant in CPO and C/RO) for energy production in the mitochondrial systems of fish. However, digestion and absorption of SFA and MUFA are known to be inferior to PUFA in fish (Sigurgisladottir *et al.* 1992; Torstensen *et al.* 2000; Ng *et al.* 2004a; Francis *et al.* 2007b). Therefore, when selecting potential VO for substituting FO in the diets of fish, particularly coldwater fish species, energy availability as well as the PUFA content must be considered.

Therefore, the VO best suited as a substitute for FO should contain high SFA and MUFA as energy sources and low amounts of LA because this fatty acid is poorly oxidized and difficult to remove using finishing diets. This will be discussed in greater detail in the following sections of this review.

1.2 Animal fats

Terrestrial animal lipid sources are processing by-products, such as lard (rendered fat from pigs), poultry fat, grease (from rendering operations) and tallow (TAL; from cattle and sheep). As seen in Table 1, the production of animal fats, including TAL, grease and lard amounted to more than 15 million tons in 2006. The availability of rendered animal fats is substantially greater than most VO (except CPO, SBO and C/RO). It is estimated that the worldwide animal rendering industry processes over 60 million tons of livestock annually with major industries located in the United States, European Union, Argentina, Australia, Brazil and New Zealand (Tacon *et al.* 2006).

The price of TAL and grease has remained relatively constant over the past 5 years, ranging from US$361 to 461 per ton in the North-Western Europe market (Table 2). With the recent rising costs of major VO, such as CPO and SBO, animal fats are perhaps the cheapest lipid resource available for the aquafeed industry. The wide availability and economical price of rendered animal

fats makes them attractive alternatives to FO in fish feed formulations. Bureau and Gibson (2004) estimated that the cost of salmonid fish feed can be reduced by US$24 per ton by using 8% TAL at the expense of FO, which results in substantial feed cost savings.

The chemical characteristics of animal fats can vary widely depending on dietary history, species and age. In general, animal fats contain high levels of SFA, ranging from 28.5% in poultry fat to 47.5% in beef TAL (Table 3). Together with the high levels of MUFA, animal fats are good sources of dietary energy for fish. Beef TAL has less than 4% PUFA, but up to approximately 20% PUFA, primarily LA, can be present in poultry fat. Although VO are completely lacking in n-3 HUFA, animal fats are reported to contain these fatty acids (Moretti & Corino 2008). In particular, there are several studies in which the presence of eicosapentaenoic acid, 20:5 n-3 (EPA) and docosahexaenoic acid, 22:6 n-3 (DHA) in the lipid fraction of swine, bovine and poultry derived products have been reported. However, the n-3 HUFA content of animal fat is extremely limited, commonly reported only at trace level, and hence these lipid sources can be considered lacking in n-3 HUFA. In general, research reports that animal fats when incorporated at no more than 50% of the dietary lipid level have no negative effects on fish growth performance as long as the EFA requirements are met (Turchini et al. 2003a,b; Bureau & Gibson 2004). The impact of animal fats on fish performance and product quality will be discussed in more detail in the following sections of this review.

1.3 Aquatic by-products

Aquatic by-products from seafood processing plants (such as fish guts, heads, blood, skin, bones, liver) have historically been considered of low value and consequently disposed of. However, with mounting economic and environmental benefits in using such by-products, some countries are using aquatic by-products for the production of valuable marine products, such as fish meal and fish silage. Fish wastes (particularly the viscera) also have great potential for use in fish offal oil production. For example, the viscera of farmed Indian major carp contain approximately 40.6–43.8% lipid (Mondal et al. 2006) and are a major source of dietary lipid for freshwater fish feeds manufactured in Myanmar (Ng et al. 2007b). Commercial channel catfish grow-out feeds in the United States use catfish oil produced from catfish processing plants as a dietary lipid source (Robinson et al. 1994). Sathivel (2002) reported that whole catfish viscera contain 30–35% crude fat and the total n-3 fatty acids in catfish visceral oil ranged from 4.3 to 20.9 mg g^{-1} dry weight. Similarly, salmonid viscera, resulting from the filleting

industry, are a potential alternative source of aquatic lipids and trout offal oil from farmed fish has been tested in the diets of Murray cod, Maccullochella peelii peelii, Mitchell, 1838 (Turchini et al. 2003a). In Pangasius catfish farming, the use of farm-made diets, including offal and other by-products from the filleting industry, is also common practice (Paripatananont 2002). However, despite the potential of these alternative sources, there are currently no official estimates with regard to the production, availability and prices of FO from the processing of aquatic by-products.

Recently, there has been increased interest in the potential use of meals and oils derived from fishery by-products for the production of certified organic farmed fish. Although organic aquaculture is still in its infancy, current organic standards in the European Union specify that fish meal and fish oil used in aquafeeds should preferentially be from scraps and by-products from fish and seafood destined for human consumption (Craig 2004). The use and recycling of these aquatic wastes and by-products as a substitute for marine FO in commercial fish feeds can markedly reduce the cost of feed, reduce wastage and increase overall aquaculture productivity and sustainability. Suitable storage, processing and quality control of FO from farmed fish wastes should be developed to promote a more eco-friendly and sustainable aquaculture industry. However, it should be noted that an ad hoc group of the Scientific Committee on Animal Health and Animal Welfare of the European Commission has recommended that the by-products of farmed finfish should not be fed to farmed finfish (European Commission 2003). This recommendation has been made in the absence of any reported fish disease outbreaks associated with the transmission of fish pathogens via fish meal and fish feeds. The group noted that the risks of transmission of potentially zoonotic bacterial and parasitic agents affecting fish do exist if farmed fish are fed untreated fish by-products. This recommendation does not include the use of by-products from the processing of fisheries by-catch, trimmings and discards in the feeds of farmed fish, which is advocated by the FAO as one of the responsible uses of fishery resources as feed inputs for aquaculture development (Tacon et al. 2006; FAO 2007b). Oils extracted from such marine-based fishery processing wastes and by-catch are high in nutritional value for use in aquafeeds, particularly in terms of n-3 HUFA content. Other fishery by-products, such as krill oil and squid oil, are rich sources of EFA, phospholipids, cholesterol and carotenoid pigments (Hertrampf & Piedad-Pascual 2000) and are considered to be high-quality feed ingredients. Their relatively high price and limited availability compared with FO does not make them, in the short term, suitable substitutes and they will not be discussed in this

review. However, an increased knowledge of the potential utilization of such raw materials, together with adequate technological development of the processing industries, which can increase availability and decrease production costs, is highly desirable. This can contribute to the long-term sustainability of the fisheries and aquaculture sector.

1.4 New alternative n-3 HUFA rich lipid sources

Recently, given the above reported limits of commonly available lipid sources that lack n-3 HUFA, a few alternative oil sources, derived from unicellular algae, pelagic organisms or benthic invertebrates containing high amounts of n-3 HUFA, have been identified and tested in aquafeeds (Hertrampf & Piedad-Pascual 2000; Carter *et al.* 2003; Olsen *et al.* 2004). For example, the potential use of DHA-rich oils derived from single-cell microalgae (i.e. *Schizochytrium* spp., *Crypthecodinium cohnii*, Biecheler, 1938 and *Phaeodactylum tricornutum*, Bohlin, 1897) have been successfully tested on gilthead sea bream (*Sparus aurata*, Linnaeus, 1758) (Atalah *et al.* 2007; Ganuza *et al.* 2008). Similarly, *Schizochytrium* spp. oil has also been successfully tested on Atlantic salmon (Miller *et al.* 2007a) and oil extracted from the calanoid copepod *Calanus finmarchicus*, Gunner, 1765 was an effective substitute for FO in the diet of Atlantic salmon (Olsen *et al.* 2004). However, the current unpredictable supply and extremely high production costs of these alternatives make their potential commercial use almost impossible on a global scale.

The oil extracted from the seeds of certain Boraginaceae plants, particularly from *Echium* sp., is rich in stearidonic acid (18:4 n-3). Recently, stearidonic acid has been the subject of several studies in animal and human nutrition (Guil-Guerrero 2007) because it is the Δ6-desaturated homologue of ALA (18:3n-3). Hence, stearidonic acid is a step further in the elongation and desaturation pathway towards the biosynthesis of n-3 HUFA. Δ6-desaturase is the first enzyme in the ALA desaturation and elongation pathway (see section 4.4) and it has been considered to be the limiting enzyme responsible for the poor bioconversion of ALA to n-3 HUFA in fish. Consequently, it has been hypothesised that echium oil could represent an effective alternative to FO in aquafeed (Bell *et al.* 2006; Tocher *et al.* 2006a; Miller *et al.* 2007b). However, the results obtained to date are slightly contrasting, ultimately suggesting that the use of echium oil is similar to the use of LO, which is rich in ALA that is easily converted to stearidonic acid *in vivo* by fish. Furthermore, unlike echium oil, LO is already commercially available.

The implementation of biotechnology for genetically modified n-3 HUFA enriched oil-seed crop production is also showing promising results (Opsahl-Ferstad *et al.* 2003; Robert 2006) and genetically engineered vegetable oils containing n-3 HUFA have been obtained. However, genetically modified organisms are considered to be a threat to the environment and to human health (Pouteau 2000; Myhr & Dalmo 2005) and it has been demonstrated that consumers strongly dislike the use of these methods in agriculture and food industries (Magnusson & Hursti 2002). Therefore, despite the potential benefits stemming from these research sectors, broad application of such strategies appears unfeasible, at least in the short to medium term.

Although it is acknowledged that potential benefits can arise from increased knowledge development and improvement in the production technique of these n-3 HUFA rich alternative lipid sources, the present review focusses on the lipid sources that are currently available and that are commonly used or have been tested in aquafeed.

1.5 Other compounds in oils and fats

Minor components in oils and fats include sterols, tocopherols, tocotrienols and carotenoids. Phytosterols and their esters are minor components of VO with values ranging from 362 to 11 276 mg kg^{-1} among the three major oils (Table 4). Beta-sitosterol is usually the dominant sterol present. Phytosterols are well known for their cholesterol lowering properties and are used as health supplements (Normen *et al.* 2000). Phytosterols, such as beta-sitosterol, are a major component found in pulp and paper mill effluents and have been reported to adversely affect the reproductive performance of fish living in these polluted waters. Smaller gonads, delayed sexual maturity and depression of sex steroids have been reported in fish exposed to these effluents (Tremblay & Van der Kraak 1999). Some fish species are also known to be masculinised when exposed to phytosterols (Nakari & Erkomaa 2003). Dietary phytosterols are reported to reduce low-density lipoprotein (LDL) cholesterol and triacylglycerols (TAG) in male brook trout (*Salvelinus fontinalis*, Mitchill, 1814) (Gilman *et al.* 2003). Similarly, in Atlantic salmon the inclusion of dietary C/RO, which is known to contain relatively high levels of β-sitosterol (Gordon & Miller 1997), affected plasma lipoproteins (Torstensen *et al.* 2004a) and reduced the total plasma cholesterol and LDL cholesterol (Jordal *et al.* 2007). Mørkøre *et al.* (2007) reported that trace amounts of phytosterols were detected in the muscle of Atlantic cod (*Gadus morhua*, Linnaeus, 1758) fed SBO-based diets. It would seem that phytosterols are poorly absorbed, and when absorbed are efficiently eliminated from the body of the fish. Cholesterol is the main sterol found in animal fats and oils and constitutes 93–97% of the sterol composition of herring and mackerel viscera (Souchet & Laplante 2007). Animal fats contain cholesterol with 580, 930 and 1090 mg kg^{-1} for poultry fat, lard and beef TAL, respectively (Higgs & Dong 2000). This is considerably less

Table 4 Minor components found in crude vegetable oils with potential physiological properties†

Component	Palm oil	Soybean oil	Canola/rapeseed oil
Phytosterols (mg kg^{-1})	362–627	1837–4089	4824–11 276
Campesterol (%)	19.8–29.1	15.8–24.2	18.2–38.6
Stigmasterol (%)	8.3–13.0	15.9–19.1	Trace
β-sitosterol (%)	50.2–62.1	51.7–57.6	45.1–57.9
Vitamin E (mg kg^{-1})	600–1000	530–1500	424–1054
α-tocopherol (%)	26.9	12.6	87.6
β-tocopherol (%)	Trace	Trace	5.1
γ-tocopherol (%)	5.8	73.9	4.8
δ-tocopherol (%)	1.4	10.2	1.0
α-tocotrienol (%)	20.2	–‡	–
β-tocotrienol (%)	Trace	–	–
γ-tocotrienol (%)	39.3	–	–
δ-tocotrienol (%)	6.2	–	–
Carotenoids (mg kg^{-1})	500–700	1.5	5–25
α-carotene (%)	29–36	–	–
β-carotene (%)	55–62	–	–
γ-carotene (%)	3–4	–	–
Chlorophyll (%)	–	100	100

†Data compiled from Gunstone *et al.* (1994) with the exception of vitamin E composition (W.-K. Ng, pers. comm., 2008).
‡Not detectable.

than the amount contained in fish oils, which can vary from 5210 mg kg^{-1} of menhaden oil to 7660 mg kg^{-1} of herring oil.

Vitamin E is a generic descriptor attributed to a group of lipid-soluble, structure-related compounds that occur naturally in α (alpha)-, β (beta)-, γ (gamma)- or δ (delta)-tocopherols and the four corresponding tocotrienols. Vitamin E is a potent anti-oxidant that inhibits lipid peroxidation in biomembranes, lipoproteins and body lipids. Research has shown that various vitamin E isoforms are deposited in the fish tissues of fish fed PO-based diets and imparted potent anti-oxidant properties (Ng *et al.* 2004b; Wang *et al.* 2006). Elevated dietary levels of vitamin E from PO resulted in a marked increase in its deposition in fish tissues, which in turn effectively prolonged the shelf life of fresh and frozen fish fillets. The various isoforms of naturally occurring vitamin E found in VO (Table 4) can be an excellent alternative to synthetic DL-α-tocopheryl acetate, which is the current industry standard source of vitamin E in fish feeds. The edible oil-processing industry is eager to preserve the natural vitamin E content during the refining of VO given the human health benefits derived from these minor components (Mag & Reichert 2002).

Carotenoids are present in the crude oils of most VO, but are found in highest concentrations in CPO (Table 4). CPO has a deep orange/red coloration because of the high content of α-carotene and β-carotene. Beta-carotene, and to a lesser extent α-carotene, is known to be a precursor of vitamin A. In tilapia, the conversion ratio of β-carotene to vitamin A was reported to be approximately 19:1 (Hu *et al.* 2006). Most fish species are unable to bioconvert β-carotene to astaxanthin, the main pigment responsible for the reddish skin and flesh coloration in fish. The carotenoid concentrations and subsequent flesh coloration of Atlantic salmon were not significantly increased by feeding fish with CPO-based diets (Ng *et al.* 2004a). However, shrimp and prawns are reported to possess the necessary enzyme pathways to biosynthesize these red carotenoids from β-carotene or zeaxanthin (Boonyaratpalin *et al.* 2001). Biosynthesis is postulated to be mediated by the enzymes C_3 and C_4 mono-oxygenase and caroten-4-ol dehydrogenase. In practice, most of the carotenoids and chlorophyll pigments present in crude plant oils are removed by bleaching during the refining process.

Most published research to date has primarily focused on the effects of dietary fatty acids from animal fats and VO when used in the diets formulated for farmed fish. The effects of the minor components found in FO and in FO alternatives requires greater consideration in future research. For example, fat-soluble vitamins, such as vitamin A and vitamin D, can be abundant in FO, particularly in fish liver oils, whereas these vitamins are lacking in many alternative vegetable sources (Hertrampf & Piedad-Pascual 2000).

Several survey studies have reported on the elevated levels of environmental pollutants in aquafeeds and farmed Atlantic salmon. These include persistent organic pollutants (POP) that can be potentially hazardous to the consumer, such as polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and furans (known as 'dioxins' in combination), polybrominated diphenyl ethers (a flame

retardant) and organochlorinated pesticides (Easton *et al.* 2002; Jacobs *et al.* 2002; Hites *et al.* 2004). These pollutants are highly persistent fat-soluble substances that are ubiquitous in the marine ecosystem and readily biomagnified in the food chain. Fish oils, extracted from marine pelagic fish species, used in high-energy fish feeds are considered to be the main source of POP in farmed salmon (World Health Organization 1999; Jacobs *et al.* 2002). The POP concentration in feed was correlated with the dioxin concentration in the fillets of farmed rainbow trout (Isosaari *et al.* 2002; Karl *et al.* 2003) and Atlantic salmon (Isosaari *et al.* 2004; Lundebye *et al.* 2004). Substitution of marine oils with VO has been shown to be effective in reducing the levels of dioxins, dioxin-like polychlorinated biphenyls and organochlorinated pesticides in fish feeds and farmed salmon (Bell *et al.* 2005; Berntssen *et al.* 2005, 2007). However, there are also possibilities of chemical contamination with the use of VO. The pesticides evaluated by Berntssen *et al.* (2007) were those included in the current European Union legislation on undesirables in feed ingredients and fish feed (European Commission 2002). Most of these persistent organochlorine pesticides have been, or are in the process of being, phased out in Europe and the USA. However, they continue to be used in tropical and subtropical regions on crops from which vegetable meal and oil are produced.

Although FO is the main contributor of POP, fish meal is the main source of metals and metalloids, such as mercury, arsenic and cadmium. Oils, including FO and VO, contain low levels of non-essential metals, such as Cd, Hg and Pb. However, the metalloid arsenic (As) is an exception to this, as high levels of As have been found in fish oils (Francesconi & Kuehnelt 2002; Schmeisser *et al.* 2005).

2 The effects of fish oil replacement on feed quality

2.1 Essential fatty acids

The lipid content of feed is important as a source of dietary energy, but is also fundamental for the supply an adequate amount of EFA (Sargent *et al.* 2002). A nutrient is commonly termed 'essential' when its biosynthesis *ex novo* is not possible; thus, it must be supplied within the diet. However, the term 'essential fatty acid' can be considered ambiguous and often inappropriately inclusive or exclusive of many PUFA (Cunnane 2003). Theoretically, the only two fatty acids that should be most rigidly termed as essential are LA (18:2 n-6) and ALA (18:3 n-3), which cannot be biosynthesised *ex novo* by fish and other vertebrates. However, it has been reported that vertebrates are able to produce LA and ALA if 14:2 n-6 and 14:3 n-3 are supplied (Cunnane 2003). However, these fatty acids

are extremely limited in nature; hence, LA and ALA can be considered to be the two basic EFA.

Almost all fish, as with all vertebrates, potentially have the ability to convert the two basic C_{18} n-6 and n-3 PUFA into the corresponding C_{20} and C_{22} n-6 and n-3 HUFA *in vivo* by an alternating succession of desaturation and elongation (Nakamura & Nara 2004). However, many species have lost this capability, most likely as the result of adapting to a HUFA-rich environment. Consequently, these species exhibit a direct requirement for HUFA (Sargent *et al.* 2002). Therefore, it is now well accepted that many aquatic species have a net requirement for the longer and more unsaturated counterparts: 20:4 n-6 (arachidonic acid (AA)) and 20:5 n-3 (EPA) and 22:6 n-3 (DHA), respectively (Sargent *et al.* 1999). With regard to the metabolism of ALA to EPA and DHA, fish can be classified into two main groups. These include: (i) species with a typical 'freshwater' fish pattern (i.e. herbivorous, omnivorous and carnivorous freshwater fish capable of bioconverting C_{18} PUFA to longer and more unsaturated homologues); and (ii) species with a 'marine' fish pattern, (i.e. the majority of marine carnivorous species that possess the necessary enzymes for the bioconversion of C_{18} PUFA, but have lost the capability to bioconvert C_{18} PUFA and therefore require HUFA as an EFA) (Sargent *et al.* 2002; Mourente *et al.* 2005a; Tocher *et al.* 2006b).

The EFA requirements of fish can vary within a species according to the development and physiological stage and among species according to trophic level and the environment in which they evolved. Moreover, the EFA requirement can increase with increases in the lipid content of the diet. In general, marine finfish and cold water freshwater fish have an essential requirement for n-3 fatty acids, whereas freshwater fish from warm waters have been reported to have requirements for both n-3 and n-6 fatty acids (De Silva & Anderson 1995). The requirements for n-6 fatty acid can be met by the inclusion of LA, which is abundantly present in almost all ingredients of both marine and/or terrestrial origin. In contrast, n-3 fatty acid requirements are only fulfilled by the sole provision of ALA in a few instances and n-3 HUFA are therefore required; hence, n-3 fatty acids can be considered to be the most limiting EFA (Sargent *et al.* 1999). Sargent *et al.* (1999) further reports that in consideration of the competitive interactions of different PUFA, dietary requirements should not be considered in isolation, and it is necessary to consider requirements in relative as well as absolute amounts. For example, juvenile gilthead sea bream (*S. aurata*) require 1.9% of a dry diet of n-3 HUFA if the DHA:EPA ratio is 0.5, whereas the requirement decreases to only 0.9% if the DHA:EPA ratio is 1 (Sargent *et al.* 2002). Therefore, it is not surprising that the range of EFA requirements recorded is relatively wide; from 5.5% of a dry diet as n-3 HUFA for

the larval stage of some marine carnivorous species to as little as 0.5% of a dry diet as C_{18} PUFA for a variety of juvenile to sub-adult freshwater carnivorous and omnivorous species (Sargent *et al.* 2002). Moreover, in some instances, fish known to have a basic requirement for only the C_{18} PUFA grow better if supplied with sufficient n-3 HUFA, making the etymological definition of 'essential fatty acid' even more confusing. In Table 5, some of the known EFA requirements for different finfish species are reported. Species have been sorted according to the natural fish environment (from freshwater to marine) and, within each environment, they have been sorted according to the fish trophic level (from herbivorous to top-order predator). It is evident, by observing Table 5, that there is a shift in dependence from C_{18} n-6 and n-3 PUFA towards n-3 HUFA as we move progressively to increasing salinity of the natural environment of the fish species and, within fish in a similar environment, as we move progressively to increasing trophic level. Basically, the higher the salinity the higher the requirements for n-3 HUFA and the lower the trophic level the lower the requirements for n-3 HUFA, and this is clearly in accordance with the adaptation of fish

metabolism to the environment and the fatty acid composition of their food sources.

Within the context of FO replacement in aquafeed, it is important to note that as previously mentioned (Table 3), VO and animal fat do not contain n-3 HUFA, although a few of these sources can contain ALA, the basic n-3 EFA. However, regardless of the dietary lipid source used, if the diet contains a relatively large amount of fish meal, EFA deficiency is unlikely. Fish meal (such as herring, anchovy or menhaden meal) usually contains 8–10% of residual fat, which commonly contains from 20 to 35% n-3 HUFA (Bimbo 2000). Hence, if fish meal is included in the diet at a level as low as 300 g kg^{-1} it will provide from 0.5 to 1% of the dry diet n-3 HUFA, and the general EFA requirements of most commonly farmed finfish species will be fulfilled regardless of the dietary lipid source included in the formulation. However, if FO is fully substituted with alternative oils lacking in EFA simultaneously with a complete or significant replacement of the fish meal fraction of the diet, the risk of a net deficiency in EFA is possible, and an appropriate source of n-3 HUFA needs to be included in the diet formulation.

Table 5 Essential fatty acid (EFA) requirements of finfish species, sorted according to trophic level and environment†

Common name	Scientific name	Trophic level	Environment	Temperature	EFA requirements (% dry diet)			
					18:2n-6	18:3n-3	n-3 HUFA	n-6 HUFA
Grass carp	*Ctenopharyngodon idella*	2.0	Freshwater	Temperate	1.0	0.5		
Common carp	*Cyprinus carpio*	3.0	Freshwater	Subtropical	1.0	0.5–1.0		
Channel catfish	*Ictalurus punctatus*	3.1	Freshwater	Subtropical	0.5	1.0–2.0	0.5–0.75	
Tilapia	*Tilapia zilli*	2.2	Freshwater, brackish	Tropical	1.0			1.0
Nile Tilapia	*Oreochromis niloticus*	2.2	Freshwater, brackish	Tropical	0.5			
Milkfish	*Chanos chanos*	2.0	Freshwater, brackish, marine	Tropical	1.0	0.5	1.0	
Chum salmon	*Oncorhynchus keta*	3.5	Freshwater, brackish, marine	Temperate	1.0	1.0		
Whitefish	*Coregonus lavaretus*	3.5	Freshwater, brackish, marine	Temperate			0.5–1.0	
Cherry salmon	*Oncorhynchus masou masou*	3.6	Freshwater, brackish, marine	Temperate		1.0	1.0	
Japanese eel	*Anguilla japonica*	3.7	Freshwater, brackish, marine	Subtropical	0.5	0.5		
European sea bass	*Dicentrarchus labrax*	3.8	Freshwater, brackish, marine	Subtropical			1.0	
Coho salmon	*Oncorhynchus kisutch*	4.2	Freshwater, brackish, marine	Temperate	1.0	1.0		
Arctic charr	*Salvelinus alpinus*	4.3	Freshwater, brackish, marine	Temperate		1.0–2.0		
Barramundi	*Lates calcarifer*	4.3	Freshwater, brackish, marine	Tropical			1.0	
Rainbow trout	*Oncorhynchus mykiss*	4.4	Freshwater, brackish, marine	Temperate	0.8–1.6	0.7–1.0	0.2–1.0	
Atlantic salmon	*Salmo salar*	4.4	Freshwater, brackish, marine	Temperate		0.5–1.0	0.5–1.0	
Turbot	*Psetta maxima*	3.1	Brackish, marine	Temperate			0.6–1.3	0.3
Gilthead sea bream	*Sparus aurata*	3.5	Brackish, marine	Subtropical			0.5–1.9	
Striped jack	*Pseudocaranx dentex*	3.9	Brackish, marine	Tropical			1.7	
Red drum	*Sciaenops ocellatus*	4.3	Brackish, marine	Subtropical			0.5–1.0	
Yellowtail flounder	*Pleuronectes ferrugineus*	3.4	Marine	Temperate			2.5	
Red sea bream	*Pagrus major*	3.7	Marine	Subtropical			0.5–1.0	
Korean rockfish	*Sebastes schlegeli*	3.7	Marine	Temperate			1.0	

†Compiled from Sargent *et al.* (2002), Higgs and Dong (2000); Ruyter *et al.* (2000a) and Froese and Pauly (2007).
n-3 HUFA, highly unsaturated fatty acids with 20 or more atoms of carbon and three or more double bonds and the first double bond at the 3rd carbon atom; n-6 HUFA, highly unsaturated fatty acids with 20 or more atoms of carbon and three or more double bonds and the first double bond at the 6th carbon atom.

Of far greater importance than the quantification of the minimal EFA content of the diet, the n-3 to n-6 ratio and the EPA to DHA to AA ratio requires careful consideration (Sargent *et al.* 1999) when FO is replaced in aquafeeds, particularly with VO rich in n-6 PUFA. Modification of the dietary n-3 to n-6 ratio will directly modify the EPA to AA ratio in fish tissues. EPA and AA are the precursors of two distinct series of highly bio-active compounds called eicosanoids. These molecules are hormone-like compounds produced by cells to act in their immediate vicinity and are involved in a variety of physiological activities, including immune and inflammatory responses, haematological and cardiovascular activity, reproduction, and renal and neural function (Tocher 2003). However, AA-derived eicosanoids have a higher activity compared with EPA-derived eicosanoids; as such, modification of the cell EPA to AA ratio will be responsible for modified physiological responses resulting from diverse activities of the two different eicosanoid series (Bell *et al.* 1997; Sargent *et al.* 1999; Tocher 2003).

2.2 Feed palatability

To assure optimal growth and feed efficiency, aquafeeds must be agreeable in flavour and acceptable in taste to the farmed fish. This guarantees the maximization of feed intake and the minimization of feed waste. Although it might be difficult to determine flavour perception and preference in fish (Lamb 2001), it is possible to quantify differences in the amounts of feed eaten (Jobling *et al.* 2001) and hence estimate feed palatability.

Dietary energy is one of the main factors that directly affects feed intake in fish (de la Higuerra 2001). In the context of FO replacement, if FO is replaced with an equal amount of an alternative lipid source, minimal differences in the total dietary energy content are expected. However, given that different oils can have different digestibilities, it is possible that the total digestible energy of feed can be partially modified, resulting in variations in feed intake. Ganga *et al.* (2005) reported a decreased leptin concentration, although not significant, in the plasma of gilthead sea bream fed VO compared with fish on a FO-based diet. Leptin is a multifunctional hormone that plays several roles in fish metabolism, but its main function pertains to the regulation of energy balance by increasing energy expenditure and decreasing food intake (Paolucci *et al.* 2001).

From a fish sensorial viewpoint, it is known that fish are primarily attracted by a variety of nitrogen-containing compounds composed of free amino acids, nucleotides and nucleosides and quaternary ammonium bases and to a minor extent by other non-nitrogenous compounds, such as glucose, lactic acid and some alcohols (de la Higuerra 2001; Kasumayan & Døving 2003). In general,

fish are attracted to compounds that are non-volatile with a low molecular weight, nitrogen containing, amphoteric and water soluble. Therefore, lipids play a very minor role in determining the palatability of aquafeeds. Nevertheless, because the raw materials used for feed production are usually not purified, it is possible that different lipid sources can contain variable amounts of water-soluble and nitrogen-containing compounds that could impact, positively or negatively, on feed palatability. This topic has received very little attention and further research is required. However, in most reported studies on FO replacement with alternative lipid sources, fish feed intake was not significantly affected, suggesting that the lipid fraction of the diet has no or little effect on feed palatability. Recently, Geurden *et al.* (2005) demonstrated for the first time that rainbow trout can discriminate between feeds with different oil sources. In particular, fish were capable of distinguishing a diet containing FO from a diet containing a plant oil, such as LO, SFO or C/RO. The authors, via the implementation of a preference test, demonstrated that rainbow trout have a general preference for the FO-based diet, whereas among the three VO tested, C/RO was the most accepted, followed by SFO, and LO was the least preferred. Although the authors speculated that nutritional factors could be responsible for this feeding behaviour, they were unable to evaluate a direct modification of the pellet palatability in terms of orosensory recognition of lipids in fish and, moreover, emphasized that dietary preferences do not necessarily reflect diet acceptance in the absence of choice (Geurden *et al.* 2005). More recently, Geurden *et al.* (2007) in a 12 week self-feeding experiment on rainbow trout reported a significant reduction in the feed demands for the LO-based diet compared with the FO, C/RO or a blend of olive and fish oil based diets. The authors concluded that the appetitive behaviour of trout is mediated by the dietary oil, and this has a practical relevance for adapting feed reward strategies (Geurden *et al.* 2007).

2.3 Feed digestibility

The principal effects of FO replacement on the overall aquafeed pellet characteristics and quality are relative to the potential modification of lipid and nutrient digestibility. It is known that lipids are generally well digested by fish (Sigurgisladottir *et al.* 1992; Olsen & Ringø 1997), with digestibility values for a variety of different oils and fats commonly over 90% (Hertrampf & Piedad-Pascual 2000). However, it is also known that individual fatty acids are absorbed at different rates.

Fish possess a non-specific lipase that is able to hydrolyse the ester bonds of fatty acids at all positions on the glycerol backbone (Patton *et al.* 1975). In fish, fatty acid

absorption in the intestine decreases with fatty acid chain length, whereas it increases relative to the degree of unsaturation (Austreng *et al.* 1980; Schwarz *et al.* 1988; Ringø 1991; Olsen & Ringø 1997; Olsen *et al.* 1998; Røsjø *et al.* 2000; Morais *et al.* 2005; Francis *et al.* 2007b). In addition, the position of the first double bond along the carbon chain of the fatty acid seems to be important in determining the fatty acid uptake. Commonly, n-3 fatty acids are absorbed at a higher rate than n-6 fatty acids and n-9 fatty acids (Francis *et al.* 2007b).

The fatty acid composition of a given lipid source, and specifically the degree of unsaturation and the chain length, is responsible for determining the lipid melting point. In turn, the melting point of dietary lipids affects the diffusion rate across the unstirred water layer (Gurr & Harwood 1991; Olsen & Ringø 1997; Morais *et al.* 2005). Consequently, the melting point of a potential alternative lipid source can be considered to be a good indicator of its potential digestibility in fish; the higher the melting point, the lower the digestibility. Thus, the relatively high melting point of PO and other oils and fats containing SFA in abundance, and the subsequent reduced fatty acid digestibility and energy availability, has been reported to be a concern over its commercial use in aquafeed, particularly in winter diets for salmonids (Torstensen *et al.* 2000; Ng *et al.* 2003, 2007a).

The combination of chain length, degree of unsaturation and the melting point of individual fatty acids is responsible for the specific preferential order of fatty acid absorption in fish. The apparent fatty acid digestibility is, indeed, higher for HUFA, followed by C_{18} PUFA, MUFA and SFA, which are the less easily digested fatty acids. Moreover, under the same degree of unsaturation, short-chain fatty acids are more easily digested than longer-chain fatty acids, as reported extensively for several species (Cravedi *et al.* 1987; Sigurgisladottir *et al.* 1992; Olsen *et al.* 1998; Johnsen *et al.* 2000; Francis *et al.* 2007b). Accordingly, Jutfelt *et al.* (2007) reported that the uptake of 18:2 n-6 in Atlantic salmon fed either a FO-based or SFO-based diet is approximately fourfold faster than the uptake of 16:0. The individual fatty acids that are most easily digested and absorbed are EPA and DHA, whereas the main C_{18} fatty acids (18:0, OA, LA and ALA) are digested less efficiently, with the lowest value recorded for 18:0, followed by OA, LA and ALA. This preferential order of absorption has been reported identically in different cold water and warm water finfish species, including various salmonids (*S. salar*, *Salvelinus alpinus*, Linnaeus, 1758 and *O. mykiss*) (Olsen & Ringø 1997; Caballero *et al.* 2002; Ng *et al.* 2003), the shortfin eel (*Anguilla australis*, Richardson, 1841) (Gunasekera *et al.* 2002) and in the Murray cod (Francis *et al.* 2007b). This might indicate higher affinities of fatty acid binding

proteins in brush-border membranes to unsaturated fatty acids or faster diffusion across brush-border membranes by unsaturated fatty acids (Jutfelt *et al.* 2007).

The modification of dietary components can also affect the digestibility of other nutrients in different fish species (De Silva & Anderson 1995). Therefore, the substitution of FO with alternative lipid sources can also be responsible for a slight, but significant, modification, not only in lipid digestibility, but also in protein, dry matter and other micronutrient digestibility. The lipid and fatty acid composition of the intestinal cells can be significantly modified by the dietary lipid and fatty acid composition (Caballero *et al.* 2003; Oxley *et al.* 2005a; Ruyter *et al.* 2006). The resulting histological and morphological modifications of the gut tissues and intestinal epithelia can be responsible for altered digestion and absorption capabilities (Caballero *et al.* 2002, 2003; Martins *et al.* 2006). In Atlantic salmon fed SFO-based diets during out-of-season parr–smolt transformation, the decreased ratio of EPA to AA in membrane polar lipids compared with FO fed fish and the resulting alteration in membrane fluidity were responsible for the disturbance of the epithelial barrier function, which affected the function of membrane-bound enzymes (Jutfelt *et al.* 2007). This diet was also responsible for modified translocation of pathogenic bacteria, with fish fed the SFO-based diet showing a higher barrier function compared with fish fed the FO-based diet. Moreover, the uptake rates of two selected amino acids (leucine and proline) were significantly increased in fish fed the SFO-based diet, suggesting a positive effect of the SFO in maintaining a high uptake rate of amino acids throughout the parr–smolt transformation (Jutfelt *et al.* 2007).

Francis *et al.* (2007b) reported that the substitution of FO with a mixed blend VO in semi-purified diets for Murray cod resulted in significantly reduced apparent lipid and protein digestibility. The authors consequently speculated that the growth retardation recorded in fish fed with the alternative lipid source could be attributable to the modification of feed digestibility. In the study, diets were formulated to be iso-proteic (500 mg g^{-1}) and iso-lipidic (170 mg g^{-1}). However, given the reduction in the apparent lipid digestibility (from 95.0% in the FO-based diet to 90.8% in the VO-based diet) and the simultaneous reduction of the apparent protein digestibility (from 96.6 to 94.3%), the fish fed the VO-based diet were actually receiving 11.5 and 7.1 mg g^{-1} of protein and lipid, respectively, less than the control fish fed with the FO-based diet (Francis *et al.* 2007b).

2.4 Feed storage properties

Aquafeeds with a high FO content are prone to oxidation and subsequent rancidity, which affects feed quality and

palatability (Bureau *et al.* 2002; Watanabe 2002). In tropical countries, aquafeed requires cold storage during transportation and to prolong quality on the farm (e.g. barramundi feeds from Australia imported into Asia) (Ng 2007). Consequently, there is the need for the addition of more anti-oxidants in FO-based aquafeeds (Autin 1997; Watanabe 2002). Some alternative lipid sources (i.e. PO, CPO and C/RO) are richer in SFA and MUFA and are, therefore, less prone to oxidation and require minor addition of anti-oxidants. Moreover, many VO also contain natural anti-oxidants, such as vitamin E and carotenoids (Ng *et al.* 2007a). In contrast, LO, containing up to 50% ALA, is extremely prone to oxidation processes (Belitz & Grosch 1999). Another important aspect that needs to be considered is that as a result of the differential melting points of different lipid sources, when FO is replaced with oils or fats with a higher melting point, there is less migration of the lipid fraction to the pellet surface (Ng *et al.* 2007a). This ultimately results in decreased loss of lipid and lipid-soluble nutrients and a reduction in oil staining of storage bags and equipment.

3 The effects of fish oil replacement on fish performance and feed efficiency

Since the early 1990s, the number of published studies on FO replacement in aquafeed has grown exponentially, covering a variety of different farmed species. Therefore, it would be near impossible to comprehensively summarise and report all studies. In the following section, an attempt is made to report the most relevant information on the effects of FO substitution on different finfish species, grouped according to trophic order and environmental characteristics, two of the most important parameters that directly affect the dietary requirements and lipid metabolism of finfish.

3.1 Cold water carnivorous species: salmonids

Several studies have investigated the effects of replacing dietary FO with different VO, such as LO (Bell *et al.* 1991a,b, 1992, 1993; Thompson *et al.* 1996; Tocher *et al.* 1997; Rosenlund *et al.* 2001; Rollin *et al.* 2003), SFO (Bell *et al.* 1992, 1993; Thompson *et al.* 1996; Tocher *et al.* 1997; Rollin *et al.* 2003), SBO (Lie *et al.* 1993; Waagbø *et al.* 1993a,b; Rosenlund *et al.* 2001), PO (Torstensen *et al.* 2000; Rosenlund *et al.* 2001; Bell *et al.* 2002), medium-chain triglyceride oil (Røsjø *et al.* 2000), C/RO (Bell *et al.* 1997, 2001, 2003a,b; Tocher *et al.* 2000; Rosenlund *et al.* 2001), poultry fat (Rosenlund *et al.* 2001) and olive oil (Tocher *et al.* 1997; Rollin *et al.* 2003), in different life stages of Atlantic salmon. There were no significant negative effects on growth reported in any of these studies,

even at complete replacement with a VO mix from start feeding until slaughter size (Torstensen *et al.* 2005). Importantly, in each of the aforementioned experiments, partial replacement of FO was used or the inclusion of dietary fish meal provided with EPA and DHA fatty acids at a minimum of 1.6% of the total dietary fatty acids.

As previously reported, the digestibility of fatty acids is affected by the dietary fatty acid composition. The quantity of dietary SFA influences the digestibility of lipids, particularly at low water temperatures (Olsen *et al.* 1999; Torstensen *et al.* 2000; Caballero *et al.* 2002; Ng *et al.* 2004a). Consequently, the replacement of FO with C/RO results in less SFA and an increase in MUFA, increasing the lipid digestibility at low water temperatures in both rainbow trout and Atlantic salmon (Caballero *et al.* 2002; Karalazos *et al.* 2007). Thus, changes observed in total liver lipids, particularly at low water temperatures, might be related to increased lipid uptake from the diet. In addition, increased protein utilisation has been reported at low temperatures when replacing dietary FO with VO (Bendiksen *et al.* 2003; Torstensen *et al.* 2005). However, whether the increased protein utilisation resulted from increased fatty acid digestibility or from increased fatty acid β-oxidation capacity at low water temperatures remains to be elucidated. Decreasing dietary protein levels at the expense of dietary lipids have also been reported to increase protein utilisation in Atlantic salmon at low water temperatures (Karalazos *et al.* 2007). In summary, the impact of dietary lipid sources and protein levels appears to be highly influenced by water temperature, both for Atlantic salmon and rainbow trout.

Depending on the species, fish are able to accumulate fat in different tissues, for example, the liver for Atlantic cod and the abdomen and flesh for Atlantic salmon and rainbow trout. Fat stores might supply energy in periods of low feed availability and, more specifically, in periods of particularly high energy demand, such as reproduction and smoltification. As most fish species have a limited capacity to use carbohydrates, a common way of reducing feed costs is to replace as much protein as possible with fat. Increased energy levels improve growth and feed utilization in most fish species. However, increased dietary energy levels also increase fat deposition in a fish's fat storage organs and thereby reduce harvest yields (Regost *et al.* 2004). Therefore, by preventing excessive fat deposition in cultivated fish, the overall feed efficiency will be increased while simultaneously reducing the costs of fish production.

Reports on the results of FO replacement with VO sources on the effects of hepatic lipid storage are contradictory. The replacement of FO with oleic acid enriched SFO and C/RO resulted in slight increases in hepatic total lipid storage (Torstensen *et al.* 2000; Bell *et al.* 2001).

However, no effects on hepatic lipid storage were found when dietary FO was replaced with PO (Torstensen *et al.* 2000; Bell *et al.* 2002) or a 1:1 C/RO:LO mix (Tocher *et al.* 2001). Furthermore, Ruyter *et al.* (2006) reported increased liver lipid when replacing FO with 100% SBO at 5°C, but not at 12°C. A long-term feeding experiment reported increased hepatic lipid stores particularly at low water temperatures in Atlantic salmon fed a VO blend (Jordal *et al.* 2007). Differences in the reported effects of dietary fatty acids on the whole body and/or liver lipid stores might also result from differences in fish size and in the duration of the feeding experiments.

In brown trout (*Salmo trutta*, Linnaeus, 1758), replacing FO with corn oil (CNO) had pronounced effects on the fatty acid profile of muscle and liver lipids, but no effect on fish growth (Arzel *et al.* 1994). Similarly, the partial substitution of FO with different VO or animal fats had no significant effects on brown trout performance (Turchini *et al.* 2003b). However, trout fed the alternative sources recorded increased fat deposition in the carcass and fillets compared with the control fish fed the FO-based diet (Turchini *et al.* 2003b). In two populations of Arctic charr (*S. alpinus*), no effect of replacing FO with echium oil was reported on growth, feed efficiency or muscle and liver lipid content (Tocher *et al.* 2006a). In addition, replacing FO with varying mixes of linseed and coconut oil in the diets of Arctic charr did not affect growth performance or negatively affect the oxidative status of the flesh or plasma (Olsen & Henderson 1997).

3.2 Other cold water carnivorous species

Atlantic cod (*G. morhua*) has been an aquaculture candidate in countries around the North Atlantic Ocean for decades. Research on Atlantic cod and on the replacement of dietary ingredients has mainly concentrated on fish meal replacement given the high protein content of Atlantic cod diets. However, some reports show that FO replacement with VO, such as echium oil, had no significant negative effect on the growth of juveniles and imparted beneficial effects on some immune parameters, including eicosanoid production, probably because of decreased levels of 20:4n-6 (Bell *et al.* 2006). In addition, in a short-term feeding trial implemented on 2 kg Atlantic cod over a 2 month period, in which FO was replaced with SBO, no negative effects on growth were recorded. However, this was enough time to significantly affect the fatty acid profile of the fish tissues (Mørkøre *et al.* 2007). In an earlier study, Atlantic cod fed cod liver oil, Greenland halibut oil or peanut oil, demonstrated that the fatty acid composition of the dietary lipid source had a strong influence on the composition of liver TAG (which constitutes the main fat depot in cod) and affected the compo-

sition of polar lipid fatty acids in liver and muscle (Lie *et al.* 1986).

Few studies are available on FO replacement in Atlantic halibut (*Hippoglossus hippoglossus*, Linnaeus, 1758). However, in a recent study (Haugen *et al.* 2006), halibut (with an initial weight of 1.6 kg) fed over a 1 year period with an FO-based diet or diets in which 50% of the FO was replaced with SBO, reported no effect on growth, muscle fibre growth or quality. However, the fatty acid composition of muscle TAG was highly affected by the dietary fatty acid composition.

Turbot (*Psetta maxima*, Linnaeus, 1758), another cold water marine species, was fed FO, SBO or LO for 13 weeks followed by an 8 week finishing period on a FO-based diet, up to market size. The incorporation of VO into the diet resulted in a slight decrease in growth compared with FO fed turbot (Regost *et al.* 2003a). However, feed and protein efficiency and whole body composition were not affected by the dietary lipid source. The total lipid content was low in the muscle of turbot (below 2%) and the ventral muscle was fatter than the dorsal muscle. Liver and muscle fatty acid composition reflected the dietary lipid source (Regost *et al.* 2003a). The flesh sensory quality of turbot fed the different treatments was significantly affected. However, following the finishing period, no differences in the sensory quality parameters were apparent (Regost *et al.* 2003b).

3.3 Temperate and warm water carnivorous marine species

European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) and gilthead sea bream (*S. aurata*) are the two main Mediterranean marine fish species reared in Europe. Despite the carnivorous nature of each species and the overlap in their environments, their feeding behaviours and dietary requirements are contrasting (Kaushik 2002; Koven 2002). Compared with other temperate and warm water marine carnivorous species, the lipid metabolism of European sea bass, having been the object of several studies, is better understood. European sea bass has extremely low rates of fatty acid bioconversion, although products (labelled fatty acids) of each of the enzymes involved in the elongation and desaturation of C_{18} fatty acids (i.e. elongase, Δ-6 desaturase and Δ-5 desaturase) have been recorded, clearly indicting the presence of such enzymes (Mourente *et al.* 2005a). Thus, this species possesses a typical 'marine' fish pattern in the metabolism of ALA to EPA and DHA, and relatively high dietary levels of n-3 HUFA (i.e. fish oil) are required.

Richard *et al.* (2006a) reported that replacing up to 60% of dietary FO with two blended VO had no significant effect on growth performance in European sea bass.

However, the final average weight of fish fed the FO-based diet was 176 g, whereas the final average weights of the fish fed the two blended VO diet were only 160 g and 143 g, equating to 9 and 19% less, respectively (Richard et al. 2006a). No significant effects on growth and feed efficiency were recorded in European sea bass fed either FO or SBO, C/RO, LO or a blended VO for 98 days (from ∼9 to ∼30 g) (Izquierdo et al. 2003). Similarly, no significant detrimental effects of complete or partial FO replacement in European sea bass diets have been reported in other short-term feeding experiments (Figueiredo-Silva et al. 2005; Martins et al. 2006). However, Mourente and Bell (2006), reported reduced growth in European sea bass fed a blend of VO compared with fish fed a FO-based diet. Accordingly, in an 8 month feeding experiment, from juvenile to commercial size fish, Montero et al. (2005) reported that European sea bass fed a diet in which the FO was replaced by up to 60% with C/RO were significantly smaller than fish fed 100% FO, 60% SBO or 60 or 80% LO. Alexis (1997) reported that the use of VO as the only source of dietary lipid significantly reduced fish performance in European sea bass and gilthead sea bream.

No significant effects on growth and feed utilisation were recorded in gilthead sea bream fed either FO or SBO, C/RO, LO or a blended VO (Izquierdo et al. 2003). Similarly, Wassef et al. (2007) reported no detrimental effects of partial replacement of FO with blended VO. Conversely, in a further study, juvenile gilthead sea bream fed diets containing 80% of the dietary lipid source as SBO or LO exhibited significant growth reduction and reduced feed efficiency. However, no differences were recorded in fish fed FO or diets where 60% of the FO was replaced by SBO, LO or C/RO (Menoyo et al. 2004; Izquierdo et al. 2005). Total substitution of FO by VO (SBO, LO or a blend of the two) produced a significant reduction in fish growth, with the lowest average fish body weight recorded in fish fed the SBO-based diet, whereas the lowest specific growth rate (SGR) was recorded for fish fed the LO-based diet. Feed efficiency was also significantly affected with food conversion ratio (FCR) values of 1.35, 1.57, 1.70 and 1.80 for fish fed the FO, LO, SBO and SBO/LO blended oils, respectively (Montero et al. 2008). In a study focussing on the combined replacement of fish meal and oil in gilthead sea bream, in which the diets contained 15% of fish meal and graded levels of a blended VO, significant growth and feed intake reductions were recorded when FO was completely replaced. However, no significant effects were recorded up to a substitution of 66% of the dietary FO (Benedito-Palos et al. 2007). In a study by Kalogeropoulos et al. (1992), in which the dietary FO was substituted by a graded inclusion of SBO, a significant effect on the

growth and feed efficiency of gilthead sea bream was reported. Following a 5 month feeding trial, juvenile gilthead sea bream (1.2 g) reached a weight of 12.27 g when fed a FO-based diet and only 9.24 g when the dietary lipid source was replaced (83%) by SBO. Moreover, by plotting the weight gain of fish against the dietary FO/SBO ratio, the authors reported that the maximal substitution level of FO with SBO was 47% (Kalogeropoulos et al. 1992).

In sharpsnout sea bream (Diplodus puntazzo, Cetti, 1777), a sparid fish similar to gilthead sea bream, but with more omnivorous feeding habits, no significant effects of FO replacement with either SBO or LO have been recorded. However, a significantly higher mortality rate (up to 66% vs 13% in other treatments) was recorded in fish fed the LO-based diet (Piedecausa et al. 2007). However, in the same species, Almaida-Pagán et al. (2007) reported that SBO and LO substitution did not negatively affect fish survival. In red sea bream (Pagrus major, Temminck & Schlegel, 1843) fish weight gain, SGR, feed intake, feed efficiency, protein and gross energy utilization and percentage survival were not affected by the substitution of 25, 48 or 70% of the dietary FO with C/RO (Huang et al. 2007). However, in red sea bream (Australian snapper, Pagrus auratus, Forster, 1801) complete substitution of FO with crude C/RO was responsible for a significant negative effect on growth and increased FCR (Glencross et al. 2003a). However, no significant differences were recorded in fish fed refined C/RO or SBO. When fish were subsequently shifted to a FO-based diet for the finishing period, the red sea bream previously fed a SBO-based diet grew significantly faster than control fish constantly fed FO (Glencross et al. 2003b). In red drum (Sciaenops ocellatus, Linnaeus, 1766), a subtropical marine perciformes of growing aquacultural interest, the replacement of FO with coconut oil or tricaprylin was responsible for significantly reduced growth, and the poorest results were obtained after the inclusion of tricaprylin (a source of medium-chain triglycerides) (Craig & Gatlin 1995). However, the inclusion of beef TAL resulted in improved growth performance even compared with FO-fed fish (Craig & Gatlin 1995). In the same species, the inclusion of SBO resulted in decreased growth performance and feed efficiency (Tucker et al. 1997). In Japanese sea bass (Lateolabrax japonicus, Cuvier, 1828), the partial substitution of FO with various alternatives resulted in no significant differences in growth patterns (Xue et al. 2006), whereas in grouper (Epinephelus coioides, Hamilton, 1822) SBO, and not CNO, SFO or peanut oil, was reported to have a negative effect on growth performance (Lin et al. 2007). However, Lin and Shiau (2007) reported significantly lower growth rates, feed efficiencies and survival rates in a different grouper species

(*Epinephelus malabaricus*, Bloch & Schneider, 1801) when dietary FO was replaced by CNO. Shapawi *et al.* (2008) found no significant effect on the growth and feed efficiency of the humpback grouper (*Cromileptes altivelis*, Valencìennes, 1828) when fish were fed fish meal based diets supplemented with either FO, SBO, PO or C/RO.

In view of the contrasting results, it is evident that, in marine carnivorous species, the effect of FO replacement is still not fully understood and has not been fully quantified. Unfortunately, feeding trials have been, to date, commonly implemented only for limited time periods and an ANOVA (with $n = 3$) is the only statistical test that has been used to discriminate among treatments. In consideration of the very large variability recorded in almost all studies, it will be useful in future experiments to have the number of replicates increased to increase the power of the statistical test. However, in light of the reported data, it seems possible to conclude that in marine carnivorous species, substitution of FO is more challenging than in other species that are better equipped to utilise dietary lipids more efficiently.

3.4 Temperate and warm water carnivorous freshwater species

In comparison to cold water species, particularly salmonids and marine carnivorous species that have received a great deal of attention from a FO substitution viewpoint, there is far less information available on freshwater carnivorous species farmed in temperate and warm water environments. Considered individually, these aquaculture industries appear to be responsible for only limited FO exploitation. However, global production of these species is quite significant. More than 1.3 millions tons of catfish, silurids and snakeheads and 2.4 million tons of other strictly freshwater species (over 50 different species, primarily perciformes that are typically farmed in their native continents), excluding cyprinids and tilapias, were produced in 2004 (FAO 2006). As previously reported, in salmonids, FO substitution is not apparently responsible for any detrimental effects on the overall zootechnical performances. However, the situation is quite the opposite for some of the carnivorous freshwater species, which are not characterised by similar optimal capabilities in utilising dietary lipid or the ability to effectively deposit storage fat in the muscle.

No significant differences in weight gain, feed conversion or total lipid content among sunshine bass (female white bass *Morone chrysops*, Rafinesque, 1820 × male striped bass *Morone saxatilis*, Walbaum, 1792) fed diets containing FO or graded levels of C/RO for 20 weeks have been reported (Wonnacott *et al.* 2004). Similarly, in a short-term feeding experiment on juvenile pike perch

(*Sander lucioperca*, Linnaeus, 1758), substitution of FO with LO or SBO did not lead to any growth reduction (Schultz *et al.* 2005; Molnár *et al.* 2006). However, Schultz *et al.* (2005) reported that the FCR was excessively high across all treatments because of the inappropriate feeding distribution. Surprisingly, the hepatosomatic index was significantly lower for fish fed the SBO-based diet, whereas higher lipid contents in the liver of fish fed both VO compared with the control treatment suggested reduced utilization of VO compared with the aquatic lipid sources (Schultz *et al.* 2005). Significantly increased body lipid deposition was reported in largemouth bass (*Micropterus salmoides*, Lacepéde, 1802) fed FO compared with fish fed VO or animal lipid sources and, although not significantly different, a trend towards growth reduction in fish fed the alternative lipid sources was observed (Subhadra *et al.* 2006). Similarly, Lane *et al.* (2006) reported no major detrimental effects of FO replacement with CNO in sunshine bass.

In juvenile Eurasian perch (*Perca fluviatilis*, Linnaeus, 1758) the growth performance was significantly affected in fish fed for 10 weeks on semi-purified diets containing either FO, olive oil, SFO or LO (Xu & Kestemont 2002). The FO-fed fish recorded a weight gain of 136.8%, whereas fish fed the VO-based diets recorded only 32.4, 43.6 and 76.8% for fish fed olive oil, SFO and LO, respectively. Likewise, feed efficiency was significantly compromised (Xu & Kestemont 2002). Using a semi-purified diet, with minimal fish meal inclusion, a series of experiments on the Australian carnivorous Murray cod (*M. peelii peelii*) clearly showed that overall fish performance was significantly affected by FO substitution with different VO, used individually or in a blended combination (Francis *et al.* 2006, 2007a,b,c). An important observation reported by Francis *et al.* (2007c) was that when the results of the same feeding trial were assessed using an ANOVA or by implementing a regression (relative to the degree of FO substitution) the achieved conclusions were clearly contrasting. This suggests that, in FO replacement studies, ANOVA can be responsible for type II errors (which is the error of failing to reject the null hypothesis 'fish oil replacement does not affect fish performances' given that the alternative hypothesis is actually true 'fish oil replacement affects fish performances').

The Brazilian freshwater siluriformes and carnivorous fish speckled surubim (*Pseudoplatystoma corruscans*, Spix & Agassiz, 1829) did not show any detrimental effects on growth among fish fed different VO or terrestrial animal fat diets (Martino *et al.* 2002). However, no control treatment of fish fed a FO-based diet was included in the experimental design. Early works on channel catfish (*Ictalurus punctatus*, Rafinesque, 1818), one of the most cultured finfish in the USA and also abundantly farmed

outside its native range, reported that channel catfish fed diets containing FO or animal fat showed better growth than fish fed diets containing only VO (Stickney & Andrews 1971, 1972; Yingst & Stickney 1979). In addition, it was suggested that the poor response to VO resulted from a limited ability to bioconvert C_{18} fatty acid to longer and more unsaturated homologues (Wilson & Moreau 1996). Subsequently, further experiments on channel catfish clearly indicated that a source of n-3 HUFA is required and that growth performance is reduced when FO is completely substituted by either VO or animal fats (Satoh *et al.* 1989; Wilson & Moreau 1996). In a different experiment, channel catfish fed TAL exhibited significantly reduced feed efficiency compared with fish fed menhaden oil or catfish oil (Li *et al.* 1994). Significantly reduced growth in channel catfish fed individual VO or terrestrial animal fats has also been reported (Fracalossi & Lovell 1994). However, no significantly different growth pattern was recorded for fish fed a blend of these oils compared with the FO-based diet (Fracalossi & Lovell 1994).

Several species of *Clarias* catfish, which are opportunistic carnivores, are commercially cultured in Africa and Asia of which *Clarias batrachus*, Linnaeus, 1758, *Clarias macrocephalus*, Günter, 1864, *Clarias gariepinus*, Burchell, 1822 and their various hybrids are the most important. The introduction of the African species *C. gariepinus* for aquaculture in many parts of Asia and its successful hybridization with Asian species, such as *C. macrocephalus*, has led to increased interest in their commercial culture. In feeding trials using various combinations of dietary lipids, fingerlings of *C. batrachus* fed a 1:1 combination of FO (cod liver oil; high in n-3 fatty acids) and SFO (high in n-6 fatty acids) grew better than fish fed FO or SFO as the sole dietary lipid source (Mukhopadhyay & Mishra 1998). *C. gariepinus* appears to have very low n-3 fatty acid requirements and has generally shown lower growth performance when fed a FO-based diet compared with a SFO-based diet (Hoffman & Prinsloo 1995; Ng *et al.* 2003). Various PO-based diets have also been used successfully in African catfish (Legendre *et al.* 1995; Lim *et al.* 2001; Ng *et al.* 2003, 2004b). The availability, lower cost, low PUFA content and high vitamin E concentration of PO makes it the VO of choice for the formulation of clariid catfish feeds in many parts of Africa and Asia where PO is planted.

3.5 Temperate and warm water carnivorous diadromous species

Eels (*Anguilla* spp.) are carnivorous catadromous fish of important aquacultural interest with a global production in 2004 of almost 250 000 tons (FAO 2006). However, there is limited information available on the potential of FO substitution in their diet. European eel (*Anguilla anguilla*, Linnaeus, 1758) elvers have been reported to grow well when fed diets containing FO or CNO (Kissil *et al.* 1987). However, in larger fish, close to commercial size, the substitution of FO with either SBO, TAL or a combination of the two resulted in significant growth reduction (Luzzana *et al.* 2003). In particular, dietary substitution affected the feed efficiency with recorded values for the FCR ranging from 1.80 for eels fed the FO diet up to 4.55 for eels fed the SBO and TAL blend (Luzzana *et al.* 2003). Gunasekera *et al.* (2002) reported decreased lipid digestibility in shortfin eel (*A. australis*) when fed different VO.

Similarly, little information is available on anadromous sturgeons, despite increased farming of these species. Linseed oil has been reported to inhibit growth and food conversion in Adriatic sturgeon (*Acipenser naccarii*, Bonaparte, 1836) (Agradi *et al.* 1993), while Xu *et al.* (1993) reported no changes in growth performance of white sturgeon (*Acipenser transmontanus*, Richardson, 1836) fed diets containing different vegetable and terrestrial animal lipid sources. In Russian sturgeon (*Acipenser gueldenstaedtii*, Brandt & Ratzeburg, 1833), SBO has been reported to be an unsuitable FO substitute because of significantly reduced growth performance (Sener *et al.* 2005). Recently, Caprino *et al.* (2008) reported that, in white sturgeon, squid oil was responsible for increased caviar production compared with a diet in which the lipid source was a blend of SBO and FO.

In barramundi/Asian sea bass (*Lates calcarifer*, Bloch, 1790), a diadromous species commonly farmed in freshwater recirculating aquaculture systems in Australia or in brackish water cage farms in many south-eastern Asian countries, the complete replacement of FO with SBO, C/RO or LO resulted in significantly reduced growth. However, partial substitution did not result in any detrimental effects (Raso & Anderson 2003).

3.6 Omnivorous and herbivorous fish

Freshwater herbivorous fish, such as carp and tilapia, represent the largest group of cultured finfish constituting approximately 75% of total global finfish aquaculture production. Despite the fact that commercial pelleted feeds for these species generally contain less than 5% dietary lipid, the sheer quantity of their production makes them a significant user of marine fish oils. Tacon *et al.* (2006) estimated that carp and tilapia consumed approximately 90 900 and 18 300 tons of FO, respectively, in 2005 assuming an average of only 1% FO in their manufactured feeds.

An understanding of the lipid and fatty acid requirements of carp and tilapia is required prior to addressing

the issue of FO replacement with alternative oils and fats in commercial feeds. Carp and tilapia are more inclined to require greater amounts of n-6 fatty acids than n-3 fatty acids for maximal growth (National Research Council 1993). High levels of n-3 PUFA have been reported to depress the growth of tilapia (Kanazawa *et al.* 1980; Huang *et al.* 1998; Ng *et al.* 2001) and carp (Du *et al.* 2008). Other researchers have found that no enhancement in growth was obtained when ALA or n-3 HUFA were supplemented into tilapia diets (Takeuchi *et al.* 1983). Growth of *Oreochromis aureus*, Steindachner, 1864 was depressed when individuals were fed more than 1% dietary ALA (Stickney & McGeachin 1983a). The fatty acid requirement for tilapia and carp was reported to range from 0.5 to 1% for both n-6 and n-3 PUFA (Takeuchi 1997). The relatively low requirement of these herbivorous fish for n-3 fatty acids coupled with the low dietary lipid requirement (Chou & Shiau 1996; Du *et al.* 2005) are positive indications for the successful replacement of FO with alternative lipid sources in the diets of carp and tilapia. Furthermore, in contrast to marine fish, these freshwater fish have been reported to be able to bio-convert larger quantities of ALA to EPA and DHA (Olsen *et al.* 1990; Cao *et al.* 1997).

Using semi-purified casein-based diets, Huang *et al.* (1998) reported that hybrid tilapia fingerlings (*Oreochromis niloticus*, Linnaeus, 1758 × *O. aureus*) fed 8% SBO or FO showed no difference in growth and feed utilization efficiency after 10 weeks on the diets. Oxidative stress (measured using the TBARS test) in fish fed SBO or FO was similar to, but significantly higher than, fish fed diets containing lard. Gaber (1996) reported good growth of Nile tilapia fed SBO-based diets. No significant differences were detected in the growth of *O. aureus* fed diets supplemented with 10% menhaden oil, catfish oil or SBO, but growth was depressed in fish fed diets supplemented with TAL (Stickney & McGeachin 1983b). Nile tilapia fed semi-purified diets supplemented with 7% TAL also showed depressed growth performance compared with fish fed with added FO, CNO or LO, which did not exhibit growth differences (Yildirim-Aksoy *et al.* 2007). Unlike TAL, poultry fat did not significantly depress the growth rate of tilapia (Viola & Arieli 1983), but the fish used in this study were larger (100–300 g). Hybrid tilapia fed semi-purified diets supplemented with VO, such as SFO, CPO, crude palm kernel oil or palm fatty acid distillates, showed comparable or slightly higher growth compared with fish fed a FO-based diet (Ng *et al.* 2001). Red hybrid tilapia fed fish-meal-based diets with various PO products as the only added oil from stocking to marketable size grew equally well compared with fish fed a FO-added diet (Bahurmiz & Ng 2007). Nile tilapia fed diets supplemented with palm-oil-laden spent bleaching clay

also grew equally well compared with fish fed the control diet containing added FO (Ng *et al.* 2006).

Common carp (*Cyprinus carpio*, Linnaeus, 1758) larvae have been reported to be able to grow and survive with very low levels of n-3 fatty acids in their diet (Radunz-Neto *et al.* 1994), with survival rates of carp larvae fed diets with peanut oil as the sole dietary lipid source not significantly different from larvae fed increasing levels of cod liver oil. At the fry stages, Mukhopadhyay and Rout (1996) reported that the Indian carp *Catla catla*, Hamilton, 1822 grew slightly better when fed semi-purified diets supplemented with SFO (high in n-6 PUFA) compared with fish fed FO. The best growth rates were observed in fry fed equal proportions of SFO and FO. Common carp fingerlings fed diets supplemented with either 10% CNO, SFO, FO or C/RO did not show any significant differences in growth and feed conversion efficiency (Steffens *et al.* 1995). A similar observation has been reported for common carp fed either 12% dietary LO or FO (Schwarz *et al.* 1988). Grass carp fingerlings fed lard or a VO blend (CNO/LO) showed better feed intake and growth performance compared with fish fed diets supplemented with HUFA-enriched FO (Du *et al.* 2008). No information is available on the characteristics and production technique of the HUFA-enriched FO used in the reported study; however, its n-3 HUFA fatty acid composition was reported to be ~15% EPA and ~30% DHA. Increasing dietary inclusion of HUFA-enriched FO from 2 to 10% significantly reduced the percentage weight gain of grass carps from 200 to 100% at the end of the feeding trial. Grass carp fingerlings fed diets with 6% HUFA-enriched FO also showed impaired mitochondrial and peroxisomal fatty acid oxidation capacity as well as hepatic pathology.

Very little information is currently available on the effects of FO replacement in the diets of other herbivorous fish species. Alava (1998) reported no significant effects of dietary lipid source (coconut oil, cod liver oil or their 1:1 combination) on the growth and survival of milkfish fry (*Chanos chanos*, Forsskål, 1775). Striped mullet (*Mugil cephalus*, Linnaeus, 1758) showed higher weight gains when fed diets containing FO as a lipid source compared with fish fed diets containing TAL or SBO (Jones & Strawn 1983).

In general, research to date has indicated that as long as the essential fatty acid requirements of herbivorous and omnivorous fish such as carp and tilapia are met, the use of VO and to a more limited extent animal fats do not significantly affect growth performance and feed utilization. However, it should be noted that, with a few exceptions, most feeding trials conducted to date have been relatively short term and the long-term effects of dietary inclusion of VO and animal fats remain to be elucidated, particularly with regard to the effects on the fish immune system.

4 The effects of fish oil replacement on lipid metabolism

4.1 General metabolism

The fatty acid composition of fish is dependent on different factors, other than dietary fatty acid composition. Digestibility (Sigurgisladottir *et al.* 1992; Torstensen *et al.* 2000), transport and uptake, elongation and desaturation processes (Bell *et al.* 2001, 2002) and β-oxidation of fatty acids (Frøyland *et al.* 2000; Torstensen *et al.* 2000) will affect the membrane and deposit lipid composition. In addition, the ratio of neutral to polar lipids affects the fatty acid composition of a given tissue. Studies of β-oxidation in fish suggest the existence of a substrate preference for SFA and MUFA over PUFA (Kiessling & Kiessling 1993; Henderson 1996). A recent report from Stubhaug *et al.* (2007) showed that, when feeding Atlantic salmon a diet with low n-3 HUFA, these fatty acids are selectively stored in the fish. Fish fed a FO-based diet stored approximately 20% EPA and 30% DHA, whereas VO-fed fish stored 70% EPA and 80% DHA during the fast growth period in seawater (Stubhaug *et al.* 2007). These results suggest a switch in the fatty acid substrate used for β-oxidation when dietary levels are low and EPA and DHA are stored in membranes rather than being used as energy substrates in VO-fed fish. Thus, cold water fish use surplus dietary fatty acids for energy production (Bell *et al.* 2003a; Stubhaug *et al.* 2007).

4.2 Lipogenesis

The transport of lipids and other lipid-soluble components from the intestine to peripheral tissues is predominantly mediated by lipoproteins (Babin & Vernier 1989). Lipoproteins are usually classified according to their density into six main classes: Chylomicrons (CM), very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and very high density lipoprotein (VHDL) (= vitellogenin (VTG)). The intestinal cells in the anterior intestine and pylorus caeca absorb dietary free fatty acids, lyso-phospholipids and monoacylglycerols from the micelles and synthesise chylomicrons and VLDL with a high lipid/protein ratio. In fish, it is not yet clearly understood which route the chylomicrons and VLDL take from the enterocytes. Sire *et al.* (1981) referred to the presence of lymphatic vessels, similar to mammalian structures, in the intestine of rainbow trout. However, a subsequent study (Kryvi & Totland 1997) showed that the existence of a fish lymphatic system has yet to be clearly established.

In fish, as in mammals, TAG are removed from the lipoprotein particles through the action of endothelial lipoprotein lipase (LPL), and the lipoprotein remnant is endocytosed by the liver parenchymal cells and the liver takes up the chylomicron remnant. The LDL is formed from VLDL as the VLDL particle is stripped of its TAG and certain apolipoproteins (Gjøen & Berg 1993). In contrast to most mammalian species where VLDL and LDL dominate the blood lipids, HDL is the dominate lipoprotein in salmonids, providing a large reservoir for cholesterol and phosphatidylcholine (Babin & Vernier 1989; Lie *et al.* 1993). When dietary fatty acids are transported to peripheral tissues by lipoproteins, LPL break the ester bonds and unesterified fatty acids can be taken up by the cells. In adipose tissue, LPL activity is promoted by insulin and depressed by catcholamines. Thus, the activity of LPL can be regulated reciprocally between muscle and adipose tissue to favour either energy production or the storage of TAG (Brindley 1985). Lipoprotein lipase is found in fish (Lindberg & Olivecrona 2002; Oku *et al.* 2002), but very little is known about how nutritional factors and hormones affect this protein in fish. In the liver and adipose tissue of red sea bream, it has been shown that LPL expression is increased by starvation, whereas dietary fatty acids can affect LPL expression in different ways (Liang *et al.* 2002). In feeding experiments with Atlantic salmon, a slight increase in hepatic total lipid storage by replacing 100% FO with either OA-enriched SFO, C/RO or SBO has been reported (Torstensen *et al.* 2000; Bell *et al.* 2001). However, no increased hepatic lipid deposition was recorded when fish were fed diets in which the FO was replaced by PO or a 1:1 C/RO:LO mix (Torstensen *et al.* 2000; Tocher *et al.* 2001; Bell *et al.* 2002). These contrasting results might be linked to hepatic LPL activity. Furthermore, a decrease in plasma and LDL cholesterol has been reported in Atlantic salmon (Jordal *et al.* 2007) and rainbow trout fed VO-based diets (Richard *et al.* 2006b), possibly because of the decreased content of dietary cholesterol in the VO-based diets. Furthermore, a reduction in cholesterol might also result from the content of phytosterols in the dietary plant oils used. However, phytosterols were not analysed in any of the aforementioned studies. Furthermore, rainbow trout fed a 100% VO mix exhibited downregulation of hepatic LDL receptor expression (Richard *et al.* 2006b).

The high dietary lipid levels used for Atlantic salmon are believed to result in low hepatic lipogenic enzyme activity, as demonstrated by a negative correlation between dietary lipid and lipogenic enzyme activity (Arnesen *et al.* 1993). Furthermore, PUFA is reported to inhibit lipogenesis in cultured rainbow trout and rat hepatocytes (Zampelas *et al.* 1995; Alvarez *et al.* 2000). The NADPH produced by the lipogenic enzymes can be used for the maintenance of the cellular red-ox state (reviewed by Kletzien *et al.* 1994), in addition to the lipogenic pathways, and PUFA is reported to affect the

4.3 Lipid catabolism

Carnivorous fish rely mainly on proteins and lipids for energy rather than carbohydrates (Van den Thillart 1986). The relative importance of dietary lipids for energy production compared with protein is species specific and in some fish species (i.e. salmonids) it is dependent on dietary lipid level, with a high dietary lipid level having a protein-sparing effect (Hemre & Sandnes 1999).

In general, red muscle, the liver and the heart are regarded as the most important tissues for β-oxidation in fish (reviewed by Henderson & Tocher 1987). Although it has a low β-oxidation capacity per gram of tissue, white muscle has been shown to be the major site of β-oxidation given the size of the tissue (Frøyland *et al.* 2000; Nanton *et al.* 2003; Stubhaug *et al.* 2005a,b). However, a fish's capacity for catabolising fatty acids varies depending on various factors, such as fish size, life stage and season, and smoltification in Atlantic salmon has a major impact on the β-oxidation capacity in the liver and red muscle (Stubhaug *et al.* 2006). The importance of mitochondrial or peroxisomal β-oxidation is dependent on the tissue. Typically in the fish liver, peroxisomal β-oxidation contributes significantly to the total β-oxidation capacity (Crockett & Sidell 1993; Nanton *et al.* 2003; Stubhaug *et al.* 2007), and contributes up to 100% of the total β-oxidation during the parr–smolt transformation (Stubhaug *et al.* 2007). The contribution from peroxisomal β-oxidation is low and is reported to be less than 10% of the total β-oxidation in both white and red muscle in Atlantic salmon. However, peroxisomal β-oxidation increases up to 60% in red muscle during parr–smolt transformation (Stubhaug *et al.* 2007).

Both natural factors, such as high-fat diets and dietary PUFA, and fibrates can induce mitochondrial and peroxisomal β-oxidation in rodents (Willumsen *et al.* 1996; Frøyland *et al.* 1997; Madsen *et al.* 1998, 1999). Increased β-oxidation capacities in rat livers as a response to dietary EPA and DHA might result from the proliferation of mitochondria and peroxisomes leading to increased expression of the enzymes involved in β-oxidation (Willumsen *et al.* 1993, 1996; Totland *et al.* 2000). Replacing dietary FO with VO and thus significantly changing the dietary and tissue fatty acid composition might influence the β-oxidation capacity. Dietary studies with Atlantic salmon have indicated that MUFA (18:1 n-9 and 22:1 n-11) and C_{18} PUFA (18:2 n-6 and 18:3 n-3) are readily oxidised when present at high concentrations (Bell *et al.* 2003a,b; Torstensen *et al.* 2004a; Stubhaug *et al.* 2007). Furthermore, EPA and DHA are also highly β-oxidised in

Atlantic salmon tissues when in dietary surplus during high growth periods (Stubhaug *et al.* 2007). Thus, although a preference for certain fatty acids over others might be present, these differences appear to be minimised when fatty acids are in dietary surplus. By altering dietary fatty acid compositions, different fatty acids will be the dominate energy substrate, with fish fed a VO-based diet retaining (i.e. not β-oxidising) more than 70% of EPA and DHA compared with FO-fed salmon, which retain only 30% of the same fatty acids (Stubhaug *et al.* 2007).

Dietary FO replacement with alternative lipid sources might also affect the β-oxidation capacity in the fish tissues. However, published data to date show only marginal effects on β-oxidation capacity by replacing FO with either C/RO (Stubhaug *et al.* 2005a,b) or a VO mix (Stubhaug *et al.* 2006). However, during the parr–smolt transformation, VO-fed Atlantic salmon had significantly decreased β-oxidation capacity compared with FO-fed fish (Stubhaug *et al.* 2007).

In vitro studies with Atlantic salmon hepatocytes have indicated that n-3 HUFA stimulate total β-oxidation through the increased uptake of fatty acids into the cells rather than stimulating the actual β-oxidation system (Torstensen & Stubhaug 2004). Similar results have been reported in an *in vivo* experiment using brown trout. Turchini *et al.* (2003b) reported significantly lower hepatic carnitine palmitoyltransferase I in fish fed a poultry fat based diet compared with fish fed a FO-based diet. Likewise, significantly lower hepatic carnitine palmitoyltransferase II in fish fed a C/RO-based diet compared with fish fed a FO-based diet has been reported (Turchini *et al.* 2003b), suggesting that n-3 HUFA can also increase fatty acid uptake into the mitochondria.

The unesterified fatty acids can either be transported into cells through a simple diffusion mechanism regulated mainly by lipid physical chemistry (Hamilton 1998) or by protein-mediated transport of fatty acids across the cell membrane. The latter theory proposes the existence of a membrane-bound fatty acid translocase (FAT/cd36) or fatty acid transport protein (FATP), which has been identified in several tissues in rodents and mammals (Van Nieuwenhoven *et al.* 1996; Frohnert & Bernlohr 2000). The FATP cloned from mice are reported to have very-long-chain acyl-CoA synthetase activity, suggesting a fatty acid uptake via esterification coupled influx (Herrmann *et al.* 2001). Studies of FATP and cd36 in fish are scarce. However, one study in rainbow trout showed that fatty acid uptake is carrier mediated in both red and white muscle (Richards *et al.* 2004). It has recently been suggested that it is the metabolic demand for fatty acids that is the driving force for fatty acid uptake through the conversion of fatty acids to acyl-CoA by the action of

acyl-CoA synthetases and regulation of the membrane-bound transport proteins (Mashek & Cloeman 2006). Mitochondrial acyl-CoA synthetase has been studied in Antarctic *Notothenioidei* fish, and it shows substrate specificity for unsaturated fatty acids over 16:0 and 22:6 n-3 (Grove & Sidell 2004). However, further studies have not been conducted on fish acyl-CoA synthetases.

When fatty acids are transported across the cell membrane, cytosolic fatty acid binding protein (FABP) might act as an acceptor for the fatty acid. The FABP are low molecular mass (14–16 kDa) soluble proteins that non-covalently bind long-chain fatty acids and other small organic hydrophobic compounds. They belong to a multi-gene family of intracellular lipid-binding proteins that also includes cellular retinol and retinoic acid binding proteins. Specific FABP have been found in various cell and tissue types, and they are named according to the location in which they were first studied, and more recently according to the gene name (Matarese *et al.* 1989; Hertzel & Bernlohr 2000). It is generally considered that FABP fulfil specialised roles in cells where they are expressed, but definitive proof has not been forthcoming. For example, heart FABP is considered to shuttle fatty acids from the plasma membrane to the mitochondrion for *β*-oxidation, whereas adipocyte FABP is considered to facilitate the efflux of lipolytically derived fatty acids (Londraville & Sidell 1996; Coe & Bernlohr 1998). Other considerations for FABP function include components in intracellular signalling pathways or as regulators of cell division. In rat heart and muscle, a correlation between fatty acid oxidation and cytosolic FABP content has been reported (Veerkamp & van Moerkerk 1993). Furthermore, peroxisome proliferator-activated receptors *α* agonist increased muscle FABP mRNA and *β*-oxidation enzyme activity in rat skeletal muscle (Furuhashi *et al.* 2002). In fish, intracellular FABP concentrations are reported to increase in response to cold acclimation (Londraville & Sidell 1996), which is correlated to increased *β*-oxidation.

In sunfish, the expression of heart FABP was twofold higher compared with the control group following injections of murine leptin (Londraville & Duvall 2002), demonstrating a central role of FABP in lipid metabolism, particularly in fish fed high-lipid diets. In Atlantic salmon, the gene expression levels of FABP3 were approximately 100-fold higher in red muscle compared with white muscle, possibly because red muscle has higher *β*-oxidation capacity and fatty acid metabolism activity (Jordal *et al.* 2006). Furthermore, the protein levels of FABP3 in red and white muscle significantly increased when replacing dietary FO with C/RO (Jordal *et al.* 2006), which again correlated with increased *β*-oxidation capacity in the fish (Stubhaug *et al.* 2005a,b). The FABP3 protein levels in Atlantic salmon muscle were not

correlated to FABP3 gene expression levels in a long-term feeding study (Jordal *et al.* 2006). However, in the liver, no effect on *β*-oxidation capacity (Stubhaug *et al.* 2005a,b), and only minor effects on liver lipid metabolism gene expression patterns related to *β*-oxidation and transport, were found by microarray analysis in salmon fed C/RO compared with FO (Jordal *et al.* 2005).

The binding of free fatty acids to FABP might also play a function in protecting the cytoplasm and cell structures from the detergent effects caused by high concentrations of free fatty acid (Londraville & Sidell 1996). The FABP carry the fatty acids to the site of their esterification and/or oxidation. Another proposed role for FABP is in the regulation of *β*-oxidation of fatty acids. In fact, altered expression of FABP results in a change of FABP levels in the cell, consequently modifying the amount of free fatty acids available for metabolism. Cold acclimation has been shown to increase *β*-oxidation in fish (Jones & Sidell 1982). Furthermore, in cold-acclimated striped bass, the levels of FABP molecules in the aerobic muscle cells significantly increased (Londraville & Sidell 1996), strongly suggesting a connection between the levels of FABP and *β*-oxidation (Londraville & Sidell 1996). However, the actual role of FABP in homoviscous adaptation remains to be elucidated.

4.4 Elongase and desaturase

Almost all fish, as with all vertebrates, have the ability to convert 18:3n-3 (ALA) into n-3 HUFA (i.e. EPA and DHA) *in vivo* by an alternating succession of desaturation and elongation (Sargent *et al.* 2002; Nakamura & Nara 2004). The first step in the fatty acid elongation and desaturation pathway is the production of 18:4 n-3 from 18:3 n-3 catalyzed by the Δ6 desaturase enzyme. The product can then be successively elongated by fatty acid elongase and further desaturated by the Δ5 desaturase enzyme to 20:5 n-3 (Sargent *et al.* 2002; Nakamura & Nara 2004). The eventual production of 22:6 n-3 requires the combination of two further elongations, a Δ6 desaturation and, finally, a chain-shortening reaction (Sprecher *et al.* 1995).

The fatty acid desaturation and elongation pathway has been extensively studied in fish at both the molecular and enzymatic levels (Henderson *et al.* 1994; Tocher *et al.* 1997, 2001, 2002a,b, 2003, 2006b; Ruyter *et al.* 2000a,b; Bell *et al.* 2001; Hastings *et al.* 2001, 2004; Agaba *et al.* 2004; Zheng *et al.* 2004, 2005a,b; Oxley *et al.* 2005b; Stubhaug *et al.* 2005a,b; Moya-Falcón *et al.* 2006).

Several of the fatty acid desaturases and elongases, critical enzymes in the pathways for the biosynthesis of the long-chain $C_{20/22}$ HUFA from the shorter-chain C_{18} PUFA, have been cloned from a range of freshwater and

marine teleosts (Hastings *et al.* 2001; Agaba *et al.* 2004; Zheng *et al.* 2004). Specifically, three cDNAs encoding the Δ6 desaturase, Δ5 desaturase enzymes and the PUFA elongase in the HUFA biosynthetic pathway have been cloned from Atlantic salmon (Hastings *et al.* 2004; Zheng *et al.* 2004). Furthermore, the Δ6 desaturase has been cloned and characterised in Atlantic cod (Tocher *et al.* 2006b). Although anadromous and marine species express elongase and Δ6 and Δ5 desaturase activity (and hence the possibility to convert 18:2 n-6 and 18:3 n-3 to AA and EPA), the extent of fatty acid bioconversion is minimal (Tocher *et al.* 2006b). The activity of the desaturases and elongases is significantly higher in salmonid hepatocytes and enterocytes (Tocher *et al.* 2001, 2002a,b, 2003; Oxley *et al.* 2005b; Stubhaug *et al.* 2005a,b). Replacing dietary FO with VO in Atlantic salmon has been shown to enhance the hepatic desaturase and elongase pathway, especially during the seawater stage (Henderson *et al.* 1994; Ruyter *et al.* 2000b; Bell *et al.* 2001; Tocher *et al.* 2001, 2002a,b, 2003; Stubhaug *et al.* 2005a,b; Zheng *et al.* 2005b; Moya-Falcón *et al.* 2006). Specifically, Atlantic salmon fed either a FO diet or a 75 or 100% VO-blend diet over a 2 year production cycle had the highest Δ6 desaturase expression during seawater transfer, and the induction of desaturase expression was significant during the seawater period, but not during the freshwater period (Zheng *et al.* 2005b). However, the quantitative significance of desaturase and elongase pathway induction in salmonids has not yet been determined. However, the reduction in dietary EPA and DHA in VO-based diets cannot be compensated by *in vivo* synthesis of EPA and DHA from the n-3 precursor (ALA), in Atlantic salmon or brown trout (Bell *et al.* 2001; Tocher *et al.* 2001, 2002a,b, 2003; Zheng *et al.* 2005b). Using the whole-body fatty acid balance method (Turchini *et al.* 2007a), which is a simple and easily implemented methodology to quantify PUFA metabolism (desaturation, elongation and β-oxidation), similar results have been reported in the freshwater, warm water, carnivorous Murray cod (Turchini *et al.* 2006a; Francis *et al.* 2007a). In particular, it has been shown that, despite the fact that Murray cod has a quite efficient capability to bioconvert ALA into n-3 HUFA and that when FO is replaced in its diet the elongase and desaturase activities are increased, a significantly lower level of n-3 HUFA is reported in its tissues compared with fish receiving a FO-based diet.

4.5 Lipid and metabolic disorder

Some of the most frequently used VO contain high levels of OA (18:1n-9), which has been shown to affect liver lipid and lipoprotein metabolism in different cell systems in fish (Ranheim *et al.* 1994; Vegusdal *et al.* 2005) and

rats (Halvorsen *et al.* 2001). Furthermore, both EPA and DHA are reported to affect hepatic TAG metabolism and β-oxidation capacity in mammals (Nossen *et al.* 1986; Willumsen *et al.* 1996; Berge *et al.* 1999; Madsen *et al.* 1999), and EPA has a plasma lipid lowering effect in rats (Frøyland *et al.* 1996, 1997). In addition, n-3 PUFA is suggested to inhibit the secretion of TAG-rich VLDL particles by inhibiting the rate-limiting enzyme diacylglycerol acyltransferase (Berge *et al.* 1999; Madsen *et al.* 1999) and by inhibiting the assembly of VLDL particles in the liver (Lang & Davis 1990; Brown *et al.* 1999; Kendrick & Higgins 1999). Several studies in humans have shown that dietary EPA and DHA decrease plasma TAG (Harris *et al.* 1983; Nestel 1990) and protect against coronary heart disease (Bang *et al.* 1971; Ruxton *et al.* 2005).

Studies on the effects of reduced n-3 HUFA and increased n-6 PUFA owing to dietary VO inclusion on salmonid heart histology are contradictory (Bell *et al.* 1993; Grisdale-Helland *et al.* 2002; Seierstad *et al.* 2005a), predominantly showing no negative effects of significantly reduced n-3 HUFA in the diet (Seierstad *et al.* 2008). However, the dietary levels of phytosterols provided from the VO used were not considered in any of these studies. The amount and type of phytosterol varies depending on the type of VO and the degree of refinement (Weihrauch & Gardner 1978). To ensure that cardiovascular diseases do not develop when different oils are used, the impact of dietary phytosterols needs to be evaluated in salmonids.

Changing the concentration of dietary n-3 HUFA in fish feeds can have both beneficial and, in some instances, detrimental effects on disease resistance. When challenged with *Aeromonas salmonicida*, Lehmann & Neumann, 1896 and *Vibrio anguillarum*, Bergeman, 1909, Atlantic salmon fed VO-based diets were less resistant to infection compared with fish fed a FO-based diet (Thompson *et al.* 1996). In contrast, Erdal *et al.* (1991) found that increasing the amount of dietary n-3 HUFA (by increasing 20:5 n-3 and 22:6 n-3) from 13 to 24% of the total fatty acids had an immuno-suppressive effect on Atlantic salmon and resulted in higher rates of mortality against *Yersinia ruckeri*, Ewing *et al.*, 1978. In line with the results from Erdal *et al.* (1991), high dietary n-3 HUFA has been reported to result in a decreased response by the unspecific immune system in Atlantic salmon (Waagbø *et al.* 1993a,b). In a study by Bransden *et al.* (2003), resistance to *V. anguillarum* was significantly impaired in salmon fed with some of the feeds in which FO was replaced by SFO at different inclusion levels. However, it remains unclear as to why some diets improved disease resistance in salmon and others did not. This suggests that an optimal dietary n-3/n-6 ratio probably exists for disease resistance, although this has not yet been identified.

Furthermore, by feeding VO-based diets, the tissue n-6 PUFA levels increase and the amount of eicosanoids produced from AA compared with EPA possibly increases. This is thought to have an impact on a number of responses, such as the stress response and smoltification (Bell & Raynard 1990; Bell *et al.* 1991a,b, 1992, 1993, 1996, 1997). Recently, in out-of-season parr–smolt-induced Atlantic salmon, Jutfelt *et al.* (2007) reported that VO-based diets can act as a stressor and can be responsible for increased basal cortisol levels compared with fish fed a FO-based diet. However, at the same time, the fish fed a SFO-based diet maintained a higher intestine barrier function, with a consequent reduction in pathogenic bacteria translocation compared with salmon fed the FO-based diet.

In a study by Gilman *et al.* (2003), male brook trout and goldfish (*Carassius auratus*, Linnaeus, 1758) were exposed to a phytosterol mixture (72% beta-sitosterol) via silastic intraperitoneal implants to elucidate the mechanisms of the actions of phytosterols on steroid depression. Beta-sitosterol, the predominant plant sterol in pulp mill effluent, has been shown to decrease plasma sex steroid and cholesterol levels and *in vitro* gonadal steroid production in fish. In male brook trout, low-density lipoprotein cholesterol and triglyceride levels decreased significantly, 43 and 50%, respectively, in phytosterol-treated fish. However, the activity of P450scc, which converts cholesterol to pregnenolone (the first step in the steroidogenic pathway), was not affected in testis mitochondria isolated from the brook trout or goldfish (Gilman *et al.* 2003).

5 The effects of fish oil replacement on the final eating quality of farmed fish

Simply finding a FO replacement that permits economically efficient growth is not a complete solution to the problem at hand (Sargent *et al.* 2002). As previously mentioned, the principal drawback of FO replacement in aquafeed is the resultant unavoidable modification of fish fillet fatty acid composition and the consequent loss of the particular health-promoting characteristic associated with the consumption of fish and seafood. These modifications can also affect the sensory qualities of fish fillets and the consumers' perception of farmed fish. Although numerous studies have reported that dietary lipid sources can affect some of the principal characteristics of the final eating qualities of farmed fish, such as sensory and organoleptic characteristics, texture, storage stability, flavour volatile compounds and pigmentation, the results are often contradictory and are not yet fully understood.

The use of high-lipid feeds for cultured fish can affect fish flesh quality by increasing the percentage of lipids stored in the edible muscle (Watanabe 1982; Arzel *et al.* 1993, 1994; Hemre & Sandnes 1999; Bendiksen *et al.* 2003). In general, increased dietary lipid results in increased muscle lipid levels. However, Hemre and Sandnes (1999) reported that lipid deposition in the muscle levelled out at 16% total muscle lipid of Atlantic salmon when the dietary lipid level increased from 38 to 47%. Contradicting results were reported on the impact of dietary fatty acid composition on body composition. In Atlantic salmon, changing the dietary fatty acid composition by replacing FO with a VO blend during both the freshwater and seawater stages did not change the body lipid stores in any major way (Nanton *et al.* 2007). However, there was a trend towards a decreased TAG/phospholipids (PL) ratio in both visceral lipid stores and myosepta in Atlantic salmon fed 100% VO; whereas muscle lipid and protein levels have been reported to be unaffected by dietary fatty acid composition (Bendiksen *et al.* 2003; Torstensen *et al.* 2005).

5.1 Modification of the fatty acid composition of fillets

There is an increasing amount of evidence to suggest that n-3 HUFA are extremely beneficial to human health (Ruxton *et al.* 2005; Sinclair *et al.* 2007). However, this topic will not be dealt with in detail in the present review. In brief, these health-promoting factors are related to the direct intake of a sufficient quantity of long-chain HUFA of the n-3 series (such as EPA and DHA), together with a balanced dietary n-6/n-3 ratio. Consequently, fish with a high concentration of EPA and DHA, together with a low n-6/n-3 ratio, are a fundamental part of a healthy human diet. With the realisation of the aforementioned properties, fish and seafood consumption is increasing globally and most governmental food agencies recommend its consumption. As fish and seafood is believed to be the major contributor of EPA and DHA in the human diet, its health beneficial properties need to be maintained (Pickova & Mørkøre 2007).

Globally, the inclusion of VO in aquafeeds as a replacement for FO is increasing. In contrast to FO, VO and terrestrial animal fats lack n-3 HUFA, such as EPA and DHA, and are characterised by a very high n-6/n-3 ratio (Table 3).

A number of studies have shown that complete or partial replacement of FO with a VO or VO blend for all or part of the production cycle of Atlantic salmon affects the fatty acid composition of the edible portion (Waagbø *et al.* 1993c; Bell *et al.* 2001, 2002, 2003a,b; Regost *et al.* 2004; Torstensen *et al.* 2004b, 2005). In addition, the dietary fatty acid composition is also mirrored in the fish's organs and lipid stores (Thomassen & Røsjø 1989; Olsen *et al.* 1999; Bell *et al.* 2001, 2003b; Caballero *et al.* 2002;

Tocher *et al.* 2003; Torstensen *et al.* 2004a,b, 2005; Nanton *et al.* 2007; Stubhaug *et al.* 2007). Similar effects have been reported in almost all finfish species studied to date, from carnivorous to herbivorous species and from marine cold water to tropical freshwater species fed with VO, terrestrial animal fats or blends (Stickney & Andrews 1972; Yingst & Stickney 1979; Viola *et al.* 1981; Stickney & McGeachin 1983a; Fracalossi & Lovell 1995; Steffens *et al.* 1995; Gaber 1996; Mukhopadhyay & Rout 1996; Cao *et al.* 1997; Steffens 1997; Huang *et al.* 1998; Martino *et al.* 2002; Glencross *et al.* 2003a; Izquierdo *et al.* 2003, 2005; Luzzana *et al.* 2003; Regost *et al.* 2003b; Turchini *et al.* 2003a, 2006a,b, 2007b,c; Menoyo *et al.* 2004; Montero *et al.* 2005; Mourente *et al.* 2005b; Francis *et al.* 2006, 2007a,b; Lane *et al.* 2006; Ng *et al.* 2006; Xue *et al.* 2006; Bahurmiz & Ng 2007; Mørkøre *et al.* 2007; Yildirim-Aksoy *et al.* 2007; Du *et al.* 2008). Thus, replacing FO with VO and therefore increasing dietary LA, OA and ALA and decreasing the marine n-3 fatty acids EPA and DHA will result in a reflection of the VO dietary lipid source in the whole fish, organs and flesh. However, the magnitude of the change in the fatty acids is dependent on the type of tissue (Bell *et al.* 2001, 2003a; Torstensen *et al.* 2004a) and the amount of PL relative to neutral lipids in the tissue. The storage TAG fraction (neutral lipids) of the lipids of different tissues more closely resembles the fatty acid make-up of the diet than does the structural PL fraction. Consequently, the extent of similarity between the fatty acid profiles of fish tissues and the dietary fatty acid profile will depend on the relative proportions of neutral lipids and polar lipids (TAG and PL) in the tissues in question (Brodtkorb *et al.* 1997; Olsen & Henderson 1997; Jobling *et al.* 2002).

The concentration of body fat (mostly neutral lipid) in farmed fish is positively correlated with the fat content of the diet. However, the fat storage location in the body differs among species (Hardy *et al.* 1987; Jobling *et al.* 2002; Sargent *et al.* 2002). As such, the degree of modification of the fatty acid composition as a result of FO substitution in the diet can be significantly different between storage and non-storage tissues. This is also apparent when considering the edible flesh among species that demonstrate a high deposition of storage fat in the fillet and species that preferentially deposit fat in other tissues, such as the liver and abdominal cavity. In a nutshell, the fattier the fish fillet, the higher the impact of FO replacement on the final fatty acid composition. The fatty acid make-up of the fillets of fatty fish, that is, salmonids, eels, tuna and halibut, will be more affected by the substitution of FO in their diet. Alternatively, this effect will be less detrimental, but still significant, in semi-lean and lean fish, such as cyprinids, perciformes and most farmed marine carnivorous and flat fish. In Table 6, the main

fatty acid composition of the diets and fish fillets of finfish species fed a control diet (FO) or a VO-based diet are reported.

A further aspect that needs to be taken into careful consideration is that not all alternative oils modify the fillet fatty acid make-up to the same extent. This is because, as previously described, the incorporation of fatty acids into fish tissue is under various metabolic influences, such as preferential incorporation, β-oxidation, lipogenic activity or fatty acid elongation and desaturation processes (Sargent *et al.* 2002; Robin *et al.* 2003) and different fatty acid and fatty acid classes are deposited into fish fillets at different rates. High correlations have been observed between the general fatty acid composition of the diets and the fatty acid compositions of the fish muscle in almost all species studied. However, the correlations between the amounts of specific individual fatty acids in the diets and the corresponding amounts in muscle are not always that obvious (Mugrditchian *et al.* 1981; Viola *et al.* 1981; Kennish *et al.* 1992; Turchini *et al.* 2003a). In particular, the mean percentage of SFA in the muscle of fish fed diets with a relatively low content of this fatty acid class have been reported to be higher than in the diet (Yu *et al.* 1977; Greene 1990; Greene & Selivonchick 1990; Turchini *et al.* 2003a). In contrast, when fish are fed with a diet containing high concentrations of SFA, the fillet deposition of SFA is significantly less than proportional. For example, Bell *et al.* (2002) reported that in the fillets of Atlantic salmon fed graded substitution levels of FO with CPO, against an increase in dietary SFA from 27.1 to 47.1%, the fillet SFA concentration varied only from 25.9 to 29.2%. As such, it seems that SFA are deposited into fish fillets at a specific physiological level, despite the dietary content.

Observing the data reported in Table 6, it is evident that, in general, the difference between a FO-based diet and a VO-based diet in the percentage of individual fatty acids or fatty acid classes is greater than the difference between the fillets of fish fed the respective diets. For example, in turbot fed a FO-based diet or a SBO-based diet the differences between the diets in the percentages of SFA, MUFA, n-6 PUFA and n-3 PUFA were −18%, −37%, +475% and −36%, whereas the differences between the fillets of fish fed the two diets were −12%, −25%, +281% and −21%, respectively (Table 6). Similarly, as a further example, in rainbow trout fed a FO-based diet or a VO blend, the differences between the dietary percentages of OA, LA, ALA and DHA were +227%, +191%, +255% and −55%, whereas the differences between the fillets of fish fed the two diets were +102%, +141%, +156% and −30%, respectively (Table 6). This observation underlines that, despite a dietary induced modification of fillet fatty acid composition, fish are making an attempt to control the extent of fatty acid

Table 6 Main fatty acid composition of the diets and fish tissues (fillet or whole body) of different finfish species fed with a control diet (FO) or a vegetable oil based diet

	SFA	MUFA	n-6PUFA	n-3PUFA	OA	LA	ALA	AA	EPA	DHA
Red sea bream (Huang *et al.* 2007)										
Diet FO	22.1	44.8	5.8	22.4	17.7	4.7	0.8	0.6	8.3	10.0
Diet C/RO (70%)	11.5	55.6	19.4	11.7	52.8	19.0	5.7	0.2	1.2	4.3
Whole body FO	24.5	45.8	5.4	20.8	22.3	4.2	0.6	0.5	5.6	11.4
Whole body C/RO (70%)	14.1	56.0	17.0	11.0	52.2	16.2	4.0	0.2	0.9	5.3
Diet difference%†	−48	24	236	−48	198	309	577	−66	−86	−57
Fish difference%‡	−42	22	217	−47	134	282	540	−60	−83	−54
Atlantic cod (Lie *et al.* 1986)										
Diet FO	18.0	48.0	2.3	31.1	16.0	1.7	1.0	0.6	10.2	16.3
Diet peanut oil (100%)	20.0	55.0	20.5	5.1	52.5	20.5	0.0	0.0	1.0	2.8
Fillet FO	21.0	22.0	3.0	53.5	12.3	1.8	0.5	1.2	14.8	35.8
Fillet peanut oil (100%)	20.0	24.0	14.0	40.6	21.4	13.2	0.6	0.8	10.3	28.4
Diet difference%	11	15	791	−84	228	1106	−100	−100	−90	−83
Fish difference%	−5	9	367	−24	74	633	20	−33	−30	−21
Turbot (Regost *et al.* 2003b)										
Diet FO	22.5	47.1	6.3	24.1	nr	5.2	1.3	0.5	7.9	10.6
Diet SBO (100%)	18.5	29.8	36.2	15.5	nr	35.6	4.6	0.3	3.7	5.6
Fillet FO	21.7	45.3	6.9	26.0	nr	5.5	1.3	0.5	6.9	12.3
Fillet SBO (100%)	19.1	34.1	26.3	20.5	nr	24.2	3.0	0.5	4.8	9.2
Diet difference%	−18	−37	475	−36	–	585	254	−40	−53	−47
Fish difference%	−12	−25	281	−21	–	340	131	0	−30	−25
Rainbow trout (Caballero *et al.* 2002)										
Diet FO	21.6	47.4	6.0	19.4	10.9	4.6	1.1	0.2	7.6	6.4
Diet VO blend (80%)	24.4	45.9	13.8	11.1	35.6	13.4	3.9	0.1	2.9	2.9
Fillet FO	22.7	47.1	5.1	18.6	16.1	3.9	0.9	0.3	4.6	9.4
Fillet VO blend (80%)	23.3	46.1	11.0	13.3	32.6	9.4	2.3	0.3	2.2	6.6
Diet difference%	13	−3	130	−43	227	191	255	−50	−62	−55
Fish difference%	3	−2	116	−28	102	141	156	0	−52	−30
Atlantic salmon (freshwater) (Torstensen *et al.* 2005)										
Diet FO	20.3	59.4	4.4	14.9	11.9	3.9	0.6	0.2	5.8	5.9
Diet VO blend (100%)	21.8	50.2	13.7	14.3	40.4	13.5	8.0	0.2	2.1	3.4
Fillet FO	21.0	51.0	4.0	21.0	14.0	3.0	0.6	0.5	3.5	13.0
Fillet VO blend (100%)	20.0	45.0	13.0	21.0	33.5	10.4	5.4	0.8	2.0	11.2
Diet difference%	7	−15	211	−4	239	246	1233	0	−64	−42
Fish difference%	−5	−12	225	0	139	247	800	60	−43	−14
Atlantic salmon (saltwater) (Torstensen *et al.* 2005)										
Diet FO	23.6	48.8	4.6	21.8	13.2	3.6	1.2	0.5	6.5	10.0
Diet VO blend (100%)	19.4	48.2	17.1	15.2	43.0	17.1	13.4	0.0	0.6	1.0
Fillet FO	21.1	48.2	4.5	23.4	14.6	3.3	1.1	0.7	4.6	12.7
Fillet VO blend (100%)	17.0	49.0	17.0	18.0	41.3	14.4	8.2	0.9	1.6	3.4
Diet difference%	−18	−1	272	−30	226	375	1017	−100	−91	−90
Fish difference%	−19	2	278	−23	183	336	645	29	−65	−73
European sea bass (Montero *et al.* 2005)										
Diet FO	31.7	28.1	6.3	31.9	11.3	3.9	0.9	0.9	13.5	11.7
Diet SBO(60%)	20.1	28.8	15.6	34.6	16.7	30.5	4.7	0.4	5.9	5.6
Fillet FO	31.3	31.9	6.8	28.4	18.5	4.6	1.2	0.8	9.2	14.1
Fillet SBO(60%)	27.8	31.2	20.7	19.4	20.1	18.9	2.9	0.5	4.9	9.4
Diet difference%	−37	2	147	8	47	679	430	−53	−57	−52
Fish difference%	−11	−2	204	−32	9	311	142	−38	−47	−33
Hybrid tilapia (Bahurmiz & Ng 2007)										
Diet FO	20.9	40.2	8.3	23.4	14.8	7.2	1.7	0.5	5.5	12.0
Diet CPO (100%)	41.2	35.7	13.3	6.1	32.6	13.2	0.8	0.1	1.6	3.0
Fillet FO	24.9	33.0	7.9	25.4	15.7	5.4	0.9	1.2	1.7	17.2
Fillet CPO (100%)	32.2	37.5	12.0	11.8	30.8	7.7	0.4	1.9	0.4	9.5
Diet difference%	97	−11	60	−74	120	83	−53	−80	−71	−75
Fish difference%	29	14	52	−54	96	43	−56	58	−76	−45

Table 6 (*Continued*)

	SFA	MUFA	n-6PUFA	n-3PUFA	OA	LA	ALA	AA	EPA	DHA
Sunshine Bass (Lane *et al.* 2006)										
Diet FO	40.1	23.9	7.5	28.5	8.5	5.4	1.4	1.5	11.8	10.1
Diet CNO (100%)	21.1	27.9	42.9	8.2	23.8	42.2	1.0	0.5	3.0	3.3
Fillet FO	29.3	31.1	12.4	27.2	19.3	9.4	1.1	2.1	8.9	13.9
Fillet CNO (100%)	25.2	34.2	24.6	16.0	24.9	21.9	1.0	1.3	4.8	8.3
Diet difference%	−47	17	475	−71	180	678	−30	−65	−74	−68
Fish difference%	−14	10	98	−41	29	133	−9	−38	−46	−40
African catfish (Ng *et al.* 2003)										
Diet FO	28.2	39.7	1.9	22.6	25.9	1.9	0.2	nr	11.9	9.0
Diet SFO (100%)	12.0	27.5	60.2	0.0	27.4	60.2	0.0	nr	0.0	0.0
Fillet FO	32.9	43.3	11.0	9.5	36.4	10.7	4.4	0.3	1.2	2.0
Fillet SFO (100%)	29.1	33.3	29.9	4.2	30.3	26.6	2.0	1.5	0.8	1.3
Diet difference%	−57	−31	3068	−100	6	3068	−100	−	−100	−100
Fish difference%	−12	−23	172	−56	−17	149	−55	400	−33	−35
Grass carp (Du *et al.* 2008)										
Diet FO	7.3	18.3	17.6	54.4	8.5	4.7	2.5	5.9	14.8	33.3
Diet VO blend (100%)	12.8	38.4	19.9	17.4	40.7	18.7	17.4	0.4	0.0	0.0
Fillet FO	19.4	39.8	18.7	21.1	30.8	12.2	1.9	3.0	4.9	12.8
Fillet VO blend (100%)	20.3	33.2	30.2	12.2	26.0	12.8	6.1	2.4	3.4	1.8
Diet difference%	75	110	13	−68	379	298	596	−93	−100	−100
Fish difference%	5	−17	61	−42	−16	5	221	−20	−31	−86

†Diet difference % = (%FA diet VO − % FA diet FO) × (%FA diet FO)$^{-1}$ × 100.

‡Fish difference % = (%FA fish VO − % FA fish FO) × (%FA fish FO)$^{-1}$ × 100.

The percentage of fish oil substituted is in parentheses. The percentage difference of the fatty acid percentage between diets and fish tissues is also reported.

AA, arachidonic acid, 20:4 n-6; ALA, α-linolenic acid, 18:3 n-3; DHA, docosahexaenoic acid, 22:6 n-3; EPA, eicosapentaenoic acid, 20:5 n-3; LA, linoleic acid, 18:2 n-6; MUFA, monounsaturated fatty acids; n-3 PUFA, polyunsaturated fatty acids with the first double bond at the 3rd carbon atom; n-6 PUFA, polyunsaturated fatty acids with the first double bond at the 6th carbon atom; OA, oleic acid 18:1 n-9; SFA, saturated fatty acids.

modification towards the maintenance of a general fish fatty acid make-up.

Making a rough generalization, this general fish fatty acid make-up can be described as one-third SFA, one-third MUFA and one-third n-3 PUFA. Within these major classes, it is important to note that in fish, SFA are generally dominated by 16:0, that MUFA are generally rich in C_{20} and C_{22} monoenes (i.e. 20:1 n-9, 22:1 n-11 and other isomers) and n-3 PUFA are fundamentally completely dominated by n-3 HUFA, such as EPA and DHA. Moreover, in the general fish fatty acid make-up, C_{18} PUFA are limited and n-6 PUFA are scarce, with LA (18:2 n-6) content usually very limited (<2%) (Table 3).

On the contrary, alternative lipid sources have a highly variable fatty acid make-up. Some are extremely rich (>50%) in SFA (e.g. PO, TAL), some in MUFA (e.g. peanut oil, olive oil, C/RO), some in n-6 PUFA (e.g. SBO, SFO) and some in C_{18} n-3 PUFA (e.g. LO). Recently, FO substitution studies have implemented a formulated blend of VO to tentatively simulate the overall fatty acid class make-up of FO. In each of these studies, it was possible to formulate blended oil with a general fatty acid make-up of one-third SFA, one-third MUFA and one-third n-3 PUFA. However, within these three major classes, it is important to note that in alternative terrestrial oils (both vegetable or animal) the primary SFA can vary from 12:0, 14:0, 16:0 to 18:0, MUFA are almost entirely represented by OA (18:1n-9) and n-3 PUFA is derived entirely from C_{18} polyunsaturated fatty acids (i.e. ALA, 18:3n-3). Moreover, in vegetable (and terrestrial animal) lipid sources, n-6 PUFA are always relatively abundant and LA (18:2 n-6) commonly accounts for anywhere from 10% up to 65% (Table 3).

In light of the above observations and from the data presented in Table 6, it is clear that when FO is replaced, the most significant modifications to the fatty acid composition of fish tissues are relative to an increase in the C_{18} PUFA content (particularly LA), a decrease in the n-3 HUFA content and a modification of the MUFA composition from C_{20} and C_{22} MUFA to C_{18} MUFA. For these reasons, the content of LA (18:2 n-6) in a potential alternative lipid source must be considered as one of the most informative (negative) parameters to be considered because this fatty acid is responsible for the most detrimental modifications to the fatty acid composition of farmed fish fillets.

As demonstrated by clinical studies, these differences in fatty acid composition have major ramifications from a human health viewpoint that ultimately could significantly affect the consumer perception of aquaculture products. Seierstad *et al.* (2005b) demonstrated that eating fish fed a diet rich in marine origin n-3 HUFA (i.e. FO) imposed favourable biochemical changes in patients with coronary heart disease compared with patients ingesting fish reared on a C/RO-based diet (rich in MUFA and LA), who did not receive health benefits of the same magnitude. However, all patient groups eating farmed salmon, despite the dietary treatment of the fish, experienced positive effects on the blood biochemical biomarkers for coronary heart disease, indicating that other components, such as selenium, anti-oxidants and/or fish protein, in farmed Atlantic salmon might also have positive health effects.

5.2 Effects on the physical characteristics of fillets

Liquid-holding capacity, *rigor mortis* and texture are important characteristics for determining fish fillet quality and involve physical and chemical interaction developed during storage and eventually in the consumer's mouth. Rørå *et al.* (2003) investigated the effects of FO substitution with SBO at 50 and 100% replacement on the final eating quality of fresh and smoked fillets of Atlantic salmon. The smoking yield increased with the inclusion of SBO, and this is likely to be related to decreased fat deposition, whereas the texture and the liquid-holding capacity of both fresh and smoked fillets were not significantly affected by the dietary oil source. Contradictory results were later reported on the same species by Regost *et al.* (2004), who reported that the liquid-holding capacity was influenced by the dietary oil source. The recorded losses of water in salmon fillets were highest in fish fed C/RO compared with fish fed two different fish oils (capelin and Peruvian fish oil) and lowest in fish fed SBO. Fat loss from fish fed capelin oil was higher than that in fish fed Peruvian fish oil or SBO. Moreover, a significant interaction between fillet water and fat loss on dietary lipid source and storage time, for both fresh and smoked products, was also reported (Regost *et al.* 2004).

In Atlantic cod, the partial substitution of FO with 40% SBO has been reported to significantly increase the contraction rate while decreasing the ATP content and pH value of the fish flesh immediately after harvesting (Mørkøre 2006). However, these differences disappeared a few hours post-mortem. Similarly, texture development has been reported to differ significantly with dietary treatment. The texture of fillets of Atlantic cod fed the alternative lipid sources was firmer immediately after slaughtering. However, the breaking force dropped to a stable level after 4 h, whereas in the fillets of fish fed the FO-based diet, the breaking force decreased continuously during the storage period. Ultimately, the breaking force was similar for both groups after 16 h of storage. The author concluded that the dietary inclusion of SBO resulted in faster energy depletion post-mortem, faster rigor contraction and faster reduction of the breaking strength (Mørkøre 2006). However, in a subsequent experiment by the same author on the same species in which FO was completely replaced by SBO, no significant effects on texture or liquid-holding capacity were observed (Mørkøre *et al.* 2007). These contrasting results show our still limited understanding of the effects of FO replacement on the physical characteristics of fish fillets.

A significantly 'softer' texture has been reported for fillets of Atlantic salmon fed C/RO compared with fish fed FO or SBO (Regost *et al.* 2004), whereas the opposite results were previously reported for brook charr (Guillou *et al.* 1995). The texture of the fillet from brook charr fed C/RO was firmer and was also preferred by panellists to that of the fish fed FO or SBO (Guillou *et al.* 1995). In turbot fed different lipid sources, and by implementing a sensorial evaluation of flesh texture, significant differences have been reported in the attributes 'moisture texture' and 'fat texture' (higher score recorded for fish fed FO) and 'exudation' (higher scores recorded in fish fed SBO) (Regost *et al.* 2003b). However, these differences changed after the finishing period on a FO-based diet, with significant differences reported for the attributes 'firmness' (highest in fish previously fed SBO) and 'moisture texture' (still higher for fish previously fed FO) (Regost *et al.* 2003b).

A texture analysis in gilthead sea bream demonstrated that the inclusion of vegetable lipid sources slightly reduced the hardness. In addition, in the same study, no differences were reported for European sea bass (Izquierdo *et al.* 2003). However, in a follow-up experiment, the same authors reported no effects of dietary lipid source on gilthead sea bream texture (Izquierdo *et al.* 2005). Similarly, dietary lipid sources have been reported to have no effects on the evaluated fillet sensory texture in different farmed species, such as European eel (Luzzana *et al.* 2003), brown trout (Turchini *et al.* 2003b), tench (*Tinca tinca*, Linnaeus, 1758) (Turchini *et al.* 2007c), Atlantic salmon (Waagbø *et al.* 1993c; Koshio *et al.* 1994) and rainbow trout (Hardy *et al.* 1987).

The current scientific literature is somewhat unclear and there are contrasting results on the effect of FO replacement on fillet texture, most likely because of species-specific variability and the time and methodology of the assessments. Our understanding of the mechanisms responsible for developing physical sensorial characteristics in the fillets of farmed fish, and in particular the specific roles of dietary lipid and fatty acids, is limited.

5.3 Effects on sensory characteristics

Organoleptic and sensory evaluations of fish products produced from farmed fish fed with fish or alternative oils have been extensively researched and are often contradictory.

Guillou *et al.* (1995) reported no sensorial differences in the fillets of brook charr fed either FO, C/RO or SBO, whereas Torstensen *et al.* (2005) reported several organoleptic differences in the flesh of Atlantic salmon fed FO or a blended VO. In particular, salmon fed the FO-based diet recorded significantly higher scores for the attributes: intensity of odour, marine oil odour, rancid odour, colour tone, marine oil flavour and rancid flavour, and recorded lower scores for the attribute described as vegetable oil flavour. However, these differences disappeared after completion of the finishing (wash-out) phase (on a FO-based diet) and no significant differences in any of the sensory descriptors were recorded (Torstensen *et al.* 2005). In brown trout, significant differences were reported in fish fed different lipid sources in the raw product, but no differences were recorded in the cooked product (Turchini *et al.* 2003b). In particular, the intensity of total odour was highest for fish fed FO or animal fat (poultry and pork) and lowest for fish fed VO (C/RO or oleine). Other experiments assessing the effect of FO replacement at relatively low replacement levels on the organoleptic attributes of salmonids fillet have reported differences in attributes such as: fish aroma (Skonberg *et al.* 1993), salmon taste and odour (Thomassen & Røsjø 1989), taste intensity, fattiness and juiciness (Waagbø *et al.* 1993c) and total odour and oily taste (Drobná *et al.* 2006). However, in other instances, no significant effects of FO replacement on the final sensory characteristic of salmonid fillets have been reported (Hardy *et al.* 1987; Koshio *et al.* 1994).

In gilthead sea bream, a significant stronger smell and taste of fish were reported in fish fed SBO, whereas no differences were reported in European sea bass subjected to a similar dietary treatment (Izquierdo *et al.* 2003). Turbot dorsal fillets of fish fed SBO-based diets recorded a significantly higher score for the attribute 'odours – potatoes' compared with fish fed FO or LO (Regost *et al.* 2003b). However, in the ventral fillet, significant differences were recorded among treatments for the odour attributes 'intensity', 'fatty fish' and 'milky' (Regost *et al.* 2003b). After the grow-out phase on the different oil based diets, turbot were then subjected to a finishing period on a FO-based diet. Surprisingly, although the differences recorded previously had disappeared, other significant differences were then recorded for the attributes 'flavour – fatty fish' and 'flavour – sweet' in the dorsal and ventral fillets, respectively (Regost *et al.* 2003b).

In European eel, a greater 'salty flavour' was recorded in fish fed a blend of SBO and TAL compared with fish fed FO (Luzzana *et al.* 2003). However, Coello *et al.* (1999) reported that different dietary lipid sources did not affect consumer perception and acceptability of eel products. In tench, a significantly higher value for the attribute 'off-flavour' in the cooked fillets of fish fed SBO compared with fish fed LO was reported and the differences were attributed to the modification of the concentration of the volatile aldehydes derived from n-3 or n-6 C_{18} PUFA in the fish fillets (Turchini *et al.* 2007c).

As for fillet texture, the reported results are unclear and contradictory. However, despite the general perception of the consumers that the final product is not negatively affected by FO replacement and in some instances even preferred, it is clear that there are significant modifications in the perceived odour and flavour attributes when FO is replaced. This is likely to result from chemical modification in the flavour compounds present in the fish fillets.

5.4 Effects on flavour compounds

Fish have complex flavour systems consisting of equally important taste and aroma/odour active components. The taste active constituents are generally non-volatile compounds, such as free amino acids, nucleotides, sugars and mineral salts, whereas the aroma active constituents are generally volatile compounds (Shahidi & Cadwallader 1997). However, it is also important to mention that the overall perceived taste of a food in the mouth is determined by the combination of the taste active constituents and the flavour volatile aroma/odour active compounds.

In marine species, the typical aroma active compounds comprise nitrogen-containing compounds, ammonia, trimethylamine (TMA) and dimethylamine (DMA), organic acids such as acetic acid, formic acid and propionic acid and sulphurated compounds (Kaway 1996). Trimethylamine oxide (TMAO) is a naturally occurring compound in marine fish that plays an important role in the overall osmoregulation process. Trimethylamine oxide is decomposed by bacterial action to TMA, which is the main odour molecule responsible for the characteristic fishy smell (Kaway 1996). In contrast, the flavour associated with fresh saltwater and freshwater fish is usually mild, delicate and pleasant and most fish have a common sweet and plant-like aroma (Durnford & Shahidi 1998). This is the result of the volatile aldehydes and alcohols that are mainly derived from the oxidative deterioration of polyunsaturated fatty acids (Kaway 1996; Durnford & Shahidi 1998). Different volatile compounds, particularly different aldehydes, originate from the

oxidation of different fatty acids (Josephon *et al.* 1984; Kaway 1996; Belitz & Grosch 1999). Therefore, it is clear that modification of the fillet fatty acid make-up, resulting from FO substitution in the diet, can directly influence the flavour volatile compound 'bouquet' of the fish fillet. The odour thresholds of aldehydes are generally lower than those of other volatile compounds (Spurvey *et al.* 1998), thus they have a great potential effect on total flavour.

There is a considerable amount of literature on fish flavour and aroma compounds relative to different species, origins, processing techniques, freshness and storage. There is also a relative abundance of studies on the sensory characteristics of farmed fish fed with FO or alternative oil-based diets. However, little information is available on the potential modification of the flavour and aroma compounds in the fillets of farmed fish fed different dietary oils.

In the fillets of brown trout fed diets containing different lipid sources, Sérot *et al.* (2002) detected 2,4-heptadienal with higher frequency in fish fed FO compared with the fillets of fish fed SBO, while hexanal, 2-hexanal and 2-nonanol seemed to contribute most to the odour of fish fed diets containing VO (SBO or LO). Similarly, in the same species, 2,4-heptadienal was recorded at a higher concentration in the fillets of trout fed a FO-based diet compared with fish fed diets based on VO or animal fat, whereas 2,4-decadienal was significantly higher in the fillets of fish fed with C/RO (Turchini *et al.* 2004). Moreover, Turchini *et al.* (2004) reported that the total amounts of volatile compounds were higher in the fillets of fish fed diets containing only FO as the lipid source and the total amounts of alcohols and aldehydes in the fillets were linearly directly related to the percentage of n-3 HUFA in brown trout flesh and to that found in the diet. These chemical observations were in accordance with the results of the sensory evaluation in which the intensity of total odour was scored higher for fish fed FO (Turchini *et al.* 2003b).

In turbot, 2,6-nonadienal, 2-pentenal and 1,3-5-ocatatriene appear to contribute strongly to the general aroma of fish fed FO-based diets (rich in n-3 HUFA), whereas hexanal and decanal showed a higher detection frequency in the fillets of animals fed VO-based diets (Sérot *et al.* 2001). The authors of this study also stated that the odorous compounds that were not derived from fatty acid oxidation, such as methional, 1-acetylpyrazine, 4-ethyl benzaldehyde and 2-acetyl-2-thiazoline, were not affected by dietary treatments (Sérot *et al.* 2001). Recently, Turchini *et al.* (2007c) reported that the relative percentages of volatile aldehydes formed by autoxidation of n-3 PUFA (2-pentenal, 2-hexenal, 2,4-heptadienal and 2,6-nonadienal) and n-6 PUFA (hexanal, 2-octenal, 2-decenal and

2,4-decadienal) in the fillets of tench were significantly correlated to the fatty acid composition of the fillet and the respective dietary treatments (graded levels of substitution of LO with SBO).

Although the fillet fatty acid composition and consequently the flavour volatile compounds are easily modified by the dietary lipid source, other fish tissues are less susceptible to variations in dietary fatty acid make-up. In particular, it has been reported that, in farmed caviar obtained from white sturgeon, the use of different dietary lipid sources is responsible for minimal modification of the overall caviar fatty acid composition and consequently the flavour volatile composition was almost unaffected (Caprino *et al.* 2008). The total amounts of the volatile compounds derived from OA, LA and ALA were almost constant across treatments in which sturgeon were fed squid oil or a blend of SBO and FO for 3 or 6 months prior to caviar collection (Caprino *et al.* 2008).

From the results reported in these few studies, it is possible to suggest some general conclusions. The volatile aldehydes formed by autoxidation of n-3 PUFA are higher in the flesh of fish fed diets containing FO, whereas the volatile aldehydes formed by autoxidation of n-6 PUFA and OA are higher in the flesh of fish fed diets containing VO. The description of the aroma of the volatile aldehydes derived from n-3 PUFA, such as 2-pentenal, 2-hexenal and 2,6-nonadienal, is generally pleasant and associated with green, apple, cucumber, grass and mushrooms, and 2,4-heptadienal is associated with green, cucumber, but also sometimes reported as oily and fatty. In contrast, the odours of the volatile aldehydes derived from the oxidation of n-6 PUFA, such as hexanal, 2-octenal, 2-decenal and 2,4-decadienal, are commonly described as tallowy, fatty, nutty, oily and associated with cod oil, fried fat and oxidized oil (Kaway 1996; Durnford & Shahidi 1998; Spurvey *et al.* 1998; Belitz & Grosch 1999; Sérot *et al.* 2001, 2002; Turchini *et al.* 2004, 2007c). Consequently, diets containing higher amounts of n-6 PUFA (LA) as a replacement for FO are responsible for increased levels of n-6 PUFA (LA) in the muscles of fish and subsequently for the increased amount of n-6-derived volatile aldehydes that are normally reported to contribute negatively to the general aroma of fish muscle. This is in general agreement with the aforementioned results of the sensorial analyses.

5.5 Effects on flesh colour

Flesh pigmentation is an important factor in the perception of flesh quality in salmonids (Bell *et al.* 1998; Refsgaard *et al.* 1998). The flesh levels of lipid soluble nutrients, such as astaxanthin, are dependent on the dietary composition (Lie 2001), and it has been shown that

both the total lipids and lipid source can affect carotenoid absorption in rodents (Clark *et al.* 2000; Clark & Furr 2001) and colour characteristics in raw and smoked Atlantic salmon fillets (Regost *et al.* 2004). However, most reports show no effects on flesh astaxanthin levels when using up to 100% VO (Bell *et al.* 2001, 2002; Torstensen *et al.* 2004b, 2005), although none of these studies have used SBO as a replacement for FO. As reported by Regost *et al.* (2004), dietary SBO inclusion resulted in reduced flesh astaxanthin, cantaxanthin and visual colour, particularly after 4 months of frozen storage. Despite the high carotenoid content found in CPO, Atlantic salmon fed CPO-based diets did not increase pigmentation in fish fillets (Ng *et al.* 2004a).

Little information is available on the potential effect of FO substitution on the fillet colour in un-pigmented fish. However, it has been reported that the substitution of FO with SBO in diets for Atlantic cod resulted in lower lightness (L^*-value) of the fish fillet (Mørkøre 2006). The white flesh of fish such as tilapia and catfish is highly valued by human consumers and the substitution of FO with alternative oils must not taint this desirable trait. The use of pigmented oils, such as CPO, does not cause the fillets of un-pigmented fish such as tilapia to become yellowish (W.-K. Ng, pers. comm., 2008).

5.6 Finishing diets

In consideration of the aforementioned modification of the final eating qualities of farmed fish when dietary FO has been replaced, there has been great interest in attempting to restore the original optimal fatty acid make-up of farmed fish fillets. The most common approach has been the potential implementation of a finishing period on a FO-based diet (wash-out or finishing diet). However, the initial concentration of fat in the tissue in question directly affects the time required for the fatty acid composition of a tissue to be influenced by a dietary change.

Jobling *et al.* (2002) reported that the proportion of some of the typical fatty acids commonly found in VO (i.e. OA, LA and ALA) were relatively high in different fish tissues following a 14 week finishing period on a FO-based diet for post-smolt Atlantic salmon previously fed a VO-based diet. Consequently, the authors suggested that it was possible to use the fatty acid profile to discriminate between fish that had different dietary histories during rearing in fresh water. Jobling *et al.* (2002) concluded that during the finishing period, the decrease in the proportion of some fatty acids typical of VO either resulted from lipid turnover and fatty acid metabolism or more likely from 'dilution' occurring from lipid deposition as the fish increased in size. In a similar study,

Regost *et al.* (2003a) reported that in turbot, following a 2 month wash-out, the influences of earlier diets with different fatty acid compositions were still present in the final fatty acid make-up of fish tissues, although a noticeable decrease in C_{18} PUFA and a significant increase in EPA and DHA were observed.

After these preliminary observations, a simple, but effective, dilution model was proposed by Robin *et al.* (2003) to tentatively describe the fatty acid deposition into fish tissues, hence providing a tool to foresee the final fatty acid make-up of a fillet of fish fed a given diet. The model is based on two assumptions: (i) that fatty acids are incorporated into different tissues without any mobilisation or turnover; and (ii) that under similar culture conditions, the same species of fish would incorporate dietary fatty acid in the similar manner, irrespective of the initial fatty acid composition of the fish tissues. The dilution model (Robin *et al.* 2003) is expressed mathematically as:

$$P_t = P_r + \frac{P_i - P_r}{Q_t/Q_i}$$

where P_t is the percentage of fatty acid in the tissues of the 'test' fish at time t, P_i is the initial percentage of fatty acid in the tissues of the 'test' fish and P_r is the percentage of the fatty acid in the tissues of the 'reference' fish at time t. Q_i is the initial total amount of fatty acids (or total fat) present and Q_t is the amount present at time t in the tissues of the 'test' fish. The 'test' fish are fish previously fed with an alternative lipid source and subsequently subjected to the finishing period, whereas the 'reference' fish are fish continuously fed with the FO-based diet.

An additional evaluation of the model by Jobling (2003) showed the positive aspects and the limits of the proposed dilution model. In particular, the principal observation of Jobling (2003) was that the model is not applicable for all fish species. In 'lean' fish that store only a small amount of fat in the fillet, the fillet lipids are primarily represented by structural, cell membrane PL. The fatty acid profiles of PL are less easily modifiable by the dietary fatty acid composition than those of the storage fats (neutral fats, TAG). As such, on 'lean' fish species the simple dilution model might not provide a satisfactory explanation of the modifications in the fatty acid make-up of fillet lipids. In conclusion, Jobling (2003) stated that, despite the reported limitations, the dilution model provides a good description of the modification of fatty acid composition of fish fillets following a change in the source of the dietary fatty acids. However, the model needs to be restricted only to fish in which the fat concentration of the fillets and fillet yield vary little over

time, and to fish species in which the fillets contain relatively large proportions of storage fats (neutral lipids), such as 'medium-fat' and 'high-fat' fish species.

In a following paper, Jobling (2004a) used the dilution model with the addition of a further step as a tool to understand individual fatty acid metabolism in fish. In particular, by plotting the observed and predicted values and comparing the regression line obtained for a specific fatty acid and the line of equity, the author showed that in Atlantic salmon, particularly for C_{18} PUFA, the slope of the plotted regression was steeper (1.197) than the line of equity. This observation implies that modification of the percentage of fillet C_{18} PUFA was the result of not only a dilution process, but also involved preferential metabolism and fatty acid turnover, and that in Atlantic salmon C_{18} PUFA are mobilised at higher rates than predicted. However, analysing the C_{18} fatty acids individually (OA, LA and ALA) and over time during the finishing period of Murray cod, Turchini *et al.* (2006b) reported that the slopes of the computed equations were lower than one (0.778, 0.844 and 0.420 for OA, LA and ALA, respectively). This indicates that in Murray cod these fatty acids, particularly ALA, were mobilised at a lower rate than predicted. In the same experiment, Turchini *et al.* (2006b) reported that trends in the modification of C_{18} fatty acids in Murray cod fillets and liver were exponentially related to time, with major modifications occurring in the first days of the finishing period. The speed of modification subsequently decreased gradually and therefore the reported trends generally conformed to the theory of the dilution model.

The dilution model has also been evaluated in sunshine bass, a 'lean' carnivorous freshwater fish by Lane *et al.* (2006). In each instance where the dilution model was implemented, the authors generally concluded that, despite the general approximations of the model, it could be useful in describing changes in the fatty acid profiles of fish following a change in the source of dietary fatty acid. However, as previously underlined by Jobling (2004b), it is important to note that erroneous conclusions can be drawn if the model is applied for the prediction of changes in fatty acid profiles in the fillets of 'lean' species. Thus, the model could be generally considered to be a useful tool to plan appropriate finishing strategies to obtain a final product with a desired fatty acid make-up.

In consideration of the fact that the health-promoting characteristics of eating fish are primarily related to the amount of n-3 HUFA present in the final product, and not simply their relative percentages, it is also important to quantify the total accumulation of EPA and DHA, as milligrams of fatty acid per fish fillet, during the finishing period. The accumulation of specific fatty acids over time has been described by exponential functions (Glencross

et al. 2003b; Turchini *et al.* 2006b). It has been reported that the key difference in the rate equations in the absolute exponential accumulation of fatty acids during finishing for fish continuously fed a FO-based diet and fish that have been previously fed with alternative oil-based diets is the increase in the index value of the exponent. Thus, in fish previously fed alternative oil diets, the accumulation of EPA and DHA is faster than in fish continuously fed a FO-based diet (Turchini *et al.* 2006b).

In addition, finishing diets can be responsible for restoring potential feed-related health problems. In some species, histological alterations, such as liver steatosis, resulting from a diet extremely rich in 18:2 n-6 or deficient in n-3 fatty acids have been reported (Caballero *et al.* 2004; Ruyter *et al.* 2006; Wassef *et al.* 2007). However, after a finishing period on a FO-based diet, a considerable reduction in cytoplasmic vacuolation and in lipid vacuole accumulation were reported (Caballero *et al.* 2004).

Recently, Turchini *et al.* (2007b) showed that finishing diets stimulate a form of compensatory growth. Fish that experienced the dietary shift from VO to FO grew faster than the control fish continuously fed a FO-based diet. This phenomenon has been termed 'lipo-compensatory growth' and still needs to be fully clarified. However, apparent growth enhancement after a dietary shift from VO to FO has also been reported in Atlantic salmon, red sea bream and Murray cod (Glencross *et al.* 2003b; Torstensen *et al.* 2004b; Turchini *et al.* 2006b).

In summary, the efficacy of a finishing period with a FO-based diet after a grow-out period during which fish have been fed with diets containing alternative lipid sources has been largely tested in Atlantic salmon (Bell *et al.* 2003a,b, 2004; Torstensen *et al.* 2004b, 2005), in temperate marine fish species (i.e. gilthead seabream, European sea bass and red seabream) (Glencross *et al.* 2003a,b; Izquierdo *et al.* 2005; Montero *et al.* 2005) and in warm water, fresh water species such as Murray cod and sunshine bass (Lane *et al.* 2006; Turchini *et al.* 2006b, 2007b). In all instances, the fatty acid make-up of fish can return to within the normal variability of fish continuously fed a FO-based diet after a period of several weeks. This finishing period needs to be, at least, sufficient to permit a duplication of fish weight. However, it has commonly been reported that the higher level of some fatty acids typical of VO, particularly LA (18:2 n-6), can persist until the end of the production cycle, despite the length of the finishing period.

In this regard, it is important to note that alternative lipid sources rich in SFA and/or MUFA, such as PO, CPO and coconut oils, TAL and animal fats, have less of a detrimental effect on the general fatty acid make-up of fish fillets than oils rich in C_{18} PUFA, such as C/RO, SBO and LO. In particular, LA is deposited at a higher

rate in fish fillets and is not easily mobilised after it has been deposited. Consequently, fish fed diets with lower concentrations of dietary C_{18} PUFA will be able to restore the original fatty acid composition of the fish fillets more effectively (Turchini *et al.* 2006b, 2007b).

In summary, although finishing diet strategies can partially ameliorate the final fatty acid make-up of fish previously fed with alternative lipid sources, they should be considered simply as a palliative solution to the problem of FO replacement in aquafeed because the final optimal fatty acid make-up of the fillets cannot be completely restored, and in attempting to do so a significant amount of FO is still required.

6 Economic and ethical aspects

It is accepted that the future and economically sustainable development of the aquaculture sector will be increasingly market driven (Josupeit *et al.* 2001) and therefore principally dependent on its ability to comply with increasing consumer expectation. The commodity price is the attribute to which consumers still pay more attention to in decision making (Vermeir & Verbeke 2006), and consequently FO replacement in aquafeed can be directly beneficial in reducing final product costs. Nevertheless, several studies have recently pointed out increasing concerns about safety and health issues (Johnsen 1991; Luten *et al.* 2003; Botonaki *et al.* 2006), and it has also been reported that environmental, organic and ethical issues are important factors that are beginning to influence the food choices of consumers (Torjusen *et al.* 2001; Pettinger *et al.* 2004). Consequently, consumers of fish and seafood products are increasingly interested in environmentally friendly produced fish, which are also expected to be safe and nourishing.

Marine FO are favoured in commercial aquafeeds as an energy source and as a major source of EFA in farmed fish that are subsequently imparted as health-beneficial n-3 fatty acids for human consumers. Although fish effectively use alternative lipid sources, such as VO, the change in the fatty acid profile of farmed fish fed these lipid sources is significant. However, Hardy (2006) correctly pointed out that comparing n-3 fatty acid levels on a percentage of total lipid basis can be misleading because farmed salmon contain more total fillet lipid than wild fish. Hardy (2006) recommended that calculations should be based on the number of grams of n-3 fatty acids in a 100 g serving of fish and marketed as such to lessen consumer confusion on the health benefits of farmed salmon fed VO-based diets.

Bureau and Gibson (2004) pointed out that because farmed fish are sold as 'generic products' as opposed to 'branded products', fish farmers have no economical or marketing incentives to maintain a certain fatty acid profile in their products. Despite some impact of alternative lipid sources on the organoleptic properties of the final product, lipid sources, such as CPO or animal fats, that contain more SFA tend to produce fish products with increased oxidative stability, thereby enhancing shelf life (Bureau & Gibson 2004; Bahurmiz & Ng 2007). Any decrease in the health benefits of consuming EPA and DHA present in FO-fed fish products can, to some extent, be partially compensated by the presence of other health benefits (i.e. vitamin-E-enriched fish products) derived from fish fed VO, such as CPO and SBO, and by the reduction of POP and other contaminants derived from FO. The deposition of tocotrienols (and other non-α-tocopherol isoforms) in fish fillets adds value to the product because the potential health benefits of tocotrienols in the human diet can include potential blood cholesterol lowering effects, anti-cancer properties, cardio-protective benefits (Sambanthamurthi *et al.* 2000) and protection against neuro-degeneration and stroke (Khanna *et al.* 2006). Further research to elucidate the potency, availability and health benefits of these natural vitamin E sources, as deposited in fish flesh, should be conducted.

Reducing FO use in aquafeeds has the added benefit of reducing the levels of POP, such as polychlorinated biphenyls and dioxins found in FO, that can be deposited in the flesh of farmed fish (Bell *et al.* 2005; Berntssen *et al.* 2005). These pollutants are known to be carcinogenic and immunosuppressive in humans. The introduction of acceptable limits for these organic pollutants by the European Union (Directive 2003/57/EC) in 2003 for various feed ingredients, including FO, means that additional costs might be incurred in the production of decontaminated FO (Tacon *et al.* 2006). With increasing consumer awareness of food safety issues, the use of VO, which generally contain much lower levels of these pollutants, can be turned into a positive benefit of eating farmed fish fed VO-based diets.

The IFFO predicted that by 2012, FO will become a strategic dietary ingredient in both agriculture and aquaculture (Jackson 2006). With demand outstripping supply and the increased cost, this might mean that FO will only be added to the diets of farmed fish at critical life stages in the culture cycle. There might come a time when consumers will have to pay a premium price for FO-fed farmed fish owing to the escalating costs and dwindling supplies of marine FO. This might be necessary for the production of specialized seafood products, such as smoked salmon, for the more discerning palate. For most consumers, the use of VO and animal fats in aquafeeds, to some degree, is inevitable if the price of farmed seafood is to be maintained at an affordable level. Despite the lack of published data and for proprietary reasons, it

is generally known that some reputable aquafeed companies are already incorporating FO alternatives into their commercial feeds. For example, SBO and CPO are commonly incorporated into aquafeeds for tropical freshwater fish and prawns, poultry fat is incorporated into tropical marine fish feeds and C/RO into salmonid feeds. The decision to use any of these alternative oils is usually dictated by their current market price (Table 2) compared with FO. At the time of publication of this review article, the market prices of CPO, SBO and C/RO have exceeded US$1000 per ton owing to rising global demands for edible oils and the expanding bio-diesel industry, making these VO presently more expensive for use in aquafeed formulations. One cheaper option available to aquafeed millers is to use by-products and waste products from VO processing, such as oleine, fatty acid distillates and residual oil found in bleaching clays. Research on the use of fatty acid distillates (Ng *et al.* 2004b; Bahurmiz & Ng 2007) and spent bleaching clays (Ng & Low 2005; Ng *et al.* 2006) from the PO refining industry has shown that these products can replace FO without compromising fish growth and feed efficiency. Despite the present higher costs of some VO, it should also be pointed out that marine FO prices have in some instances increased even more, surpassing US$2000 per ton because of very high demand from the aquafeed and other industries worldwide.

7 Conclusions

7.1 Fish oil replacement and fish growth

With regard to fish performance, it is possible to conclude that, if the EFA requirements are met, a significant portion (60–75%) of dietary FO can be substituted with alternative lipid sources without significantly affecting growth performance, feed efficiency and feed intake in almost all finfish species studied. However, different species respond in different ways to FO replacement in their diets and the above generalisation needs to be considered carefully before dietary recommendations are put forward.

In salmonids, as a result of a recent effort by European researchers, it has been shown that partial or even complete replacement of FO with alternative dietary lipid sources can be implemented without any apparent detrimental effects on fish performance over the entire production cycle. However, it is important to note that in most feeding trials, practical diets containing large amounts of fish meal were implemented (an important source of residual n-3 HUFA), and the long-term effects of the simultaneous substitution of the fish meal and FO fractions require further elucidation. Salmonids such as Atlantic salmon and rainbow trout currently account for

66.4% of the total FO used in aquaculture. However, and quite fortunately, salmonids have a lipid metabolism characterized by a 'freshwater' fish pattern in their metabolism of ALA to EPA and DHA. In addition, they are able to store fat at high concentrations in their fillets and have an extremely efficient protein-sparing capability (i.e. a highly efficient lipid utilisation capability). These characteristics are peculiar to salmonids and consequently FO replacement in these species seems to be, from a merely growth and performance viewpoint, easily and effectively implemented. However, the effects of FO replacement in other cultured species diverge slightly from the effects reported for salmonid species.

The lipid metabolism of salmonids, and in particular Atlantic salmon, is probably the most studied among farmed species and is often considered as the basic reference when other finfish species are examined. However, this assumption/generalization can lead to possible misinterpretation of the results obtained. Most studies on FO replacement in other farmed species have been, to date, implemented over a relatively short feeding period (8–16 weeks). One way ANOVA (at $P < 0.05$ and $n = 3$; often with low power) is the most commonly used statistical test in fish nutrition studies. Although few statistically significant differences have been observed in fish performance, in almost all reported studies the control fish (fed a FO-based diet) were larger than the fish fed the experimental treatments in which FO was replaced. This observation suggests that the experimental design and statistical tests commonly implemented in FO replacement studies have limited power and type II errors can be a common occurrence in these experiments. Therefore, if a larger number of replicates (Ruohonen *et al.* 2001), a longer experimental period (Shearer 2000), the use of a larger P value to detect significance differences (Shearer & Storebakken 2002) or a different statistical test (i.e regression) (Francis *et al.* 2007c) were to be implemented, the conclusions of these same experiments could be markedly different.

The use of purified or semi-purified diets or the simultaneous substitution of the fish meal fraction of the diet could be useful in future experimentation to better understand and quantify the effects of combined FO and fish meal replacement. A perfect example of this area of research has been conducted by Drew *et al.* (2007) on rainbow trout. No differences in growth performance were observed in fish fed a 100% FO-based or 100% VO-based diet containing 40% fish meal. However, when the fish meal fraction was reduced to 20%, a significant growth reduction in fish fed the VO-based diet was observed. Hence, long-term investigations on concurrent FO and fish meal substitution are required for the realisation of economical, eco-friendly aquafeeds.

7.2 Fish oil replacement and fish fatty acid composition

The most important, yet unsolved, drawback of FO replacement in aquafeed is the resultant unavoidable modification to the final n-3 HUFA make-up of the fish fillets. Therefore, for the production of farmed fish rich in n-3 HUFA, a direct source of dietary n-3 HUFA is required. This requirement, in a vicious circle, is currently derived only from wild fisheries (marine FO). Although fish are theoretically able to biosynthesize n-3 HUFA via the desaturation and elongation of ALA (found in LO and other VO), their lipid metabolism has long been adapted to the great abundance of n-3 HUFA in their natural diets and the ability to effectively use their own n-3 HUFA biosynthetic capability is insufficient.

The methods adopted globally to combat fatty acid modification of farmed fish are numerous and varied in approach. In brief, they encompass: (i) the implementation of new n-3 HUFA rich alternative lipid sources; (ii) the restoration of an optimal fatty acid profile with a finishing diet following a VO grow-out diet; (iii) the use of genetically modified n-3 HUFA rich grain crops; and (iv) the farming of transgenic fish with superior n-3 HUFA biosynthetic capabilities. Although each of these approaches has contributed valuably to the advancement of our knowledge of fish lipid metabolism, a panacea remains far from reach.

New alternative n-3 HUFA sources, although proving to be a suitable alternative to FO, are relatively limited in supply, highly expensive or continue to rely on a limited natural resource. Similarly, the implementation of a finishing strategy requires an extended time period (~16 weeks and/or at least duplication of fish body weight) and is reliant on a large quantity of FO to restore an optimal fatty acid profile. The incorporation of biotechnology in aquaculture is eminently possible and the approach of transgenic grain crops and transgenic fish has produced outstanding results, but has concurrently attracted significant negative publicity from consumer groups in relation to the use of this technology in food production.

It is clear that, despite this being one of the most significant problems currently facing the global agro-food industry, an acceptable solution to the problem of global n-3 HUFA (FO) shortages remains to be found.

In light of the above considerations and with regard to the modification of fish fatty acid make-up and the potential bioconversion of ALA to n-3 HUFA, the suggestion made by Sargent *et al.* (2002, p. 193) that 'the problem, therefore, is how to switch on the recalcitrant genes rather than to introduce them by genetic engineering', is extremely pertinent and needs to be carefully considered in planning future research activities.

As far as the effects on the final eating quality (fatty acid composition, nutritional characteristics and sensorial/organoleptic qualities) of farmed seafood are concerned, it is extremely important to note that not all alternative lipid sources are similar. Linseed oil, despite its high cost, recently attracted much attention for its high ALA content, which in fish can be potentially desaturated and elongated to EPA and DHA. However, it is now readily accepted that the increase in the transcription rate and the activity of the enzymes involved in fatty acid biosynthesis in a n-3 HUFA deficient environment is insufficient and unlikely to fulfil the gap observed in fatty acid composition resulting from decreased HUFA intake. Consequently, when FO is replaced by LO, the final fatty acid make-up of the fish is depleted in the n-3 HUFA beneficial fatty acids and the ALA content is greatly increased. Theoretically, ALA is, in humans, a precursor for EPA and DHA; thus, its intake might improve the dietary n-3/n-6 ratio, but ALA itself is not responsible for any health benefit in humans. Furthermore, ALA is extremely prone to oxidation, with detrimental effects on fish shelf life.

Optimal potential substitutes for FO should be characterised by good availability, competitive pricing and by minimal content of LA (18:2 n-6), which can be considered to be the most detrimental fatty acid from both a nutritional and sensorial viewpoint. As such, preference should be given to VO and animal fats rich in SFA, or to a lesser extent oils rich in MUFA such as PO, CPO and coconut oil or animal fats such as TAL and pork lard. In contrast, the use of SBO, SFO, C/RO, CNO and poultry by-product fat should be minimised because of the high LA content.

7.3 Closing remarks

The current finfish aquaculture industry is a dynamic food industry that is responsible for the potential fulfilment of the ever-increasing global demand for fish. However, aquaculture expansion is limited by its dependence on the use of FO, which has not increased in production globally over the past few decades. Therefore, it is imperative to find a way to uncouple the dependence of aquaculture on wild fisheries; thus, overcoming the barrier to the expansion of the aquaculture industry as a sustainable means of producing high-quality, nourishing fish. With regard to the global supply and demand of n-3 HUFA, it is important to note that the current finfish aquaculture industry is a net consumer of n-3 HUFA. Consequently, the aim of research in this sector should be to design eco-friendly, cost-effective aquafeed that ensures the optimal utilisation of fisheries-derived raw products and guarantees maximum fish growth, health and the maintenance of product quality.

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9 References

Agaba M, Tocher DR, Dickson C, Dick JR, Teale AJ (2004) A zebrafish cDNA encoding a multifunctional enzyme involved in the elongation of polyunsaturated, monounsaturated and saturated fatty acids. *Marine Biotechnology* **6**: 251–261.

Agradi E, Abrami G, Serrini G, McKenzie D, Bolis C, Bronzi P (1993) The role of dietary N-3 fatty acid and vitamin E supplements in growth of sturgeon (*Acipenser naccarii*). *Comparative Biochemistry and Physiology A* **105**: 187–195.

Alava VR (1998) Effect of salinity, dietary lipid source and level on growth of milkfish (*Chanos chanos*) fry. *Aquaculture* **167**: 229–236.

Alexis MN (1997) Fish meal and fish oil replacers in Mediterranean marine fish diets. *CIHEAM – Options Mediterraneennes* **22**: 183–204.

Almaida-Pagán PF, Hernández MD, García García B, Madrid JA, De Costa J, Mendiola P (2007) Effects of total replacement of fish oil by vegetable oils on n-3 and n-6 polyunsaturated fatty acid desaturation and elongation in sharpsnout seabream (*Diplodus puntazzo*) hepatocytes and enterocytes. *Aquaculture* **272**: 589–598.

Alvarez MJ, Diez A, Lopez-Bote C, Gallego M, Bautista JM (2000) Short-term modulation of lipogenesis by macronutrients in rainbow trout (*Onchorhynchus mykiss*) hepatocytes. *British Journal of Nutrition* **84**: 619–628.

Arnesen P, Krogdahl Å, Kristiansen IØ (1993) Lipogenic enzyme activities in liver of Atlantic salmon (*Salmo salar*, L). *Comparative Biochemistry and Physiology B* **105**: 514–546.

Arzel J, Cardinal M, Cornet J, Metailler R, Guillaume JC (1993) Nutrition of brown trout (*Salmo trutta*) reared in seawater, effect of dietary lipid on growth performances, body composition and fillet quality. *Special Publication of the European Aquaculture Society* **19**: 309.

Arzel J, Martinez Lopez FX, Metailler R, Stephan G, Viau M, Gandemer G *et al.* (1994) Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*) reared in seawater. *Aquaculture* **123**: 361–375.

Atalah E, Hernández-Cruz CM, Izquierdo MS, Rosenlund G, Caballero MJ, Valencia A *et al.* (2007) Two microalgae *Crypthecodinium cohnii* and *Phaeodactylum tricornutum* as alternative source of essential fatty acids in starter feeds for seabream (*Sparus aurata*). *Aquaculture* **270**: 178–185.

Austreng E, Skrede A, Eldegard A (1980) Digestibility of fat and fatty acids in rainbow trout and mink. *Aquaculture* **19**: 93–95.

Autin M (1997) Commercial aquafeed manufacture and production. *Cahiers Options Mediterraneennes* **22**: 79–104.

Babin PJ, Vernier JM (1989) Plasma lipoproteins in fish. *Journal of Lipid Research* **30**: 467–489.

Bahurmiz OM, Ng WK (2007) Effects of dietary palm oil source on growth, tissue fatty acid composition and nutrient digestibility of red hybrid tilapia, *Oreochromis* sp., raised from stocking to marketable size. *Aquaculture* **262**: 382–392.

Bang HO, Dyerberg J, Nielsen AB (1971) Plasma lipid and lipoprotein pattern in Greenlandic west-coast eskimos. *Lancet* **1**: 1143–1145.

Basiron Y (2007) Palm oil production through sustainable plantations. *European Journal of Lipid Science and Technology* **109**: 289–295.

Belitz HD, Grosch W (1999) *Food Chemistry*. Springer-Verlag, Berlin.

Bell JG, Raynard RS (1990) Effects of a high linoleic acid diet on the fatty acid composition of leucocytes from Atlantic salmon (*Salmo salar*). *Transactions of the Biochemical Society* **18**: 911–912.

Bell JG, Raynard RS, Sargent JR (1991a) The effect of dietary linoleic acid on the fatty acid composition of individual phospholipids and lipoxygenase products from gills and leucocytes of Atlantic salmon (*Salmo salar*). *Lipids* **26**: 445–450.

Bell JG, Mc Vicar AH, Park MT, Sargent JR (1991b) High dietary linoleic acid affects the fatty acid compositions of individual phospholipids from tissues of Atlantic salmon (*Salmo salar*): association with stress susceptibility and cardiac lesions. *The Journal of Nutrition* **121**: 1163–1172.

Bell JG, Sargent JR, Raynard RS (1992) Effects of increasing dietary linoleic acid on phospholipid fatty acid composition and eicosanoid production in leucocytes and gill cells of Atlantic salmon (*Salmo salar*). *Prostaglandins, Leukotrienes and Essential Fatty Acids* **45**: 197–206.

Bell JG, Dick JR, Mc Vicar AH, Sargent JR, Thompson KD (1993) Dietary sunflower, linseed and fish oils affect phospholipid fatty acid composition, development of cardiac lesions, phospholipase activity and eicosanoid production in Atlantic salmon. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **49**: 665–673.

Bell JG, Farndale BM, Dick JR, Sargent JR (1996) Modification of membrane fatty acid composition, eicosanoid production, and phospholipase A activity in Atlantic salmon (*Salmo salar*) gill and kidney by dietary lipid. *Lipids* **31**: 1163–1171.

Bell JG, Tocher DR, Farndale BM, Cox DI, McKinney RW, Sargent JR (1997) The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr–smolt transformation. *Lipids* 35: 515–525.

Bell JG, McEvoy J, Webster JL, McGhee F, Millar RM, Sargent JR (1998) Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar*). *Journal of Agriculture and Food Chemistry* 46: 119–127.

Bell JG, McEvoy J, Tocher DR, McGhee F, Campbell PJ, Sargent JR (2001) Replacement of fish oil with rape seed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *The Journal of Nutrition* 131: 1535–1543.

Bell JG, Henderson RJ, Tocher DR, McGhee F, Dick JR, Porter A *et al.* (2002) Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. *The Journal of Nutrition* 132: 222–230.

Bell JG, McGhee F, Campbell PJ, Sargent JR (2003a) Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". *Aquaculture* 218: 515–528.

Bell JG, Tocher DR, Henderson RJ, Dick JR, Crampton VO (2003b) Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *The Journal of Nutrition* 133: 2793–2801.

Bell JG, Henderson RJ, Tocher DR, Sargent JR (2004) Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid composition in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. *Lipids* 39: 223–232.

Bell JG, McGhee F, Dick JR, Tocher DR (2005) Dioxin and dioxin-like polychlorinated biphenyls (PCBs) in Scottish farmed salmon (*Salmo salar*): effects of replacement of dietary marine fish oil with vegetable oils. *Aquaculture* 243: 305–314.

Bell JG, Strachan F, Good JE, Tocher DR (2006) Effect of dietary echium oil on growth, fatty acid composition and metabolism, gill prostaglandin production and macrophage activity in Atlantic cod (*Gadus morhua* L.). *Aquaculture Research* 37: 606–617.

Bendiksen EÅ, Berg OK, Jobling M, Arnesen AM, Måsøval K (2003) Digestibility, growth and nutrient utilisation of Atlantic salmon (*Salmo salar* L.) in relation to temperature, feed fat content and oil source. *Aquaculture* 224: 283–299.

Benedito-Palos L, Saera-Vila A, Calduch-Giner JA, Kaushik S, Pérez-Sánchez J (2007) Combined replacement of fish meal and oil in practical diets for fast growing juveniles of gilthead sea bream (*Sparus aurata* L.): networking of systemic and local components of GH/IGF axis. *Aquaculture* 267: 199–212.

Benzie IFF (1996) Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *International Journal of Food Science and Nutrition* 47: 233–261.

Berge RK, Madsen L, Vaagenes H, Tronstad KJ, Göttlicher M, Rustan AC (1999) In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. *The Biochemical Journal* 343: 191–197.

Berntssen MHG, Lundebye AK, Torstensen BE (2005) Reducing the levels of dioxins and dioxin-like PCBs in farmed Atlantic salmon by substitution of fish oil with vegetable oil in the feed. *Aquaculture Nutrition* 11: 219–231.

Berntssen MHG, Torstensen BE, Julshamn K, Lundebye A-K (2007) Organochlorine pesticides in farmed Atlantic salmon reared on vegetable oil- or fish oil-based feeds. EAS Congress Proceedings, Aquaculture Europe 2007 – "Competing Claims"; 25–27 October 2007, Istanbul, Turkey.

Bimbo AP (2000) Fish meal and oil. In: Martin RE, Carter EP, Flick GJ, Davis LM (eds) *Marine and Freshwater Products Handbook*, pp. 541–581. Technomic Publishing, Lancester.

Boonyaratpalin M, Thongrod S, Supamattaya K, Britton G, Schlipalius LE (2001) Effects of beta-carotene source, *Dunaliella salina*, and astaxanthin on pigmentation, growth, survival and health of *Penaeus monodon*. *Aquaculture Research* 32: 182–190.

Botonaki A, Polymeros K, Tsakiridou E, Mattas K (2006) The role of food quality certification on consumers' food choices. *British Food Journal* 108: 77–90.

Bransden MP, Carter CG, Nichols PD (2003) Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comparative Biochemistry and Physiology B* 135: 611–625.

Brindley DN (1985) Absorption and metabolism of fat. In: Padley FB, Podmore J (eds) *The Role of Fats in Human Nutrition*, pp. 35–47. Ellis Horwood, Chichester.

Brodtkorb BT, Rosenlund G, Lie Ø (1997) Effects of 20:5n-3 and 22:6n-3 on tissue lipid composition in juvenile Atlantic salmon, *Salmo salar*, with emphasis on brain and eye. *Aquaculture Nutrition* 3: 175–187.

Brown AM, Castle J, Hebbachi AM, Gibbons GF (1999) Administration of n-3 fatty acids in the diets of rats directly to hepatocyte cultures results in different effects on hepatocellular ApoB metabolism and secretion. *Arteriosclerosis, Thrombosis and Vascular Biology* 19: 106–114.

Bureau D, Gibson J (2004) Animal fats as aquaculture feed ingredients: nutritive value, product quality and safety. *Aquafeed International* 7: 32–37.

Bureau DP, Gibson J, El-Mowafi A (2002) Review: Use of animal fats in aquaculture feeds. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Gaxiola-Cortés MG, Simoes N (eds) *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola*; 3–7 Sep 2002, Cancún, Quintana Roo, México.

Caballero MJ, Obach A, Rosenlund G, Montero D, Gisvold M, Izquierdo M (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition

and histology of rainbow trout, *Onchorhynchus mykiss*. *Aquaculture* **214**: 253–271.

Caballero MJ, Izquierdo M, Kjørsvik E, Montero D, Socorro J, Fernández AJ et al. (2003) Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. *Aquaculture* **214**: 325–340.

Caballero MJ, Izquierdo MS, Kjørsvik E, Fernández AJ, Rosenlund G (2004) Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. *Journal of Fish Diseases* **27**: 531–541.

Cao JM, Liu YJ, Lao CL, Tian LX, Liang GY (1997) Effect of dietary fatty acids on tissue lipid content and fatty acid composition of grass carp. *Acta Zoonurimenta Sinica* **9**: 36–44.

Caprino F, Moretti VM, Bellagamba F, Turchini GM, Busetto ML, Giani I et al. (2008) Fatty acid composition and volatile compounds of caviar from farmed white sturgeon (*Acipenser transmontanus*). *Analytica Chimica Acta* **617**: 139–147.

Carter CG, Bransden MP, Lewis TE, Nichols PD (2003) Potential of Thraustochytrids to partially replace fish oil in Atlantic salmon feeds. *Marine Biotechnology* **5**: 480–492.

Chou BS, Shiau SY (1996) Optimal dietary lipid level for growth of juvenile hybrid tilapia, *Oreochromis niloticus × Oreochromis aureus*. *Aquaculture* **143**: 185–195.

Clark RM, Furr HC (2001) Absorption of canthaxanthin by the rat is influenced by total lipid in the intestinal lumen. *Lipids* **36**: 473–475.

Clark RM, Yao L, She L, Furr HC (2000) A comparison of lycopene and astaxanthin absorption from corn oil and olive oil emulsions. *Lipids* **35**: 803–806.

Coe NR, Bernlohr DA (1998) Physiological properties and functions of intracellular fatty acid-binding proteins. *Biochimica et Biophysica Acta* **1391**: 287–306.

Coello MT, Sánchez MJ, Vicario IM, Heredia FJ, Martín M (1999) Assessing the acceptability of eel (*Anguilla anguilla*) fed three different diets. *Journal of the Science of Food and Agriculture* **79**: 2087–2093.

Craig SR (2004) Organic aquaculture feeds. *Aqua Feeds: Formulation & Beyond* **1** (3): 11–13.

Craig SR, Gatlin MI (1995) Coconut oil and beef tallow, but not tricaprylin, can replace menhaden oil in the diet of red drum (*Sciaenops ocellatus*) without adversely affecting growth or fatty acid composition. *The Journal of Nutrition* **125**: 3041–3048.

Cravedi JP, Choubert G, Delous G (1987) Digestibility of chloraphenicol, oxolinic acid and oxytetracycline in rainbow trout and the influence of these antibiotics on lipid digestibility. *Aquaculture* **60**: 133–141.

Crockett E, Sidell BD (1993) Peroxisomal beta-oxidation is a significant pathway for catabolism of fatty acids in a marine teleost. *The American Physiology Society* **33**: 1004–1009.

Cunnane SC (2003) Problems with essential fatty acids: time for a new paradigm? *Progress in Lipid Research* **42**: 544–568.

De Silva SS, Anderson TA (1995) *Fish Nutrition in Aquaculture*. Chapman & Hall, London.

Drew MD, Ogunkoya AE, Janz DM, Van Kessel AG (2007) Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **167**: 260–268.

Drobná Z, Zelenka J, Mrkvicová E, Kladroba D (2006) Influence of dietary linseed and sunflower oil on sensory characteristics of rainbow trout (*Oncorhynchus mykiss*). *Czech Journal of Animal Science* **51**: 475–482.

Du ZY, Liu YJ, Tian LX, Wang JT, Wang Y, Liang GY (2005) Effect of dietary lipid level on growth, feed utilization and body composition by juvenile grass carp (*Ctenopharyngodon idella*). *Aquaculture Nutrition* **11**: 139–146.

Du ZY, Clouet P, Huang LM, Degrace P, Zheng WH, He JG et al. (2008) Utilization of different dietary lipid sources at high level in herbivorous grass carp (*Ctenopharyngodon idella*): mechanism related to hepatic fatty acid oxidation. *Aquaculture Nutrition* **14**: 77–92.

Durnford E, Shahidi F (1998) Flavour of fish meat. In: Shahidi F (ed.) *Flavor of Meat, Meat Products and Seafood*, pp. 130–158. Blackie Academic and Professional, London.

Easton MDL, Luszniak D, Von der Geest E (2002) Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. *Chemosphere* **46**: 1053–1074.

Erdal JI, Evensen O, Kaurstad OK, Lillehaug A, Solbakken R, Thorud K (1991) Relationship between diet and immune response in Atlantic salmon (*Salmo salar* L.) after feeding various levels of ascorbic acid and omega-3 fatty acids. *Aquaculture* **98**: 363–379.

European Commission (2002) European Commission Council Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. *Official Journal of the European Communities*. L 140/10.

European Commission (2003) *The Use of Fish By-products in Aquaculture*. Report of the Scientific Committee on Animal Health and Animal Welfare. European Commission, Brussels.

FAO (Food and Agriculture Organization of the United Nations) (2006) *FISHSTAT Plus: Universal Software for Fishery Statistical Time Series, Version 2.3*. FAO Fisheries Department, Fishery Information, Data and Statistical Unit, Rome.

FAO (Food and Agriculture Organization of the United Nations) (2007a). *State of World Aquaculture 2006*. FAO Fisheries Technical Paper No. 500. FAO Fisheries Department; Food and Agriculture Organization of the United Nations, Rome.

FAO (Food and Agriculture Organization of the United Nations) (2007b) *Use of Wild Fish and/or Other Aquatic Species to Feed Cultured Fish and its Implications to Food Security and Poverty Alleviation*. FAO Expert Workshop. FAO Fisheries Department, Food and Agriculture Organization of the United Nations and Marine Export Development

Authority, Government of India, 16–18 November 2007, Kochi, India.

Figueiredo-Silva A, Rocha E, Dias J, Silva P, Rema P, Gomes E et al. (2005) Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (Dicentrarchus labrax) and rainbow trout (Oncorhynchus mykiss) juveniles. Aquaculture Nutrition 11: 147–155.

Fracalossi DM, Lovell RT (1994) Dietary lipid sources influence responses of channel catfish (Ictalurus punctatus) to challenge with the pathogen Edwardsiella ictaluri. Aquaculture 119: 287–298.

Fracalossi DM, Lovell RT (1995) Growth and liver polar fatty acid composition of year-1 channel catfish fed various lipid sources at two water temperatures. The Progressive Fish Culturist 57: 107–113.

Francesconi KA, Kuehnelt D (2002) Arsenic compounds in the environment. In: Frankenberger WT Jr (ed.) Environmental Chemistry of Arsenic, pp. 51–94. Marcel Dekker, New York.

Francis DS, Turchini GM, Jones PL, De Silva SS (2006) Effects of dietary oil source on the growth and muscle fatty acid composition of Murray cod, Maccullochella peelii peelii. Aquaculture 253: 547–556.

Francis DS, Turchini GM, Jones PL, De Silva SS (2007a) Dietary lipid source modulates in vivo fatty acid metabolism in the freshwater fish, Murray cod (Maccullochella peelii peelii). Journal of Agricultural and Food Chemistry 55: 1582–1591.

Francis DS, Turchini GM, Jones PL, De Silva SS (2007b) Effects of fish oil substitution with a mix blend vegetable oil on nutrient digestibility in Murray cod, Maccullochella peelii peelii. Aquaculture 269: 447–455.

Francis DS, Turchini GM, Jones PL, De Silva SS (2007c) Growth performance, feed efficiency and fatty acid composition of juvenile Murray cod, Maccullochella peelii peelii, fed graded levels of canola and linseed oil. Aquaculture Nutrition 13: 335–350.

Froese R, Pauly D (2007) FishBase. [Cited 7 Nov 2007.] Available from URL: http://www.fishbase.org.

Frohnert BI, Bernlohr DA (2000) Regulation of fatty acid transporters in mammalian cells. Progress in Lipid Research 39: 83–107.

Frøyland L, Vaagenes H, Asiedu DK, Garras A, Lie Ø, Berge RK (1996) Chronic administration of eicosapentaenoic acid and docosahexaenoic acid as ethyl esters reduced plasma cholestrol and changed the fatty acid composition in rat blood and organs. Lipids 31: 169–178.

Frøyland L, Madsen L, Vaagenes H, Totland GK, Auwerx J, Kryvi H et al. (1997) Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. Journal of Lipid Research 38: 1851–1858.

Frøyland L, Lie Ø, Berge RK (2000) Mitochondrial and peroxisomal beta-oxidation capacities in various tissues from Atlantic salmon Salmo salar. Aquaculture Nutrition 6: 85–89.

Furuhashi M, Ura N, Murakami H, Hyakukoku M, Yamaguchi K, Higashiura K et al. (2002) Fenofibrate improves insulin sensitivity in connection with intramuscular lipid content, muscle fatty acid-binding protein, and beta-oxidation in skeletal muscle. Journal of Endocrinology 174: 321–329.

Gaber MMA (1996) Effect of oil source on growth, feed conversion and body composition of Nile tilapia (Oreochromis niloticus). Annals of Agricultural Science 34: 1549–1562.

Ganga R, Bell JG, Montero D, Robaina L, Caballero MJ, Izquierdo MS (2005) Effect of dietary lipids on plasma fatty acid profiles and prostaglandin and leptin production in gilthead seabream (Sparus aurata). Comparative Biochemistry and Physiology B 142: 410–418.

Ganuza E, Benítez-Santana T, Atalah E, Vega-Orellana O, Ganga R, Izquierdo MS (2008) Crypthecodinium cohnii and Schizochytrium sp. as potential substitutes to fisheries-derived oils in seabream (Sparus aurata) microdiets. Aquaculture 277: 109–116.

Geurden I, Cuvier A, Gondouin E, Olsen RE, Ruohonen K, Kaushik S et al. (2005) Rainbow trout can discriminate between feeds with different oil sources. Physiology & Behaviour 85: 107–114.

Geurden I, Corraze G, Boujard T (2007) Self-feeding behaviour of rainbow trout, Oncorhynchus mykiss, offered diets with distinct feed oils. Applied Animal Behavioural Science 108: 313–326.

Gilman CI, Leusch FDL, Breckenridge WC, MacLathcy DL (2003) Effects of a phytosterol mixture on male plasma lipoprotein fractions and testis P450scc activity. General and Comparative Endocrinology 130: 172–184.

Gjøen T, Berg T (1993) Interaction of low density lipoproteins with liver cells in rainbow trout. Fish Physiology and Biochemistry 10: 465–473.

Glencross BD, Hawkins WE, Curnow JG (2003a) Evaluation of canola oils as alternative lipid resources in diets for juvenile red seabream, Pagrus auratus. Aquaculture Nutrition 9: 305–315.

Glencross BD, Hawkins WE, Curnow JG (2003b) Restoration of the fatty acid composition of red seabream (Pagrus auratus) using a fish oil finishing diet after grow-out on plant oil based diets. Aquaculture Nutrition 9: 409–418.

Globefish (2006) Fish Info Network. [Cited 9 May 2007.] Available from URL: http://www.globefish.org.

Gordon MH, Miller LAD (1997) Development of steryl ester analysis for the detection of admixtures of vegetable oils. Journal of the American Oil Chemists' Society 74: 505–510.

Greene DHS (1990) Lipid metabolismin fish. In: Stansby ME (ed.) Fish Oils in Nutrition, pp. 226–246. Nostrand Reinhold, New York.

Greene DHS, Selivonchick DP (1990) Effects of dietary vegetable, animal and marine lipids on muscle lipid and haematology of rainbow trout (Oncorhynchus mykiss). Aquaculture 89: 165–182.

Grisdale-Helland B, Ruyter B, Rosenlund G, Obach A, Helland SJ, Sandberg MG et al. (2002) Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen

consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. *Aquaculture* **207**: 311–329.

Grove TJ, Sidell BD (2004) Fatty acyl CoA synthetase from Antarctic notothenioid fishes may influence substrate specificity of fat oxidation. *Comparative Biochemistry and Physiology B* **139**: 53–63.

Guil-Guerrero JL (2007) Stearidonic acid (18:4n-3): metabolism, nutritional importance, medical uses and natural sources. *European Journal of Lipid Science and Technology* **109**: 1226–1236.

Guillou A, Soucy P, Khalil M, Adambounou L (1995) Effects of dietary vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook charr (*Salvelinus fontinalis*). *Aquaculture* **136**: 351–362.

Gunasekera RM, Leelarasamee K, De Silva SS (2002) Lipid and fatty acid digestibility of three oil types in the Australian shortfin eel, *Anguilla australis*. *Aquaculture* **203**: 335–347.

Gunstone FD, Harwood JL, Padley FB (1994) *The Lipid Handbook*. The University Press, Cambridge.

Gurr MI, Harwood JL (1991) *Lipid Biochemistry*, 4th edn. Chapman & Hall, London.

Halvorsen B, Rustan AC, Madsen L, Reseland J, Berge RK, Sletnes P *et al.* (2001) Effects of long-chain monounsaturated and n-3 fatty acids on fatty acid oxidation and lipid composition in rats. *Annals of Nutrition and Metabolism* **45**: 30–37.

Hamilton JA (1998) Fatty acid transport: difficult or easy? *The Journal of Lipid Research* **39**: 467–481.

Hardy RW (2006) Fish oil replacement shift fatty acid content of farmed fish. *Global Aquaculture Advocate* **9**: 48–49.

Hardy RW, Scott TM, Harrell LW (1987) Replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens. *Aquaculture* **65**: 267–277.

Harris WS, Connor WE, McMurphy MP (1983) The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils. *Metabolism* **32**: 179–184.

Hastings N, Agaba M, Tocher DR, Leaver MJ, Dick JR, Sargent JR *et al.* (2001) A vertebrate fatty acid desaturase with delta 5 and delta 6 activities. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 14 304–14 309.

Hastings N, Agaba MK, Tocher DR, Zheng X, Dickson CA, Dick JR *et al.* (2004) Molecular cloning and functional characterization of fatty acyl desaturase and elongase cDNAs involved in the production of eicosapentaenoic and docosahexaenoic acids from alpha-linolenic acid in Atlantic salmon (*Salmo salar*). *Marine Biotechnology* **6**: 463–474.

Haugen T, Kiessling A, Olsen RE, Rora MB, Slinde E, Nortvedt R (2006) Seasonal variations in muscle growth dynamics and selected quality attributes in Atlantic halibut (*Hippoglossus hippoglossus* L.) fed dietary lipids containing soybean and/or herring oil under different rearing regimes. *Aquaculture* **261**: 565–579.

Hemre GI, Sandnes K (1999) Effect of dietary lipid level on muscle composition in Atlantic salmon *Salmo salar*. *Aquaculture Nutrition* **5**: 9–16.

Henderson RJ (1996) Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archives of Animal Nutrition* **49**: 5–22.

Henderson RJ, Sargent JR (1985) Chain-length specificities of mitochondrial and peroxisomal β-oxidation of fatty acids in livers of rainbow trout (*Salmo gairdneri*). *Comparative Biochemistry and Physiology B* **82**: 79–85.

Henderson RJ, Tocher DR (1987) The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* **26**: 281–347.

Henderson RJ, Park MT, Sargent JR (1994) The desaturation and elongation of 14C-labelled polyunsaturated fatty acids by pike (*Esox lucius* L.) in vivo. *Fish Physiology and Biochemistry* **14**: 223–235.

Herrmann T, Buchkremer F, Gosch I, Hall AM, Bernlohr DA, Stremmel W (2001) Mouse fatty acid transport protein 4 (FATP4): Characterization of the gene and functional assessment as a very long chain acyl-CoA synthetase. *Gene* **270**: 31–40.

Hertrampf JW, Piedad-Pascual F (2000) *Handbook on Ingredients for Aquaculture Feeds*. Kluwer Academic Publishers, Dordrecht.

Hertzel AV, Bernlohr DA (2000) The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends in Endocrinology and Metabolism* **11**: 175–180.

Higgs DA, Dong FM (2000) Lipids and fatty acids. In: Stickney RR (ed.) *Encyclopedia of Aquaculture*, pp. 476–496. John Wiley & Sons, New York.

de la Higuerra M (2001) Effects of nutritional factor and feed characteristics on feed intake. In: Houlihan D, Boujard T, Jobling M (eds) *Food Intake in Fish*, pp. 250–268. Blackwell Publishing, Oxford.

Hites RA, Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ (2004) Global assessment of organic contaminants in farmed salmon. *Science* **303**: 226–229.

Hoffman LC, Prinsloo JF (1995) The influence of different dietary lipids on the growth and body composition of the African sharptooth catfish, *Clarias gariepinus* (Burchell). *South African Journal of Science* **91**: 315–320.

Hu CJ, Chen SM, Pan CH, Huang CH (2006) Effects of dietary vitamin A or beta-carotene concentrations on growth of juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture* **253**: 602–607.

Huang CH, Huang MC, Hou PC (1998) Effect of dietary lipids on fatty acid composition and lipid peroxidation in sarcoplasmic reticulum of hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. *Comparative Biochemistry and Physiology B* **120**: 331–336.

Huang SSY, Oo AN, Higgs DA, Brauner CJ, Satoh S (2007) Effect of dietary canola oil level on the growth performance and fatty acid composition of juvenile red sea bream, *Pagrus major*. *Aquaculture* **271**: 420–431.

Isosaari P, Vartiainen T, Hallikainen AKR (2002) Feeding trial on rainbow trout: comparison of dry fish feed and Baltic herring as a source of PCDD/Fs and PCBs. *Chemosphere* **48**: 795–804.

Isosaari P, Kiviranta H, Lie O, Lundebye A, Ritchie G, Vartiainen T (2004) Accumulation and distribution of polychlorinated dibenzo-p-dioxin, dibenzofuran, and polychlorinated biphenyl congeners in Atlantic salmon (*Salmo salar*). *Environmental Toxicology and Chemistry* **23**: 1672–1679.

Izquierdo MS, Obach A, Arantzamendi L, Montero D, Robaina L, Rosenlund G (2003) Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. *Aquaculture Nutrition* **9**: 397–407.

Izquierdo MS, Montero D, Robaina L, Caballero R, Rosenlund G, Ginés R (2005) Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* **250**: 431–444.

Jackson A (2006) The importance of fishmeal and fish oil in aquaculture diets. *International Aquafeed* (November–December) 16–19.

Jacobs MN, Covaci A, Schepens P (2002) Investigation of selected persistent organic pollutants in farmed Atlantic salmon (*Salmo salar*), salmon aquaculture feed, and fish oil components of the feed. *Environmental Science & Technology* **36**: 2797–2805.

Jobling M (2003) Do changes in Atlantic salmon, *Salmo salar* L., fillet fatty acids following a dietary switch represent wash-out or dilution? Test of a dilution model and its application *Aquaculture Research* **34**: 1215–1221.

Jobling M (2004a) ''Finishing'' feeds for carnivorous fish and the fatty acid dilution model. *Aquaculture Research* **35**: 706–709.

Jobling M (2004b) Are modifications in tissue fatty acid profiles following a change in diet the result of dilution? Test of a simple dilution model. *Aquaculture* **232**: 551–562.

Jobling M, Covés D, Damsgård B, Kristiansen HR, Koskela J, Petursdottir TE *et al.* (2001) Techniques for measuring feed intake. In: Houlihan D, Boujard T, Jobling M (eds) *Food Intake in Fish*, pp. 49–87. Blackwell Science, Oxford.

Jobling M, Larsen AV, Andreassen B, Olsen RE (2002) Adiposity and growth of post-smolt Atlantic salmon *Salmo salar* L. *Aquaculture* **33**: 533–541.

Johnsen PB (1991) Aquaculture product quality issues – market position opportunities under mandatory seafood inspection regulations. *Journal of Animal Science* **69**: 4209–4215.

Johnsen RI, Grahl-Nielsen O, Roem A (2000) Relative absorption of fatty acids by Atlantic salmon *Salmo salar* from different diets, as evaluated by multivariate statistics. *Aquaculture Nutrition* **6**: 255–261.

Jones PL, Sidell BD (1982) Metabolic responses of striped bass (*Morone saxatilis*) to temperature acclimation. II. Alterations in metabolic carbon sources and distributions of fiber types in locomotory muscle. *Journal of Experimental Zoology* **219**: 163–171.

Jones FV, Strawn K (1983) Growth and food utilization of caged Atlantic croaker and striped mullet reared on various lipid diets in a heated water system. *Journal of the World Mariculture Society* **14**: 590–594.

Jordal A-EO, Torstensen BE, Tsoi S, Tocher DR, Lall SP, Douglas S (2005) Small scale cDNA microarray analysis of expression of genes for lipid metabolism in liver of atlantic salmon (*Salmo salar* L.) – effect of dietary rapeseed oil replacement. *The Journal of Nutrition* **35**: 2355–2361.

Jordal A-EO, Hordvik I, Pelsers M, Bernlohr DA, Torstensen BE (2006) FABP3 and FABP10 in Atlantic salmon (*Salmo salar* L.), general effects of dietary fatty acid composition, and life cycle variations. *Comparative Biochemistry and Physiology B* **145**: 147–158.

Jordal A-EO, Lie Ø, Torstensen BE (2007) Complete replacement of dietary fish oil with a vegetable oil blend affect liver lipid and plasma lipoprotein levels in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition* **13**: 114–130.

Josephon DB, Lindsaly RC, Stuiber DA (1984) Variations in the occurrence of enzymatically derived volatile aroma compounds in salt and freshwater fish. *Journal of Agricultural and Food Chemistry* **32**: 1344–1361.

Josupeit H, Lem A, Lupin H (2001) Aquaculture products: quality, safety, marketing and trade. In: Subasinghe RP, Bueno P, Phillips MJ, Hough C, McGladdery SE, Artur JR (eds) *Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium*, pp. 249–257; 20–25 Feb 2000, Bangkok, Thailand. NACA, Bangkok and FAO, Rome.

Jutfelt F, Olsen RE, Björnsson BT, Sundell K (2007) Parr–smolt transformation and dietary vegetable lipids affect intestinal nutrient uptake, barrier function and plasma cortisol levels in Atlantic salmon. *Aquaculture* **273**: 298–311.

Kalogeropoulos N, Alexis MN, Henderson RJ (1992) Effects of dietary soybean and cod-liver oil levels on growth and body composition of gilthead bream (*Sparus aurata*). *Aquaculture* **104**: 293–308.

Kanazawa A, Teshima S, Sakamoto M, Awal MA (1980) Requirements of *Tilapia zillii* for essential fatty acids. *Bulletin of the Japanese Society for Scientific Fisheries* **46**: 1353–1356.

Karalazos V, Bendiksen EÅ, Dick JR, Bell JG (2007) Effects of dietary protein, and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (*Salmo salar* L.) reared at low water temperatures. *Aquaculture Nutrition* **13**: 256–265.

Karl H, Kuhlmann H, Ruoff U (2003) Transfer of PCDDs and PCDFs into the edible parts of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), via feed. *Aquaculture Research* **34**: 1009–1014.

Kasumayan AO, Døving KB (2003) Taste preferences in fishes. *Fish Fisheries* **4**: 289–347.

Kaushik S (2002) European sea bass, *Dicentrarchus labrax*. In: Webster CD (ed.) *Nutrient Requirements and Feeding of Finfish for Aquaculture*, pp. 28–39. CABI Publishing, Auburn.

Kaway T (1996) Fish flavour. *Critical Reviews in Food Science and Nutrition* **36**: 257–298.

Kendrick JS, Higgins JA (1999) Dietary fish oils inhibit early events in the assembly of very low density lipoproteins and target ApoB for degradation within the rough endoplasmatic reticulum of hamster hepatocytes. *The Journal of Lipid Research* **40**: 504–514.

Kennish JM, Sharp-Dahl JL, Chambers KA, Thrower F, Rice SD (1992) The effect of herring diet on lipid composition, fatty acid composition, and cholesterol levels in the muscle tissue of pen-reared chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* **108**: 309–322.

Khanna S, Roy S, Parinandi NL, Maurer M, Sen CK (2006) Characterization of the potent neuroprotective properties of the natural vitamin E alpha-tocotrienol. *Journal of Neurochemistry* **98**: 1474–1486.

Kiessling K-H, Kiessling A (1993) Selective utilization of fatty acids in rainbow trout (*Onchorhychus mykiss* Walbaum) red muscle mitochondria. *Canadian Journal of Zoology* **71**: 248–251.

Kissil GW, Youngson A, Cowey CB (1987) Capacity of the European eel (*Anguilla anguilla*) to elongate and desaturate dietary linoleic acid. *The Journal of Nutrition* **117**: 1379–1384.

Kletzien RF, Harris PK, Foellmi LA (1994) Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue specific regulation by hormones, nutrients and oxidant stress. *The FASEB Journal* **8**: 174–181.

Koshio S, Ackman RG, Lall SP (1994) Effects of oxidized herring and canola oils in diets on growth, survival and flavor of Atlantic salmon, *Salmo salar*. *Journal of Agricultural and Food Chemistry* **42**: 1164–1169.

Koven W (2002) Gilt-head sea bream, *Sparus aurata*. In: Webster CD (ed.) *Nutrient Requirements and Feeding of Finifsh for Aquaculture*, pp. 64–78. CABI Publishing, Auburn.

Kryvi H, Totland GH (1997) Fordøyelsessystemet. In: Kryvi H, Totland GH (eds) *Fiskeanatomi*, pp. 127–143. Høyskoleforlaget, Kristiansand.

Lamb CF (2001) Gustation and feeding behaviour. In: Houlihan D, Boujard T, Jobling M (eds) *Food Intake in Fish*, pp. 108–130. Blackwell Science, Oxford.

Lane RL, Trushenski JT, Kohler CC (2006) Modification of fillet composition and evidence of differential fatty acid turnover in sunshine bass *Morone chrysops × M. saxatilis* following change in dietary lipid source. *Lipids* **41**: 1029–1038.

Lang CA, Davis RA (1990) Fish oil fatty acids impair VLDL assembly and/or secretion by cultured rat hepatocytes. *The Journal of Lipid Research* **31**: 2079–2086.

Legendre M, Kerdchuen N, Corraze G, Bergot P (1995) Larval rearing of an African catfish, *Heterobranchus longifilis* (Teleostei, Clariidae): effect of dietary lipids on growth, survival and fatty acid composition of fry. *Aquatic Living Resources* **8**: 355–363.

Li MH, Wise DJ, Johnson MR, Robinson EH (1994) Dietary menhaden oil reduced resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri*. *Aquaculture* **128**: 335–344.

Liang X-F, Ogata HY, Oku H (2002) Effect of dietary fatty acids on lipoprotein lipase gene expression in the liver and visceral adipose tissue of fed and starved red sea bream *Pagrus major*. *Comparative Biochemistry and Physiology A* **132**: 913–919.

Lie Ø (2001) Flesh quality – the role of nutrition. *Aquaculture Research* **32**: 341–348.

Lie Ø, Lied E, Lambertsen G (1986) Liver retention of fat and of fatty acids in cod (*Gadus morhua*) fed different oils. *Aquaculture* **59**: 187–196.

Lie Ø, Sandvin A, Waagbø R (1993) Influence of dietary fatty acids on the lipid composition of lipoproteins in farmed atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* **12**: 249–260.

Lim PK, Boey PL, Ng WK (2001) Dietary palm oil level affects growth performance, protein retention and tissue vitamin E concentration of African catfish, *Clarias gariepinus*. *Aquaculture* **202**: 101–112.

Lin YH, Shiau SY (2007) Effects of dietary blend of fish oil with corn oil on growth and non-specific immune responses of grouper, *Epinephelus malabaricus*. *Aquaculture Nutrition* **13**: 137–144.

Lin HZ, Liu YJ, He JG, Zheng WH, Tian LX (2007) Alternative vegetable lipid sources in diets for grouper, *Epinephelus coioides* (Hamilton): effects on growth, and muscle and liver fatty acid composition. *Aquaculture Research* **38**: 1605–1611.

Lindberg A, Olivecrona G (2002) Lipoprotein lipase from rainbow trout differs in several aspects from the enzyme in mammals. *Gene* **292**: 213–223.

Londraville RL, Duvall CS (2002) Murine leptin injections increase intracellular fatty acid-binding protein in green sunfish (*Leopomis cyanellus*). *General and Comparative Endocrinology* **129**: 56–62.

Londraville RL, Sidell BD (1996) Cold acclimation increase fatty acid-binding protein concentration in aerobic muscle of striped bass, *Morone saxatilis*. *Journal of Experimental Zoology* **175**: 36–44.

Lundebye AK, Berntssen MHG, Lie Ø, Ritchie G, Isosaari P, Kiviranta H *et al.* (2004) Dietary uptake of dioxins (PCDD/PCDFs) and dioxin-like PCBs in Atlantic salmon (*Salmo salar*). *Aquaculture Nutrition* **10**: 199–207.

Luten JB, Oehlenschläger J, Ólafsdóttir G (2003) *Quality of Fish from Catch to Consumer. Labelling, Monitoring and Traceability*. Wageningen Academic Publishers, Wageningen.

Luzzana U, Scolari M, Campo Dall'Orto B, Caprino F, Turchini G, Orban E *et al.* (2003) Growth and product quality of European eel (*Anguilla anguilla*) as affected by dietary protein and lipid sources. *Journal of Applied Ichthyology* **19**: 74–78.

Madsen L, Frøyland L, Dyrøy E, Helland K, Berge RK (1998) Docosahexaenoic and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation. *The Journal of Lipid Research* **39**: 583–593.

Madsen L, Rustan AC, Vaagenes H, Berge K, Dyrøy E, Berge RK (1999) Eicosapentaenoic and docosahexaenoic acid affect

mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. *Lipids* **34**: 951–963.

Mag TK, Reichert RD (2002) A new recommended calculation of vitamin E activity: implications for the vegetable oil industry. *Inform* **13**: 836–839.

Magnusson MK, Hursti UKK (2002) Consumer attitudes towards genetically modified foods. *Appetite* **39**: 9–24.

Malaysian Palm Oil Board (2005) *Malaysian Oil Palm Statistics 2005*. Malaysian Palm Oil Board, Selangor.

Martino RC, Cyrino JEP, Ports L, Trugo LC (2002) Performance and fatty acid composition of surubim (*Pseudoplatystoma coruscans*) fed diets with animal and plant lipids. *Aquaculture* **209**: 233–246.

Martins DA, Gomes E, Rema P, Dias J, Ozório ROA, Valente LMP (2006) Growth, digestibility and nutrient utilization of rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*) juveniles fed different dietary soybean oil levels. *Aquaculture International* **14**: 285–295.

Mashek DG, Cloeman RA (2006) Cellular fatty acid uptake: the contribution of metabolism. *Current Opinion in Lipidology* **17**: 274–278.

Matarese V, Stone RL, Waggoner DW, Bernlohr D (1989) Intracellular fatty acid trafficking and the role of cytosolic lipid binding proteins. *Progress in Lipid Research* **28**: 245–272.

Menoyo D, Izquierdo MS, Robaina L, Ginés R, Lopez-Bote CJ, Bautista JM (2004) Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. *British Journal of Nutrition* **92**: 41–52.

Miller MR, Nichols PD, Carter CG (2007a) Replacement of fish oil with thraustochytrid *Schizochytrium* sp. L oil in Atlantic salmon parr (*Salmo salar* L) diets. *Comparative Biochemistry and Physiology A* **148**: 382–392.

Miller MR, Nichols PD, Carter CG (2007b) Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega-3 long-chain polyunsaturated fatty acid concentrations. *Comparative Biochemistry and Physiology B* **146**: 197–206.

Mittaine JF (2005) IFFO fishmeal, fish oil outlook 2005–2006. *Global Aquaculture Advocate* **8**: 52–55.

Molnár T, Szabó A, Szabó G, Szabó C, Hancz C (2006) Effect of different dietary fat content and fat type on the growth and body composition of intensively reared pikeperch *Sander lucioperca* (L.). *Aquaculture Nutrition* **12**: 173–182.

Mondal K, Kaviraj A, Mukhopadhyay PK (2006) Fish wastes in urban and suburban markets of Kolkata: problems and potentials. *Aquaculture Asia* **11**: 22–25.

Montero D, Robaina L, Caballero R, Ginés R, Izquierdo MS (2005) Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time-course study on the effect of a re-feeding period with a 100% fish oil diet. *Aquaculture* **248**: 121–134.

Montero D, Grasso B, Izquierdo M, Real F, Tort L, Caballero MJ *et al.* (2008) Total substitution of fish oil by vegetable oils in gilthead sea bream (*Sparus aurata*) diets: effects on hepatic Mx expression and some immune parameters. *Fish and Shellfish Immunology* **24**: 147–155.

Morais S, Koven W, Rønnestad I, Dinis MT, Conceição LEC (2005) Dietary protein:lipid ratio and lipid nature affects fatty acid absorption and metabolism in a teleost larva. *British Journal of Nutrition* **93**: 813–820.

Moretti VM, Corino C (2008) Omega-3 and trans fatty acids. In: Nollet LML, Toldra F (eds) *Handbook of Processed Meats and Poultry Analysis*, pp. 233–271. CRC Press, Crystal Bay.

Mørkøre T (2006) Relevance of dietary oil source for contraction and quality of pre-rigor filleted Atlantic cod, *Gadus morhua*. *Aquaculture* **251**: 56–65.

Mørkøre T, Netteberg C, Johnsson L, Pickova J (2007) Impact of dietary oil source on product quality of farmed Atlantic cod, *Gadus morhua*. *Aquaculture* **267**: 236–247.

Mourente G, Bell JG (2006) Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comparative Biochemistry and Physiology B* **145**: 389–399.

Mourente G, Dick JR, Bell JG, Tocher DR (2005a) Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and β-oxidation of [1-^{14}C]18:3n-3 (LNA) and [1-^{14}C]20:5n-3 (EPA) in hepatocytes and enterocytes of European sea bass (*Dicentratchus labrax* L.). *Aquaculture* **248**: 173–186.

Mourente G, Good JE, Bell JG (2005b) Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): effects on flesh fatty acid composition, plasma prostaglandins E2 and F2, immune function and effectiveness of a fish oil finishing diet. *Aquaculture Nutrition* **11**: 25–40.

Moya-Falcón C, Hvattum E, Tran TN, Thomassen MS, Skorve MS, Ruyter B (2006) Phospholipid molecular species, B-oxidation, desaturation and elongation of fatty acids in Atlantic salmon hepatocytes: effects of temperature and 3-thia fatty acids. *Comparative Biochemistry and Physiology B* **145**: 68–80.

Mugrditchian DS, Hardy RW, Iwaoka WT (1981) Linseed oil and animal fat as alternative lipid sources in dry diets for chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* **25**: 161–172.

Mukhopadhyay PK, Mishra S (1998) Effect of feeding different lipid souces on growth, feed efficiency and tissue fatty acid composition of *Clarias batrachus* fry and fingerling. *Journal of Applied Ichthyology* **14**: 105–107.

Mukhopadhyay PK, Rout SK (1996) Effects of different dietary lipids on growth and tissue fatty acid changes in fry of the carp *Catla catla* (Hamilton). *Aquaculture Research* **27**: 623–630.

Myhr AI, Dalmo RA (2005) Introduction of genetic engineering in aquaculture: ecological and ethical implications for science and governance. *Aquaculture* **250**: 542–554.

Nakamura MT, Nara TY (2004) Structure, function, and dietary regulation of delta-6, delta-5, and delta-9 desaturases. *Annual Review of Nutrition* **24**: 345–376.

Nakari T, Erkomaa K (2003) Effects of phytosterols on zebrafish reproduction in multigeneration test. *Environmental Pollution* **123**: 267–273.

Nanton DA, Lall SP, Ross NW, McNiven MA (2003) Effect of dietary lipid level on fatty acid beta-oxidation and lipid composition in various tissues of haddock, *Melanogrammus aeglefinus*. *Comparative Biochemistry and Physiology B* **135**: 95–108.

Nanton DA, Vegusdal A, Benze-Røraa AM, Ruyter B, Baeverfjord G, Torstensen BE (2007) Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. *Aquaculture* **265**: 230–243.

National Research Council (1993) *Nutrient Requirements of Fish*. National Research Council, National Academy Press, Washington.

Naylor RL, Goldburg RJ, Primavera J, Kautsky N, Beveridge MCM, Clay J *et al.* (2000) Effects of aquaculture on world food supplies. *Nature* **405**: 1017–1024.

Nestel PJ (1990) Effects of n-3 fatty acids on lipid metabolism. *Annual Review of Nutrition* **10**: 149–167.

Ng WK (2007) Replacing marine fish oil in aquafeeds with tropical palm oil products. *Aquaculture Asia Magazine* **12**: 38–41.

Ng WK, Low SY (2005) Evaluation of spent bleaching clay from palm oil refining as a feed ingredient in the diets of red hybrid tilapia, *Oreochromis* sp. *Journal of Applied Aquaculture* **17**: 87–97.

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

Ng WK, Lim PK, Boey PL (2003) Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, *Clarias gariepinus*. *Aquaculture* **215**: 229–243.

Ng WK, Sigholt T, Bell JG (2004a) The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. *Aquaculture Research* **35**: 1228–1237.

Ng WK, Wang Y, Ketchimenin P, Yuen KH (2004b) Replacement of dietary fish oil with palm fatty acid distillate elevates tocopherol and tocotrienol concentrations and increases oxidative stability in the muscle of African catfish, *Clarias gariepinus*. *Aquaculture* **233**: 423–437.

Ng WK, Koh CB, Zubir BD (2006) Palm oil-laden spent bleaching clay as a substitute for marine fish oil in the diets of Nile tilapia, *Oreochromis niloticus*. *Aquaculture Nutrition* **12**: 459–468.

Ng WK, Tocher DR, Bell JG (2007a) The use of palm oil in aquaculture feeds for salmonid species. *European Journal of Lipid Science and Technology* **109**: 394–399.

Ng WK, Soe M, Phone H (2007b) Aquafeeds in Myanmar: a change from farm-made to factory-made feeds. *Aquaculture Asia* **12**: 7–12.

Normen L, Dutta P, Lia A, Andersson H (2000) Soy sterol esters and β-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. *American Journal of Clinical Nutrition* **71**: 908–913.

Nossen JØ, Rustan AC, Gloppestad SH, Målbakken S, Drevon CA (1986) Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. *Biochimica et Biophysica Acta* **879**: 56–65.

Oku H, Ogata HY, Liang X-F (2002) Organization of the lipoprotein lipase gene of the red sea bream *Pagrus major*. *Comparative Biochemistry and Physiology B* **131**: 775–785.

Olsen RE, Henderson RJ (1997) Muscle fatty acid composition and oxidative stress indices of Arctic charr, *Salvelinus alpinus* (L.), in relation to dietary polyunsaturated fatty acid levels and temperature. *Aquaculture Nutrition* **3**: 227–238.

Olsen RE, Ringø E (1997) Lipid digestibility in fish: a review. *Recent Research Development in Lipid Research* **1**: 199–265.

Olsen RE, Henderson RJ, McAndrew BJ (1990) The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by *Tilapia (Oreochromis) nilotica* in vivo. *Fish Physiology and Biochemistry* **8**: 261–270.

Olsen RE, Henderson RJ, Ringø E (1998) The digestion and selective absorption of dietary fatty acids in Arctic charr, *Salvelinus alpinus*. *Aquaculture Nutrition* **4**: 13–21.

Olsen RE, Løvaas E, Lie Ø (1999) The influence of temperature, dietary polyunsaturated fatty acids, alfa-tocopherol and spermine on fatty acid compostion and indices of oxidative stress in juvenile Artic char, *Salvelinus alpinus* (L.). *Fish Physiology and Biochemistry* **20**: 13–29.

Olsen RE, Henderson RJ, Sountama J, Hemre G-I, Ringø E, Melle W *et al.* (2004) Atlantic salmon, *Salmo salar*, utilizes wax ester-rich oil from *Calanus finmarchicus* effectively. *Aquaculture* **240**: 433–449.

Opsahl-Ferstad H-G, Rudi H, Ruyter B, Refstie S (2003) Biotechnological approaches to modify rapeseed oil composition for applications in aquaculture. *Plant Science* **165**: 349–357.

Oxley A, Tocher DR, Torstensen BE, Olsen RE (2005a) Fatty acid utilisation and metabolism in caecal enterocytes of rainbow trout (*Oncorhynchus mykiss*) fed dietary fish or copepod oil. *Biochimica et Biophysica Acta* **1737**: 119–129.

Oxley A, Torstensen BE, Rustan AC, Olsen RE (2005b) Enzyme activities of intestinal triacylglycerol and phosphatidylcholine biosynthesis in Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology B* **141**: 77–87.

Paolucci M, Rocco M, Varricchio E (2001) Leptin presence in plasma, liver and fat bodies in the lizard *Podarcis sicula*: fluctuations throughout the reproductive cycle. *Life Sciences* **69**: 2399–2408.

Paripatananont T (2002) Snakehead and Pangasius catfish. In: Webster CD (ed.) *Nutrient Requirements and Feeding of Finfish for Aquaculture*, pp. 396–401. CABI Publishing, Auburn.

Patton JS, Nevenzel JC, Benson AA (1975) Specificity of digestive lipases in hydrolysis of wax esters and triglycerides in anchovy and other selected fish. *Lipids* **10**: 575–583.

Pettinger C, Holdsworth M, Gerber M (2004) Psycho-social influences on food choice in Southern France and Central England. *Appetite* **42**: 307–316.

Pickova J, Mørkøre T (2007) Alterante oils in fish feeds. *European Journal of Lipid Science and Technology* **109**: 256–263.

Piedecausa MA, Mazón MJ, García García B, Hernández MD (2007) Effects of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus puntazzo*). *Aquaculture* **263**: 211–219.

Pike IH, Barlow SM (2003) Impact of fish farming on fish stocks. In: *International Aquafeed – Directory and Buyers' Guide 2003*, pp. 24–29. Turret West, Bartham Press, Luton.

Pouteau S (2000) Beyond substantial equivalence: ethical equivalence. *Journal of Agricultural and Environmental Ethics* **13**: 273–291.

Radunz-Neto J, Corraze G, Charlon N, Bergot P (1994) Lipid supplementation of casein-based purified diets for carp (*Cyprinus carpio* L.) larvae. *Aquaculture* **128**: 153–161.

Ranheim T, Gedde-Dahl A, Rustan AC, Drevon CA (1994) Effect of chronic incubation of CaCo-2 cells with eicosapentaenoic acid (20:5n-3) and oleic acid (18:1n-9) on triacylglycerol production. *The Biochemical Journal* **303**: 155–161.

Raso S, Anderson TA (2003) Effects of dietary fish oil replacement on growth and carcass proximate composition of juvenile barramundi (*Lates calcarifer*). *Aquaculture Research* **34**: 813–819.

Refsgaard HHF, Brockhoff PB, Jensen BH (1998) Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar*). *Journal of Agricultural and Food Chemistry* **46**: 808–812.

Regost C, Arzel J, Robin JH, Rosenlund G, Kaushik SJ (2003a) Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*). 1. Growth performance, flesh fatty acid profile and lipid metabolism. *Aquaculture* **217**: 465–482.

Regost C, Arzel J, Cardinal M, Rosenlund G, Kaushik SJ (2003b) Total replacement of fish oil by soybean or linseed oil with a return to fish oil in Turbot (*Psetta maxima*). 2. Flesh quality properties. *Aquaculture* **220**: 737–747.

Regost C, Jakobsen JV, Rørå AMBF (2004) Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. *Food Research International* **37**: 259–271.

Richard N, Mourente G, Kaushik S, Corraze G (2006a) Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). *Aquaculture* **261**: 1077–1087.

Richard N, Kaushik S, Larroquet L, Panserat S, Corraze G (2006b) Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). *British Journal of Nutrition* **96**: 299–309.

Richards JG, Bonen A, Heigenhauser GJ, Wood CM (2004) Palmitate movement across red and white muscle membranes of rainbow trout. *American Journal of Physiology* **286R**: 46–53.

Ringø E (1991) Hatchery-reared landlocked Arctic charr, *Salvelinus alpinus* (L.), from Lake Takvatn reared in fresh and sea water. II. The effect of salinity on the digestibility of protein, lipid and individual fatty acids in a capelin roe diet and commercial feed. *Aquaculture* **93**: 135–142.

Robert SS (2006) Production of eicosapentaenoic and docosahexaenoic acid-containing oils in transgenic land plants for human and aquaculture nutrition. *Marine Biotechnology* **8**: 103–109.

Robin JH, Regost C, Arzel J, Kaushik SJ (2003) Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. *Aquaculture* **225**: 283–293.

Robinson EH, Weirich CR, Li MH (1994) *Feeding Catfish. Bulletin 1019*. Office of Agricultural Communications, Division of Agriculture, Forestry and Veterinary Medicine, Mississippi State University, Mississippi.

Rollin X, Peng J, Pham D, Ackman RG, Larondelle Y (2003) The effects of dietary lipid and strain difference on polyunsaturated fatty acid composition and conversion in anadromous and landlocked salmon (*Salmo salar* L.) parr. *Comparative Biochemistry and Physiology B* **134**: 349–366.

Rørå AMB, Regost C, Lampe J (2003) Liquid holding capacity, texture and fatty acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or soybean oil. *Food Research International* **36**: 231–239.

Rosenlund G, Obach A, Sandberg MG, Standal H, Tveit K (2001) Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.). *Aquaculture Research* **32**: 323–328.

Røsjø C, Nordrum S, Olli JJ, Krogdahl Å, Ruyter B, Holm H (2000) Lipid digestibility and metabolism in Atlantic salmo (*Salmo salar*) fed medium-chain triglycerides. *Aquaculture* **190**: 65–76.

Ruohonen K, Kettunen J, King J (2001) Experimental design in feeding experiments. In: Houlihan D, Boujard T, Jobling M (eds) *Food Intake in Fish*, pp. 88–107. Blackwell Publishing, Oxford.

Ruxton CHS, Calder PC, Reed SC, Simpson MJA (2005) The impact of long-chain *n*-3 polyunsaturated fatty acids on human health. *Nutrition Research Reviews* **18**: 113–129.

Ruyter B, Røsjø C, Einen O, Thomassen MS (2000a) Essential fatty acids in Atlantic salmon: effects of increasing dietary doses of n-6 and n-3 fatty acids on growth, survival and fatty acid composition of liver, blood and carcass. *Aquaculture Nutrition* **6**: 119–127.

Ruyter B, Roesjoe C, Maesoeval K, Einen O, Thomassen MS (2000b) Influence of dietary n-3 fatty acids on the desaturation and elongation of [1-^{14}C] 18:2 n-6 and [1-^{14}C] 18:3 n-3 in Atlantic salmon hepatocytes. *Fish Physiology and Biochemistry* **23**: 151–158.

Ruyter B, Moya-Falcón C, Rosenlund G, Vegusdal A (2006) Fat content and morphology of liver and intestine of Atlantic salmon (*Salmo salar*): effects of temperature and dietary soybean oil. *Aquaculture* **252**: 441–452.

Sambanthamurthi R, Sundram K, Tan YA (2000) Chemistry and biochemistry of palm oil. *Progress in Lipid Research* **39**: 507–558.

Sargent JR, Bell JG, McEvoy J, Tocher DR, Estevez A (1999) Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* **177**: 191–199.

Sargent JR, Tocher DR, Bell JG (2002) The lipids. In: Halver JE, Hardy RW (eds) *Fish Nutrition*, pp. 181–257. Academic Press, Elsevier, San Diego.

Sathivel S (2002) *Production, Process Design and Quality Characteristics of Catfish Visceral Oil. Dissertation Abstracts International Part B: Science and Engineering*, Vol. 63. Louisiana State University, Baton Rouge.

Satoh SW, Poe E, Wilson RP (1989) Effect of dietary n-3 fatty acids on weight gain and liver polar lipid fatty acid composition of fingerling channel catfish. *The Journal of Nutrition* **119**: 23–28.

Schmeisser E, Goessler W, Kienzl N, Francesconi KA (2005) The direct measurement of lipidsoluble arsenic species in biological samples with HPLC–ICPMS. *Analyst* **130**: 948–955.

Schultz C, Knaus U, Wirth M, Rennert B (2005) Effects of varying dietary fatty acid profile on growth performance, fatty acid, body and tissue composition of juvenile pike perch (*Sander lucioperca*). *Aquaculture Nutrition* **11**: 403–413.

Schwarz FJ, Kirchgessner M, Steinhart H, Runge G (1988) Influence of different fats with varying additions of alpha-tocopheryl acetate on growth and body composition of carp (*Cyprinus carpio* L.). *Aquaculture* **69**: 57–67.

Seierstad SL, Poppe TT, Koppang EO, Svindland A, Rosenlund G, Frøyland L *et al.* (2005a) Influence of dietary lipid composition on cardiac pathology in farmed Atlantic salmon, *Salmo salar*. *Journal of Fish Diseases* **28**: 677–690.

Seierstad SL, Seljeflot I, Johansen O, Hansen R, Haugen M, Rosenlund G *et al.* (2005b) Dietary intake of differently fed salmon; the influence on markers of human atherosclerosis. *European Journal of Clinical Investigation* **35**: 52–59.

Seierstad SL, Svindland A, Larsen S, Rosenlund G, Torstensen BE, Evensen Ø (2008) Development of intimal thickening of coronary arteries over the lifetime of Atlantic salmon, *Salmo salar* L., fed different lipid sources. *Journal of Fish Diseases* **31**: 401–413.

Sener E, Yildiz M, Savas E (2005) Effects of dietary lipids on growth and fatty acid composition in Russian sturgeon (*Acipenser gueldenstaedtii*) juveniles. *Turkish Journal of Veterinary and Animal Sciences* **29**: 1101–1107.

Sérot T, Regost C, Prost C, Robin JH, Arzel J (2001) Effect of dietary lipid sources on odour-active compounds in muscle of turbot (*Psetta maxima*). *Journal of the Science of Food and Agriculture* **81**: 1339–1346.

Sérot T, Regost C, Arzel J (2002) Identification of odour-active compounds in muscle of brown trout (*Salmo trutta*) as affected by dietary lipid sources. *Journal of the Science of Food and Agriculture* **82**: 636–643.

Shahidi F, Cadwallader KR (1997) Flavor and lipid chemistry of seafoods: an overview. In: Shahidi F, Cadwallader KR (eds) *Flavor and Lipid Chemistry of Seafoods*, pp. 1–5. American Chemical Society, Washington.

Shapawi R, Mustafa S, Ng WK (2008) Effects of dietary fish oil replacement with vegetable oils on growth and tissue fatty acid composition of humpback grouper, *Cromileptes altivelis* (Valenciennes). *Aquaculture Research* **39**: 315–323.

Shearer KD (2000) Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. *Aquaculture Nutrition* **6**: 91–102.

Shearer KD, Storebakken T (2002) Evaluation of protein ingredient replacement studies. Proceedings of the 10th International Symposium on Nutrition and Feeding in Fish; Feeding for Quality, 2–7 Jun 2002, Rhodes, Greece.

Sigurgisladottir S, Lall SP, Parrish CC, Ackman RG (1992) Cholestane as a digestability marker in the absorbtion of polyunsaturated fatty acid ethyl esters in Atlantic salmon. *Lipids* **27**: 418–424.

Sinclair AJ, Begg DP, Mathai M, Weisinger RS (2007) Omega 3 fatty acids and the brain: review of studies in depression. *Asia Pacific Journal of Clinical Nutrition* **16**: 391–397.

Sire MF, Lutton C, Vernier JM (1981) New views on intestinal absorption of lipids in teleostean fishes: an ultrastructural and biochemical study in the rainbow trout. *Journal of Lipid Reserach* **22**: 81–94.

Skonberg DI, Rasco BA, Dong FM (1993) Effects of feeding high monounsaturated sunflower oil diets on sensory attributes of salmonid fillets. *Journal of Aquatic Food Product Technology* **2**: 117–133.

Souchet N, Laplante S (2007) Seasonal and geographical variations of sterol composition in snow crab hepatopancreas and pelagic fish viscera from Eastern Quebec. *Comparative Biochemistry and Physiology B* **147**: 378–386.

Sprecher H, Luthria DL, Mohammed BS, Baykousheva SP (1995) Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. *The Journal of Lipid Research* **36**: 2471–2477.

Spurvey S, Pan BS, Shahidi F (1998) Flavour of shellfish. In: Shahidi F (ed.) *Flavor of Meat, Meat Products and Seafood*, pp. 159–196. Blackie Academic and Professional, London.

Steffens W (1997) Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. *Aquaculture* **151**: 97–119.

Steffens W, Wirth M, Rennert B (1995) Effects of adding various oils to the diet on growth, feed conversion and chemical composition of carp (*Cyprinus carpio*). *Archives of Animal Nutrition* **47**: 381–389.

Stickney RR, Andrews JW (1971) Combined effects of dietary lipids and environmental temperature on growth, metabolism and body composition of channel catfish (*Ictalurus punctatus*). *The Journal of Nutrition* **101**: 1703–1710.

Stickney RR, Andrews JW (1972) Effects of dietary lipids on growth, food conversion, lipid and fatty acid composition of channel catfish. *The Journal of Nutrition* **102**: 249–258.

Stickney RR, McGeachin RB (1983a) Responses of *Tilapia aurea* to semipurified diets of differing fatty acid composition. Proceedings of the International Symposium on Tilapia in Aquaculture, pp. 346–355; 8–13 May 1983, Nazareth. Tel Aviv University Press, Nazareth.

Stickney RR, McGeachin RB (1983b) Effects of dietary lipid quality on growth and food conversion of tilapia. *Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies* **1983**: 352–357.

Stubhaug I, Frøyland L, Torstensen BE (2005a) β-oxidation capacity of red and white muscle and liver in Atlantic salmon (*Salmo salar* L.) – effects of increasing dietary rapeseed oil and olive oil to replace capelin oil. *Lipids* **40**: 39–47.

Stubhaug I, Tocher DR, Bell JG, Dick JR, Torstensen BE (2005b) Fatty acid metabolism in Atlantic salmon (*Salmo salar* L.) hepatocytes and influence of dietary vegetable oil. *Biochimica et Biophysica Acta* **1734**: 277–288.

Stubhaug I, Lie Ø, Torstensen BE (2006) β-oxidation capacity in liver increases during parr–smolt transformation of Atlantic salmon (*Salmo salar* L.) fed vegetable and fish oil. *Journal of Fish Biology* **69**: 504–517.

Stubhaug I, Lie Ø, Torstensen BE (2007) Fatty acid productive value and β-oxidation capacity in Atlantic salmon tissues (*Salmo salar* L.) fed on different lipid sources along the whole growth period. *Aquaculture Nutrition* **13**: 145–155.

Subhadra B, Lochmann R, Rawles S, Chen R (2006) Effect of dietary lipid source on the growth, tissue composition and hematological parameters of largemouth bass (*Micropterus salmonides*). *Aquaculture* **255**: 210–220.

Tacon AGJ, Hasan MR, Subasinghe RP (2006) Use of fishery resources as feed inputs to aquaculture development: trends and policy implications. FAO Fisheries Circular No. 1018. FAO Fisheries Department, Food and Agriculture Organization of the United Nations, Rome.

Takeuchi T (1997) Essential fatty acid requirements of aquatic animals with emphasis on fish larvae and fingerlings. *Reviews in Fisheries Science* **5**: 1–25.

Takeuchi T, Satoh S, Watanabe T (1983) Requirement of *Tilapia nilotica* for essential fatty acids. *Bulletin of the Japanese Society of Scientific Fisheries* **49**: 1127–1134.

Thomassen MS, Røsjø C (1989) Different fats in feed for salmon: influence on sensory parameters, growth rate and fatty acids in muscle and heart. *Aquaculture* **79**: 129–135.

Thompson KD, Tatner MF, Henderson RJ (1996) Effects of dietary (n-3) and (n-6) polyunsaturated fatty acid ratio on the immune response of Atlantic salmon, *Salmo salar* L. *Aquaculture Nutrition* **2**: 21–31.

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* **11**: 107–184.

Tocher DR, Bell JG, Dick JR, Sargent JR (1997) Fatty acyl desaturation in isolated hepatocytes from Atlantic salmon (*Salmo salar*): stimulation by dietary borage oil containing gamma-linolenic acid. *Lipids* **32**: 1237–1247.

Tocher DR, Bell JG, Dick JR, Henderson RJ, McGhee F, Michell D *et al.* (2000) Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr–smolt transformation and the effects of dietary linseed and rapeseed oils. *Fish Physiology and Biochemistry* **23**: 59–73.

Tocher DR, Bell JG, MacGlaughlin P, McGhee F, Dick JR (2001) Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: effects of dietary vegetable oil. *Comparative Biochemistry and Physiology B* **130**: 257–270.

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

Tocher DR, Fonseca-Madrigal J, Bell JG, Dick JR, Henderson RJ, Sargent JR (2002b) Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* **26**: 157–170.

Tocher DR, Bell JG, Dick JR, Crampton VO (2003) Effects of dietary vegetable oil in Atalntic salmon hepatocyte fatty acid desaturation and liver fatty acid composition. *Lipids* **38**: 723–732.

Tocher DR, Dick JR, MacGlaughlin P, Bell JG (2006a) Effect of diets enriched in Δ6 desaturated fatty acids (18:3n-6 and 18:4n-3), on growth, fatty acid composition and highly unsaturated fatty acid synthesis in two populations of Arctic charr (*Salvelinus alpinus* L.). *Comparative Biochemistry and Physiology B* **144**: 245–253.

Tocher DR, Zheng X, Schlechtriem C, Hastings N, Dick JR, Teale AJ (2006b) Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl delta 6 desaturase of Atlantic cod (*Gadus morhua* L.). *Lipids* **41**: 1003–1016.

Torjusen H, Lieblein G, Wandel M, Francis CA (2001) Food system orientation and quality perception among consumers and producers of organic food in Hedmark County, Norway. *Food Quality and Preference* **12**: 207–216.

Torstensen BE, Stubhaug I (2004) Beta-oxidation of 18:3n-3 in Atlantic salmon (*Salmo salar* L.) hepatocytes treated with different fatty acids. *Lipids* **39**: 1–8.

Torstensen BE, Lie Ø, Frøyland L (2000) Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L) –

effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. *Lipids* **35**: 653–664.

Torstensen BE, Frøyland L, Lie Ø (2004a) Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein composition and lipogenic enzyme activities. *Aquaculture Nutrition* **10**: 175–192.

Torstensen BE, Frøyland L, Ørnsrud R, Lie Ø (2004b) Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. *Food Chemistry* **87**: 567–580.

Torstensen BE, Bell JG, Rosenlund G, Henderson RJ, Graff IE, Tocher DR *et al.* (2005) Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. *Journal of Agricultural Food Chemistry* **53**: 10 166–10 178.

Totland GK, Madsen L, Klemetsen B, Vaagenes H, Kryvi H, Frøyland L *et al.* (2000) Proliferation of mitochondria and gene expression of carnitine palmitoyl transferase and fatty acyl-CoA oxidase in rat skeletal muscle, heart and liver by hypolipidemic fatty acids. *Biology of the Cell* **92**: 1–13.

Tremblay L, Van der Kraak G (1999) Comparison between the effects of the phytosterol beta-sitosterol and pulp and paper mill effluents on sexually immature rainbow trout. *Environmental Toxicology and Chemistry* **18**: 329–336.

Tucker JWJ, Lellis WA, Vermeer GK, Roberts DE, Woodward PN (1997) The effects of experimental starter diets with different levels of soybean or menhaden oil on red drum (*Sciaenops ocellatus*). *Aquaculture* **149**: 323–339.

Turchini GM, Gunasekera RM, De Silva SS (2003a) Effect of crude oil extracts from trout offal as a replacement for fish oil in the diets of the Australian native fish Murray cod (*Maccullochella peelii peelii*). *Aquaculture Research* **34**: 697–708.

Turchini GM, Mentasti T, Frøyland L, Orban E, Caprino F, Moretti VM *et al.* (2003b) Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). *Aquaculture* **225**: 251–267.

Turchini GM, Mentasti T, Caprino F, Panseri S, Moretti VM, Valfré F (2004) Effects of dietary lipid sources on flavour volatile compounds of brown trout (*Salmo trutta* L.) fillet. *Journal of Applied Ichthyology* **20**: 71–75.

Turchini GM, Francis DS, De Silva SS (2006a) Fatty acid metabolism in the freshwater fish Murray cod (*Maccullochella peelii peelii*) deduced by the whole-body fatty acid balance method. *Comparative Biochemistry and Physiology B* **144**: 110–118.

Turchini GM, Francis DS, De Silva SS (2006b) Modification of tissue fatty acid composition in Murray cod (*Maccullochella peelii peelii*, Mitchell) resulting from a shift from vegetable oil diets to a fish oil diet. *Aquaculture Research* **37**: 570–585.

Turchini GM, Francis DS, De Silva SS (2007a) A whole body, in vivo, fatty acid balance method to quantify PUFA metabolism (desaturation, elongation and beta-oxidation). *Lipids* **42**: 1065–1071.

Turchini GM, Francis DS, De Silva SS (2007b) Finishing diets stimulate compensatory growth: results of a study on Murray cod, *Maccullochella peelii peelii*. *Aquaculture Nutrition* **13**: 351–360.

Turchini GM, Moretti VM, Mentasti T, Orban E, Valfré F (2007c) Effects of dietary lipid source on fillet chemical composition, flavour volatile compounds and sensory characteristics in the freshwater fish tench (*Tinca tinca* L.). *Food Chemistry* **102**: 1144–1155.

Van den Thillart G (1986) Energy metabolism of swimming trout (*Salmo gairdneri*). *Journal of Comparative Physiology B* **156**: 511–520.

Van Nieuwenhoven FA, Van der Vusse GJ, Glatz JFC (1996) Membrane-associated and cytoplasmic fatty acid-binding proteins. *Lipids* **31**: 223–227.

Veerkamp JH, van Moerkerk HTB (1993) Fatty acid-binding protein and its relation to fatty acid oxidation. *Molecular and Cellular Biochemistry* **123**: 101–106.

Vegusdal A, Gjøen T, Berge RK, Thomassen MS, Ruyter B (2005) Effect of 18:1n-9, 20:5n-3 and 22:6n-3 on lipid accumulation and secretion by Atlantic salmon hepatocytes. *Lipids* **40**: 477–486.

Vermeir I, Verbeke W (2006) Sustainable food consumption: exploring the consumer "attitude – behavioral intention" gap. *Journal of Agricultural and Environmental Ethics* **19**: 169–194.

Viola S, Arieli Y (1983) Nutrition studies with tilapia hybrids. 2. The effects of oil supplements to practical diets for intensive aquaculture. *Bamidgeh* **35**: 44–52.

Viola S, Rappaport U, Arieli Y, Amidan G, Mokady S (1981) The effects of oil-coated pellets on carp (*Cyprinus carpio*). *Aquaculture* **26**: 49–65.

Waagbø R, Sandnes K, Joergensen J, Engstad R, Glette J, Lie Ø (1993a) Health aspects of dietary lipid sources and vitamin E in Atlantic salmon (*Salmo salar*). 2. Spleen and erythrocyte phospholipid fatty acid composition, nonspecific immunity and disease resistance. *Fiskeridirektoratets Skrifter. Serie Ernaering* **6**: 63–80.

Waagbø R, Sandnes K, Lie Ø, Nilsen ER (1993b) Health aspects of dietary lipid sources and vitamin E in Atlantic salmon (*Salmo salar*). 1. Erythrocyte total lipid fatty acid composition, haematology and humoral immune response. *Fiskeridirektoratets Skrifter. Serie Ernaering* **6**: 47–62.

Waagbø R, Sandnes K, Torrisen OJ, Sandvin A, Lie Ø (1993c) Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E. *Food Chemistry* **46**: 361–366.

Wang Y, Yuen KH, Ng WK (2006) Deposition of tocotrienols and tocopherols in the tissues of red hybrid tilapia, *Oreochromis* sp., fed a tocotrienol-rich fraction extracted from crude palm oil and its effect on lipid peroxidation. *Aquaculture* **253**: 583–591.

Wassef EA, Wahby OM, Sakr EM (2007) Effect of dietary vegetable oils on health and liver histology of gilthead seabream (*Sparus aurata*) growers. *Aquaculture Research* **38**: 852–861.

Watanabe T (1982) Lipid nutrition in fish. *Comparative Biochemistry and Physiology B* **73**: 3–15.

Watanabe T (2002) Strategies for further development of aquatic feeds. *Fisheries Science* **68**: 242–252.

Weihrauch JL, Gardner JM (1978) Sterol content of foods of plant origin. *Journal of the American Dietetic Association* **73**: 39–47.

Willumsen N, Hexeberg S, Skorve J, Lundquist M, Berge RK (1993) Docosahexaenoic acid shows no triglyceride-lowering effects but increases the peroxisomal fatty acid oxidation in liver of rats. *The Journal of Lipid Research* **34**: 13–22.

Willumsen N, Vaagenes H, Asiedu D, Lie Ø, Rustan AC, Berge R (1996) Eicosapentaenoic acid but not docosahexaenoic acid (both as ethyl esters) increases mitochondrial fatty acid oxidation and upregulates 2,4-dienoyl-CoA reductase gene expression. A potential mechanism for the hypolipidemic action of fish oil in rats. *Lipids* **31**: 579–592.

Wilson RP, Moreau Y (1996) Nutrient requirements of catfishes (Siluridei). *Aquatic Living Resources* **9**: 103–111.

Wonnacott EJ, Lane RL, Kohler CC (2004) Influence of dietary replacement of menhaden oil with canola oil on fatty acid composition of sunshine bass. *North American Journal of Aquaculture* **66**: 243–250.

World Health Organization (1999) *Food Safety Issues Associated with Products from Aquaculture*. World Health Organisation Report of a Joint FAO/NACA (WHO Study Group). WHO Technical Report Series 883. World Health Organization, Geneva.

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS *et al.* (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science* **3**: 787–790.

Xu X, Kestemont P (2002) Lipid metabolism and FA composition in tissues of Eurasian perch *Perca fluviatilis* as influenced by dietary fats. *Lipids* **37**: 297–304.

Xu R, Hung SSO, German JB (1993) White sturgeon tissue fatty acid compositions are affected by dietary lipids. *The Journal of Nutrition* **123**: 1685–1692.

Xue M, Luo L, Wu X, Ren Z, Gao P, Yu Y *et al.* (2006) Effects of six alternative lipid sources on growth and tissue fatty acid composition in Japanese sea bass (*Lateolabrax japonicus*). *Aquaculture* **260**: 206–214.

Yildirim-Aksoy M, Lim C, Davis DA, Shelby R, Klesius PH (2007) Influence of dietary lipid source on the growth performance, immune response, and resistance of Nile tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* challenge. *Journal of Applied Aquaculture* **19**: 29–49.

Yingst WL, Stickney RR (1979) Effects of dietary lipid on fatty acid composition of channel catfish fry. *Transactions of the American Fisheries Society* **11**: 90–93.

Yu TC, Sinnhuber RO, Putnam GB (1977) Effect of dietary lipids on fatty acid composition of body lipid in rainbow trout (*Salmo gairdneri*). *Lipids* **12**: 495–499.

Zampelas A, Morgan LM, Furlonger N, Williams CM (1995) Effects of dietary fatty acid composition on basal and hormonestimulated hepatic lipogenesis and on circulating lipids in the rat. *British Journal of Nutrition* **74**: 381–392.

Zheng X, Seiliez I, Hastings N, Tocher DR, Panserat S, Dickson CA *et al.* (2004) Characterization and comparison of fatty acyl D6 desaturase cDNAs from freshwater and marine teleost fish species. *Comparative Biochemistry and Physiology B* **139**: 269–279.

Zheng X, Tocher DR, Dickson CA, Bell JG, Teale AJ (2005a) Highly unsaturated fatty acid synthesis in vertebrates: new insights with the cloning and characterization of a delta6 desaturase of Atlantic salmon. *Lipids* **40**: 13–24.

Zheng XZ, Torstensen BE, Tocher DR, Dick JR, Henderson RJ, Bell JG (2005b) Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). *Biochimica et Biophysica Acta* **1734**: 13–24.