Chapter 6
Safe and Nutritious Aquaculture Produce: Benefits and Risks of Alternative Sustainable Aquafeeds

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Abstract It is estimated that by 2010 >85% of globally available fish oil (FO) and ~50% of fish meal (FM) will be consumed by aquaculture so, it is vital that reliance on marine raw materials is reduced and that sustainable aquafeeds are developed using more terrestrial plant products. In addition, levels of persistent organic pollutants (POPs), principally dioxins/furans and polychlorinated biphenyls (PCBs), in some European FO may breach new EU limits and prevent their use in aquafeeds. Current evidence suggests that salmonids can be grown on diets where 100% of the FO is replaced by vegetable oils (VO), and that bass and bream fed up to 60% VO showed no detrimental effects on growth. However, use of VO can result in reductions of the n-3 highly unsaturated fatty acids, DHA and EPA, of between 50% and 65%, although these values can be restored to 70–100% of the values in fish fed FO by the use of FO-containing finishing diets. Such high levels of FO replacement can only be used if essential fatty acid levels are maintained by inclusion of adequate FM levels. Simultaneous reductions in FM *and* FO will require considerable care if fish health and welfare, as well as product quality, are to be maintained. The efficacy of n-3 highly unsaturated fatty acids (HUFA), principally EPA and DHA, in the prevention or modulation of many of the inflammatory conditions prevalent in the developed world is well established. However, there is concern that the levels of POPs (dioxins, PCBs and PBDEs), as well as the presence of toxic metals, (e.g., Pb, As, Cd and Hg), present a potential risk to human health. The nutrients, as well as contaminants, found in fish flesh are derived largely from the feed and, thus, farmed fish can be tailored to provide optimal levels of fatty acids, and selected vitamins and minerals for human consumption.

Keywords Sustainable aquafeeds, vegetable oils, plant proteins, micronutrients, n-3 fatty acids, organic contaminants

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6.1 Introduction

The rapid growth of aquaculture production worldwide, estimated to be around 10.5% per annum over the last 10 years, (Tacon 2003), has meant that demand for extruded aquafeeds has increased in parallel with production. The culture of carnivorous species in Europe, principally Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), has meant that aquafeed formulations have contained relatively large amounts of both fish meal (FM) and fish oil (FO) derived from marine feed grade fisheries. Until recently this has been regarded as sound practice as fish meal is rich in essential amino acids, is readily accepted and digested by fish and has been readily available and relatively cheap. In addition, FO meets the high requirements of fish for n-3 essential fatty acids and, while freshwater fish and salmonids can convert 18:3n-3 to 20:5n-3 and 22:6n-3, but thrive on 20:5n-3 and 22:6n-3, marine fish cannot perform this conversion and have an absolute requirement for 20:5n-3 and 22:6n-3 (Sargent et al. 2002).

Fish meal and oil are obtained from feed grade fisheries that have effectively reached their sustainable limits and production of FM and FO has remained relatively stable for the last 20 years (Pike 2005). Previous estimates that global FO demand would outstrip production by 2010 or earlier (Barlow and Pike 2001) have now been revised but current estimates suggest that by 2010 more than 85% of the fish oil will be consumed in aquafeed production (Tacon 2004). Therefore, it is vital to reduce dependence on marine raw materials and that sustainable aquafeeds are developed using more terrestrial plant products. An additional reason to include more plant products, especially vegetable oils (VO), is because levels of persistent organic pollutants, principally dioxins/furans and polychlorinated biphenyls (PCBs), in some Northern hemisphere FO may breach new EU limits and prevent their use in aquafeeds (Easton et al. 2002; Foran et al. 2005; EC, 2006b).

Over the last 5–10 years considerable research has been conducted on testing replacements for FO and FM in a range of cultured freshwater, anadromous and marine finfish species. A large amount of data on FO and FM replacement has arisen from the EU Framework 5 projects, Researching Alternatives to Fish Oil in Aquaculture (RAFOA, www.rafoa.stir.ac.uk) and Perspectives on Plant Protein Alternatives (PEPPA, www.st-pee.inra.fr/ici/stpee/nut/peppa/peppa.htm). RAFOA established that salmonids could be cultured on diets where 100% of the FO is replaced by single VO, or a VO blend and that for bass and bream replacement of up to 60% of FO showed no detrimental effects on growth. PEPPA established that trout and bream can be cultured with 75% replacement of FM, by plant products with no loss of growth performance. However, such high levels of FO replacement can only be achieved provided essential fatty acid (EFA) levels are maintained by the inclusion of adequate FM levels. Likewise, high levels of FM replacement were achieved in feeds using FO as oil source and, thus, future studies seeking to replace both FM and FO may prove more challenging.

Fish have proved fairly tolerant of changes in lipid and protein sources, in terms of growth and survival, provided EFA and essential amino acid requirements are met. However, there are potential detrimental effects, in the edible flesh, on biologically important fatty acid concentrations, due to replacement of FO with VO, and also in micronutrient concentrations, when FO and FM are replaced. High dietary VO inclusion can result in reductions of flesh DHA and EPA of ~65% in salmon fed 100% VO or up to 50% in bass and bream fed 60% VO. However, flesh EPA and DHA values can be restored to 70–100% of the values in fish fed FO, for the whole grow out period, by the use of FO-containing finishing diets in the pre-harvest period (Bell et al. 2004a; Torstensen et al. 2005; Mourente et al. 2005; Izquierdo et al. 2005).

Significant replacement of FO and FM also leads to changes in dietary supply, bioavailability and requirement of micronutrients for the farmed fish. Greatest focus has been on mineral bioavailability aspects related to the inherent anti-nutrient factors (ANF) in plant derived raw materials (Francis et al. 2001). Besides the risks for suboptimal micronutrient nutrition for the fish, by lower gross nutrient concentrations and bioavailability, this also implies subsequent alteration in product composition and quality, since several vitamins and minerals in fish flesh are tailored through diet (Baker 2001; Lie 2001). There has been less focus on these secondary consequences of changes in feed ingredients, which especially includes nutrients with antioxidant properties and those normally associated with the benefits of seafood consumption, such as vitamins B_{12}, D, E, carotenoids (astaxanthin and canthaxanthin), iodine and selenium. Changes in product composition will, like lipid retention, also depend on fish species (lean or fat), as well as their feed intake and growth rate.

The importance of n-3 highly unsaturated fatty acids (HUFA), principally EPA and DHA, in human nutrition was first recognised in the 1970s by Dyerberg and Bang who suggested that the diet of Greenland Inuit populations resulted in reduction, or absence, of disease conditions which were prevalent in developed societies (Dyerberg et al. 1975; Bang et al. 1980). Over the last 50 years the prevalence of diseases with an inflammatory pathology has increased dramatically especially pathologies of the cardiovascular system (Kris-Etherton et al. 2002; Wang et al. 2003). However, more recent research has implicated many more disease conditions with an inflammatory pathology that may respond to n-3 HUFA supplementation. These include asthma (Broughton et al. 1997), rheumatoid arthritis (Calder and Zurier 2001), Alzheimer's disease (Morris et al. 2003), Crohn's disease (Belluzzi and Miglio 1998), lupus (Kelley et al. 1985), cancer (Hardman 2002), diabetes (Lombardo and Chicco 2006), psoriasis (Ziboh 1998), schizophrenia (Peet et al. 2001), bipolar disorder (Noaghiul and Hibbeln 2003) and autism (Bell et al. 2004b). In recent times, many countries in the developed world, as well as the World Health Organisation and NATO, have produced recommendations on combined EPA and DHA intake for improved human health which are generally in the range of 0.3–0.5 g day^{-1}. In 2004, ISSFAL (www.issfal.org.uk) updated their recommendation for n-3 HUFA intake suggesting that consuming 500 mg of EPA + DHA day^{-1}, or 3.5 g $week^{-1}$ should provide for good cardiac health in adults.

Nowadays, the efficacy of n-3 HUFA in the prevention or modulation of many of the inflammatory conditions prevalent in the developed world is well established (Connor 2000). However, while the increased consumption of fish is widely recommended, there is currently concern that the levels of persistent organic pollutants (POPs) including dioxins, furans, polychlorinated biphenyls (PCB) and polybrominated dephenylether (PBDE) flame retardants, as well as the presence of toxic metals, including Pb, As, Cd and Hg, present a potential risk to human health (Jacobs et al. 2002a; Hites et al. 2004a,b; MAFF 1999). In 2001, the EU introduced new limits on dioxins and furans in fish feeds and fish for human consumption (SCAN 2000; SCF 2001). These values were 2.25 ng dioxin toxic equivalents (TEQ) kg^{-1} in feed and 4.0 ng TEQ kg^{-1} in fish. Currently, the EU has set limits for the 12 dioxin-like (DL) PCBs, in addition to the values for the 17 dioxins/furans assigned toxic equivalency factors, with combined values of 8 ng TEQ kg^{-1} in fish (EC, 2006a).

In this chapter we will discuss why there is a need to develop aquafeeds that are less reliant on marine raw materials including the effects of including plant meals and oils on the nutritional quality of farmed fish in terms of n-3 HUFA content, as well as vitamin and mineral content. In addition, the importance of n-3 HUFA for human health, current recommended intake levels of n-3 HUFA and the concentrations of n-3 HUFA and other nutrients provided by farmed fish will be discussed. Finally, the concentrations of POPs in farmed fish, as well as mechanisms by which these concentrations can be reduced by manipulation of feed ingredients will be discussed.

6.2 Why Do We Need Alternatives to Fishmeal and Fish Oil?

Food-grade capture fisheries have maintained a fairly stable supply over the last 20 years, although increasing demand has meant that the shortfall in sea food availability for human consumption has been met by increasing aquaculture production (Tidwell and Allan 2002; FAO 2005). In 2003, production of Atlantic salmon and marine-cultured rainbow trout in Europe was ~824,000 t with bass and bream contributing ~150,000 t (FAO 2005). Salmonids are the biggest consumers of extruded aquafeeds in Europe and to produce this tonnage of fish required over 1 million tonnes of extruded feed (Tacon 2005). In 2003, aquaculture utilised 79% of global FO production and 42% of FM production and, of this, salmonid culture used 52 and 27% of FO and FM production, respectively (Tacon 2004).

Therefore, as the supplies of FO and FM have remained static at ~6 million tonnes and ~1 million tonnes per annum, respectively, for around 20 years, the continued increased demand for these products for aquaculture is unsustainable. It is therefore vital that reliance on marine raw materials is reduced and that sustainable aquafeeds are developed using more terrestrial plant products.

In addition to a potential shortfall in the supply of FM and FO, concerns have recently been raised regarding the sustainability and ethical arguments for utilising

fish species that could be used directly as food for humans, in animal feeds (Tacon 1997; Goldburg and Naylor 2005). Furthermore, there is also heightened public and non-governmental organisations (NGO) awareness over the management of feed grade fisheries and the potential impact on marine ecosystems particularly effects on sea birds and marine mammals (Huntington et al. 2004).

A further reason to substitute FO for VO in fish diets is that carnivorous fish have evolved to use oil for the energy required to fuel growth and reproduction. Thus, in effect, most of the oil consumed by fish is effectively "burned" to supply energy and fish do not seem to discriminate greatly between which fatty acids are retained and which are metabolised. Therefore, once essential fatty acid requirements have been met the excess n-3 PUFA in FO are stored as triacylglycerols in body stores and then used for energy production (Sargent et al. 2002). For that reason, it might be more productive to feed VO for energy production, in place of FO, for most of the production cycle and not waste valuable n-3 HUFA in the production of energy for growth. Further, the use of FO finishing diets can still be used to increase n-3 HUFA in fish body oils at a point where the fish has undergone most of its growth increment but has not yet reached the reproductive phase.

A final reason for seeking substitutes for FM and FO relates to the levels of POPs present in some geographical sources of marine raw materials. It is generally established that the lowest levels of contaminants are found in pelagic fish species from South America, while the highest levels are found in those caught in Northern Europe (Easton et al. 2002; FIN 2004). In addition, due to tightening of EU legislation in 2001, a significant proportion of Northern European FO and FM, particularly some of those originating from the Baltic and Barent seas, would no longer be eligible for inclusion in animal and aquafeeds (SCAN 2000; FIN 2004; EC, 2006b).

6.3 Fish Oil and Fish Meal Substitution

6.3.1 Effects of Fish Oil Replacement on Growth Performance

Over the last five years a large number of studies have investigated a range of FO substitutes in a wide range of species. However, all of these earlier studies fed the experimental diets for only a small part of the growth cycle for each species, usually only for 8–20 weeks. By contrast, the studies undertaken as part of the EU project, RAFOA, were unique in that they were long term trials from juvenile stages or covered the whole cycle from first feeding to harvest (www.rafoa.stir.ac.uk).

In salmon smolts, replacement of FO with increasing quantities (25–100%) of linseed oil (LO), rapeseed oil (RO) and olive oil (OO) had no effect on growth or survival (Torstensen et al. 2004a; Bell et al. 2004a) and confirms reports of similar studies using either low (Hardy et al. 1987; Bell et al. 1991; Waagbø et al. 1991, 1993) or high energy diets in Atlantic salmon (Bell et al. 2001a, 2002; Rosenlund et al. 2001; Torstensen et al. 2000, 2004b). These studies show similar growth responses to other

studies that have used RO (Bell et al. 2001a, 2003a,b; Rosenlund et al. 2001) or LO (Bell et al. 1997, 2003a; Tocher et al. 2000) as full, or partial, replacement of FO and suggest that the energy requirements of salmon can be satisfied by VO with variable fatty acid compositions. Furthermore, the lack of any negative growth response suggests that the contribution of EPA and DHA from dietary fishmeal is sufficient to satisfy EFA requirements of salmon up to 100% replacement of FO with VO.

In a full production cycle trial in salmon, a dietary fatty acid composition was formulated by mixing RO, LO and palm oil (PO) to provide similar levels of the different fatty acid classes (saturated, monounsaturated and polyunsaturated n-3 fatty acids) to capelin oil. It was hoped this balance might be better physiologically for fish health and welfare. Thus, at high levels of FO replacement (75 or 100%) a balanced fatty acid composition should be less stressful physiologically compared to the more extreme fatty acid compositions obtained by replacing FO with a single VO. Thus, when salmon were fed either 75 or 100% of the VO blend for the whole production cycle, at two different geographical locations, growth was high in all treatments. However, for the 100% VO group in Norway, significantly higher final mean weight was found compared to the FO group (Torstensen et al. 2005). The higher mean weight after 22 months post-first feeding (PFF) correlated with higher protein sparing in the 100% VO group compared to the FO group indicating that, during the late autumn and winter period of the sea water growth phase, the fatty acid composition of the 100% VO diet favoured protein growth and spared dietary protein from energy production (Torstensen et al. 2005). Previously, dietary lipid content, but not dietary oil source (Torstensen et al. 2000; Bendiksen et al. 2003), has been shown to affect protein utilisation, growth rate, muscle lipid level and feed conversion (Watanabe 1977; Arzel et al. 1993, 1994).

In rainbow trout fed the same single VOs as described for salmon, for 12 weeks, or the 75% and 100% VO blend, for 62 weeks, there were no significant effects of diet on final weight, SGR, TGC or FCR (Kaushik and Corraze 2004, Richard et al. 2006). This data supports earlier studies with rainbow trout and other salmonids where no detrimental effects on growth were observed with different FO substitutes, including soybean oil (SO), RO, OO, PO, LO and lard (Dosanjh et al. 1988; Greene and Selivonchick 1990; Guillou et al. 1995; Caballero et al. 2002; Figueiredo-Silva et al. 2005; Fonseca-Madrigal et al. 2005). No increase in final weight of rainbow trout fed the 100% VO blend was observed. In salmon, the beneficial growth effect during the winter period in Norway may have been due to increased fatty acid digestibility, and thereby increased protein sparing, which lead to improved growth at low water temperatures of less than 5°C. By contrast, the rainbow trout trial was conducted at a constant 17°C where any differences in digestibility at low temperature would not be apparent (Ng et al. 2004a).

In sea bass and sea bream, replacement of up to 60% of FO with VO had no detrimental effects on growth or feed conversion (Izquierdo et al. 2003, 2005; Mourente et al. 2005). However, replacement with 80% linseed oil or 100% of a VO blend in sea bream did reduce growth rates (Izquierdo et al. 2003, 2005) although, with the VO blend, growth reduction was not seen in fish over 250 g suggesting that EFA requirements in larger fish were less stringent than in smaller fish.

6.3.2 Effects of Fish Meal Replacement on Growth Performance

The investigation of fish meal substitutes to supply dietary protein in aquafeeds has been conducted for many years, and although often driven by the desire for more cost effective raw materials, more recently the focus has been to introduce more sustainable aquaculture practices. Generally, despite lower protein content, lower levels of some essential amino acids and the presence of anti-nutritional factors, replacement of around 30% of fish meal can be achieved without loss of growth performance, depending on the degree of product refinement (Teskeredzic et al. 1995; Medale et al. 1998; Glencross et al. 2004; Morris et al. 2005). Some trials have been conducted with fish meal-free diets but generally these have resulted in loss of growth performance (Kaushik et al. 1995; Watanabe et al. 1998). In recent studies in rainbow trout, replacement of FM above 75% resulted in growth reduction as well as some changes in sensory properties, even though amino acid contents were optimized by addition of crystalline amino acids (de Francesco et al. 2004). However, this was a long term trial of almost 6 months and growth rates only became depressed in the high plant protein group after 12 weeks, which is similar to the length of many trials conducted on FM replacement and emphasizes the need to conduct longer term trials (de Francesco et al. 2004).

Generally, Atlantic salmon appear less able to cope with high levels of plant proteins than rainbow trout which may be related to different digestive capacity as well as sensitivity to ANFs (Refstie et al. 2000; Glencross et al. 2004; Mundheim et al. 2004). Salmon fed a range of plant protein concentrations, provided by full-fat soya meal and maize gluten (2:1 w:w), from 15% to 65% showed a linear decrease in growth with each addition of plant protein (Mundheim et al. 2004), although there were no significant differences in SGR, TGC or FCR.

Diets replacing all fish meal with maize gluten and soy protein showed significant growth reduction in European sea bass (Dias 1999) compared to studies utilizing lower levels of replacement (Tibaldi et al. 1999; Tulli et al. 1999). A more recent study, in which sea bass were fed up to 98% of dietary protein as plant meals, showed no reduction of growth over a 12 week period (Kaushik et al. 2004), and, unlike previous studies (Gomes et al. 1995; Dias 1999), there was no reduction of voluntary feed intake in the study by Kaushik et al. (2004). In gilthead sea bream, previous studies have indicated that about one third of the fish meal could be replaced without reducing the levels of indispensable amino acids or reduction in growth rate (Pereira and Oliva-Teles 2002; Gomez-Requeni et al. 2003). However, in a more recent study over 12 weeks, sea bream showed a slight growth reduction when 50 and 75% of protein was provided by plant sources but a 30% growth reduction was seen at 100% plant protein inclusion and this was associated with a marked reduction in feed intake (Gomez-Requeni et al. 2004). By contrast, a recent longer term trial showed that 75% plant protein inclusion did not result in growth reduction (Sitja-Bobadilla et al. 2005).

In turbot (*Psetta maxima*), studies with either maize gluten or lupin have shown promising results as potential fish meal substitutes although reduced feed intake was observed (Burel et al. 2000a,b). In an attempt to provide a more balanced

amino acid composition Fournier et al. (2004) fed a mixture of lupin, wheat and maize gluten with supplementary crystalline amino acids and showed that growth rate in turbot was only compromised when fed 90 or 100% replacement of fish meal over 12 weeks.

The inclusion of high levels of plant proteins can be limited by the presence of ANFs including protease inhibitors, phytates, glucosinolates, tannins, lectins, phytoestrogens and antivitamins among others (Francis et al. 2001). At levels of individual product inclusion in fish feeds many of these factors should not affect growth performance and some can be reduced or eliminated by solvent extraction, steam extrusion or enzymatic treatment. These anti-nutritional factors can reduce growth by affecting palatability and reduction of feed intake or by limited digestibility. Besides, these direct effects on nutrient supply and utilisation, indirect toxic effects with organ damage and endocrine disruption are evident for some ANFs. Increased use of plant proteins in aquafeeds requires more information on which factors are present in specific plant meals so that measures to limit their effects can be achieved by appropriate processing techniques.

A further concern regarding plant proteins is the presence of genetically modified (GM) products, currently used in terrestrial animal production, especially those derived from soya, canola and maize (Pusztai and Bardocz 2006). However, studies conducted with Atlantic salmon suggest that while short transgenic sequences (~120 bp) can be detected in gut tissues, no transgenic fragments have been found in liver, muscle or brain (Sanden et al. 2004). For this reason, there should be no danger of transgenic plant material entering the human food chain from consumption of farmed salmon flesh.

6.3.3 Flesh Fatty Acid Compositions Including Success of Finishing Diets

Numerous studies, in a wide range of fish species, have shown that flesh fatty acid compositions are closely correlated to dietary fatty acid compositions and that feeding high levels of VO will strongly influence flesh fatty acid compositions (Bell et al. 2004a; Izquierdo et al. 2003; Caballero et al. 2002; Mourente et al. 2005; Visentainer et al. 2005; Glencross et al. 2003). However, the influence of dietary lipid on flesh fatty acids is also related to the lipid content of the flesh and the ratio of neutral to polar lipid present, since the correlation with diet is closest in lipid rich flesh, which is high in neutral lipid, especially triacylglycerols (Sargent et al. 2002). In this regard, the rank order of flesh lipid content would be salmon > trout > sea bream > sea bass > cod and flesh lipid deposition tends to increase with fish weight, especially in salmonids (Hemre and Sandnes 1999; Torstensen et al. 2001). Several studies with salmon have shown a clear linear relationship between dietary and flesh fatty acid compositions where a number of VO including RO, PO, SO and blends of RO and LO have been used, along with FO, in diet formulations (Rosenlund et al. 2001; Torstensen et al. 2001, 2004a; Bell et al. 2001a, 2002, 2003a).

The data from studies with salmon, and similar studies with other species, confirm that individual fatty acids, within a blend of fatty acids, are selectively retained or metabolised depending on their concentration in the diet and the biological function of each specific fatty acid. One of the most striking effects, in all species, is the preferential deposition and retention of DHA in flesh lipids, regardless of the concentration present in the diet. This selectivity presumably reflects the specificity of the fatty acyl transferase enzymes that incorporate the individual fatty acids into flesh triacylglycerols and phospholipids, a phenomenon that has been observed in previous studies with salmon fed different combinations of VO (Torstensen et al. 2000; Bell et al. 2001a, 2002, 2003a; Rosenlund et al. 2001) as shown in Table 6.1.

In comparison to DHA, the other PUFA and HUFA seem to be directed more towards metabolism, presumably being largely catabolised for energy production rather than deposition, especially when present at high concentrations. When present at lower concentrations, only EPA appeared to be selectively retained as demonstrated by higher flesh values compared to diet values, specifically in fish fed 100% LO (Table 6.1). In contrast, both 18:2n-6 and especially 18:3n-3 were selected against in terms of deposition in flesh. The tendency towards preferential metabolism of C_{18} PUFA by β-oxidation has been observed not only in fish (Bell et al. 2001b; Bell et al. 2003c) but also in humans, in whom 18:3n-3 was preferred over 18:2n-6 as an oxidative substrate (DeLany et al. 2000). However, it should also be noted that both 18:2n-6 and 18:3n-3 are substrates for Δ6-desaturase, and salmon hepatocytes reportedly favour desaturation and elongation of 18:3n-3 over 18:2n-6 (Bell et al.,1997; Ruyter et al. 2003). In addition to PUFA, the long chain monoene fatty acids (20:1 & 22:1), found in high latitude FO, are thought to be important catabolic substrates (Sargent et al. 2002). This appears to be confirmed in the present studies, particularly so in the salmonids fed capelin oil, as 20:1 and especially 22:1, were selected against in terms of flesh deposition. The literature suggests that 22:1n-11 and 18:2n-6 are preferred substrates for β-oxidation, along

Table 6.1 The differences (Δ) between diet and flesh total lipid fatty acid values for salmon fed 100% fish oil, 50% linseed oil (LO), 100% LO, 33% rapeseed oil (RO) and 100% RO diets

Fatty acid	Δ 100% FO	Δ 50% LO	Δ 100% LO	Δ 33% RO	Δ̃100% RO
16:0	0.8	1.4	2.2	0.8	1.7
18:1n-9	4.1	2.5	1.6	−1.8	−6.8
18:2n-6	−0.3	−1.2	−2.0	−1.0	−3.3
18:3n-3	−0.1	−5.5	−11.7	−0.9	−2.1
20:1n-9	−1.3	−0.3	0.5	−0.2	0.9
22:1n-11	−3.4	−1.7	0.0	−1.2	−0.7
20:5n-3	−1.6	−1.0	0.3	−1.7	−0.4
22:6n-3	3.1	1.9	1.6	1.3	2.1

Data from Bell et al. 2003, 2004. Fatty acid concentrations are g/100 g fatty acid in flesh and diet. Negative Δ values indicate lower values in flesh compared with diet whereas positive values indicate accumulation in flesh relative to diet.

with 16:0, 16:1 and 18:1n-9 (Henderson 1996; Kiessling and Keissling 1993; Frøyland et al. 2000), although it should be noted that much of this work was done using tissue homogenates, isolated cells or mitochondria. In our recent studies, 16:0 was selectively deposited in flesh, suggesting that it may not be readily used as a catabolic substrate (Bell et al. 2003a, 2004a; Table 6.1). By comparison, 18:1n-9 was selectively deposited except when present in high concentrations, e.g., where salmonids were fed > 33% RO or OO (Torstensen et al. 2004a,b) and in sea bass and bream fed 60% RO and 60% OO (Izquierdo et al. 2005; Mourente et al. 2005). It is generally accepted that DHA is selectively retained due to the biological importance of this fatty acid in cell membrane functional integrity, especially in neural, reproductive and immune tissues (Sargent et al. 2002). The selective deposition of 16:0 and 18:1n-9, rather than mobilisation, may reflect the structural importance of both these fatty acids in membrane phospholipids, where they are often located in the *sn*-1 position, especially in PC and PE, with PUFA and HUFA being favoured in the *sn*-2 position (Bell and Dick 1991; Sargent et al. 2002).

The health benefits of fish consumption, related to the n-3 HUFA content, are now widely recognised (Simopoulos 1999; Connor 2000) and it is important that aquaculture maintains a healthy product image by producing seafood that is comparable with those from capture fisheries. However, evidence from several studies suggests that when fish are cultured on diets containing VO, especially at levels over 50%, then there are significant reductions in flesh EPA and DHA (Table 6.2, Bell et al. 2004a; Torstensen et al. 2004a,b; Menoyo et al. 2004; Mourente et al. 2005). To overcome this, fish can be placed on a FO finishing diet for a period prior to harvest to restore n-3 HUFA levels. In general, the ability to restore EPA and DHA concentrations was more easily achieved than the dilution or wash out of the 18:2n-6 and 18:3n-3 (Bell et al. 2004a; Torstensen et al. 2004b, 2005; Mourente et al. 2005; Izquierdo et al. 2005). In salmon and trout, although DHA and EPA levels were still significantly lower, after 24 and 12 weeks on a FO finishing diet, respectively, the values attained were at least 80% of the values in fish fed FO, in fish previously fed VO compared to fish fed FO throughout. In salmon, the DHA and EPA were largely restored after 16 weeks with only further small increases up to 24 weeks (Bell et al. 2004a) while for sea bass and bream restoration of EPA and DHA could be largely achieved in 14 weeks (Mourente et al. 2005; Izquierdo et al. 2005).

In trials where fish were fed what were considered maximal levels of VO (60–100% of total added oil) for the whole production cycle, fish were exposed to high dietary VO for an extended period of time of 50–100 weeks. However, the blend of RO, LO and PO used was selected to balance the saturated, monounsaturated and polyunsaturated fatty acids with the same levels as found in either capelin oil or anchovy oil. This generally resulted in lower levels of 18:2n-6 and 18:3n-3 than were found when either LO or RO were used as single FO substitutes. In salmon, flesh DHA and EPA levels were restored to ~90%, in fish fed 75% VO, and ~65%, in fish fed 100% VO, of values in fish fed FO throughout, after 24 weeks on a FO finishing diet (Torstensen et al. 2005). The difference between the two VO treatments can be explained, in part, by the seasonal differences in the finishing diet period between the 75% VO trial in Scotland (March–September when water

Table 6.2 Selected flesh fatty acid compositions (weight % of total fatty acids) in salmon, sea bass and sea bream fed diets containing different levels of rapeseed (RO) or linseed oils (LO) relative to fish oil (FO) from juvenile to commercial harvest weight

Species & Diet/Fatty acid	18:2n-6	18:3n-3	20:5n-3	22:6n-3
Salmon				
100% FO	3.9	0.8	4.3	8.1
100% LO	13.1	38.7	1.3	3.1
100% RO	15.0	5.1	1.7	4.9
Sea bass				
100% FO	3.0	1.0	9.6	20.2
60% LO	5.7	8.4	5.7	14.4
60% RO	8.5	2.7	5.0	9.4
Sea bream				
100% FO	5.5	0.8	9.1	7.3
60% LO	10.4	17.7	3.5	4.6
60% RO	12.9	3.4	3.6	4.9

Data from Bell et al. 2004; Torstensen et al. 2004; Mourente et al. 2005; Izquierdo et al. 2005.

temperatures and growth were high) and the 100% VO trial in Norway (January–May when water temperatures and growth were low; Torstensen et al. 2005).

While restoration of flesh DHA and EPA levels could be reasonably easily achieved, for all species, in around 14–24 weeks, the reduction of 18:2n-6 and 18:3n-3 was less easy (Bell et al. 2004a; Torstensen et al. 2005; Mourente et al. 2005; Montero et al. 2005). In salmon previously fed 100% LO or RO 18:2n-6 levels were still 75% higher compared to fish fed FO, after 24 weeks on the finishing diet. By comparison, the 18:3n-3 remaining in flesh of salmon previously fed 100% LO was 1344% higher after 24 weeks on the FO finishing diet (Bell et al. 2004a; Torstensen et al. 2004a,b). Comparable values for sea bream flesh were 18:2n-6 120% higher, in fish previously fed 60% RO and 18:3n-3 1525% higher in fish fed 80% LO, compared to fish fed FO throughout (Izquierdo et al. 2005). By contrast, for salmon in the whole life cycle trials, 18:3n-3 was only 160% or 360% higher, for fish fed the 75% or 100% VO diet, respectively, while 18:2n-6 was 60% higher than the FO fish after 24 weeks on the FO finishing diet (Torstensen et al. 2005). Thus, residual C_{18} PUFA levels were much lower when using a VO blend with lower 18:2n-6 and 18:3n-3 levels than when single VO high in C_{18} PUFA were used. A recent study with Atlantic salmon introduced the concept of a dilution model that allowed changes in flesh fatty acids to be accurately predicted when fish were switched from a VO diet to a FO diet. The model described by Jobling (2003) supports the findings of the RAFOA studies such that relatively constant levels of DHA and EPA were found after feeding salmonids a FO finishing diet for 16–24 weeks.

6.4 n-3 Highly Unsaturated Fatty Acids (HUFA) and Human Health

The efficacy of EPA and DHA in preventing or attenuating inflammatory disease in humans was first recognized in the early 1970s when epidemiological studies indicated a low incidence of cardiovascular disease in Inuit populations in Greenland and that coastal populations had different disease patterns from inland dwellers (Bang and Dyerberg 1972; Dewailly et al. 2001a,b). The reason for the differences in disease patterns were attributed to higher fish and n-3 HUFA intake in coastal populations. Historically, the human genome has changed little since Paleolithic times when humans were hunter-gatherers and consumed a diet where the ratio of n-6/n-3 PUFA was estimated to be around 1:1 (Leaf and Weber 1987; Simopoulos 1999). Thus, over the past 10,000 years the human genome will have changed little such that the nutritional input in the developed world in the 21st century will be very different to that which our genetic composition is best suited. The changes in our lipid intake from Paleolithic times to the present day are shown in Fig. 6.1 (Leaf and Weber 1987). This demonstrates the increase in total fat intake towards the end of the Agricultural revolution and similar increases in saturated fat and n-6 PUFA, with decreased n-3 PUFA during the Industrial revolution. It is also noteworthy that increased consumption of cereal grains at this time resulted in a greatly elevated starch intake that resulted in increased lipogenesis. The n-6/n-3 PUFA ratio increased steadily from around 1:1 in the early 19th century such that the ratio in the developed world now ranges from 5:1 to 25:1. The most dramatic increases have been due to increased production and use of n-6-rich seed oils which became established following the First World War and have subsequently dominated agricultural production. They gained popularity in human nutrition due to the improvement in serum lipid and cholesterol profile induced by n-6 PUFA compared to saturated fat (Keys et al. 1957). Unfortunately, the dominance of n-6 PUFA in the human food chain, due to direct consumption of vegetable oils as well as the use of oilseeds for the production of farm animals, has seen a steady decline in n-3 PUFA and HUFA in the food chain in the 20th century (Simopoulos 1999).

Over the last 50 years the prevalence of diseases with an inflammatory pathology has increased dramatically especially pathologies of the cardiovascular system (Simopoulos 1991; Zheng et al. 2001). Recent evidence suggests that supplementation with EPA and DHA can reduce death from coronary heart disease (CHD) by 25% and of sudden cardiac death by 45% (Marchioli et al. 2002), and that the risk of CHD can be predicted by a so called “Omega-3 index” based on combined blood fatty concentrations of DHA + EPA (Harris and von Schacky 2004). Using a dose response study, the authors studied the effectiveness of increasing DHA + EPA supplementation on red blood cell DHA + EPA content and thereby correlating Omega-3 index with CHD risk factors identified in earlier epidemiological studies and randomised controlled trials (Harris and von Schacky 2004). However, when considering a healthy intake of n-3 HUFA it is also vital to consider n-6 PUFA and HUFA intake as the n-3 and n-6 fatty acids compete during metabolic conversions

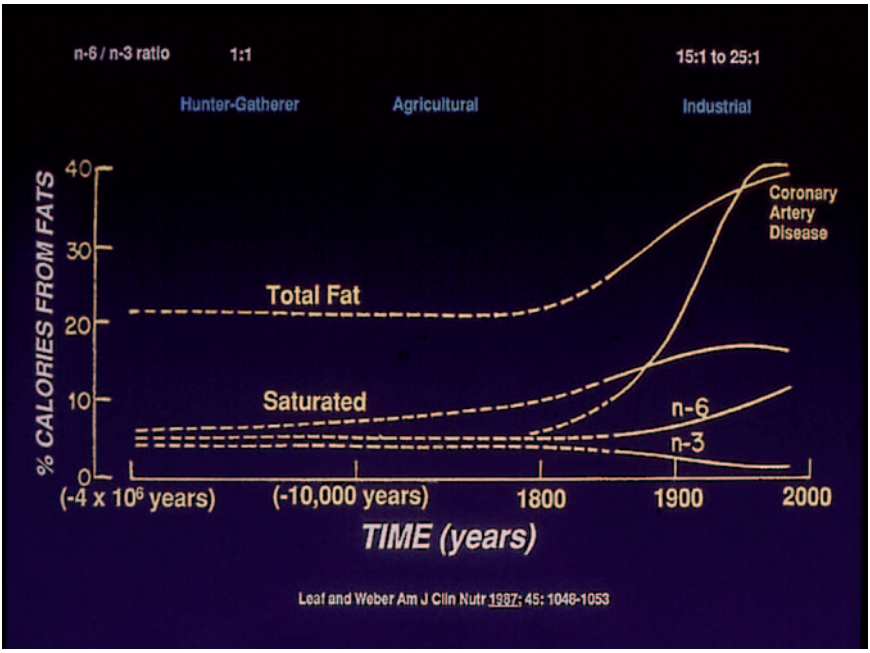

Fig. 6.1 Changes in fat intake from the Paleolithic era to the present day

including desaturation and elongation, acylation into phospholipids and production of eicosanoids (Lands et al. 1992; Lands 1992). The competitive interaction between ARA and EPA for incorporation into membrane phospholipids has been demonstrated in fish (Bell et al. 1989) and in mammals (Lands 2003) and is the basis for the anti-inflammatory activity of EPA and marine fish oils. The importance of maintaining low levels of tissue n-6 HUFA, especially ARA, is shown in the data of Lands (2003) where the positive correlation between tissue n-6 HUFA and CHD mortality is clearly demonstrated (Fig. 6.2).

However, while there has been a great deal of research conducted on the benefits of fish and fish oils on cardiovascular disease, there is also more recent research which has investigated many more disease conditions with an inflammatory pathology that may respond to n-3 HUFA supplementation. The fatty acid composition of inflammatory and immune cells is closely linked to the dietary fatty acid composition and thus provides a link between diet, inflammation and immune function (Calder 2001; Yaqoob 2004). In conditions with an inflammatory or auto-immune component, results following supplementation with marine fish oils have generally been positive. In the case of rheumatoid arthritis improvements observed included reduced stiffness and joint pain, increased grip strength and reduced reliance on non-steroidal anti-inflammatory drugs (James and Cleland 1997; Calder and Zurier 2001). Dietary supplementation with n-3 fatty acids has also shown benefits for dyslipidemia, insulin resistance, glucose homeostasis, diabetes and obesity in

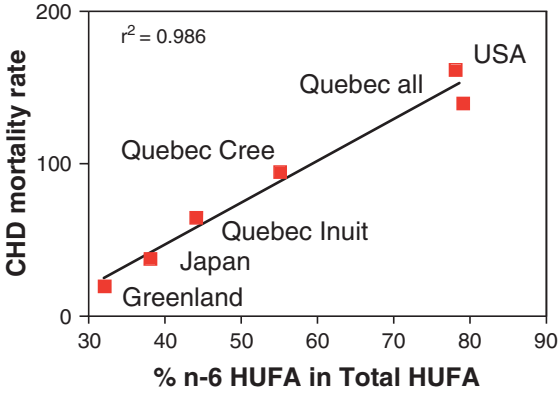

Fig. 6.2 Coronary heart disease (CHD) mortality rates versus tissue n-6 HUFA concentrations in subjects from the USA, Japan, Greenland, Quebec total population, Quebec Inuit and Quebec Cree populations (Lands, 2002)

animal and human studies (Storlien et al. 1998; Lombardo and Chicco 2006). In inflammatory conditions of the bowel, consumption of oily fish reduced relapses in patients with Crohn's disease (Belluzzi and Miglio 1998) and in ulcerative colitis, dependence on steroids was reduced as well as improved colon histology following supplementation with n-3 HUFA (Rogers 1998). In a study with patients suffering from Crohn's or ulcerative colitis, reduced disease activity, serum cholesterol and joint pain index were observed when patients were given an infusion of seal oil (Arslan et al. 2002). In patients with the inflammatory skin condition, psoriasis, improvements in itching, scaling, lesion thickness and erythema were observed following supplementation with EPA + DHA (Ziboh 1998). In childhood asthma, increased dietary n-3 HUFA tended to reduce the asthma severity, while increased n-6 PUFA had the opposite effect (Hodge et al. 1996; Haby et al. 2001). However, supplementation studies gave mixed results with some individuals reporting no effects, while other reported reduced symptoms (Broughton et al. 1997; Hodge et al. 1998). Another inflammatory condition that shows potential for response to n-3 HUFA supplementation is cystic fibrosis, where low blood DHA levels have been found (Roulet et al. 1997).

The proliferation of certain tumour cells is known to be increased by ARA, while this effect can be reversed by inhibitors of eicosanoid production (Horrocks and Yeo 1999). Subsequently, administration of n-3 HUFA has been shown to promote programmed cell death (apoptosis) in leukaemia and lymphoma cell cultures as well as in animal models (Fernandes et al. 1996; Heimli et al. 2002). In addition, animal studies have demonstrated that consumption of n-3 HUFA can reduce cancer growth rates, increase the efficacy of chemotherapy and reduce associated side effects of treatment and of the cancer (Hardman 2002; Hardman et al. 2002). The proposed mechanisms involve suppression of nuclear factor-κB activation and alteration in expression of specific genes including suppression of

cyclooxygenase-2 expression and that of other genes that are implicated in tumour promotion (Hardman 2002). In a recent study, proliferation and gene expression in a B-lymphocyte cell line were investigated when supplemented with EPA or DHA (Verlengia et al. 2004). Cell proliferation was enhanced by both n-3 HUFA while production of key immunomodulatory cytokines, including IL-10, TNF-α and INF-γ was reduced. In addition, altered expression of specific genes including those involved with cytokines, signal transduction, transcription, cell cycle, defence and repair, apoptosis, cell adhesion, cytoskeleton and hormones was observed with EPA almost 3 times more active than DHA at the same concentration (Verlengia et al. 2004).

Although the health benefits of increased fish, and thereby n-3 HUFA, intake have been regarded as beneficial for CHD for over 35 years, more recent focus has turned to the role of essential HUFA in normal neural function and the prevention and treatment of neuropsychiatric disorders (Young and Conquer 2005). It should be no surprise that both ARA and DHA are vital for neural function as these two HUFAs comprise around 30% of the dry weight of brain and retinal tissue (Sastry 1985). By contrast, EPA is not particularly enriched in neural tissues and its role is more likely involved in an anti-inflammatory capacity, as an inhibitor of ARA-derived eicosanoid production and activity as well as inhibiting phospholipase A_2 (PLA_2) activity (Finnen and Lovell 1991). Reduced blood concentrations of n-3 HUFA have been observed in numerous neurodevelopmental and neurodegenerative disorders including Attention Deficit/Hyperactivity Disorder (ADHD) (Stevens et al. 1995), Alzheimer's disease and dementia (Coorigan et al. 1998), schizophrenia (Richardson et al. 2003), bipolar, unipolar and post-natal depression (Peet et al. 1998; Frasure-Smith et al. 2004; Hibbeln 2002). More recently other neurological disorders have also been implicated with abnormal blood n-3 HUFA levels, although the evidence is less well founded at the present time. These include dyslexia (Taylor et al. 2000), autism spectrum disorders (Bell et al. 2004b), dyspraxia (Richardson 2004), obsessive compulsive disorder (Fox et al. 2004) and aggression (Iribarren et al. 2004).

6.5 18:3n-3 and Human Health

While the data from section 6.3.3 above suggests that fish cultured using diets containing a significant proportion of VO have reduced levels of n-3 HUFA in their flesh lipids, they also contain significant levels of α-linolenic acid (18:3n-3; ALA) as well as linoleic acid (18:2n-6; LA). However, while the results of clinical trials with 18:3n-3 have been less clear than those with n-3 HUFA, there is still good evidence that diets that provide increased tissue levels of 18:3n-3 may also be beneficial to human health (Sanderson et al. 2002). Benefits of increased 18:3n-3 intake for various cardiovascular disorders, as well as for both breast and prostate cancer has been reported in the literature (Billman et al. 1999; Singh et al. 1997; Ferreti and Flanagan 1996; Maillard et al. 2002; Newcomer et al. 2001). Therefore, producing fish that provide moderate doses of EPA, DHA and 18:3n-3, but with low

18:2n-6 can be of significant value in human nutrition. In addition, even when salmon were cultured on 100% LO for the whole marine grow-out phase, flesh EPA and DHA concentrations were 0.12 g and 0.28 g, respectively, while ALA was 3.48 g and LA 1.12 g per 100 g of salmon flesh (Bell et al. 2004a). These values are not very different to those suggested by Simopoulos et al. (1999, 2000), who recommended 0.22 g each/day of DHA and EPA, 2.22 g/d for ALA and < 6.67 g/day of LA. The studies conducted as part of the EU RAFOA project, as well as numerous other studies on FO replacement, have allowed us to advance our knowledge on how farmed fish flesh can be "tailored" to deliver ratios of DHA/EPA/18:3n-3/18:2n-6 that are beneficial to human health.

6.6 Recommended Intake of EPA and DHA for Human Health and Concentrations Provided by Farmed Fish

There is now a considerable weight of scientific evidence to support the widely recognised belief that n-3 HUFA intake, especially EPA and DHA, have wide ranging benefits for human health (SACN/COT 2004). But how much fish and n-3 HUFA do we need to consume to provide realistic benefits? Over the past 20 years studies in many countries have sought to recommend beneficial intake values for n-3 HUFA. One of the earliest, established by the Committee on Medical Aspects of Food Policy (COMA), recommended a daily intake of n-3 HUFA of 200 mg/day against an estimated daily intake, in the UK in 1994, of 100 mg/day (DH 1994). By comparison, the recommendation of the Scientific Advisory Committee on Nutrition and the Committee on Toxicity recommended increasing the intake up to 450 mg/day (SACN/COT 2004), against national intake values of 282 mg/day of which 244 mg were from EPA + DHA (Givens and Gibbs 2006). In terms of protection against CVD, Singh et al. (1997) suggested that 2 g n-3 HUFA/day reduced mortality in patients who had suffered a previous myocardial infarction. However, ISSFAL suggested in 2004 that an intake of 500 mg/day or 3.5 g/week of EPA + DHA should provide optimal cardiac health in humans (www.issfal.org.uk).

So how much fish produced by European aquaculture, whether cultured using predominantly marine raw materials or alternative feeds with increased vegetable oil inclusion, requires to be consumed to meet these recommended intake values? A moderate intake (~200 g/week) of Atlantic salmon, grown on FO containing diets, can provide the ISSFAL weekly recommended intake (3.5 g) of EPA + DHA and around 460 g/week of a salmon grown on 75% VO or 750 g/week of salmon grown on 100% VO would meet the ISSFAL intake value for EPA + DHA (Fig. 6.3). However, following the FO finishing diet period 200 g of salmon fed FO exceeds the weekly intake value or 260 g of salmon previously fed 75% VO or 290 g of salmon previously fed 100% VO would meet the recommended EPA + DHA intake value (Torstensen et al. 2005; Fig. 6.3). It is also worth noting that the capelin oil used in the RAFOA trials, during both the grow out and finishing diet phases, contains relatively low levels of EPA + DHA and using other oils with higher n-3

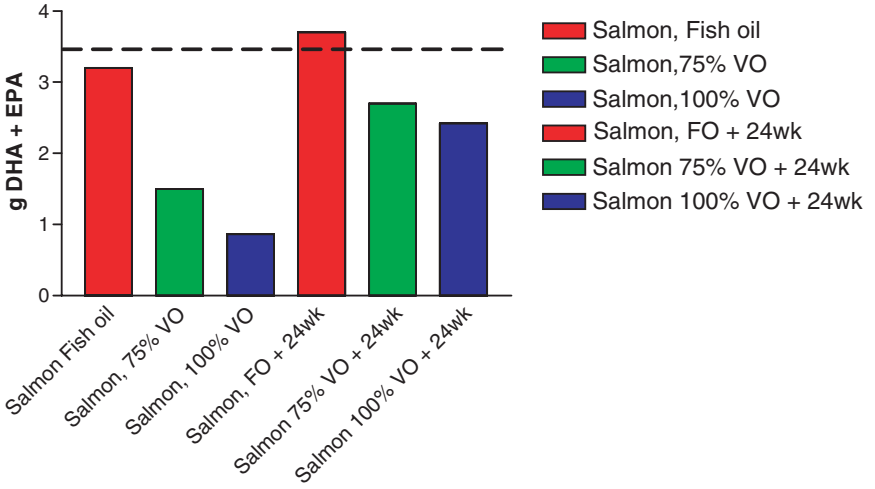

Fig. 6.3 Flesh EPA + DHA (g) in 200 g of salmon fed fish oil (FO), 75% vegetable oil (VO) or 100% VO for the whole production cycle (25 months) and following 24 weeks on a fish oil finishing diet. The dotted line shows the ISSFAL recommended intake of EPA + DHA of 3.5 g/week

HUFA levels would produce higher n-3 HUFA levels in market sized fish. The values found in the RAFOA trials were slightly higher than those reported for wild salmon but lower than those reported for farmed salmon in recent published reports (SACN/COT 2004; EFSA 2005).

By comparison, 200 g of rainbow trout, grown on FO diets, can provide ~60% of the ISSFAL weekly recommended intake (3.5 g) of EPA + DHA and around 340 g/week would be needed to fully meet the ISSFAL intake (Richard et al. 2006). Five hundred and sixty grams of trout grown on 75% VO or 650 g of trout grown on 100% VO would meet the ISSFAL weekly intake value for EPA + DHA (Fig. 6.4). However, following the FO finishing diet period 300 g of trout fed FO, 340 g of trout previously fed 75% VO or 350 g of trout previously fed 100% VO would meet the recommended EPA + DHA intake value (Fig. 6.4). The values of ~1.0 g EPA + DHA/100 g wet flesh reported in the RAFOA studies are similar to recently reported literature values (SACN/COT 2004; EFSA 2005).

For the marine species, 200 g of sea bream, grown on FO diets, can provide 35% of the ISSFAL weekly recommended intake (3.5 g) of EPA + DHA and around 580 g/week would be needed to fully meet the ISSFAL recommendation. Slightly more than 1 kg of sea bream grown on 60% VO or 2.3 kg of sea bream grown on 100% VO would need to be consumed to meet the ISSFAL weekly intake value for EPA + DHA (Fig. 6.5). Following the FO finishing diet period 800 g of sea bream fed FO would supply the weekly intake value or 950 g of sea bream fed 60% VO or 930 g of sea bream fed 100% VO would meet the recommended EPA + DHA intake value (Fig. 6.5). The increase in the required consumption of fish fed FO in the post-finishing diet period is due to the higher lipid deposition in larger bream where more of the lipid deposited is saturates and monoenes rather than HUFA (Izquierdo et al. 2005).

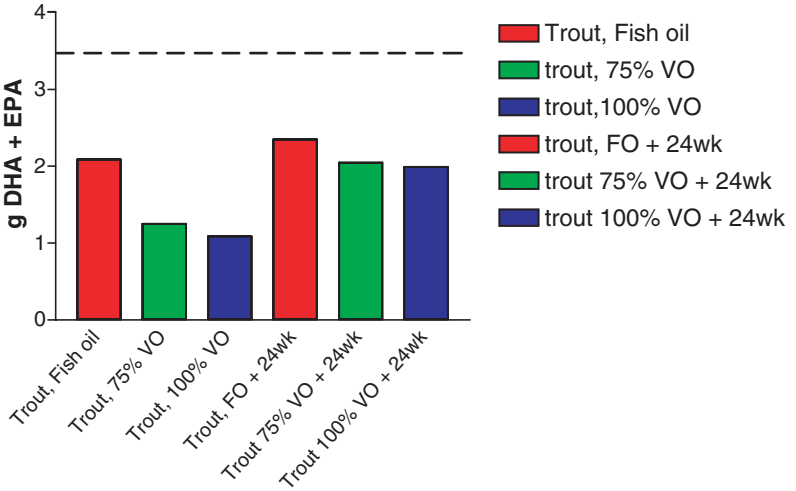

Fig. 6.4 Flesh EPA + DHA (g) in 200 g of rainbow trout fed fish oil (FO), 75% vegetable oil (VO) or 100% VO for the whole production cycle (62 weeks) and following 23 weeks on a fish oil finishing diet. The dotted line shows the ISSFAL recommended intake of EPA + DHA of 3.5 g/week

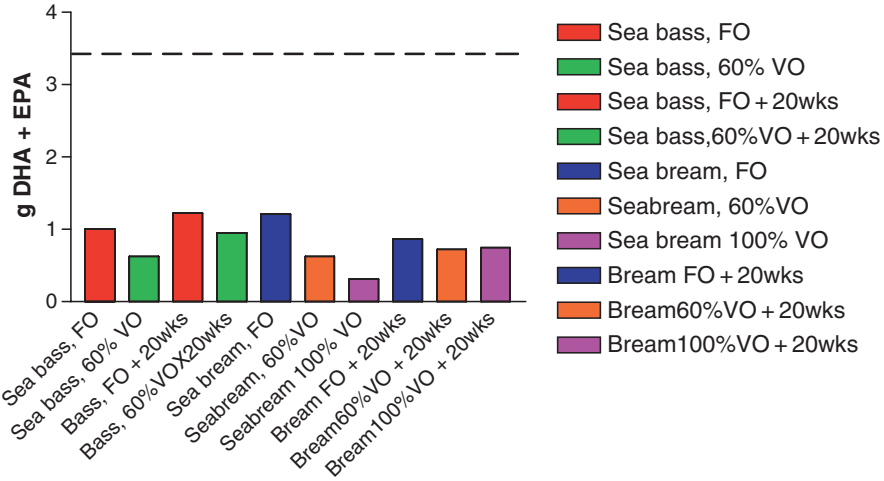

Fig. 6.5 Flesh EPA + DHA in 200 g of sea bass or sea bream fed 100% fish oil (FO), 60% vegetable oil (VO) or 100% VO for the whole production (64 weeks, bass; 40 weeks, bream) cycle and following 20 weeks on a fish oil finishing diet. The dotted line shows the ISSFAL recommended intake of EPA + DHA of 3.5 g/week

A very similar situation was seen in sea bass where 200 g of flesh, from fish grown on FO diets, can provide 29% of the ISSFAL weekly recommended intake (3.5 g) of EPA + DHA and around 690 g/week would be needed to fully meet the ISSFAL recommendation. As with bream, just over 1 kg of sea bass grown on 60% VO would meet the ISSFAL weekly intake value for EPA + DHA (Fig. 6.5). Following a FO finishing diet period 570 g of sea bass fed FO would supply the weekly intake value or 740 g of sea bass fed 60% VO would meet the recommended EPA + DHA intake value (Fig. 6.5). In contrast to bream, but similar to salmon and trout, the concentrations of EPA + DHA increase in the post-finishing diet period. This is due to a lower lipid deposition, seen in larger fish, in bass compared to bream (Mourente et al. 2005; Izquierdo et al. 2005).

While these results clearly demonstrate that fish with oily flesh, such as salmon and trout, can provide a higher dietary intake of EPA and DHA, compared with leaner fish, the contribution of beneficial fatty acids from the leaner species is still significant. Even in very lean white fish species, such as cod and plaice, where flesh lipid values may only be 1% of wet weight, the EPA + DHA content of ~0.25 g/100 g can still make an important contribution to the human diet (SACN/COT 2004; EFSA 2005). This is especially important as the HUFA in lean fish are concentrated in the phospholipid fraction which is thought to be more easily digested and deposited in cell membranes than HUFA supplied as triglyceride oils. For this reason, the advice of the UK Food Standards Agency that we should consume two portions of fish per week, of which one should be lean and the other oily, is sound nutritional advice (www.food.gov.uk).

6.7 Health Benefits of Consuming Fish Protein

In addition to the widely documented benefits of the n-3 HUFA, found in fish oils, there is also considerable evidence to support the role of fish protein, peptides and amino acids as valuable nutrients for animal and human health, especially cardiovascular health. Feeding rats cod protein reduced hepatic triacylglycerol (TAG) concentrations and reduced TAG secretion rates compared to rats fed casein (Demonty et al. 2003) while a mixture of cod protein and menhaden oil resulted in 50% lower plasma TAG compared to rats fed casein and beef tallow (Demonty et al. 2003). In rats fed fish, soybean or casein as protein source, the plasma cholesterol concentrations were reduced for fish and soya compared to casein (Iritani et al. 1985) while the former two also increased excretion of bile salts and cholesterol in faeces. In another study, feeding fish protein to rats, rabbits and humans resulted in reduced plasma cholesterol concentrations in all cases (Jacques 1990). In addition, feeding cod and soy proteins resulted in lower fasting plasma glucose and insulin levels, in rats, compared to those fed casein (Lavigne et al. 2000). Fish protein has also been shown to affect blood coagulation by increasing fibrinolysis in rats (Murata et al. 2004).

Fish protein also contains bioactive peptides that can be released by fermentation. Feeding a fermented fish protein concentrate (FPC) to mice for 7 days resulted in enhanced phagocytic activity of peritoneal macrophages (Duarte et al. 2006). In addition, intestinal IgA + cells were increased along with concentrations of IL-4, IL-6 and IL-10 in mice fed FPC.

Clearly consumption of fish protein and hydrolysates can have a wide range of benefits for cardiovascular function as well as enhancing non-specific immune defence and glucose metabolism. There is also more recent evidence that supplementing fish protein hydrolysates, from blue whiting, cod, plaice and salmon, can have anti-proliferative activity against human breast cancer cell lines grown in vitro (Picot et al. 2006).

6.8 Micronutrients in Feed and Fish

The intuitive opinion, that fish and seafood are healthy and nutritious relies not only on the content of n-3 HUFA, as discussed above, but also on the balanced content of micronutrients. Selected vitamins and minerals, present in high concentrations in seafood, are of special interest for their roles in prevention of life style disorders, such as CHD (vitamin B12, McCully 1991; selenium, Suadicani et al. 1992), osteoporosis (calcium, vitamin D), cancer (selenium, Albanes et al 1999), impaired vision (vitamin A), neurological and neuropsychiatric disorders (B vitamins), developmental disorders (vitamin A) and iodine deficiency disorders (iodine). For these reasons, national food administrations routinely advise people to increase their seafood consumption (SACN/COT 2004; Norwegian Scientific Committee for Food Safety 2006).

Aquaculture research has shown that fish flesh reflects the feed content of selected micronutrients and this allows the opportunity to tailor products both according to market preferences and as healthy added value food (Baker 2001; Lie 2001). Conversely, changes in feed micronutrient compositions and their bioavailabilities, arising from the use of FM and FO substitutes, could constitute an element of risk both for suboptimal micronutrient supply for the fish, as well as introduce undesirable changes in the composition of the fish for the consumer.

6.8.1 Essential Minerals and Trace Elements

A vegetable FM substitute like soybean meal often contains lower concentrations of minerals and in less bioavailable forms for the fish, for example, phosphorous in the form of phytate (Table 6.3). Phytate is also regarded an ANF, since it seems to impact on the digestibility of nutrients by chelating essential minerals, especially zinc, magnesium and phosphorous (Richardson et al. 1985; Storebakken et al. 1998; Francis et al. 2001; Denstadli et al. 2006), and are thereby potentially harmful

by affecting growth and health of the fish. This effect could be overcome, for example, by enzymatic treatment (phytase) of the soy product before inclusion in fish feed (Storebakken et al. 1998; Vielma et al. 1998). As reviewed by Francis et al. (2001) several other ANFs from plant derived FM substitutes impact on element bioavailability. Strategies to overcome this problem include actions to eliminate these ANFs or compensate for their reduced element bioavailability. Despite the fact that elevated feed levels of the essential trace elements like copper, zinc, manganese and iron are reflected in respective target organs in the fish, they are not easily enriched in the fillet (Lall 2002; Lorentzen 1998; Maage 1994).

Table 6.3 Vitamin and mineral concentration ranges in South American and herring fish meal compared to soya meal and known requirements for cold water fish including the possibility to tailor fish fillets by dietary supplementation. (Data from Hertrampf and Piedad-Pascual, 2000; INRA, 1986; Lall, 2002; Halver, 2002)

Micronutrient	Fish meal (mg/kg)	Soya meal (mg/kg)	Requirement (mg/kg)	Possibility to tailor fillet?
Vitamins				
Thiamine (B1)	0.7–1.9	10	1–15	
Riboflavin (B2)	6.6–7.3	2.6	7–30	
Niacin	95–126	23	14–200	
Pantothenic acid	9–31	16	25–50	
Pyridoxine	3.5–3.7	10	3–20	
Folic acid	0.16–0.50	3.5	6–10	Yes
Cobalamin (B12)	0.18–0.25	–	0.02	
Biotin	0.26–0.42	0.3	1.0–1.5	
Choline	4400	2000	1500–4000	
Inositol	700–800[a]	–	200–900	
Ascorbic acid (C)	0	–	30–150	Yes
Vitamin A	3.9–8.9	–	0.30–0.75	(Yes)[d]
Vitamin D	0.01–0.18[b]	–	0.013–0.06	Yes
Vitamin E	3–4	55	30–100	Yes
Vitamin K	–	–	10	(Yes)
Minerals				
Calcium (%)	20–40	2.5	2.7–3.4	
Phosphorous (%)	19–26	5.7	3–9	
Magnesium (%)	–	2.9	0.4–0.6	
Potassium	7–12	–	8	
Iron	150–246	90	30–170	
Zinc	111–120	40	15–67	
Copper	5–11	15	3–5	
Manganese	2–10	25	2.4–13	
Selenium	1.4–2.2	0.5	0.15–0.25	Yes
Iodine	5–90[c]	0.05	0.6–1.1	Yes

[a] Waagbø et al. (1998); [b] Horvli and Lie, (1994); [c] Lall, (2002); [d] including through retention of provitamin A carotenoids, like astaxanthin and canthaxanthin in salmonids muscle

Besides being rich in calcium, fish products contain considerable amounts of iodine (I) and selenium (Se) that can contribute a significant part of the human recommended daily intakes. It is possible to tailor fillet Se concentration by dietary supplementation in salmonids, however both the retained form and efficacy of retention depends on the dietary chemical form of the element (Bell and Cowey 1989; Lorentzen et al. 1994). It also appears that the bioavailability of Se, as observed in rats (Ørnsrud and Lorentzen 2002), and the benefits of Se in human nutrition and health depend upon the chemical form supplied (Drake 2006). For example, the oxidative nature of selenite seems to exert higher cancer chemopreventive effects than the amino acid forms, selenomethionine and Se-methyl-selenocysteine, which lack oxidation capability (Drake 2006). FM contains considerably more Se compared to soybean meal (Table 6.3). Recently, Polatajko et al. (2006) reviewed the complexity of chemical Se species in biological samples. In this regard, speciation of Se in feed and food is necessary to evaluate risks and benefits of using FM substitutes, in addition to considering the total Se content (Table 6.3).

Iodine deficiency disorders, such as goitre, hypothyroidism, cretinism and related mental effects occur frequently in humans, especially in developing countries, with estimates of 1 billion at risk (Hetzel and Clugston 1999). Fish and seaweed are among the food items with the highest naturally occurring iodine contents, however, there is large variation among fish species and even between individuals. Despite relatively low levels in salmonids, it has recently been demonstrated that it is possible to increase fillet iodine levels three fold in adult Atlantic salmon farmed in seawater (from 0.25 to 0.9 mg I kg^{-1} wet weight) by feeding diets supplemented with high levels of an iodine salt (0–80 mg I kg^{-1}) (Julshamn et al. 2006). Freshwater char (*Salvelinus* sp.) seem to respond in a similar range when using a marine algae in the feed (fillet conc. 0.14 to 0.54 mg I kg^{-1} wet weight) (Schmid et al. 2003). Other species show higher levels of fillet iodine, for example, wild caught Atlantic cod (*Gadus morhua*) from the Barents Sea, range between 0.34 and 12.7 mg I kg^{-1} (Julshamn et al. 2001), and may be an even better species for tailoring muscle iodine content than Atlantic salmon. Replacing FM with plant meals may introduce variations in fillet iodine content, even though the minimum iodine requirement for growth of fish (1 mg/kg diet; National Research Council 1993) may easily be covered through uptake from seawater and diet (Lall 2002).

6.8.2 Fish Oil and Fish Meal Substitutes – Consequences for Lipid Soluble Vitamins A and D

Fish are among the few natural sources of vitamins A and D, originating from the lower trophic levels in the marine food web. Fish species with oily flesh, like the salmonids, contain considerable amounts of vitamin D in their fillets (Ostermeyer and Schmidt 2006) and less vitamin A, while lean fish species normally have higher concentrations of fat soluble vitamins in their liver stores (for example, cod liver oil used as human vitamin A and D supplements). FO-based aquafeeds normally supply

sufficient amounts of the lipid soluble vitamins A and D to support fish growth and product quality, even at elevated levels that have been of concern for fish bone health (Graff et al. 2002; Ørnsrud et al. 2002). Both FM (Table 6.3) and FO contain considerable amounts of these vitamins, so there should be no risks for vitamin deficiencies by use of vegetable feed substitutes. However, the benefits of vitamin rich seafood would be reduced, since vitamin supplementations to gain similar feed levels would not currently be supported by EC legislation (EC directive 1970).

6.8.3 Concerns on B-vitamins in Feed and Seafood

For water soluble B-vitamins, storage capacities in farmed fish are normally limited and the muscle tissue will easily reach saturation level at moderate feed intake levels. The possibility to manipulate the product through dietary means is therefore limited. However, several fold variations in B vitamins in fish fillets may be seen between species and relative to fillet muscle type (red or white muscle) and lipid content, as well as relative to environmental factors, sexual maturation and annual cycle (Brækkan 1959; Sandnes et al. 1998; Waagbø unpublished data). As a traditional major protein raw material, FM supplies many of these vitamins in adequate amounts and in readily available forms providing essential requirements for growth and muscle saturation, including biotin (Mæland et al. 1998), vitamin B12 (Mæland A, Sandnes, K and Waagbø R, unpublished data), panthotenic acid (Sandnes et al. 1998) and riboflavin (Brønstad et al. 2002). Table 6.3 illustrates differences in gross vitamin content in FM and soybean meal, the latter representing an important candidate among FM substitutes. Besides observed differences in content, vitamins from plant raw materials may occur in other chemical forms (pyridoxine, riboflavin, niacin, folic acid, vitamin B12) or together with anti-nutrients that results in lower bioavailabilities than vitamins from animal derived raw materials (Machlin 1991). Even though this information is derived from feeding studies or *in vitro* experiments in humans and terrestrial animals, this may also be true for carnivorous fish species. Thus, care should be taken to fulfill the optimal supply of these vitamins in aquafeeds containing FM substitutes through micronutrient supplementation or by using selected vitamin-rich raw materials.

6.8.4 Antioxidant Vitamins and Pigments

The success of micronutrient tailoring of farmed fish fillet depends on the ability of the fish species to handle the dietary intakes, through absorption, retention, metabolism and excretion. The concentrations of the antioxidant vitamins E and C in the fish fillet are important for ensuring the oxidative storage stability of the highly susceptible HUFAs as well as vitamins available to fish consumers (Hamre et al. 1998; Ng et al. 2004b; Waagbø et al. 1993; Yildiz et al. 2006). In a multivariate

23-week feeding study on the impact of dietary pro- and antioxidants on product quality and health of adult Atlantic salmon, feed vitamins E (α-tocopherol acetate) and C (ascorbate polyphosphate) supplementations of 69 and 430 mg/kg, and 52 and 1940 mg/kg, respectively, increased fillet α-tocopherol three fold (from 12–31 μg/g) and vitamin C two fold (15–31 μg/g), respectively (Waagbø unpublished data). In the same study, flesh astaxanthin varied two fold (1.3–2.5 μg/g), when salmon were fed diets containing 11 or 48 mg astaxanthin/kg (Hamre et al. 2004). Even though the flesh concentrations of antioxidant vitamins reflected the respective dietary levels, fillet α-tocopherol alone was the major determinant of oxidative stability after an *in vitro* oxidative challenge of muscle tissue (Hamre et al. 2004). The antioxidant nutrients occur normally at low concentrations in feed ingredients (Table 6.3), and even more may be lost through heat treatment and refining procedures. Therefore, these are routinely supplied in fish feed production through stabilized additives. There are no indications of increased requirement for these antioxidants when using FM and FO substitutes. Indeed, some vegetable oils contain plant derived antioxidants (vitamin E, carotenoids and xanthophylls), as well as n-3 PUFA less susceptible to oxidation which can reduce the oxidative challenge in feed and tissues (Hertrampf and Piedad-Pascual 2000; Ng et al. 2004b).

6.9 The Impact of Vegetable Oil Inclusion on Organic Contaminant Concentrations in Salmon Flesh

Polychlorinated dibenzodioxins and polychlorinated dibenzofurans, collectively known as dioxins, can arise from natural processes such as forest fires and incomplete combustion of organic matter, as well as from industrial processes. The dioxin-like polychlorinated biphenyls (DL-PCBs) are synthetic products used in electrical transformers, heat exchange fluids, hydraulic oils and plastic manufacturing. Although production of PCBs is now banned, they have been deposited in the oceanic benthos, due to industrial activity over the last century, and they are widely distributed across the marine biota (North Sea Task Force 1993). Dioxins and DL-PCBs are highly lipophilic with biological half-lives of several decades, which means they can accumulate in predators at the top of the food chain (Froescheis et al. 2000). However, levels of both dioxins and PCBs in the environment have been declining since the 1950s, although, due to their persistent nature, they will remain in the biota for a considerable period (Brevik et al. 1990; Bignert et al. 1998).

There are around 210 known dioxin and furan congeners and, of these, 17 have been shown to be toxic although individual congeners have different levels of toxicity. For this reason the World Health Organisation (WHO) have established toxic equivalency factors (TEFs), according to their relative toxicity, enabling the calculation of toxic equivalents (TEQs; Van den Berg et al. 1998). Similarly, of the 209 PCB congeners 12 have known dioxin-like toxicity and have been assigned WHO-TEQs. In 2001 the EU introduced new limits on dioxins and furans in fish feeds and fish for human consumption (SCAN 2000; SCF 2001). These values are

2.25 ng dioxin toxic equivalents (TEQ)/kg feed and 4.0 ng TEQ/kg fish. The EU has recently revised dioxin limits and assigned new limits for the 12 dioxin-like (DL) PCBs, such that combined values of 8 ng TEQ/kg for fish products and 7 ng/TEQ for fish feeds have now been introduced (EC 2006a,b).

The polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in a wide variety of household furniture and electrical equipment since their introduction in the 1980s and global annual production currently exceeds 70 kt, with around 75% produced in North America (Hites et al. 2004b). Numerous PBDE congeners exist with different levels of bromination ranging from the tetra through to the deca-brominated products. The less brominated congeners tend to be more persistent in the environment and in 2004 the EU banned production of the tetra to nona-brominated products in favour of the less persistent deca-brominated congeners (Covaci et al. 2003; SACN/COT 2004). However, due to their lipophilic and persistent characteristics the PBDEs are currently increasing in the environment including fish products (FSA 2004; EFSA 2005).

As described above replacement of significant amounts of either FO or FM can be achieved without loss of growth performance or affects on fish health (Kaushik et al. 2004; Torstensen et al. 2005; Mourente et al. 2005). However, significant inclusion of dietary VO results in reduction of the flesh content of n-3 HUFA content, although this could potentially be offset by reduced levels of organic contaminants, as vegetable oils generally contain lower levels of these pollutants than most marine fish oils (SACN/COT 2004).

In the RAFOA II trial in salmon fed 100% FO, the flesh dioxin/furan content was 0.58 and the DL-PCBs 1.18 ng TEQ/kg making a total of 1.76 ng TEQ/kg that was well within existing and proposed EU limit values for dioxins and DL-PCBs (EC, 2006a). By contrast, salmon produced on diets containing 75% VO had reduced values of 0.21 and 0.42 ng TEQ/kg, for dioxins/furans and DL-PCBs, respectively, compared to fish fed FO. The total of 0.63 TEQ/kg flesh for dioxin + DL-PCBs represents a 64% reduction compared to fish fed FO (Fig. 6.6). The flesh dioxin + DL-PCB concentration in fish fed 100% FO of 1.76 ng TEQ/kg is lower than those reported by Lundebye et al. (2004) of 2.5 and by Bell et al. (2005) of 2.01 ng TEQ/kg. Indeed, all of these values are lower than those reported for Scottish farmed salmon by Hites et al. (2004a) of ~3 ng TEQ/kg. The value of 0.63 ng TEQ/kg for dioxin + DL-PCBs in salmon fed 75% VO for the whole production cycle is slightly lower than that reported by Bell et al. (2005) for salmon grown for the whole production cycle on diets containing 100% VO (0.68 ng TEQ/kg) but higher than the value found by Berntssen et al. (2005) in fish from the RAFOA II study conducted in Norway and fed 100% VO (0.30 ng TEQ/kg). The differences found between these studies are due to different dioxin + DL-PCB concentrations in the oils used in the dietary formulations. In the two RAFOA studies capelin oil was used whereas the study of Bell et al. (2005) used a mixture of capelin and herring oils as the FO component. The concentration of dioxins and DL-PCBs in capelin are generally lower than in herring, although there is considerable geographical and seasonal variation for both species (Lundebye-Haldorsen and Lie 1999; SCAN 2000). The dioxin + DL-PCB concentrations found in salmon flesh in

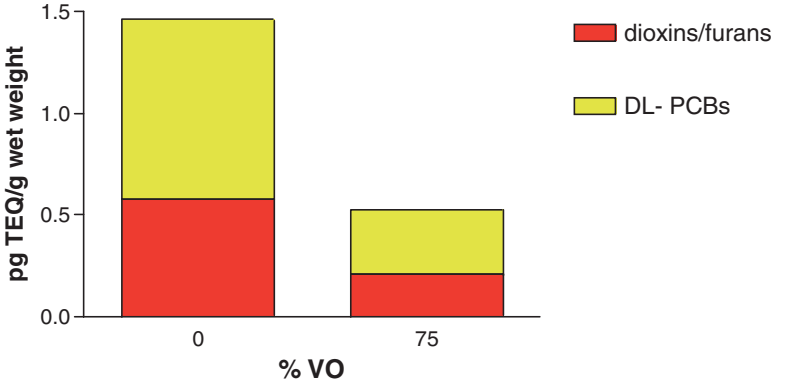

Fig. 6.6 Concentrations of flesh dioxins/furans and dioxin-like PCBs (pg TEQ/g wet weight) in salmon fed diets containing 100% fish oil (FO) or 75% vegetable oil blend (VO) for 24 months post-first feeding

the two RAFOA studies and in the study by Bell et al. (2005), where 75–100% of FO was replaced by VO, are similar or less than those found by Hites et al. (2004a) in wild Pacific salmon. In conclusion, these values confirm that replacing marine fish oils with VO in aqua feed formulations can significantly reduce dioxin and DL-PCB concentrations in farmed salmon flesh. While the reduction in flesh concentrations of dioxins and PCBs arising from the use of VO in aqua feeds is to be welcomed, the reduction in n-3 HUFA that accompanies the use of VO is potentially detrimental. However, careful use and choice of FO towards the end of the production cycle, including the use of FO "finishing diets" can largely restore n-3 HUFA levels in fish pre-market (Bell et al. 2004a; Torstensen et al. 2005; Mourente et al. 2005; Montero et al. 2005). In addition, there is the possibility that, by using more FO at the end of the production cycle to increase n-3 HUFA, organic contaminant concentrations might be elevated. In the study by Bell et al. (2005), a 16–24 week finishing diet period successfully restored flesh DHA and EPA concentrations to >80% of the value seen in fish fed FO throughout the production cycle. However, the flesh dioxin and DL-PCB concentrations in the fish previously cultured on 100% VO diets, following the 24 week finishing diet period, were still 60 and 47% lower, respectively, than the values seen in fish cultured on FO throughout (Bell et al. 2005). This suggests that finishing diets can still be used to successfully restore n-3 HUFA levels while producing fish with significantly reduced contaminant concentrations.

While over 40 PBDE congeners have been identified (Covaci et al. 2003), the contribution to overall tissue concentrations in fish is largely due to six congeners namely PBDEs 28, 47, 99, 100, 153 and 154 (Jacobs et al. 2002b; Hites et al. 2004b). In salmon flesh from the RAFOA II study in Scotland, the concentrations of the 6 major PBDE congeners were 3819 ng/kg in fish fed FO compared to 1083 ng/kg in fish fed 75% VO (Fig. 6.7). The flesh PBDE concentration found in the RAFOA fish fed FO was similar to that observed by Hites et al. (2004b) for

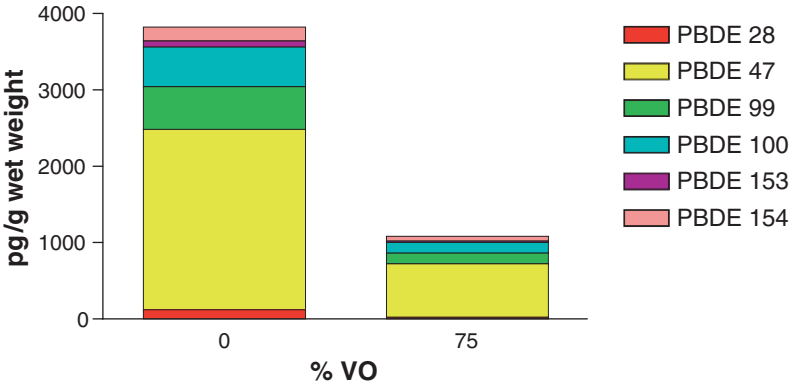

Fig. 6.7 Concentrations of the six principal PBDE congeners (pg/g wet weight) in flesh of salmon fed diets containing 100% fish oil (FO) or 75% vegetable oil blend (VO) for 24 months post-first feeding

farmed salmon sourced in Europe in 2002 and also similar to wild chinook salmon from British Columbia but was higher than farmed Atlantic salmon from Chile or North America. However, the flesh concentration in RAFOA fish fed FO was lower than the concentration found in salmon obtained in Scotland in 1999 by Jacobs et al. (2002b). The flesh concentration in salmon fed 75% VO was only 28% of that found in fish fed FO and the levels in the former were comparable to salmon sourced in Chile but still significantly higher than wild chum, pink, coho and sockeye sourced from British Columbia and Alaska (Hites et al. 2004b). In comparison to dioxins and DL-PCBs there are no TEQ values assigned to any PBDE congeners or any tolerable daily intake (TDI) values calculated at the present time. However, there is some evidence from studies with mice and rats, fed high doses (30 mg/kg/day) of PBDE during gestation, that neurodevelopmental and thyroid hormone defects could result (Fowles et al. 1994; Zhou et al. 2002). Similar effects on thyroid hormones, as well as increased oxidative stress and altered retinol concentrations, were seen in American kestrels when eggs were dosed and nestlings were fed doses of PBDE congeners between 100 and 1500 ng/g with these concentrations being similar to values found in Great Lakes trout and Great Lakes gull eggs respectively (Fernie et al. 2005). However, the values used in this dosing study are 33–500 times greater than those found in salmon fed diets containing FO and the recent SACN/COT (2004) report stated that levels found in fish in the UK were unlikely to present any risk to human health. In addition, the ban on production of penta- to nono-BDEs, introduced in EU countries in 2004, should prevent any future increases in PBDEs in the European food chain.

Following the recent SACN/COT (2004) report the UK Food Standards Agency (FSA, www.food.gov.uk) revised their advice to consumers regarding consumption of fish. The new recommendation suggests a weekly intake of up to four 140 g portions of oily fish per week for men, boys and women over reproductive age, up

to a maximum of 8 pg TEQ/kg body wt/day of dioxins and PCBs. However, girls and women of reproductive age are advised to consume up to two 140 g portions of oily fish/week up to a maximum of 2 pg TEQ/kg body wt/day of dioxins and PCBs. The latter recommendation is in agreement with current EU guidelines. Thus, based on the data from the RAFOA studies, consuming 2 × 140 g/week portions of salmon fed FO or 75% VO would account for 39 and 14% of the suggested maximal weekly intake of dioxins + DL-PCBs, respectively, for women of child bearing age, while providing 128 and 60% of the ISSFAL recommended EPA + DHA intake, respectively (Fig. 6.8a & b). By comparison, consuming 4 × 140 g portions/week of salmon fed FO or 75 and 100% VO would account for 20 and 7% of the suggested weekly maximal intake of dioxins + DL-PCBs, respectively, for men, boys and women over child bearing age while providing 256 and 128% of ISSFAL recommended EPA + DHA intake, respectively (Fig. 6.8a & b).

The cautionary caveat, that women of childbearing age should consume less oily fish, is unfortunate but understandable given that unborn children and young infants may be particularly sensitive to environmental pollutants. However, they are also the most likely to benefit from the positive effects of increased intake of n-3 HUFA. We feel that the data presented here confirms that farmed fish can be regarded as a safe and healthy food option for human consumers. However, future strategies for aquaculture production must aim to reduce current dependence on fish oil and fish meal while taking all possible steps to reduce contaminant levels in fish and, at the same time ensure that we preserve current levels of n-3 HUFA in farmed fish. This can be achieved by judicious use of terrestrial plant products to replace marine raw materials during the main grow out phase of production but should also include the investigation of cleaned or decontaminated FO as a means of restoring n-3 HUFA without increasing the contaminant burden. Such decontamination processes are

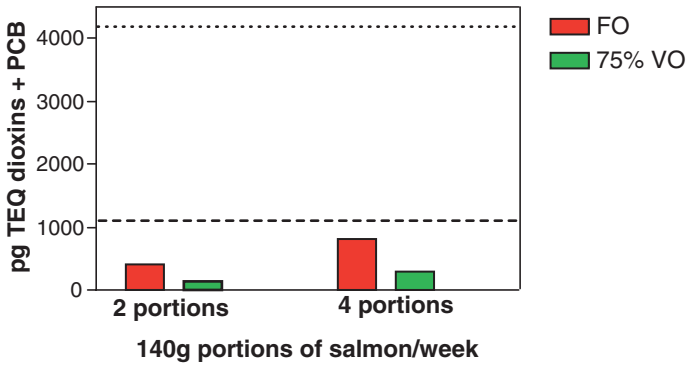

Fig. 6.8a Amount of dioxin + DL-PCBs present in 2 or 4 × 140 g portions of salmon, produced using 100% fish oil (FO) or 75% vegetable oil blend (VO) diets for the full production cycle

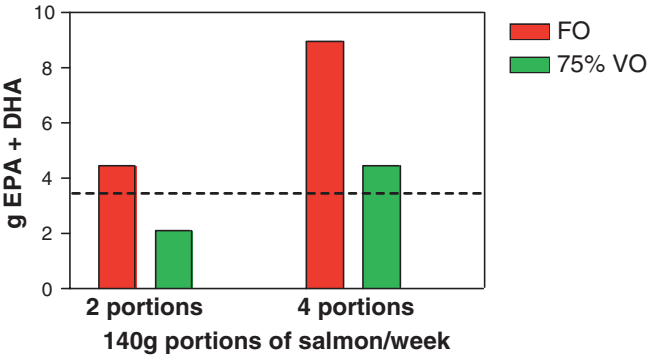

Fig. 6.8b Amount of EPA + DHA (g) provided by 2 or 4 × 140 g portions of salmon, produced using 100% fish oil (FO) or 75% vegetable oil blend (VO) diets for the full production cycle

currently being developed (Breivik and Thorstad 2004; Maes et al. 2005) and, with economy of scale, the cost implications for the industry are minor. The future of aquaculture production, as well as the improved health and well being of human consumers, depends on the investigation and implementation of new sustainable aquafeeds that are safe and nutritionally optimal for both fish and consumers.

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List of abbreviations Bell

ADHD – Attention Deficit/Hyperactivity Disorder
ALA – alpha-linolenic acid
ANF – anti-nutritional factors

ARA – arachidonic acid
CHD – coronary heart disease
CVD – cardio-vascular disease
DHA – docosahexaenoic acid
DL – dioxin-like
DL-PCBs – dioxin-like polychlorinated biphenyls
EFA – essential fatty acid
EPA – eicosapentaenoic acid
FCR – Feed conversion ratio
FM – fish meal
FO – fish oil
FPC – fish protein concentrate
HUFA – highly unsaturated fatty acids
IL-10 – interleukin 10
INF-γ – interferon-gamma
LA – linoleic acid
LO – linseed oil
OO – olive oil
PBDE – polybrominated dephenylether
PC – phosphatidylcholine
PCBs – polychlorinated biphenyls
PE – phosphatidylethanolamine
PFF – post-first feeding
PLA_2 – phospholipase A_2
PO – palm oil
POPs – persistent organic pollutants
PUFA – polyunsaturated fatty acid
RO – rapeseed oil
SO – soybean oil
SGR – specific growth rate
TAG – triacylglycerol
TDI – tolerable daily intake
TEQ – toxic equivalents
TEFs – toxic equivalency factors
TGC – thermal growth coefficient
TNF-α – tumour necrosis factor-alpha
VO – vegetable oils