SUSTAINABLE ALTERNATIVES TO FISH MEAL AND FISH OIL IN FISH NUTRITION: EFFECTS ON GROWTH, TISSUE FATTY ACID COMPOSITION AND LIPID METABOLISM

THESIS SUBMITTED TO THE UNIVERSITY OF STIRLING
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by

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To my family

Declaration

I hereby declare that this thesis has been composed entirely by myself and has not been submitted for any other degree. Except where specifically acknowledged the work described in this thesis is the result of my own investigations.

Vasileios Karalazos

Abstract

Traditionally, fish meal (FM) and fish oil (FO) have been used extensively in aquafeeds, mainly due to their excellent nutritional properties. However, various reasons dictate the use of sustainable alternatives and the reduction of the dependence on these commodities in fish feeds. Hence, the aim of the present thesis was to investigate the effects of the replacement of FM and FO with two vegetable oils (VO) and an oilseed meal on the growth performance, feed utilization, nutrient and fatty acids (FA) digestibility and tissue FA composition and metabolism in three commercially important European fish species. Specifically, in Experiment I crude palm oil (PO) was used to replace FO in diets for rainbow trout. In Experiments II and III FO was replaced with rapeseed oil (RO) in diets for Atlantic salmon at various dietary protein/lipid levels aiming also at further reductions of FM by using low protein (high lipid) diet formulations. In Experiments II and III the fish were reared at low and high water temperatures, respectively, in order to elucidate, also, the potential effects of temperature. Lastly, the effects of the replacement of FM with full fat soya meal (FFS) in Atlantic cod were investigated in Experiment IV. The results of the present thesis showed no negative effects on growth performance and feed utilization in rainbow trout when FO was replaced with PO. The dietary inclusion of RO improved the growth of Atlantic salmon, possibly, due to changes in the nutrient and FA digestibilities and FA catabolism while, the growth and feed utilization were unaffected by the dietary protein/lipid level. However, the growth of Atlantic cod was affected negatively by the replacement of FM with FFS. The proximate composition of the fish whole body was in most cases unaffected by dietary treatments. The changes in dietary formulations affected the dietary FA compositions and resulted in significant changes in the fish tissue FA compositions. It was clearly shown that the fish tissue total lipid FA composition reflects the FA composition of the diet, although specific FA were selectively utilized or retained in the tissues by the fish. These may have serious implications not only for fish metabolism and growth but also for the quality of the final product, especially in terms of possible reductions of n-3 HUFA.

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Abbreviations and Acronyms

AA	Amino Acid(s)
ADC	Apparent Digestibility Coefficient
ANF	antinutritional factors
ANOVA	Analysis of Variance
ARA	arachidonic acid (20:4n-6)
conc.	concentrated
CSI	Cardiosomatic Index
DE	Digestible Energy
DHA	docosahexaenoic acid (22:6n-3)
DM	dry matter
DP	Digestible Protein
EAA	Essential Amino Acid(s)
EPA	eicosapentaenoic acid (20:5n-3)
FA	fatty acid(s)
FAME	fatty acid methyl esters
FCR	Feed Conversion Ratio
FFS	full fat soy bean meal
FM	fish meal
FO	fish oil
HSI	Hepatosomatic Index
HUFA	Highly Unsaturated Fatty Acid(s)
K	Condition Factor
NQC	Norwegian quality cuts
PCB	Poly-chlorinated biphenyls
PO	crude palm oil
POP	Persistent Organic Pollutants
PPV	Protein Productive Value
prot.	protein
PUFA	Poly-Unsaturated Fatty Acid(s)
RO	rapeseed oil
SBM	soy bean meal
SGR	Specific Growth Rate
TGC	Thermal Growth Coefficient
VO	vegetable oil(s)
VSI	Viscerosomatic Index

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Chapter 1. General Introduction

1.1 Lipids in fish nutrition

1.1.1 General

Lipids are, along with proteins, the major organic components of fish and in most cases of their feeds, also; carbohydrates are, at least quantitatively, less important. In fish, the lipids and their constituent fatty acids (FA) along with their metabolic derivatives, such as the eicosanoids, play significant roles in various functions of the organism, including growth, health, reproduction etc (Sargent, et al., 2002; Tocher, 2003). Dietary lipids provide energy and essential fatty acids (EFA) to the fish and they also assist the absorption of fat-soluble vitamins (NRC, 1993). Lipids are of great importance not only to fish nutrition but also to human nutrition. It is well documented that the n-3 highly unsaturated fatty acids (HUFA) have numerous beneficial effects on human health and, undoubtedly, fish constitute the best sources of these nutrients in human diets (De Deckere, et al., 1998; Simopoulos, 1999; ISSFAL, 2000; Simopoulos, 2000; Simopoulos, 2003).

1.1.2 Lipids and fatty acids – a brief overview

Lipids can be classified into several groups or "lipid classes", with distinctive properties, functions and roles. The main lipid classes in fish tissues and their feeds are triacylglycerols (TAG), phosphoglycerides or phospholipids (PL), sphingolipids, sterols (mainly cholesterol) and wax esters (Sargent, et al., 2002). TAG are a major class of the neutral lipids and consist of three fatty acids esterified in the *sn*-1, *sn*-2 and *sn*-3 positions of L-glycerol, usually saturated or monounsaturated FA located in the *sn*-1 and *sn*-3 and PUFA in *sn*-2 (Sargent, et al., 2002). TAG is the main form in which

lipids are stored in fish tissues. Specifically, in most fish species, including salmonids, the primary storage sites are the mesenteric adipose tissue, the adipose tissue within the white muscle and to a smaller extent the liver, although the latter can be the major lipid storage site for many marine fish species (Zhou, et al., 1995, 1996; Nanton, et al., 2007). The gadoids, which includes Atlantic cod, store large amounts of lipid (up to 70% w/w) in their livers (Henderson and Tocher, 1987; Tocher, 2003). PL are a major class of polar lipids and consist of two fatty acids esterified to L-glycerol 3-phosphate, the two FA usually being saturated or monounsaturated in the *sn*-1 position and PUFA in the *sn*-2 position (Sargent, et al., 2002). PL, along with their constituent FA are essential structural components of cellular membranes and play a significant role in their functions and properties (Henderson and Tocher, 1987; Sargent, et al., 2002; Tocher, 2003).

The FA are major constituents of the lipid classes mentioned above (apart from cholesterol). The FA found in fish lipids contain a single carboxyl group and a straight carbon chain, ranging from 14 to 24 carbon atoms, predominantly with even carbon numbers. Depending on the degree of unsaturation, they are classified as saturated and unsaturated FA, with the latter being further divided into monounsaturated or monoenes (one double bond), and polyunsaturated FA with two or more double bonds (PUFA). In fish, the double bonds are largely in the cis configuration and in most cases interrupted by a methylene group. Lastly, the position of the double bonds, and especially the position of the first double bond in relation to the methyl terminus, is of great significance. Hence, two major PUFA families occur, i.e. the n-3 and the n-6, where the first double bond is in the third and the sixth carbon atom from the methyl end, respectively. PUFA with carbon chain lengths of more than 20 carbon atoms with three

or more double bonds are defined as highly unsaturated fatty acids (HUFA) (Sargent, et al., 2002).

The FA may be found under a variety of names including trivial and systematic names, names using the Δ nomenclature and shorthand abbreviations or "n" (or "ω") nomenclature. The latter has proved popular in biology and nutrition and is used in the present study. Briefly, it consists of the number of carbon atoms, the number of double bonds and the position of the first double bond counting from the terminal methyl group, allowing the entire structure to be defined since the double bonds are interrupted by methylene groups in cis configuration. For example, stearic acid is designated as 18:0, oleic acid as 18:1n-9, α-linolenic acid as 18:3n-3, linoleic as 18:2n-6, eicosapentaenoic (EPA) as 20:5n-3, docosahexaenoic (DHA) as 22:6n-3 etc.

1.1.3 Biosynthesis of fatty acids

Fish, like all known organisms, can synthesize *de novo* the saturated FA 16:0 and 18:0 (Sargent, et al., 2002). The fatty acid synthesis occurs in cytoplasm utilizing acetyl-CoA and involving the oxidation of NADPH. The pathway is catalyzed by the fatty acid synthase (FAS). From the endogenously synthesized FA, 16:0 and 18:0, fish can then produce 16:1n-9 and 18:1n-9, respectively (Figure 1.1). The desaturation is carried out by the microsomal Δ^9 desaturase. The desaturases, including Δ^4, Δ^5, Δ^6 and Δ^9 desaturase, introduce double bonds in the 4^{th}, 5^{th}, 6^{th}, and 9^{th} carbon atom counting from the carboxylic end, respectively. It should be noted that there is no evidence for existence of a Δ4 desaturase in fish but the final step in the desaturation pathway is catalysed by Δ6 as shown in Figure 1.1. This second Δ6 is sometimes called Δ6*. However, since these enzymes cannot introduce double bonds beyond C_9, thus, linoleic (18:2n-6) and linolenic (18:3n-3) acids cannot be synthesized. Hence, these FA are

referred to as essential fatty acids (EFA) and must be obtained through the feed (NRC, 1993). Elongation of FA occurs in fish, involving condensation of acyl-CoA groups with malonyl-CoA, and resulting finally in a two carbons extended FA. However, it is not well known to what extent 16:1n-9 and 18:1n-9 can be further elongated in fish to produce long-chain monounsaturated FA (Sargent, et al., 2002).

Linoleic (18:2n-6) and linolenic (18:3n-3) acids, once obtained from the feed, can be further elongated and desaturated to produce HUFA, such as arachidonic acid (ARA, 20:4n-6), EPA and DHA (Figure 1.1) (Sprecher, et al., 1995; Sprecher, 2000). The respective pathways have been well described in fish and they involve elongation of the FA in several steps and desaturation by Δ^5 and Δ^6 desaturases (Buzzi, et al., 1996, 1997a, 1997b; Sargent, et al., 2002; Tocher, 2003). The extent to which n-3 HUFA are produced by the elongation and desaturation of 18:3n-3 depends on many parameters including the fish species, life stage and fish size, water temperature and feed, including the dietary lipid source, of the fish (Tocher, et al., 2000; Tocher, et al., 2001; Tocher, et al., 2002; Tocher, et al., 2003a; Tocher, et al., 2003b; Tocher, et al., 2004; Stubhaug, et al., 2005b).

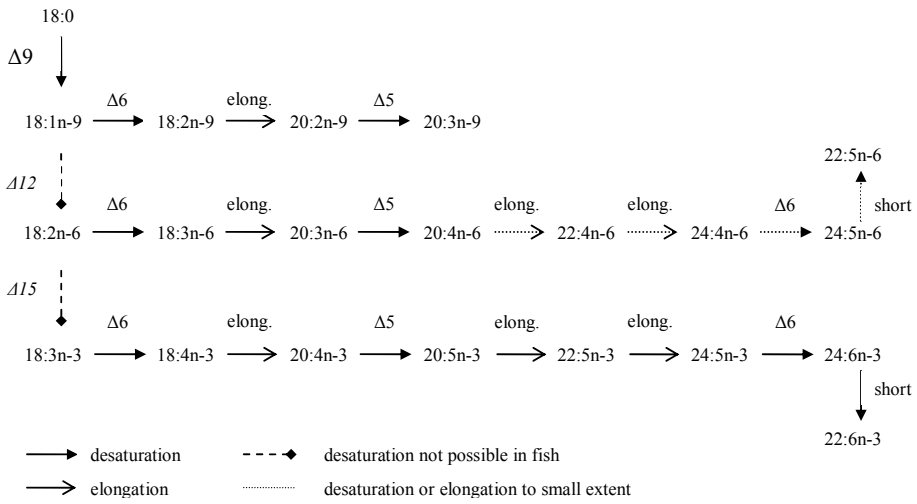

Figure 1.1. Pathways of desaturation and elongation of C_{18} FA to HUFA.
Modified from Tocher (2003)

1.1.4 β-oxidation of fatty acids and energy production

As mentioned previously, one of the major roles of lipids and specifically of their constituent FA in all organisms, including fish, is to provide energy. The metabolic energy is provided by the catabolism of the FA via β-oxidation occurring mainly in the inner space of the mitochondria (matrix) or in the peroxisomes. As the name reveals, the β-oxidation process involves the sequential removal of 2-carbon units by oxidation at the β-carbon position of the fatty acyl-CoA molecule. Each cycle of β-oxidation generates one mole of NADH, one mole of $FADH_2$ and one mole of acetyl-CoA. After further oxidation of the acetyl-CoA to CO_2 in the tricarboxylic acid cycle three moles of NADH, one mole of $FADH_2$ and one mole of ATP are produced.

It is well documented that specific FA are preferentially utilized via β-oxidation in fish; specifically saturated and monounsaturated FA, such as 16:0 18:1n-9, 20:1n-9 and 22:1n-11 are readily catabolized, whereas EPA is usually oxidized to a lesser extent and DHA is mainly retained in tissues rather than catabolized (Henderson, 1996; Sargent, et al., 2002; Tocher, 2003). However, Stubhaug et al. (2005a; 2005b) demonstrated that EPA and DHA are still significant substrates for β-oxidation, especially when present to excess as is the case in fish oils (FO). Hence, feeding the fish with different oils, for example replacing FO with vegetable oils (VO), may affect their β-oxidation activity, while other factors, such as the size of the fish or the water temperature, are also of importance (Tocher, et al., 2002; Tocher, et al., 2004; Torstensen and Stubhaug, 2004; Stubhaug, et al., 2005a; Stubhaug, et al., 2005b).

1.2 Overview of aquaculture in Europe and the world

1.2.1 General

Numerous fish species are cultured in Europe and throughout the world. However, the most important species in European aquaculture are Atlantic salmon (*Salmo salar* Linnaeus, 1758) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), accounting for more than 80% of the total European production (Failler, 2006), although it is noteworthy that they are of great importance for other, non-European, countries, also. Moreover, the introduction and expansion of new species and cultures is important for the development and the long term viability of the sector. Culture of Atlantic cod (*Gadus morhua* Linnaeus, 1758) is a fairly new but fast growing in Europe. Under that perspective the present study focused on these three species.

1.2.2 Atlantic salmon

The farming of Atlantic salmon is a relatively new aquaculture activity, starting in Norway in the 1960's (FAO, 2004b). In the late 1970's Atlantic salmon culture started growing steadily and in the 1990's this growth was rapid, as it is clearly demonstrated in Figure 1.2. In 2004 the global production was more than 1.2 million tonnes and its value was more than 4 billion (4.09×10^9) US$ (FAO, 2007a). The major producing countries, apart from Norway, include Scotland (UK), Faeroe Islands, Ireland, Chile, Canada, USA and Australia.

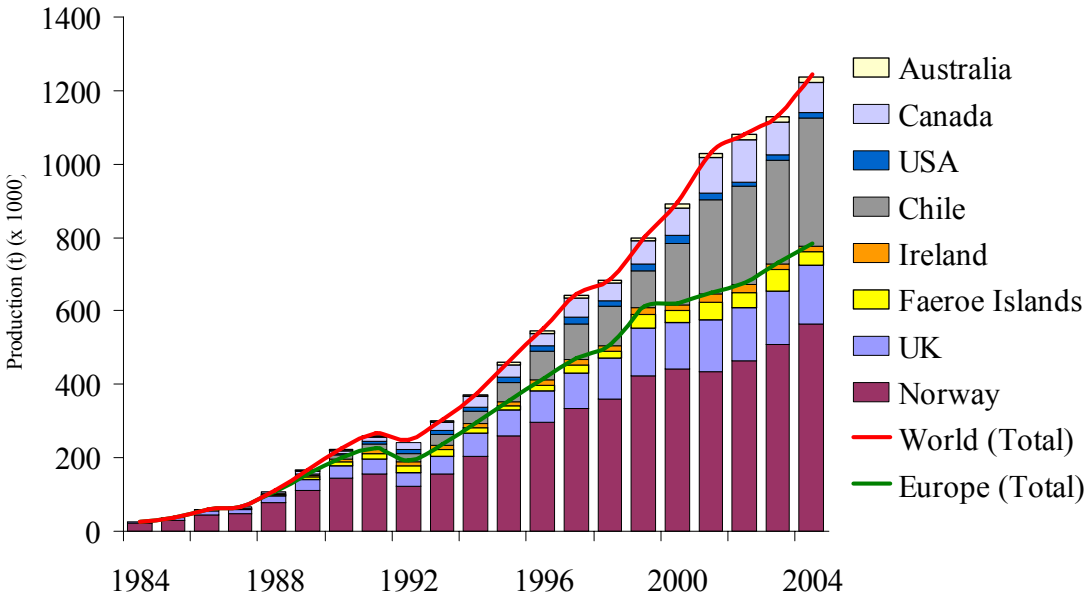

Figure 1.2. Total aquaculture production for Atlantic salmon from 1984 to 2004 including the production of the major producing countries
(Source: FAO, 2007a)

Atlantic salmon belongs to the family of Salmonidae (Salmonids) and is an anadromous species, which in the wild is found on both European and North American sides of the North Atlantic (FAO, 2004b). The spawning and smoltification take place in freshwater and is followed by an ongrowing period at sea. During the ongrowing period, which can take up to 4 years, wild Atlantic salmon feed on pelagic species such as herring, sprat and squid (FAO, 2004b). In culture, Atlantic salmon are reared in freshwater facilities to the fingerling or smolt stage and then they are transferred to seawater where fish are ongrown in sea cages (Purser and Forteath, 2003). The ongrowing phase can be up to two years until the fish reach the desired market size, which may vary from 2 to 6kg (FAO, 2004b).

Ongrowing Atlantic salmon are fed with pelleted, extruded diets in varying sizes (diameter of the pellet) depending on the size of the fish (Helland, et al., 1991; NRC, 1993; Lovell, 2002). The diets of Atlantic salmon contain fish meal FM and FO,

traditionally used as the main sources of protein and lipid, respectively, although other protein and lipid sources of plant or rarely of animal origin, grain derived products for binding and micronutrient premixes are also included (Helland, et al., 1991; NRC, 1993; Lovell, 2002; FAO, 2004b). Atlantic salmon feeds contain high amounts of protein, lipid and energy (Helland, et al., 1991; Lovell, 2002). However, the trend over the last 20 years has been to increase the dietary lipid level at the expense mainly of protein but also of carbohydrates (Einen and Roem, 1997; Hillestad, et al., 1998; Lovell, 2002; FAO, 2004b; Solberg, 2004). Currently, Atlantic salmon diets contain up to 400 g kg^{-1} of lipid although these levels may vary significantly between the producing countries (Solberg, 2004). In general, Atlantic salmon can use dietary protein and lipids for energy very efficiently but the use of carbohydrates is poor (Lovell, 2002). Regarding the essential fatty acid (EFA) requirements of Atlantic salmon, it is surprising that they have not been defined yet; however, it has been suggested that they would be about 1.0% of 18:3n-3 or 0.5 – 1.0% of n-3 HUFA although 18:2n-6 may also be needed for optimal growth (Sargent, et al., 2002). Lastly, feed additives such as pigments (carotenoids), either synthetic or natural, are usually included in the diets to produce the characteristic salmon pink flesh pigmentation (NRC, 1993; Purser and Forteath, 2003).

1.2.3 Rainbow trout

Rainbow trout also belong to the family of Salmonidae (Salmonids) and is one of the oldest fish species to be farmed successfully in Europe and North America. It is a native species to the Pacific drainages of North America, however, since more than 200 years ago, it has been introduced to all continents apart from Antarctica (FAO, 2005a). The aquaculture production of rainbow trout has grown rapidly from the 1950's and in

the last 30 years it has increased 5-fold as shown clearly in Figure 1.3 (FAO, 2007a). In 2004 the global production was 504,876 tonnes with a total value of almost 1.7 billion (1.69×10^9) US$ (FAO, 2007a). Figure 1.3 shows that the major producing countries include UK, Norway, France, Italy, Spain, Denmark, Germany, USA, Chile and lately Iran. It is noteworthy that Europe is the biggest producer of rainbow trout, producing more than half of the global production in 2004.

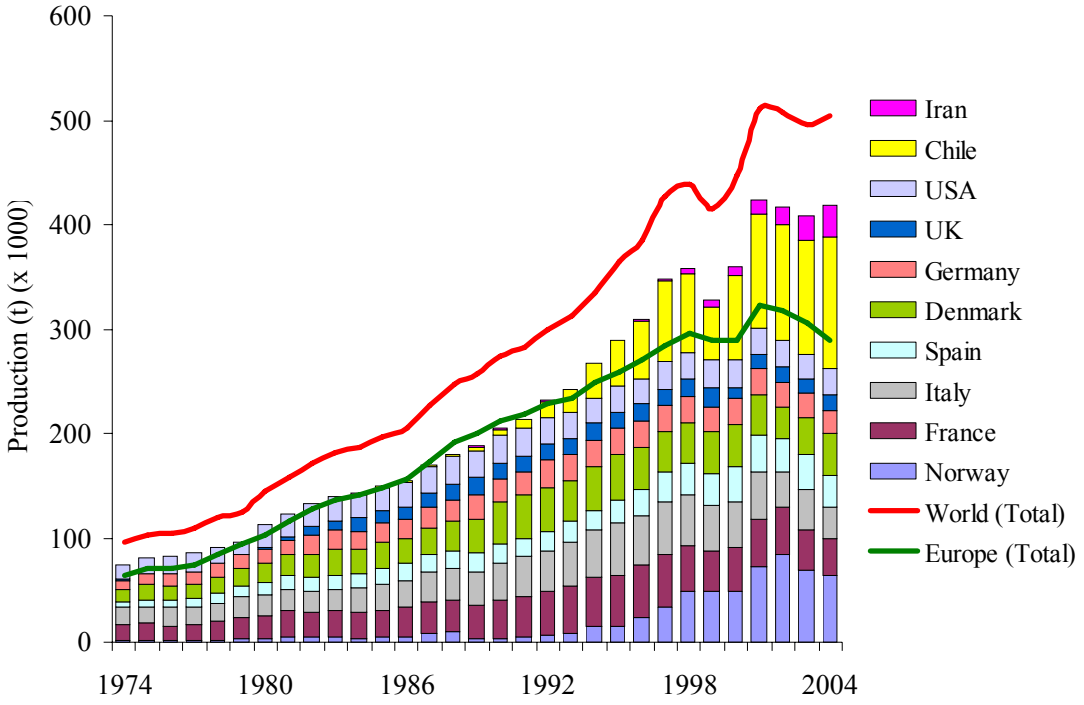

Figure 1.3. Total aquaculture production for Rainbow trout from 1974 to 2004 including the production of the major producer countries
(Source: FAO, 2007a)

Rainbow trout is a fast growing fish which can tolerate variable environmental conditions and husbandry techniques and ranges from anadromous to freshwater strains

(FAO, 2005a). In the wild, the natural feed of rainbow trout may vary widely from plankton for fry, to insects, crustaceans and even small fish for larger trout (Cho and Cowey, 1991; FAO, 2005a). In aquaculture, the feeding and nutrition of rainbow trout is to a great extent similar to that of Atlantic salmon. Specifically, the diets for trout are usually extruded and varying in size (diameter of the pellet) depending on the size of the fish, with high protein, lipid and energy content, while the main ingredients include FM and FO along with other protein and lipid sources (usually of plant origin), grains and micronutrient premixes (Cho and Cowey, 1991; NRC, 1993; Lovell, 2002; FAO, 2005a). Such diets are utilized by the fish very efficiently, often at feed conversion ratios (FCR) of 1 (FAO, 2005a). Rainbow trout dietary requirements for EFA are about 0.7-1% of dry diet for 18:3n-3 and 0.4-0.5% for n-3 HUFA (NRC, 1993; Sargent, et al., 2002).

1.2.4 Atlantic cod

The intensive culture of Atlantic cod is a fairly recent achievement of aquaculture, beginning in Canada and Norway in the mid 1980's. However, as shown in Figure 1.4, in this relatively short time, the culture of Atlantic cod has grown rapidly increasing from 169 t in 2000 to 3,812 t in 2004 with a total value of global production in 2004 of 13 million (0.13×10^6) US$ (FAO, 2007a). After 1990, Atlantic cod is intensively produced only in European countries. Norway is the major producing country, accounting for 83% of the global production, while Iceland and, to a much lesser extent, UK are also contributing to the Atlantic cod culture (FAO, 2007a).

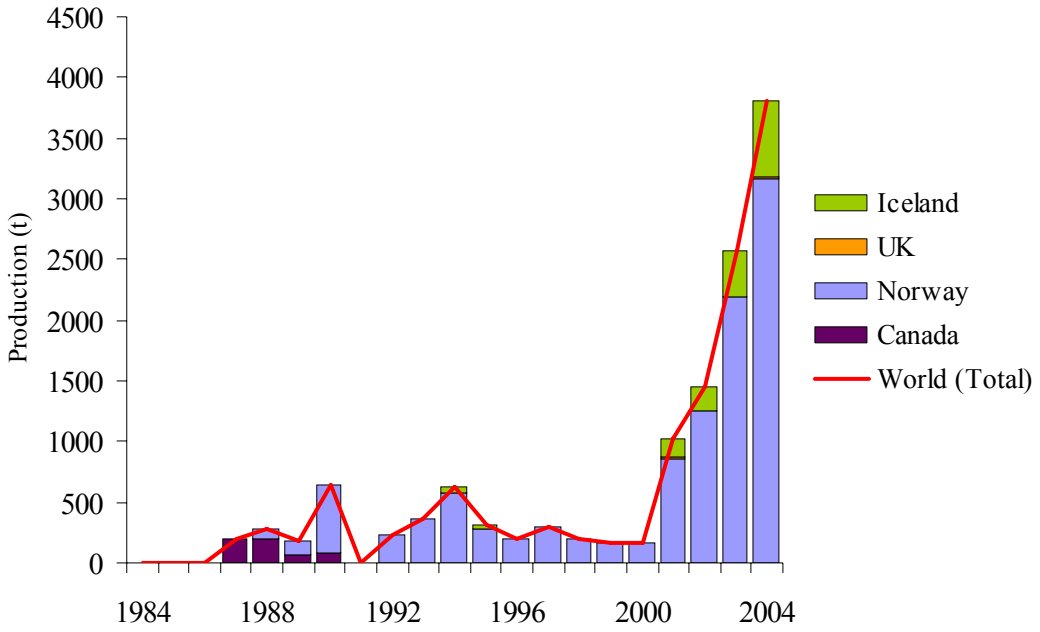

Figure 1.4. Total aquaculture production of Atlantic cod from 1984 to 2004 including the production of the major producing countries
(Source: FAO, 2007a)

Atlantic cod belong to the family of Gadidae. In the wild they are distributed throughout the northern Atlantic, the Baltic sea, and the Barents sea (FAO, 2001). It is a voracious and omnivorous species and its feed consists of plankton for larvae and post-larvae, invertebrates for juveniles, and invertebrates and fish for larger fish, with fish becoming more important than crustaceans in the diet of larger individuals (FAO, 2001). In aquaculture, Atlantic cod is fed extruded diets with high protein / low oil content, and has a high dependence on marine FM and FO (Lie, et al., 1988; Morais, et al., 2001; Lall and Nanton, 2002; Rosenlund, et al., 2004; Karlsen, et al., 2006). However, compared to other species little is yet known about the nutrition and feeding of Atlantic cod, including its nutritional requirements. Atlantic cod in larval and juvenile stages require about 1% (of dry diet) of DHA however, for older fish the requirements for EFA have yet to be defined quantitatively (Sargent, et al., 2002).

1.2.5 Current and future status of aquaculture

The growth of world aquaculture has been rapid during the last fifty years increasing by approximately 10% per annum in the last decade (Brugère and Ridler, 2004). In the early 1950s, the total global production (including aquatic plants) was less than a million tonnes, whereas in 2004 it was 59.4 million tonnes at a value of 70.3 billion US$ (FAO, 2007a). The aquaculture fin fish production grew from less than half a million tonnes in 1950 to 28.2 million tonnes in 2004 with a value of almost 38 billion US$ (FAO, 2007a). Although, much of this growth is due to the very large increase in China's aquaculture production, it should be noted that the increase in the world aquaculture production, if China is excluded, has also been significant and about 6% per annum from 1970-2000 (Brugère and Ridler, 2004).

It has been estimated that the growth of aquaculture will continue over forthcoming decades as the demand for and the consumption of aquaculture products increases (Delgado, et al., 2003; Brugère and Ridler, 2004; Failler, 2006; FAO, 2006b). Specifically, by 2020 global aquaculture will expand by 1.9%, while the highest output forecast is to increase by 3.3% (Brugère and Ridler, 2004). The growth for the 15 members of the pre-2004 European Union between 2000 and 2020 is similar to that of the world's estimated growth, however Norway's aquaculture production (based on salmonids) is estimated to increase at a much higher rate of 6.3% (Brugère and Ridler, 2004). Regarding the production of the species in focus in the present study, both salmonids and cod output is expected to rise significantly over forthcoming decades, although no specific data are available for cod production at the present time (Brugère and Ridler, 2004).

1.3 Fish meal and fish oil in fish feeds

1.3.1 General

FM is a protein-rich light brown flour or meal derived from processing (cooking, pressing, drying, grinding) fresh raw fish (usually small pelagic fish not suitable as food fish or bycatch) and residues and by-products from fish processing plants (fish offal or fish trimmings) (FIN, 2006c; FAO, 2007b). FM have been used in animal nutrition for many centuries (Hertrampf and Piedad-Pascual, 2000). Today, FM is mainly used in feeds for domestic livestock (poultry, pigs, cattle, etc.) and in aquaculture feeds, largely for carnivorous aquatic species and is also used as a taste attractant for omnivorous and herbivorous species (FAO, 2007b). There are four different products sold as FM: "High quality" which is usually used for small-scale aquaculture units (trout farms) or marine species, "LT (low temperature)" which is highly digestible and used in salmon and piglet production, "Prime" and "Fair Average Quality (FAQ)" which is a lower protein content feed ingredient for pigs and poultry (FIN, 2006c).

The oil obtained from the total fish body or from fish waste by extraction is called FO (FAO, 2007b). FO are used in the food industry, e.g. for the manufacture of edible fats, in feeds for terrestrial and aquatic animals, and in industrial products (Hertrampf and Piedad-Pascual, 2000; FAO, 2007b).

1.3.2 Raw material sources and production

The sources of FM and FO include a variety of fish species and may be fish caught for the sole purpose of FM production, by-catches from another fishery, or fish

trimmings/offals from the food fish processing industry (FAO, 1986). The fish species that are commonly used for the production of FM and FO include anchovies, blue-whiting, herrings, mackerels, capelin, pilchard, menhaden, sardines, sandeel, Norway pout, sprat, cod and others (Hertrampf and Piedad-Pascual, 2000; Tacon, 2005; FIN, 2006a). These fish can be divided into three categories in terms of their potential use for human consumption: fish that are not suitable for human consumption, like sandeel and Norway pout; fish with potential use for human consumption but mainly used for fishmeal, like blue whiting, sprat and capelin; and fish that are primarily intended for human consumption but any surplus within the Total Allowable Catch (TAC) may be used for fishmeal, e.g. herring and mackerel (FIN, 2006a). The process of FM and FO production follows the principal of separating the main fractions of the raw material (fish), that is to separate the solids from water and oil (FAO, 1986; Hertrampf and Piedad-Pascual, 2000).

1.3.3 Nutritional properties

FM is a highly nutritious feedstuff primarily used as a high quality protein source but also as a rich source of energy, EFA vitamins and minerals (FAO, 1986; NRC, 1993). However, the chemical composition of different FM may vary significantly depending on the source fish used for its production (Hertrampf and Piedad-Pascual, 2000). For instance, the protein content ranges from less than 60% to more than 70% in different types of FM while the fat varies from 5% to 10% (NRC, 1993). FO are an almost unique source of EFA and especially n-3 HUFA, which comprise 10% to 25% of total FA. Moreover, FO are excellent energy sources due to their high content of 16:0 and monoenes such as 18:1 and, in the case of N. Hemisphere FO, 20:1 and 22:1 (NRC, 1993). Mainly due to their high nutritional value, FM and FO

have, traditionally, been the major protein and oil sources in aquafeeds especially for carnivorous species (Sargent and Tacon, 1999).

1.3.4 Organic contaminants and human nutrition and health issues

In recent years the contamination of FM and FO with Persistent Organic Pollutants (POP) has been raised in the media with potential implications for fish nutrition, to the quality of farmed fish as final products and consequently to public health. The POPs include dioxins and furans, dioxin-like-poly-chlorinated biphenyls (PCBs), polybrominated diphenylether flame retardants and others. Numerous studies have reported on the levels of contaminants and their accepted limits, as regulated by organisations such as the EU and the WHO, in FM and FO, in the farmed and wild fish, and especially in oily fish like Atlantic salmon, herring and mackerel, as well as their potential effects on human health (NRC, 1993; SCAN, 2000; SCF, 2001; Hendricks, 2002; Jacobs, et al., 2002a; Jacobs, et al., 2002b; Hites, et al., 2004; Lundebye, et al., 2004; Bell, et al., 2005; Tacon, 2005; Bethune, et al., 2006; FIN, 2006b; Mozaffarian and Rimm, 2006).

1.3.5 Current and future production, consumption and demand

As described previously (paragraph 1.3.2), the production of FM and FO mainly relies on wild stocks and specifically on pelagic species. Since 1950, when the first data from FAO are available, there has been a seven-fold increase in the global capture of the pelagic species destined for production of FM and FO, with a total production for the top pelagic species of 3.7 million tonnes in 1950 to 24.9 million tonnes for 2004 (FAO, 2007a). However, since the middle of the 1990's, with the exception of 1998 when the El Niño-Southern Oscillation (ENSO) phenomenon occurred, the global

fisheries capture has been relatively stable, ranging between 20 and 25 million tonnes (FAO, 2007a). This is clearly demonstrated in Figure 1.5 presenting the fisheries capture for the top pelagic fish species destined for FM and FO production from 1984 to 2004.

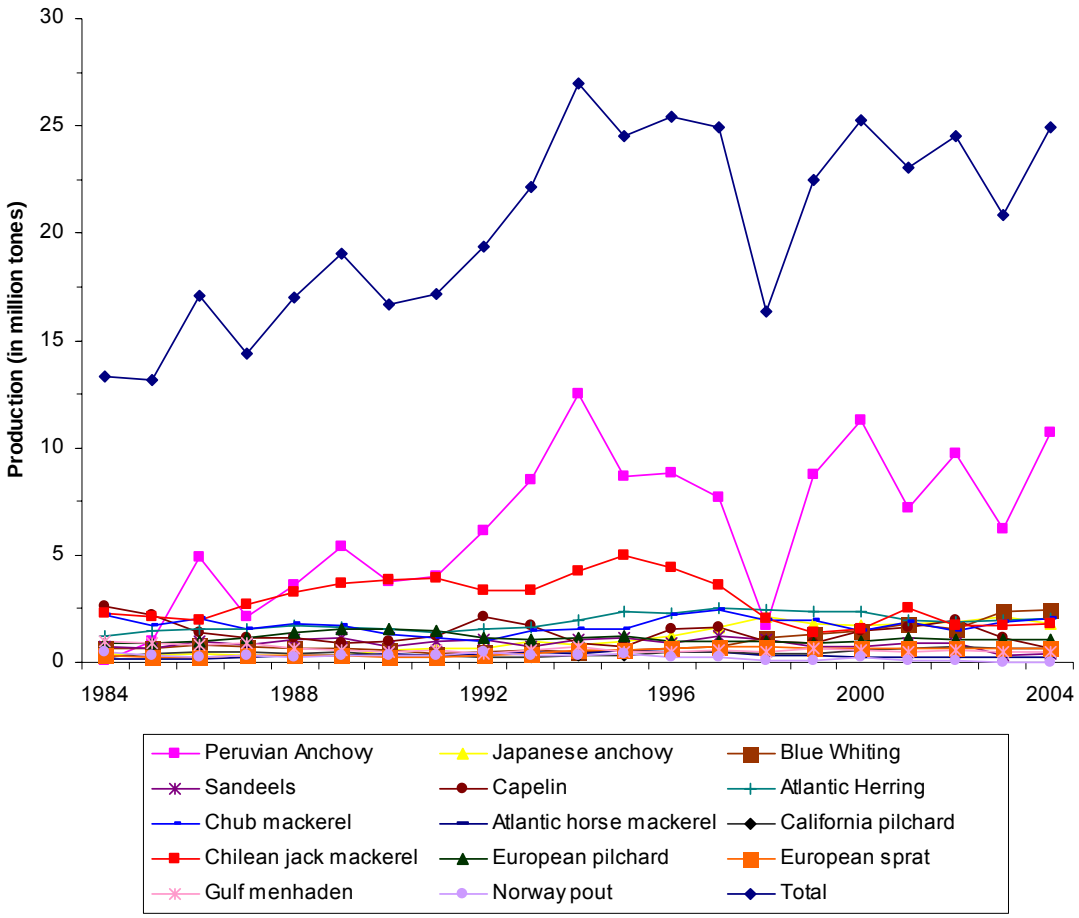

Figure 1.5. Total fisheries capture for the top pelagic fish species destined for FM and FO production from 1984 to 2004 including capture amounts for each species
(Source: FAO, 2007a)

Moreover, it has been estimated that the resources of wild feed grade fisheries will remain static in the next decade (Pike and Barlow, 2003). Tacon (2005, pp. 31), summarizing the status of exploitation of the major pelagic fish stocks based on data from FAO's review of the state of world marine fishery resources (FAO, 2005c), underlines that 52% of the world stocks are fully exploited and of the remaining, approximately 17% are over exploited, 7% depleted and 1% recovering and, hence, there is no room for further expansion. It is also noted that the catches from the fully exploited wild stocks cannot be further expanded as they are already at, or very close to, their maximum sustainable production limit (Tacon, 2005).

Lastly, natural phenomena have affected, in previous years, the global fisheries capture. For instance, the El Niño – Southern Oscillation (ENSO), which occurred in 1997 – 1998, caused a dramatic decline in Southern hemisphere catches and consequently reduced the global production as demonstrated clearly in Figure 1.5 (FAO, 2007a). The El Niño – Southern Oscillation is a periodical phenomenon and, hence, the risk of such negative effects on fisheries in the future is apparent.

The relatively stable production of FM and FO in the last 20 years is demonstrated in Figure 1.6. The global production of FM, that is excluding meals from crustaceans and aquatic mammals, ranged from 5,810,228 tonnes in 1984 to 6,572,967 tonnes in 2004. The production of FO varied from 1,506,479 tonnes in 1984 to 1,085,674 tonnes in 2004. The negative effect of the El Niño in 1998 mainly on the production of fish meal is also clearly shown.

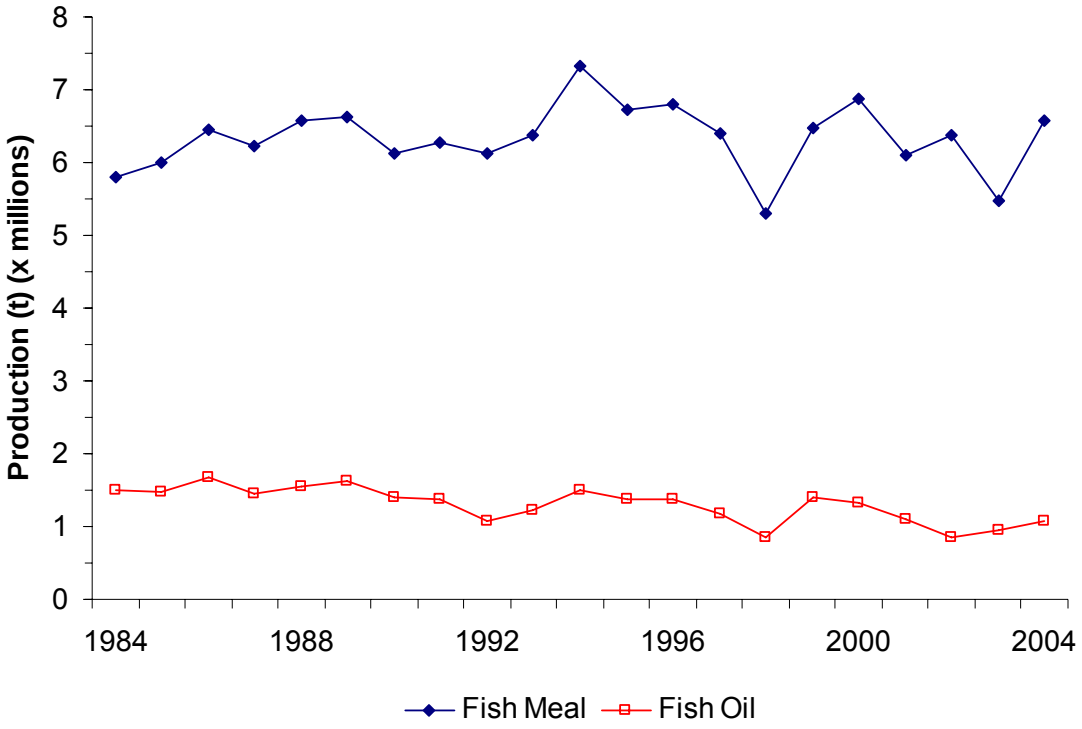

Figure 1.6. Global production of fish meal and fish oil from 1984 to 2004
(Source: FAO, 2004a)

The aquaculture sector is at present the biggest consumer of FM and FO, consuming, in 2003, about 2,936,000 tonnes of FM and 802,000 tonnes of FO (Tacon, 2005). Figure 1.7 shows the estimated use of FM and FO in animal and aquafeeds in 2002 and 2012 based on the data from the International Fishmeal and Fish Oil Organisation (IFFO) (Pike, 2005). In 2002, aquaculture's share for FM and FO was estimated at 46% and 81%, respectively, and is expected to increase in the future.

Lastly, the largest proportion of the total FM and FO used in aquafeeds in 2003, was used by salmonids as is clearly demonstrated in Figure 1.8 (Tacon, 2005). Specifically, salmon species used 19.5% and 51% and trout species 7.4% and 15.4% of the total FM and FO in aquafeeds, respectively. The consumption of FM and FO for marine fish, which include Atlantic cod, is also high.

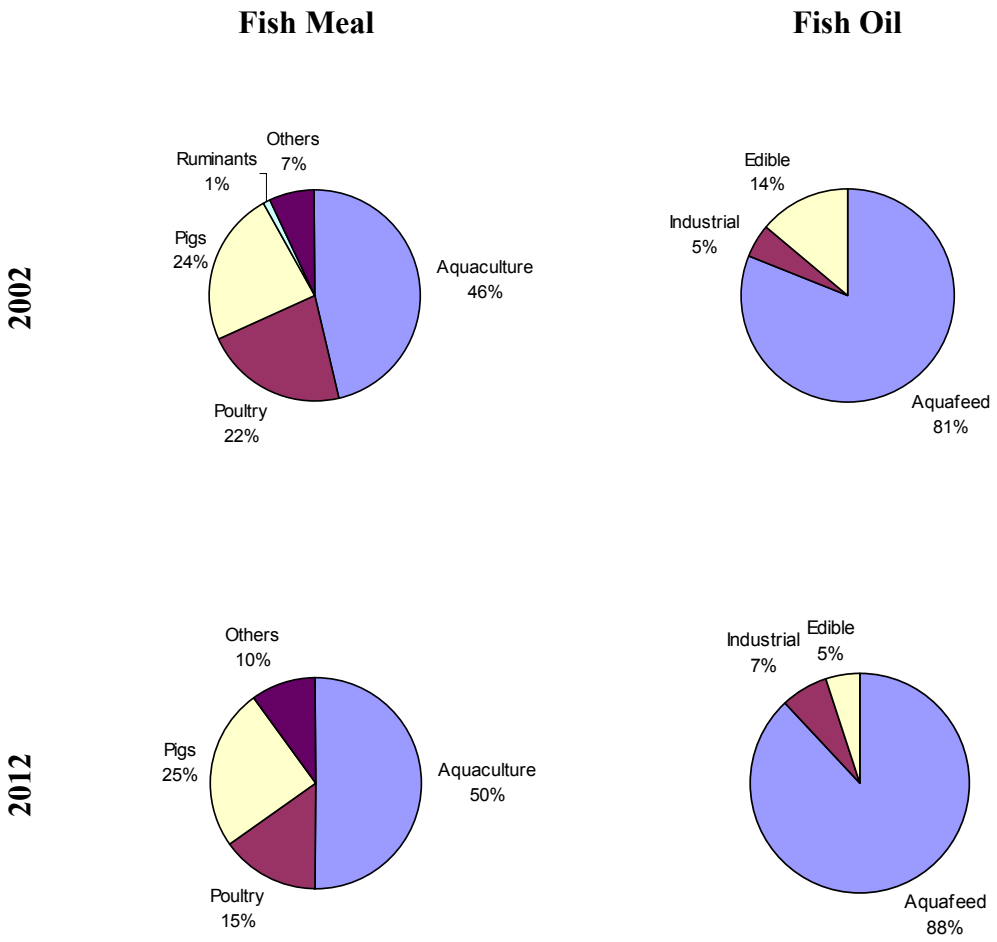

Figure 1.7. Estimated use of fishmeal and fish oil in animal and aquaculture feeds in 2002 and 2012

(Source: Pike, 2005).

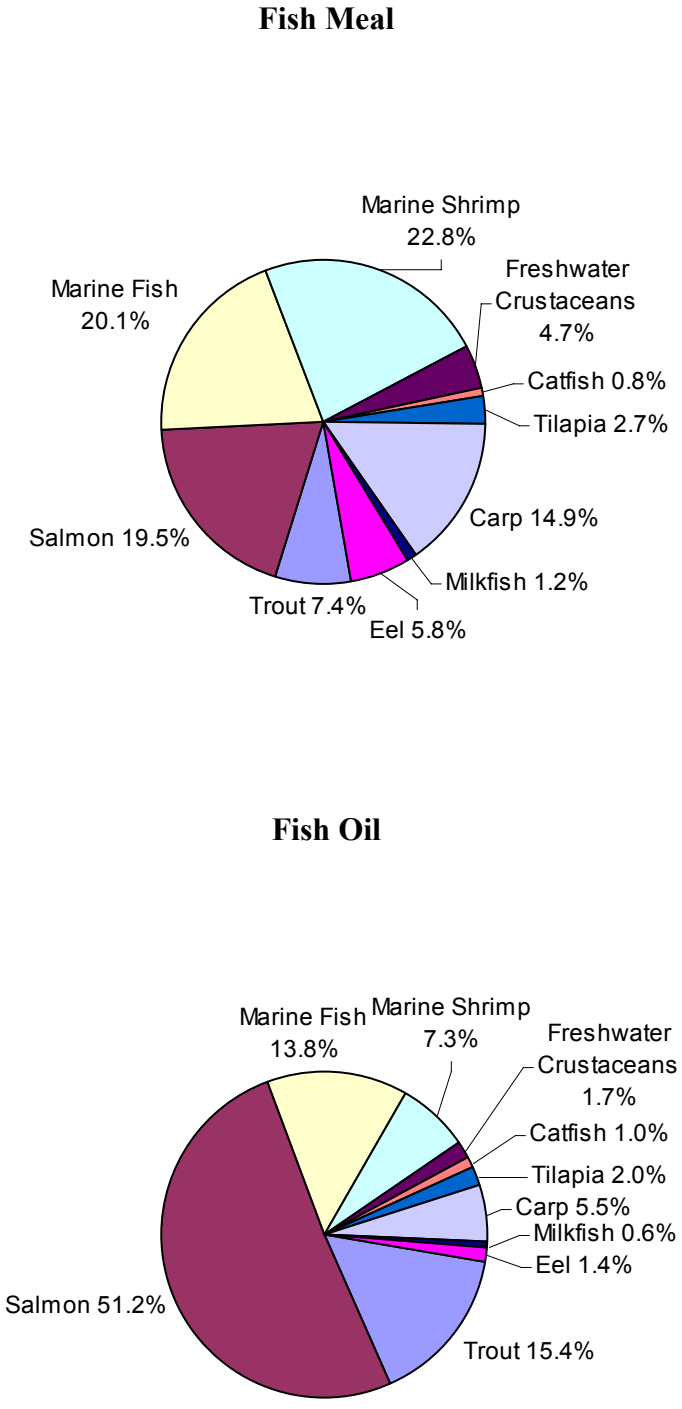

Figure 1.8. Estimated global use of fishmeal and fish oil in aquafeeds in 2003 by major species

(Source: Tacon, 2005)

1.3.6 Replacement of fish meal and fish oil

Clearly, aquaculture is heavily dependent on FM and FO. Specifically, there is a strong dependence on FM and FO for the salmon and trout industry, and Atlantic cod culture also relies to a great extent on these commodities, especially FM. Given the estimated significant growth of aquaculture in the next decade, while the production from wild feed grade fisheries will remain static, this could be risky and even harmful to the viability, growth and profitability of the sector (Sargent and Tacon, 1999; Tidwell and Allan, 2002; Pike and Barlow, 2003; Tacon, 2004; Pike, 2005). It is noteworthy that the prices of FM and FO are projected to rise significantly by 2020 under a variety of possible scenarios (Delgado, et al., 2003). Furthermore, other issues like the contamination of FM and FO with organic pollutants, make the use of some FM and FO for aquafeeds problematic. This is emphasized by the increasing international and national demand for safer and higher quality aquatic products (FAO, 2006b). Hence, there is a growing, pressing need for sustainable alternatives to FM and FO and for the reduction of the dependence on FM and FO for fish feeds. In fact, the need for reducing the FM and FO share in aquafeeds has been underlined in numerous reviews, reports and scientific papers and it presents a considerable challenge for the future development of aquaculture (Naylor, et al., 1998; Sargent and Tacon, 1999; Delgado, et al., 2003; Tacon, 2005; FAO, 2006b; Trushenski, et al., 2006).

1.4 Sustainable alternatives to fish meal and fish oil

1.4.1 Substitution with plant alternatives

There are numerous protein and lipid sources with a potential use in aquafeeds as substitutes for FM and FO, respectively, including animal by products, plant proteins

and oils, marine products from lower trophic levels (Olsen, et al., 2004) and transgenic plants (Robert, 2006). However, the use of ingredients of plant origin as sustainable alternatives to marine meals and oils in aquafeeds is of great potential. Specifically, plant ingredients have high global availability at competitive prices, compared to FM and FO, and they have nutritional properties that can largely satisfy the nutritional requirements of the fish (NRC, 1993). However, their use does present some problems and several challenges have to be met before successful replacement of FM and FO, with plant meals and oils, is achieved. These are further discussed in the following section (1.4.2). There is a large number of plant ingredients that have been studied or used as substitutes for FM and FO in aquafeeds; however, the studies presented in this thesis focused on two VO and an oilseed meal, namely crude palm oil, rapeseed oil and full fat soy bean meal, respectively.

1.4.2 Challenges and limitations

Any dietary changes using alternative plant-derived materials must ensure that firstly, the growth of the fish is not compromised. Moreover, as feed accounts for 40-60% of the total production cost, any negative effects on feed efficiency should also be avoided. Lastly, the quality of the final product, including its highly beneficial properties for human health, should not be compromised.

1.4.2.1 Growth performance

As previously described (paragraph 1.3.3), FM is a high quality protein source providing the essential amino acids (EAA) for optimum fish growth, while both FM and FO are excellent energy sources for fish. Hence, any reduction in the dietary levels of

these ingredients should take into account the potential losses in macro- and micro-nutrients and energy content (NRC, 1993).

When selecting potential VO for substituting FO in fish diets, their FA composition must be considered. Studies investigating mitochondrial β-oxidation suggested that saturated and monounsaturated FA are preferred over PUFA for energy production in fish (Henderson, 1996). Hence, to minimise any reduction in growth rate, potential substitutes for FO should provide sufficient energy in the form of the preferred FA. Moreover, the replacement of FO with VO can be also challenging due to the fact that VO lack the n-3 HUFA which are abundant in FO; these FA and especially EPA and DHA are essential for optimal growth and development in salmon (Sargent, et al., 2002). The n-3 HUFA in FO is generally around 20-30% of total lipids, while in VO contain no HUFA and, in most cases, only moderate levels of the C18 PUFA, 18:3n-3. On the other hand fish oils contain low levels of n-6 PUFA whereas VO are usually rich sources of C18 PUFA (NRC, 1993; Hertrampf and Piedad-Pascual, 2000; Hardy, et al., 2001).

1.4.2.2 Quality of the final product and implications for human nutrition

The FA composition of fish tissues reflects the FA composition of the diets (Sargent, et al., 2002). Hence, replacement of FO with VO results in reductions of EPA, DHA and the n-3/n-6 FA ratio, with a direct effect on the nutritional quality of the end product (Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2003a). This is important for the human consumer as EPA and DHA and a high n-3/n-6 FA ratio have been associated with numerous beneficial effects for human health and any reduction of these HUFA in farmed fish would be undesirable (De Deckere, et al., 1998; Horrocks and Yeo, 1999; Simopoulos, 1999; Hunter and Roberts, 2000; ISSFAL, 2000;

Simopoulos, 2003). Hence, substitution of FO with VO should be such that the catabolism of EPA and DHA is minimised and avoids excess deposition of 18:2n-6 (Bell, et al., 2001a; Sargent, et al., 2002). Lastly, reductions in tissue EPA and DHA but also in 20:1n-9 and 22:1n-11 may affect the retention of oil in tissue adipocytes and consequently its physicochemical properties during storage and processing, influencing the quality of the final product (Sargent, et al., 2002).

1.4.2.3 Antinutritional factors

The use of plant materials in aquafeeds may be, to some extent, challenging or even problematic, due to the presence of antinutrients or antinutritional factors (ANF). These substances are naturally occurring toxins or antimetabolites, whose main function in plants is as natural insecticides or generally, as parts of survival mechanisms that prevent grazing and consumption of plants by insects and herbivores (Hendricks, 2002). Common antinutritional factors include trypsin inhibitors, hemagglutinatinins (lectins), phytic acid, gossypol, phytoestrogens, cyclopropenoic FA, glucosinolates, erucic acid, alkaloids and thiaminase (NRC, 1993; Hendricks, 2002). The presence of such substances in aquafeeds may affect their nutritional value that may result in a variety of physiological effects on fish (Francis, et al., 2001).

1.4.3 Palm oil in rainbow trout diets

Palm oil (PO) is derived from the oil palm (*Elaeis guineensis* family: Arecaceae). PO is a fruit flesh oil, however, a seed oil (palm kernel oil) is also produced (Hertrampf and Piedad-Pascual, 2000). PO has a high availability as its production provides one of the largest VO tonnages in the world, along with soybean oil (FAO, 2005b; U.S. Department for Agriculture, 2007). Interestingly, it is also predicted to

exceed soybean oil production within the next few years to become the most abundant VO in the world (Gunstone, 2001).

Crude palm oil has a very high content of 16:0 and 18:1n-9 (43.5% and 36.6% of total lipid FA, respectively) and relatively low levels of 18:2n-6 (9.1%) (NRC, 1993; Ng, 2002a, 2002b). This FA composition makes PO a good potential candidate to replace FO in diets for rainbow trout and that is firstly, for providing energy to the fish for sufficient growth. Previous studies have shown that in rainbow trout liver, 22:1n-11 and 16:0 were the best substrates for mitochondrial β-oxidation while in red muscle 16:0, 16:1, 18:1n-9 and 18:2n-6 were the preferred substrates (Henderson and Sargent, 1985; Kiessling and Kiessling, 1993). Moreover, the relatively low levels of 18:2n-6 should avoid excess deposition of this FA in the fish tissues, although the lack of n-3 PUFA and HUFA, as is the case for many VO, could restrict its use at high levels as a respective decrease in fish flesh n-3 HUFA would be undesirable.

The use of PO in the diets of Atlantic salmon and rainbow trout has been investigated in several studies with regards to the growth and feed utilization efficiency, and changes in tissue FA composition and FA metabolism, giving promising results (Torstensen, et al., 2000; Rosenlund, et al., 2001; Bell, et al., 2002; Caballero, et al., 2002; Ng, et al., 2003; Ng, et al., 2004).

1.4.4 Rapeseed oil in Atlantic salmon feeds

Rapeseed oil (RO) is extracted from rapeseed (*Brassica napus* – family Brassicacea), an oil seed which contains 40%-50% oil, and is used both in animal feeds and also as a food-grade oil, especially in middle and northern Europe (Hertrampf and Piedad-Pascual, 2000; Beare-Rogers, et al., 2001). It is rich in monounsaturated FA and

especially in 18:1n-9 (NRC, 1993). It may also have a relatively high content (up to 40 – 50%) of erucic acid (22:1n-9) although, in current varieties, grown for food and feed use, this has been eliminated through selective breeding (Hertrampf and Piedad-Pascual, 2000; Beare-Rogers, et al., 2001; Gunstone and Herslöf, 2004). RO has a high availability, as it is the third largest production of VO in the world, after soy and palm oil (FAO, 2005b; U.S. Department for Agriculture, 2007).

RO is considered to be a good sustainable substitute for FO in diets for Atlantic salmon, not only because of its high availability and marketing potential but also for its nutritional properties, although its FA composition differs significantly to that of marine oils. Its content of 18:2n-6 and 18:3n-3 is moderate and at a ratio of 2:1 and thus, should result only in modest deposition of these FA in fish tissues and, perhaps, enhance the endogenous conversion of 18:3n-3 to 20:5n-3 and 22:6n-3. As discussed in paragraph 1.4.2.2, accumulation of 18:2n-6 may result in low n-3/n-6 ratios that are undesirable for the quality of the final product as it may compromise the health benefits of eating fish and hence, such an effect would be unacceptable to producers and consumers. Secondly, RO contains high levels of monounsaturated FA, especially 18:1n-9, which are preferred substrates for energy production by Atlantic salmon and hence, growth rates of the fish should not be compromised (Henderson and Sargent, 1985; Kiessling and Kiessling, 1993; Bell, et al., 2001a; Sargent, et al., 2002).

In fact, RO has been successfully used in a number of previous studies with Atlantic salmon, replacing up to 100% of FO either as a single replacer or in blends with other VO including linseed oil, PO, olive oil and others. RO inclusion up to 100% showed no negative effects on growth or feed conversion and utilization and a correlation between dietary and tissue FA composition, although the endogenous

hepatic desaturation and elongation of C_{18} FA to HUFA is enhanced by RO (Tocher, et al., 2000; Bell, et al., 2001a; Rosenlund, et al., 2001; Tocher, et al., 2001; Bell, et al., 2003a; Bell, et al., 2003b; Bendiksen, et al., 2003b; Bendiksen and Jobling, 2003; Tocher, et al., 2003a; Tocher, et al., 2003b; Torstensen, et al., 2004a; Torstensen, et al., 2004b; Moya-Falon, et al., 2005; Torstensen, et al., 2005; Stubhaug, et al., 2007). However, a number of these studies used small fish and focused mainly on the effects of the replacement of FO only, while moderate dietary lipid levels were used. Lastly, it has been shown that water temperature may play a significant role in the effects of the replacement of FO with RO (Bendiksen, et al., 2003b; Bendiksen and Jobling, 2003; Jobling and Bendiksen, 2003). Hence, there is scope for the investigation of the interactive effects of dietary RO inclusion at various dietary protein/lipid levels on growth and FA composition of large on-grown Atlantic salmon cultured at different temperatures.

1.4.5 Soybean meal in Atlantic cod diets

Soybean meal is produced as a by-product after the removal of the oil from soy beans (*Glycine max*); when whole unextracted soy beans are used the product is called "full fat soybean meal" (FFS) (Hertrampf and Piedad-Pascual, 2000). Soybean meals have a very high availability; soy beans, soybean meal and soybean oil are the oilcrop, oilmeal and VO, respectively, with the largest production tonnages in the world (U.S. Department for Agriculture, 2007). The amino-acid (AA) profile of soybean meal could meet most of the EAA requirements of fish, and is considered to be one of the best among all protein-rich plant feedstuffs, although levels of the EAA methionine and histidine are significantly lower than in FM (NRC, 1993). However, it may contain several anti-nutritional factors, such as trypsin inhibitors, hemagglutinatinins (lectins)

and others, that could affect its nutritional value (NRC, 1993; Hertrampf and Piedad-Pascual, 2000; Francis, et al., 2001).

Although FFS is included in dietary formulations largely as a protein source, having a crude protein content of ~38%, it also contributes to the dietary fat, containing about 18% lipid (NRC, 1993). More than 50% of the total FA (FA) of the soya beans is 18:2n-6, more than 25% is 18:1n-9, less than 10% is 16:0 and around 6% is 18:3n-3, while it does not contain any HUFA such as EPA and DHA (Hertrampf and Piedad-Pascual, 2000; Beare-Rogers, et al., 2001).

Due to its high availability and nutritional value, FFS, along with other soy products, is considered a good potential protein substitute for FM in aquafeeds. Such products have been used to replace FM in diets for various fish species (van den Ingh, et al., 1991; Oliva-Teles, et al., 1994; Olli and Krogdahl, 1994; Refstie, et al., 1998; Alexis and Nengas, 2001; Refstie, et al., 2001; Grisdale-Helland, et al., 2002; Glencross, et al., 2004; Kaushik, et al., 2004; Mundheim, et al., 2004) including Atlantic cod (Von der Decken and Lied, 1993; Hansen, et al., 2006; Refstie, et al., 2006). However, the results of these studies are variable, and in some cases contradictory, probably due to the variable ANF content that soybeans contain (Francis, et al., 2001). In general, it could be said that relatively little is known about the replacement of FM with soya products in diets for Atlantic cod.

1.4.6 Energy dense / High lipid diets

The use of high lipid or energy dense diets is mainly designed to increase the growth of the fish for a given amount of feed intake by maximizing the utilization of lipids with a consequent protein-sparing effect (Sargent, et al., 2002). The protein

sparing effect is briefly the utilization by the fish of as much of the available dietary protein as possible for conversion into muscle protein instead of energy production. Salmonids can utilize lipids efficiently, therefore the use of high lipid diets in salmon allows protein sparing and subsequently improved growth (Frøyland, et al., 1998; Hillestad, et al., 1998; Bendiksen, et al., 2003b).

Traditionally, salmon diets contain high proportions of protein, most of it provided by FM. However, it is crucial for the aquafeed industry to optimise the use of feed protein and to improve the protein utilisation in salmon diets. Clearly, high lipid or energy dense dietary formulations have a potential in commercial aquaculture, as they would allow less dependence on FM, reduce the cost of the feed and also reduce the environmental impact through reduced nitrogenous waste output from salmon culture (Halver and Hardy, 2002). Thus, as mentioned in paragraph 1.2.2, the trend in Atlantic salmon feeds is currently for high dietary lipid levels.

Several studies have investigated the use of low protein / high lipid diets showing promising growth results and protein sparing, although an excessive fat deposition in the fish carcass was coincident with high lipid feeds (Einen and Roem, 1997; Bendiksen, et al., 2003b; Azevedo, et al., 2004a; Solberg, 2004). Although, in most of the earlier studies relatively high protein/lipid ratios were investigated, high inclusions of dietary lipid of more than 400 g kg^{-1} have also been studied in Atlantic salmon (Hemre and Sandnes, 1999). However, to what extent the dietary protein / lipid ratio can be reduced and what the optimum levels of dietary protein / lipid are for Atlantic salmon is largely unknown.

1.5 General objectives

The overall objective of the present study was to investigate the effects of substitution of FM and/or FO with sustainable alternatives of plant origin and the use of low protein/high lipid diets on the growth, fish tissue proximate and FA compositions, FA metabolism and nutrient and FA digestibility of commercially important European fish species. Specifically the objectives were:

1) To investigate the effects of dietary PO on growth, feed efficiency, whole body proximate composition and tissue FA compositions in rainbow trout

2) To elucidate the interactive effects of dietary fat and protein contents and oil source on growth and feed efficiency, tissue proximate and FA composition in Atlantic salmon at low water temperatures.

3) To elucidate the interactive effects of dietary fat and protein contents and oil source on growth and feed efficiency, tissue proximate and FA composition, β-oxidation capacity, and nutrient and FA digestibility in Atlantic salmon at high water temperatures.

4) To investigate the effects of the partial replacement of FM and FO with FFS on growth, feed efficiency and tissue FA composition in Atlantic cod.

Chapter 2. General Materials and Methods

2.1 Materials

All reagents, butylated hydroxytoluene (BHT) and 17:0 free fatty acid standard were obtained from Sigma Chemical Co. (Poole, U.K.). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England. Thin-layer chromatography (TLC) plates, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All gases were obtained from the British Oxygen Company (BOC, Glasgow, UK).

2.2 Experimental diets

The experimental diets for Experiments I, II and III (described in Chapters 3, 4 and 5, respectively) were produced at the BioMar TechCentre (Brande, DK) as practical-type extruded pellets. The experimental diets for Experiment IV (Chapter 6) were produced by Fiskeriforskning (Fyllingsdalen, Norway). The diets were formulated to meet all the known nutritional requirements of the fish species that were studied, specifically of rainbow trout, Atlantic salmon and Atlantic cod (NRC, 1993). The formulations, proximate and fatty acid compositions of the experimental diets used in each individual experiment, along with details regarding the feeding practices, are described in the methodology of the appropriate chapter for each experiment. Prior to each feeding trial or during an initial acclimatisation period the fish were fed commercial extruded feeds.

2.3 Experimental animals and husbandry

The experiments were conducted in accordance with either the British Home Office (experiments I and IV) or the Norwegian Government (experiments II and III) guidelines regarding research on experimental animals.

The experimental animals used in this study were rainbow trout for experiment I, Atlantic salmon for experiments II and III and Atlantic cod for experiment IV. The fish for experiments I and IV were held in enclosed tanks at the aquarium facilities of the Institute of Aquaculture of the University of Stirling (Stirling, Scotland) and at the Scottish Association for Marine Science (SAMS) (Ardtoe, Scotland), respectively. The fish for experiments II and III were held in sea cages at Gildeskål Research Station (GIFAS) (Inndyr, Norway) and Fjord Research Station AS (Helgeland, Dønna, Norway), respectively. The specific experimental conditions for each individual dietary trial are described in the specific methodology section of the appropriate chapter.

2.4 Sampling procedures

2.4.1 Feed sampling

At the beginning of each experiment sufficient amounts of the diets required to complete the analyses under investigation were collected. The diets were kept at -20^{o}C until analysed.

2.4.2 Fish sampling

Prior to any experimental procedure (e.g. weighing and measuring) all fish were anaesthetized using MS222 (Tricaine, Sigma, Poole, UK). After the experimental

procedure the fish were placed in clean aerated water and allowed to recover (usually within 5 min) before being returned to the original housing.

Measurements of fish weight and length were made throughout the experiments. The fish were weighed to the nearest 0.1g and total (fork) length was measured to the nearest millimetre.

Where fish required to be sacrificed for tissue sampling they were first anaesthetized and then killed with a sharp blow to the head such that death was instantaneous. Tissue samples (i.e livers, gills, hearts, kidneys, pyloric caeca) were dissected out and immediately frozen in liquid nitrogen or dry ice, as described in the methodology of the appropriate chapter for each experiment. For flesh samples, Norwegian quality cuts (NQC) were taken, and skinned and de-boned before being frozen in liquid nitrogen or dry ice. Muscle and liver samples for β-oxidation capacity analyses were taken as described above. Briefly, liver samples were dissected out and immediately frozen in liquid nitrogen and NQC steaks were taken and frozen immediately, with red and white muscle sections being separated at a later stage, prior to analyses.

All samples were placed in appropriate containers i.e. plastic tubes, centrifuge tubes, beakers, plastic bags etc and stored at -20^{o}C until further analyses. Where samples were required to be transferred from the experimental sites to the Institute of Aquaculture, University of Stirling labs for analyses they were placed frozen in polystyrene boxes containing an excessive amount of dry ice to ensure that they were always kept frozen until delivered.

2.5 Proximate analysis of diets, tissues and faeces

The chemical compositions of the experimental diets, fish tissues and faeces samples were determined by proximate analyses based on methods described in AOAC (1995).

2.5.1 Moisture

Thermal drying to constant weight in an oven at 110 °C for 24h was applied to determine moisture content of feeds and fish whole body and tissue samples. Specifically, the feed samples were ground to a powder using a coffee grinder or mortar and pestle. Then 1.0 to 5.0g of sample was placed into a pre-weighed dish and dried in an oven at 110°C. Regarding the whole fish samples, they were either blended in a food processor or blender or sliced open and then cut into pieces and weighed onto a pre-weighed dish. Then they were dried in the oven as described above, allowing a longer drying time and until constant weight was obtained. After removing the samples from the oven they were placed in a desiccator to cool and then reweighed.

The moisture content of the samples was calculated as:

$$\text{Moisture, \%} = \frac{\text{sample weight (g)} - \text{dried sample weight (g)}}{\text{sample weight (g)}} \times 100$$

2.5.2 Freeze drying

The faeces samples were freeze dried before analysis using a CHRIST Alpha 1-4 LSC (Osterode am Harz, Germany) freeze dryer. Briefly, the faeces were placed into a pre-weighed dish and their weight was recorded. Then they were freeze dried at -50°C under vacuum to constant weight. The vapour pressure above ice was 1.030 mbar

giving a freeze drying of -20^{o}C. The dry matter (and consequently the moisture) was quantified gravimetrically by reweighing the dish or flask and the moisture content of the faeces was determined per weight of sample freeze-dried.

2.5.3 Crude protein

Crude protein content was determined by Kjeldahl analysis (nitrogen x 6.25) using a Kjeltec Autoanalyser (Tecator). Briefly, 200mg of sample were weighed into a Kjeldahl digestion tube and 2 mercury Kjeltabs and 5ml conc. sulphuric acid were added. The tube was then placed into the digestion block. After 1 hour the tubes were removed from the block and left to cool inside a fume cupboard for at least 15 minutes. Then, 20ml de-ionised water and 5ml sodium thiosulphate solution were added to the digestion tube and mixed thoroughly. The tubes were distilled using the Kjeltec auto analyser and the titration values were recorded. All samples were analysed in triplicates. Similarly, for each batch of samples, 3 urea standard tubes and 3 blank tubes were prepared and analysed.

The protein content of the samples was calculated as:

$$\text{Protein},\% = \frac{(\text{sample titre} - \text{blank titre}) \times 1750.875}{\text{sample weight (mg)}}$$

where 1750.875 is a multiplication factor to convert titre vol. to % protein based on standardised protein factor.

2.5.4 Acid hydrolysis

Diet samples were acid hydrolysed using a Soxtec System 1047 hydrolysing unit (Tecator Application note 92/87) as follows. Sample of 1.0 –3.0g, celite of the same weight and 100ml 3M hydrochloric acid solution were added into a hydrolysis tube. The tube was then inserted into the hydrolysing unit were an appropriate glass thimble was placed. The sample was boiled gently for 1 hour and then 100ml de-ionised water were added to the tube. The solution was then filtered through the glass thimbles and the tube washed with 5 x 50ml hot deionised water using a spray gun. The tubes and the glass thimble were then removed from the unit. The residue of the sample was cleaned from the tube with cotton wool and placed in the glass thimble. The thimble was dried at 100°C for 3 hours (or 60°C overnight) before the sample lipid was extracted on the Tecator Soxtec Unit.

2.5.5 Crude fat

Crude fat was determined by exhaustive Soxhlet extraction using petroleum ether on a Soxtec System HT6 (Tecator application note 67/83). Prior to the fat extraction the diet samples were acid hydrolysed as described in paragraph 2.5.4. Briefly, 1g of the sample was put into a Soxhlet extraction thimble. A Soxhlet extraction cup, containing 5 glass balls, was weighed and 40ml of petroleum ether (40-60°C, BP) were added. The thimble was moved to the Soxtec System and boiled in the solvent for 20 minutes and then rinsed for 2 hours. After the extraction the solvent was evaporated off, the extraction cup was removed and placed into an oven at 110°C. After 1 hour the extraction cup was removed from the oven and left to cool inside a desiccator. Fat was quantified gravimetrically by reweighing the extraction cup and

crude fat content was determined per weight of sample extracted. All samples were analysed in triplicate.

2.5.6 Ash

The ash content of diets, fish tissues and faeces was determined by dry ashing approximately 1g of sample in a porcelain crucible in a muffle furnace at 600 °C overnight. After ashing, the samples were removed from the furnace, cooled to room temperature in a desiccator and then reweighed.

The ash content was calculated as:

$$\text{Ash, \%} = \frac{\text{Ash Weight (g)}}{\text{Sample Weight (g)}} \times 100$$

2.5.7 Gross energy

The gross energy content of diets and faeces was determined using an adiabatic bomb calorimeter using the Gallenkamp Autobomb system. Briefly, 1g of dried sample was pressed to make a firm pellet and placed into a crucible. Nickel firing wire was fixed between the electrodes and then a cotton string was wound from the pellet around the firing wire and the shorter electrode. The electrode assembly was then put into the calorimetry bomb. The water jacket of the bomb was filled with tap water and the calorimeter vessel was filled with water at 21-23°C and weighed to exactly 3kg. The calorimeter vessel was placed into the water jacket. Before firing the calorimetry bomb the thermometer reading was recorded as the initial temperature. The bomb was then fired and when the temperature stabilised it was again recorded (final temperature).

The energy content (kJ/g) of the sample was calculated as:

$$\text{Gross Energy} = \frac{\left[(\text{Final Temp.} - \text{Initial Temp.}) \times 10.82\right] - 0.0896}{\text{Sample Weight (g)}}$$

where 10.82 is the factor of heat capacity of the system and 0.0896 represents the combined energy values (expressed in kJ) for the wire and the cotton thread used in the analysis.

2.5.8 Yttrium oxide (Y_2O_3)

Yttrium oxide (Y_2O_3) was determined by a spectrophotometric method after acid digestion by Dr Andrew Fisher at the University of Plymouth. The diet or faeces samples were digested in concentrated nitric acid and then analysed by inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.6 Lipid extraction and analysis

2.6.1 Total lipid extraction of diets, tissues and faeces

Total lipids of diet, tissue and faeces samples were extracted by homogenisation in chloroform/methanol (C/M) (2:1, v/v) containing butylated hydroxytoluene (0.01% w/w, BHT) as antioxidant, according to Folch et al. (1957). Specifically, approximately 1g of diet, tissue or faeces sample was added to a boiling tube with 20 ml of C/M) (2:1,v/v). The sample was then homogenised using an Ultraturrax^{TM} , and the stoppered tube was left on ice for approximately 1 hour. After that, 5 ml of 0.88% (w/v) KCl (0.25 volumes of KCl) were added to the homogenised sample, mixed thoroughly on a vortex mixer and left to stand on ice. After 15 minutes the tubes were centrifuged at 400 x g

for 2-3 minutes. After the layers had separated the top layer was removed by aspiration. The bottom layer was transferred to pre-weighed tubes through 11 cm filter papers (Whatman no. 1) pre-washed with C/M 2:1. The solvent was evaporated under a stream of oxygen-free nitrogen (OFN), on a nitrogen evaporator, to dryness and the samples were desiccated in vacuo overnight. Lipids were quantified gravimetrically by reweighing the test tube and total lipid content was determined per weight of material extracted. Lastly, the total lipids were re-dissolved in C/M (2:1) + 0.01% (w/v) BHT at a concentration of 10 mg/ml and transfered to 2 ml glass vials. The total lipids were stored under nitrogen in a freezer at -20^{o}C.

2.6.2 Preparation and purification of fatty acid methyl esters

Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification as described by Christie (1982) and FAME extracted and purified as described by Tocher & Harvie (1988). Specifically, total lipid (normally 1 mg but up to 50 mg) was added into a small test tube along with 17:0 free fatty acid standard (prepared to a concentration of 1 mg/ml in C/M (2:1, v/v)) at 10% of the total lipid mass. The organic solvent was evaporated on the nitrogen evaporator and then 1 ml of toluene and 2 ml of the methylating reagent were added and thoroughly mixed. The methylation reagent was prepared as a 1% (v/v) solution of conc. sulphuric acid in methanol. The tube was flushed with nitrogen, stoppered and incubated overnight (16 hr) at 50^{o}C in a hot-block. After incubation the tube was removed from the hot-block. When cool, 2 ml 2% (w/v) $KHCO_3$ and 5 ml iso-hexane/diethyl ether (1:1, v/v) + 0.01% (w/v) BHT were added, mixed and centrifuged at 350-400 x g_{ave} for 2 minutes. The upper organic layer was transferred to another test tube and a further 5 ml iso-hexane/diethyl ether (1:1, v/v) (no BHT) were added to the original tube. The tube was

mixed and centrifuged as before and the upper layer was added to the other tube as before. The combined solvent was evaporated under nitrogen and the FAME were re-dissolved in 100 µl of iso-hexane.

Methyl esters were purified by TLC on 20 x 20 cm plates. The FAME were loaded on the plate using a 100 µl glass Hamilton syringe. The plate was chromatographed in iso-hexane/diethyl ether/acetic acid (90:10:1, v/v/v). Then, it was removed from the tank and the solvent was allowed to evaporate in the fume cupboard. The edge of the plate was then sprayed with 1% (w/v) iodine in chloroform to visualise the FAME. The FAME bands were marked and then scraped from the TLC plate into a test tube using a straight edged scalpel blade. FAME were eluted from the silica with 10 ml of iso-hexane/diethyl ether (1:1, v:v), then mixed and centrifuged as described above, to sediment the silica. The solvent was transferred to a clean small test tube and then evaporated to dryness under nitrogen. The samples were transferred to 2 ml glass vials in 1 ml of iso-hexane, evaporated to dryness and re-dissolved in iso-hexane to a concentration of 1 mg/ml. The FAME were stored under nitrogen or argon at -20^{o}C until GLC analysis.

2.6.3 Separation and identification of fatty acid methyl esters

FAME were separated and quantified by gas-liquid chromatography using a Carlo Erba Vega 8160 (Milan, Italy) chromatograph equipped with on-column injection (cold) and using a 30 m x 0.32 mm id, 0.25µm film thickness, capillary column (CP wax 52CB; Chrompak Ltd., London, U.K.). Hydrogen was used as carrier gas at a flow rate of 2.0 ml/min (constant flow mode). The temperature programming was from 50^{o}C to 150^{o}C at 40^{o}C/min and then to 225^{o}C at 2^{o}C/min. The fatty acid peaks were detected by flame ionisation detection (FID). The volume of the sample injected was 1 µl of 1

mg/ml FAME/iso-hexane. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman, 1980). Peak data was processed using Chromcard for Windows (version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy).

2.7 β-oxidation capacity

Liver and red and white muscle were weighed and homogenized in 20% (w/v) ice-cold buffered sucrose solution containing 0.25M sucrose, 0.04M potassium phosphate buffer (pH 7.4), 0.15M KCl, 40mM KF and 1mM N-acetyl cysteine. The resulting total homogenates were then centrifuged at 1880 × *g* for 10 min at 2°C. The resulting post-nuclear fractions were collected, and portions were used immediately to determine β-oxidation capacity. The latter was determined as acid-soluble products using radiolabelled [1-^{14}C]-palmitoyl-CoA as a substrate as described by Frøyland *et al.* (1995).

Briefly, 250 µL of assay medium were added to 2ml eppendorf tubes. Then 10 µL of [1-^{14}C]palmitoyl-CoA substrate (0.1 µCi/100 µM) were added to each tube. The samples were pre-incubated at incubation (room) temperature for 2 min. The reaction was started by the addition of homogenate (30-50 µl for liver and red muscle and 300-500 µl for white muscle, homogenized in 20% (w/v) ice-cold buffered sucrose solution as described above) and the reaction continued for 10 min. The reaction was stopped by addition of 150 µL of 1.5 M KOH. Then 25 µL FAF-BSA (100mg/ml) were added, the tubes were vortexed and 500 µL of ice-cold 4M $HClO_4$ (perchloric acid) were added. The tubes were centrifuged at 1880 x g for 10 min. Aliquots of 500 µL were placed in scintillation vials, 2.5 ml of scintillant added and the radioactivity was determined in a scintillation counter.

The protein content of the samples was determined according to the Lowry method (Lowry, et al., 1951). Specifically, the preparation of standards and samples was done as follows. For the standards 0, 20, 40, 60, 80 and 100 µl of the BSA standard were added to 10 ml test tubes in triplicates. Then the volume in each tube was made up to 100 ml with distilled water and 100 µl of NaOH/SDS solution were added to each tube. For the samples, 100 µl of cell samples collected were put in an eppendorf tube and 400 µl of NaOH/SDS were added. The tubes were heated at 60^{o}C for 1h. After cooling for 10 min, 50 µl portions of the digest were transferred from each sample into a plastic tube. The standards and samples were then assayed. Specifically, 1 ml of 2% (w/v) NaK tartrate solution and 1ml of 1% (w/v) $CuSO_4$ solution were added to 100 ml of 2% (w/v) $NaCO_3$ solution and mixed (volumes were adjusted as appropriate). Then 2.5 ml of this mixture were added to each standard and sample test tube. The tubes were mixed and left at room temperature for 15 min. Just prior to use, Folin reagent was diluted 1:1 with distilled water and 250 µl of the diluted reagent were added to each test tube and mixed. The tubes were then left at room temperature for 30 min before measuring at 660 nm in a UV/vis spectrophotometer. The calibration curve of protein (0, 20, 40, 60, 80 and 100 mg) versus absorbance at 660 nm (A_{660}) was prepared and protein content of samples was determined using the A_{660} values.

2.8 Calculations

The following formulae were applied to the data:

Feed Conversion Ratio:

$$\text{FCR} = \frac{\text{feed intake (g)}}{\text{wet weight gain (g)}}$$

Specific Growth Rate:

$$\text{SGR, \%/day} = 100 \times \frac{\ln(W_1) - \ln(W_o)}{\text{number of days}}$$

Thermal Growth Coefficient:

$$\text{TGC} = 1000 \times \frac{\sqrt[3]{W_1} - \sqrt[3]{W_0}}{\text{days} \times \text{Temp.}\,(^{o}\text{C})}$$

Condition Factor:

$$K = 100 \times \frac{W}{L^3}$$

Hepatosomatic Index:

$$\text{HSI, \%} = 100 \times \frac{W_{\text{liver}}}{W}$$

Viscerosomatic Index:

$$\text{VSI, \%} = 100 \times \frac{W_{\text{viscera}}}{W}$$

Cardiosomatic Index:

$$\text{CSI, \%} = 100 \times \frac{W_{\text{heart}}}{W}$$

Protein productive value, (g protein gain x g protein ingested^{-1}):

$$PPV = \frac{P_1 W_1 - P_0 W_0}{P_F \times \text{cumulative feed intake}}$$

In the above formulae W is the weight of the sampled fish in grams; W_0 and W_1 are the initial and the final fish mean weights in grams; W_{liver}, $W_{viscera}$ and W_{heart} are the weights in grams of the liver, viscera and heart, respectively, of the sampled fish; L is the fork length of the sampled fish in cm; P_0 and P_1 are the initial and final protein concentrations of the fish; P_F is the protein concentration of the feed on a dry matter basis; cumulative feed intake was determined in grams on a dry matter basis.

The Apparent Digestibility Coefficient (ADC) of nutrients and FA was calculated as:

$$ADC\,\% = 100 \times \left[1 - \left(\frac{F}{D} \times \frac{Di}{Fi}\right)\right]$$

Where D is the concentration of the nutrient or FA (or kJ/g gross energy) in the diet, F is the concentration of the nutrient or FA (or kJ/g gross energy) in the faeces, Di is the concentration of the inert marker in the diet and Fi is the concentration of the inert marker in the faeces.

2.9 Statistics

All the data are presented as means ± SD (n = 3). The statistical analyses for experiments I and II and IV were performed using SPSS 13 (SPSS Inc, 2004) while for

experiment III SPSS 14 (SPSS Inc, 2005) was used. The graphs were created using Prism 4 (Graphpad Software Inc., San Diego, USA).

Regarding experiments I and IV, the significant differences between dietary treatments were determined by one-way ANOVA followed, where appropriate, by Tukey's post-hoc test to rank the groups (P=0.05).

The experimental designs for the experiments II and III were factorial with 2 factors (2 x 3). Hence, the effects of the two factors and their interactions were analysed by two-way ANOVA. When significant interactions of the two factors were observed, the main effects were not further discussed but instead, multiple comparison testing was performed to look at the simple main effects, that is the main effect of one factor at a given level of the other (Zar, 1999, pp. 260-261). The analysis of the simple main effects was done for both factors. Where appropriate Tukey's post-hoc test was conducted to rank the groups (P=0.05).

Data which were identified as non-homogeneous (Levene's test) were subjected to square root, log or arcsin transformation before analysis. Differences were regarded as significant when $P < 0.05$ (Zar, 1999).

Chapter 3. Effects of dietary crude palm oil on rainbow trout (*Oncorhynchus mykiss)*

3.1 Introduction

As previously discussed, FM and FO have been the main sources of protein and oil for rainbow trout diets over the last 30 years (Lovell, 2002). Such feed formulations have been shown to be nutritionally beneficial for the fish, providing sufficient essential amino-acids and FA, along with highly digestible energy, but are also cost effective and readily available (NRC, 1993). Furthermore, the FA composition of the marine oils and meals, being rich in n-3 HUFA, reflects on the fish tissues, providing a nutritious and health beneficial final product for human consumers (De Deckere, et al., 1998; ISSFAL, 2000; Sargent, et al., 2002). However, various reasons, including the stable or limited supply of these commodities in the near future, along with increased demand from the aquaculture industry, make their use problematic and a need to find sustainable alternatives is growing (Pike and Barlow, 2003; Tacon, 2004).

The replacement of FO with VO in aquafeeds has been thoroughly described in the "General Introduction" section. Briefly, VO can be used successfully as sustainable oil sources for aquafeeds, while they are readily available around the world and at competitive prices. However, such a replacement could be challenging as VO lack the n-3 HUFA that are abundant in FO, with potential negative effects on fish growth as well as on the quality of the final product (Bell, et al., 2001a; Bell, et al., 2003a).

PO is one of the most abundant VO in the world, and thus its high availability and competitive price make it a good candidate to replace FO in aquafeeds (U.S. Department for Agriculture, 2007). Moreover, due to its FA composition, PO has a high potential for use in feeds for rainbow trout. Specifically, PO content of 16:0 and 18:1n-9 is high while 18:2n-6 is relatively low (NRC, 1993; Ng, 2002a, 2002b). As previously described, 16:0 and 18:1n-9 are good substrates for β-oxidation (Kiessling and

Kiessling, 1993), and hence PO should be a good energy source for the fish, while the low content of 18:2n-6 should only result in moderate accumulation of this FA in the fish tissues. However, the lack of n-3 HUFA could be challenging in terms of the changes on the FA composition of the flesh and the consequent effects on the quality of the final product, especially at high levels of substitution.

Previous studies have investigated the effects of the inclusion of PO in diets for salmonids. Specifically, graded substitution of FO with PO, up to total replacement of FO, or blends of dietary oils including PO had no significant effects on growth and feed utilization both in rainbow trout (Caballero, et al., 2002; Ng, et al., 2003) and Atlantic salmon (Rosenlund, et al., 2001; Bell, et al., 2002; Ng, et al., 2004). However, the FA compositions of muscle and liver have been shown to be affected by the altered dietary FA composition, due to the inclusion of PO, and linear correlation between dietary and tissue FA have also been suggested, although specific FA are specifically retained or utilized by the fish (Torstensen, et al., 2000; Rosenlund, et al., 2001; Bell, et al., 2002; Caballero, et al., 2002; Ng, et al., 2003; Ng, et al., 2004; Tocher, et al., 2004). Nevertheless, little is known to the effects of the dietary inclusion of PO on other tissues of rainbow trout, such as the heart, gills and kidneys.

The aim of the present study was to investigate the effects of graded inclusion of PO at expense of FO in diets for rainbow trout on growth, feed utilization, and FA composition of various tissues, namely muscle, liver, heart, kidney and gills.

3.2 Materials and methods

3.2.1 Fish and facilities

Rainbow trout (*Oncorhynchus mykiss*) were randomly distributed into twelve circular tanks of 100 L capacity (40 fish/tank) at the Institute of Aquaculture (University of Stirling, Scotland). The fish had a mean initial weight of about 27 g and were obtained from a local fish hatchery (Almondbank, Perthshire, Scotland). The tanks were supplied with flow-through freshwater at 1 L min^{-1}. During the experimental period (10 weeks) the water temperature was constant at 13^{o}C and the fish were subjected to a photoperiod regime of 12 h light: 12 h dark. Prior to the experiment, the fish were fed on a commercial trout pellet diet during an initial one-week acclimatisation period. The experiment was conducted in accordance with British Home Office guidelines regarding research on experimental animals.

3.2.2 Experimental feeds and feeding

The four experimental diets were formulated to provide approx. 472 g kg^{-1} crude protein and 224 g kg^{-1} crude lipid, containing varying contents of crude PO added at the expense of marine FO (Table 3.1). The diets were produced at the BioMar Tech Center (Brande, Denmark) as practical type extruded pellets (3 mm diameter). PO replaced 0% (P0), 25% (P25), 50% (P50) and 100% (P100) of the added FO and hence, it was included at 0, 50, 100 and 200 g kg^{-1} of the diet, respectively. PO was thoroughly mixed with the FO before the oil mixtures were used to coat the extruded pellets. The FA compositions of the four diets are shown in Table 3.2. The diets were formulated to meet all the known nutritional requirements of salmonid fish (NRC, 1993). Each of the

four experimental diets was fed to satiety three times daily, for ten weeks, to randomly assigned triplicate tanks of fish. Feed consumption was recorded daily.

3.2.3 Sampling procedure

At the beginning of the trial, all fish were individually weighed, fork length was measured and 3 fish were randomly sampled for whole body proximate composition determination. After 4 weeks fish were bulk weighed and two fish per tank were sampled for whole body proximate composition. At the end of the experimental period (10 weeks), all fish were anaesthetised and weight and length were recorded. Two fish per tank were sampled for whole body proximate composition and another two for tissues and blood samples. All fish were killed by a blow to the head and samples were taken as described in the General Materials and Methods section. Livers, hearts, gills and kidneys were dissected out and immediately frozen in liquid nitrogen. For flesh samples, Norwegian quality cuts were taken, and skinned and de-boned before being frozen in liquid nitrogen. Diet samples were taken at the beginning of the trial and stored in -20^{o}C until analysed.

3.2.4 Proximate analysis

The nutrient composition, including moisture, crude protein, crude fat and ash contents, of diets and whole body samples was determined by proximate analysis according to AOAC (1995), modified as described by Bell et al (2001a) and described in detail in "General Materials and Methods" section (paragraph 2.5).

3.2.5 Lipid extraction and fatty acid analyses

Total lipids of tissue and diet samples were extracted by homogenization according to Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification as described by Christie (1982) and FAME extracted and purified as described by Tocher & Harvie (1988). FAME were separated and quantified by gas-liquid chromatography and individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman, 1980). The methods followed for the lipid extraction and FA analyses of diets and tissues are thoroughly described in paragraph 2.6.

3.2.6 Calculations and statistical analysis

Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Thermal Growth Coefficient (TGC), Protein Productive Value (PPV), Condition Factor (K) and hepato-somatic index (HSI) were calculated using the formulae presented in paragraph 2.8.

All the data are presented as means ± SD (n = 3) and were analysed by one-way ANOVA. Statistical analyses were performed using SPSS 13 (SPSS Inc, 2004) as described in paragraph 2.9. Differences were regarded as significant when $P < 0.05$ (Zar, 1999).

3.3 Results

3.3.1 Diet proximate and fatty acid composition

The four experimental diets contained graded amounts of PO, namely 25%, 50% and 100% of the added oil, at the expense of FO, while one containing only FO (0%

PO) was used as control. The analysed proximate composition of the experimental diets is shown in Table 3.1. All diets contained approximately 47% protein, 22% lipid, 7% ash and 7% moisture irrespective of the PO content. The gross energy of all diets was approximately 23 kJ / g.

The inclusion of PO resulted in significant changes in the FA compositions of the diets as shown in Table 3.2. The control diet, P0, contained 34.7% monoenes (mainly 18:1n-9, but also significant amounts of 16:1n-7, 22:1 and 20:1n-9, 29.3% saturated FA, of which more than half was 16:0, 26.9% n-3 PUFA mainly DHA and EPA, and 7.2% n-6 PUFA largely in the form of 18:2n-6. Graded inclusion of PO resulted in increased 16:0, 18:1n-9 and 18:2n-6 (2-fold, 3-fold and 2-fold between P0 and P100, respectively). Total saturated, monoenes and n-6 PUFA also increased. On the other hand, 14:0, most of the monoenes, 20:2n-6, ARA and all the n-3 PUFA, including EPA, DHA and total n-3 PUFA, decreased drastically (9-fold and 6-fold between P0 and P100, for EPA and DHA, respectively).

3.3.2 Growth performance

The growth performance and feed efficiency, including mean weights and lengths, SGR, TGC and FCR, of rainbow trout fed the experimental diets for 4 and 10 weeks is shown in Table 3.3. In general, the dietary inclusion of PO, up to100% of the added oil and the total replacement of FO, had no significant effects on the growth performance of the fish. Specifically, the final weight varied from 70.1 to 81.0g and the total length between 16.8 and 17.9 cm. SGR was good for all treatments and for both the growth periods (start to 4 weeks and 4 – 10 weeks). The overall SGR ranged from 1.34 – 1.59. Similarly, TGC varied from 1.22 to 1.47. No significant effects of the PO inclusion were shown by ANOVA either on SGR or TGC. In the first 4 weeks of the

feeding trial the FCR was approximately 0.72, while the overall FCR was much higher, varying from 1.44 to 1.79. Nevertheless, no significant differences were shown between the four dietary treatments.

3.3.3 Whole body proximate composition

The whole body proximate composition at the beginning of the dietary trial and after 4 and 10 weeks of feeding on the experimental diets is shown in Table 3.4. The initial composition of the carcass was 75.1% moisture, 66.3% protein, 23.0% lipid and 9.8% ash. After 4 weeks the moisture, protein, lipid and ash content varied between 70.8% - 73.9%, 55.5% - 59.7%, 29.3% - 35.0% and 8.2% - 9.5%, respectively. ANOVA showed only a significant effect on moisture content, while the protein, lipid and ash contents were not affected by the PO inclusion. Lastly, at the end of the experimental period (after 10 weeks) the moisture was approximately 70.6%, protein 55.0%, lipid 35.8% and ash 7.8%. The graded inclusion of PO did not affect significantly the proximate composition of the whole body at 10 weeks.

3.3.4 Tissue fatty acid compositions

3.3.4.1 Muscle fatty acid composition

The FA compositions of muscle from rainbow trout fed the four experimental diets for 10 weeks are shown in Table 4.5. The graded inclusion of PO resulted in significant alterations in the FA composition of the fish muscle, mainly between the fish fed the P0 and P100 diets, reflecting the dietary FA composition. Regarding the saturated FA, 14:0 was decreased from 4.2% to 1.4% for P0 and P100, respectively, while 16:0 was slightly, but significantly, increased (22.4% - 26.5% for P0 and P100,

respectively). However, there were no significant differences in the total saturated FA being approximately 32%. Reflecting the dietary FA compositions, all monounsaturated FA were significantly decreased, with the exception of 18:1n-9 which was significantly increased from 11.6% to 26.9% for P0 and P100, respectively. The increase in 18:1n-9, being the most abundant monounsaturated FA, resulted in a respective, significant increase in total monoenes. All n-6 PUFA increased with dietary inclusion of PO. 18:2n-6 increased from 4.5% to 9.0%, 20:2n-6 from 0.4% to 0.7%, 20:3n-6 from 0.2% to 0.6% and 20:4n-6 from 0.7% to 0.9%, for P0 and P100, respectively, resulting in an 2-fold increase for the total n-6 PUFA (6.1% to 11.9% for P0 and P100, respectively). There was a significant decrease, especially between P0 and P100 groups, in the n-3 PUFA, including 18:3n-3, EPA, DHA and total n-3 PUFA. It should be noted, though, that the decrease in DHA was significant only for the P100 group (24.5% vs. 17.5%, for P0 and P100, respectively). Lastly, the n-3 / n-6 ratio was decreased from 5.8% to 1.8%, for P0 and P100, respectively.

3.3.4.2 Liver fatty acid composition

The liver FA compositions, and the changes arising from the dietary inclusion of PO, are presented in Table 3.6. The total saturated FA ranged from 29.4% - 32.5% while 16:0 had the highest proportion (approximately 21.7%) of all saturated FA. Noticeably, there were no significant differences in any of the individual saturated FA, or in the total saturated FA. Similarly, the monounsaturated FA were only partially affected. Specifically, when PO was included in the diets 18:1n-9 was significantly increased by 2-fold (12.1% vs. 24.6%, for P0 and P100, respectively) and 18:1n-7 and 24:1n-9 were significantly decreased, while there were no significant differences shown in any of the other monoenes or the total monoenes. The n-6 PUFA, including the total

n-6 PUFA, were significantly increased with graded inclusion of PO. 18:2n-6 was increased 2-fold, from 2.6% to 5.5% for P0 and P100, respectively. 20:2n-6 increased almost 3-fold and 20:3n-3 5-fold between P0 and P100, while ARA was unaffected by the dietary treatments (approx. 1.9%). EPA and the total n-3 PUFA were significantly decreased when PO replaced FO in the diets of trout. Specifically, EPA was decreased from 5.0% to 1.0% for P0 and P100, respectively. It is worth mentioning that DHA was not significantly affected by the dietary changes although it was slightly reduced from 31.2% for P0 to 22.3% for P100. Lastly, the n-3/n-6 ratio was reduced from 6.4 to 1.9 between P0 and P100.

3.3.4.3 Gill fatty acid composition

The FA composition of gills from rainbow trout fed the four experimental diets is shown in Table 3.7. In gills the graded inclusion of PO resulted in a significant reduction in 14:0 (4.5% vs. 1.8% for P0 and P100, respectively), in a significant increase in 16:0 (19.2% vs. 24.7% for P0 and P100, respectively), while 18:0 and total saturated FA were not significantly affected. Similarly to the other tissues, most monoenes were significantly reduced when PO replaced FO with only 18:1n-9 (16.3% vs. 34.8% for P0 and P100, respectively) and total monoenes (39.4% vs. 46.9% for P0 and P100, respectively) were significantly increased. The dietary inclusion of PO resulted also in significant increases in 18:2n-6 (6.3% - 10.1% for P0 and P100, respectively), 20:2n-6 (0.4% - 0.6% for P0 and P100, respectively), 20:3n-6 (0.2% - 0.4% for P0 and P100, respectively) and total n-6 PUFA (8.1% - 12.1% for P0 and P100, respectively), while there was no significant effect on 20:4n-6. All n-3 PUFA were significantly reduced with the dietary inclusion of PO. Specifically, EPA was

reduced from 4.9% to 1.6% and DHA from 13.6% to 6.7% for P0 and P100, respectively.

3.3.4.4 Heart fatty acid composition

The heart FA compositions from fish fed the four experimental diets for 10 weeks is presented in Table 3.8. The saturated FA were largely unaffected by the graded inclusion of PO, with total saturates comprising approximately 32% and 16:0 24.3% of the total lipid. 18:1n-9 and total monoenes were increased (10.5% vs. 27.1%, for 18:1n-9 and 23.0% vs. 34.2% for total monoenes, for P0 and P100, respectively) while 16:1n-7, 20:1n-9 and 22:1 were decreased when PO replaced FO. The n-6 PUFA were significantly increased, including 18:2n-6 (3.7% vs. 8.8% for P0 and P100, respectively), 20:2n-6 (0.3% - 0.7% for P0 and P100, respectively), 20:3n-6 (0.3% - 0.8% for P0 and P100, respectively) and total n-6 PUFA (6.2% vs. 12.7% for P0 and P100, respectively). No significant effect was shown on ARA which varied from 1.3% – 1.5%. With the exception of DHA, which was only slightly but not significantly reduced (26.8% vs. 18.5% for P0 and P100, respectively), all n-3 PUFA were significantly decreased. 18:3n-3 was reduced 2-fold (0.8% vs. 0.4% for P0 and P100, respectively) and EPA was decreased by more than 4-fold (6.3% vs. 1.5% for P0 and P100, respectively). The n-3/n-6 ratio was decreased from 6.1 (P0) to 1.7 (P100). It should be noted that most of the aforementioned differences in the FA compositions were significant only when PO was included at 100% of the added dietary oil.

3.3.4.5 Kidney fatty acid composition

The FA composition of kidney from rainbow trout fed diets containing graded amounts of PO are shown in Table 3.9. Similar to the FA compositions of other tissues, most of the differences caused by the inclusion of PO were significant largely between

P0 and P100 groups. Regarding the saturated FA, 14:0 was significantly decreased (4.1% vs. 1.2%, for P0 and P100, respectively), 16:0 was slightly but significantly increased (22.6% vs. 27.5%, for P0 and P100, respectively), whereas 18:0 and total saturated were not affected by the graded inclusion of PO. 18:1n-9 was more than doubled between P0 and P100 (14.2% vs.31.0%), total monoenes were increased from 31.6% (P0) to 39% (P100); however, all other monoenes were significantly reduced. Significant increases were shown for the n-6 PUFA with dietary inclusion of PO. 18:2n-6 increased from 5.1% to 9.3%, 20:2n-6 from 0.4% to 0.7%, 20:3n-6 from 0.2% to 0.6% and 20:4n-6 from 1.3% to 1.9%, for P0 and P100, respectively. All n-3 PUFA decreased significantly when FO was replaced by PO in the diets of trout. 18:3n3-3 was reduced from 0.9% to 0.3%, between P0 and P100, EPA from 6.4% to 1.8%, DHA from 17.8% to 11.5% and total n-3 PUFA from 28.5% to 14.2%. The n3/n-6 ratio was reduced from 3.9 to 1.1, for P0 and P100, respectively.

Table 3.1. Feed formulations and proximate compositions (% of wet weight) of the experimental diets

	P0	P25	P50	P100
Components				
Fishmeal[1]	34.3	34.3	34.3	34.3
Hi Pro Soya[2]	12.7	12.7	12.7	12.7
Wheat gluten[2]	10.0	10.0	10.0	10.0
Corn gluten[3]	10.0	10.0	10.0	10.0
Wheat[4]	10.0	10.0	10.0	10.0
Fish oil[1]	20.0	15.0	10.0	0.0
Palm oil[5]	0.0	5.0	10.0	20.0
Methionine	0.21	0.21	0.21	0.21
Lysine	0.79	0.79	0.79	0.79
Micronutrients[6]	2.41	2.41	2.41	2.41
Vitamin E[7]	0.015	0.011	0.0076	0.0
Ytrium oxide	0.02	0.02	0.02	0.02
Analyzed composition				
Moisture	6.5	8.2	7.2	6.9
Crude protein	47.9	46.5	47.8	46.7
Crude lipid	22.3	21.5	22.5	23.3
Ash	7.1	7.1	7.0	7.0
Gross energy (kJ/g)	22.8	23.6	23.8	23.6

[1]Norsemeal Ltd., London, UK; [2]Cargill, Swinderbury, UK; [3]Cerestar UK Ltd., Manchester, UK; [4]J.D. Martin, Tranent, UK; [5]United Plantations Bhd, Jenderata Estate, Teluk Intan, Malaysia; [6]Vitamins, minerals and astaxanthin (Carophyl pink®), BioMar A/S, Brande, Denmark; [7]Roche, Basel, Switzerland.

Table 3.2. Fatty acid composition (% of total fatty acids) of the experimental diets

Fatty acid	P0	P25	P50	P100
14:0	6.4	4.6	3.7	1.6
16:0	18.6	26.7	30.6	37.9
18:0	3.4	3.8	4.2	4.2
Total saturated[1]	29.3	35.8	39.1	44.1
16:1n-7	6.7	4.5	3.5	1.0
18:1n-9	11.1	20.2	24.2	35.9
18:1n-7	2.5	1.9	1.8	0.6
20:1n-9	5.4	3.5	2.8	0.4
22:1[2]	7.6	4.7	2.7	0.4
24:1n-9	0.7	0.4	0.3	0.1
Total monoenes[3]	34.7	35.6	35.5	38.5
18:2n-6	5.9	8.1	9.1	11.8
20:2n-6	0.3	0.2	0.1	n.d.[*]
20:3n-6	0.1	0.1	tr.[**]	n.d.
20:4n-6	0.6	0.4	0.3	0.2
Total n-6 PUFA[4]	7.2	9.0	9.9	12.1
18:3n-3	1.3	1.0	0.9	0.6
18:4n-3	2.9	1.9	1.4	0.2
20:4n-3	0.7	0.4	0.3	0.1
20:5n-3	9.2	6.0	4.7	1.2
22:5n-3	1.2	0.8	0.7	0.3
22:6n-3	11.3	7.9	6.4	2.7
Total n-3 PUFA[5]	26.9	18.4	14.6	5.1
Total PUFA	36.0	28.5	25.4	17.4
(n-3) / (n-6)	3.7	2.0	1.5	0.4

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7; [4]Includes 18:3n-6, 22:4n-6 & 22:5n-6; [5]Includes 20:3n-3 & 22:4n-3.

[*] n.d.: Not detected; [**] tr.: Detected as traces

Table 3.3. Growth and performance of rainbow trout fed the experimental diets for 4 and 10 weeks

	P0	P25	P50	P100
Weight (g)				
Start	27.2 ± 0.7	26.6 ± 0.5	27.8 ± 0.4	27.3 ± 1.1
4 weeks	43.9 ± 5.2	40.9 ± 1.7	44.0 ± 0.7	42.3 ± 1.7
10 weeks	76.1 ± 4.5	81.0 ± 2.9	76.7 ± 7.1	70.1 ± 8.5
Length (cm)				
10 weeks	17.5 ± 0.4	17.9 ± 0.5	17.3 ± 0.6	16.8 ± 0.4
SGR				
0-4 weeks	1.48 ± 0.31	1.35 ± 0.12	1.43 ± 0.04	1.37 ± 0.23
4-10 weeks	1.46 ± 0.43	1.79 ± 0.18	1.45 ± 0.20	1.32 ± 0.36
Overall	1.47 ± 0.11	1.59 ± 0.08	1.45 ± 0.13	1.34 ± 0.15
TGC				
0-4 weeks	1.24 ± 0.29	1.11 ± 0.11	1.20 ± 0.04	1.14 ± 0.20
4-10 weeks	1.44 ± 0.41	1.78 ± 0.18	1.45 ± 0.23	1.28 ± 0.37
Overall	1.35 ± 0.11	1.47 ± 0.08	1.34 ± 0.14	1.22 ± 0.16
FCR				
0-4 weeks	0.71 ± 0.17	0.73 ± 0.06	0.72 ± 0.10	0.72 ± 0.11
Overall	1.50 ± 0.21	1.44 ± 0.12	1.79 ± 0.62	1.68 ± 0.36
Mortalities	3	0	5	3

Values are mean ± S.D (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

Table 3.4. Whole body proximate composition (g $100g^{-1}$, dry weight) of rainbow trout fed experimental palm oil diets for 4 and 10 weeks

	Start	4 weeks				10 weeks			
		P0	P25	P50	P100	P0	P25	P50	P100
Moisture	75.1 ± 1.3	70.8 ± 1.6 [a]	73.9 ± 0.2 [b]	71.1 ± 1.3 [a]	70.8 ± 0.4 [a]	71.0 ± 0.5	70.5 ± 0.5	70.5 ± 0.5	70.4 ± 0.6
Protein	66.3 ± 2.1	55.5 ± 2.2	59.7 ± 0.8	56.1 ± 2.6	55.6 ± 0.4	55.5 ± 1.7	55.1 ± 2.8	54.7 ± 0.3	55.0 ± 0.3
Lipid	23.0 ± 3.1	34.3 ± 3.0	29.3 ± 0.7	33.6 ± 4.0	35.0 ± 0.5	35.2 ± 0.7	35.8 ± 2.5	36.2 ± 1.0	35.8 ± 0.6
Ash	9.8 ± 0.9	8.3 ± 0.2	9.5 ± 0.9	8.5 ± 1.1	8.2 ± 0.1	8.0 ± 0.4	7.6 ± 0.2	7.6 ± 0.5	7.8 ± 0.3

Values are mean ± S.D (n=3). Values within a row for each period with a different superscript letter are significantly different ($P<0.05$)

Table 3.5. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of muscle from rainbow trout fed the experimental diets for 10 weeks

Fatty Acid	P0	P25	P50	P100
Total lipid	17.5 ± 0.5	14.8 ± 0.2	16.6 ± 3.1	16.6 ± 2.3
14:0	4.2 ± 0.2 [a]	3.0 ± 0.3 [b]	2.7 ± 0.2 [b]	1.4 ± 0.1 [c]
16:0	22.4 ± 0.3 [b]	24.5 ± 1.9 [a,b]	24.2 ± 1.3 [a,b]	26.5 ± 1.3 [a]
18:0	4.3 ± 0.1 [a]	4.1 ± 0.2 [a,b]	3.8 ± 0.3 [b]	3.9 ± 0.2 [a,b]
Total saturated[1]	31.8 ± 0.5	32.3 ± 2.6	31.2 ± 1.9	32.3 ± 1.7
16:1n-7	5.2 ± 0.1 [a]	4.2 ± 0.3 [b]	4.0 ± 0.3 [b]	2.3 ± 0.3 [c]
18:1n-9	11.6 ± 0.7 [d]	16.4 ± 0.9 [c]	20.2 ± 1.9 [b]	26.9 ± 1.6 [a]
18:1n-7	2.4 ± 0.1 [a]	2.1 ± 0.1 [b]	2.0 ± 0.0 [b]	1.5 ± 0.2 [c]
20:1n-9	3.6 ± 0.4 [a]	2.8 ± 0.3 [b]	2.6 ± 0.1 [b]	2.0 ± 0.1 [c]
22:1[2]	3.5 ± 0.3 [a]	2.5 ± 0.2 [b]	2.3 ± 0.2 [b]	1.0 ± 0.2 [c]
24:1n-9	0.7 ± 0.0 [a]	0.7 ± 0.1 [a]	0.6 ± 0.0 [a]	0.5 ± 0.0 [b]
Total monoenes[3]	27.2 ± 1.7 [c]	28.9 ± 1.6 [b,c]	32.0 ± 2.4 [a,b]	34.6 ± 1.2 [a]
18:2n-6	4.5 ± 0.2 [d]	5.7 ± 0.2 [c]	6.8 ± 0.4 [b]	9.0 ± 0.4 [a]
20:2n-6	0.4 ± 0.0 [b]	0.4 ± 0.0 [b]	0.4 ± 0.0 [b]	0.7 ± 0.1 [a]
20:3n-6	0.2 ± 0.0 [b]	0.2 ± 0.0 [b]	0.3 ± 0.0 [b]	0.6 ± 0.1 [a]
20:4n-6	0.7 ± 0.0 [b]	0.7 ± 0.0 [b]	0.6 ± 0.0 [b]	0.9 ± 0.1 [a]
Total n-6 PUFA[4]	6.1 ± 0.2 [c]	7.5 ± 0.3 [b]	8.5 ± 0.5 [b]	11.9 ± 0.7 [a]
18:3n-3	0.8 ± 0.0 [a]	0.7 ± 0.0 [b]	0.7 ± 0.0 [b]	0.4 ± 0.0 [c]
18:4n-3	1.1 ± 0.0 [a]	0.8 ± 0.0 [b]	0.7 ± 0.0 [b,c]	0.4 ± 0.2 [c]
20:4n-3	0.9 ± 0.1 [a]	0.7 ± 0.0 [b]	0.7 ± 0.0 [b]	0.3 ± 0.1 [c]
20:5n-3	5.9 ± 0.2 [a]	4.7 ± 0.3 [a,b]	4.2 ± 0.8 [b]	2.1 ± 0.5 [c]
22:5n-3	1.5 ± 0.0 [a]	1.2 ± 0.1 [b]	1.0 ± 0.0 [c]	0.5 ± 0.1 [d]
22:6n-3	24.5 ± 1.5 [a]	23.3 ± 1.7 [a]	21.0 ± 2.8 [a,b]	17.5 ± 2.5 [b]
Total n-3 PUFA[5]	34.9 ± 1.3 [a]	31.3 ± 1.5 [a]	28.2 ± 3.7 [a,b]	21.2 ± 3.3 [b]
Total PUFA	41.0 ± 1.1 [a]	38.8 ± 1.8 [a,b]	36.8 ± 3.2 [a,b]	33.1 ± 2.6 [b]
(n-3) / (n-6)	5.8 ± 0.5 [a]	4.2 ± 0.1 [b]	3.4 ± 0.7 [b]	1.8 ± 0.4 [c]

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7; [4]Includes 18:3n-6, 22:4n-6 & 22:5n-6; [5]Includes 20:3n-3 & 22:4n-3.

Table 3.6. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of liver from rainbow trout fed the experimental diets for 10 weeks

Fatty Acid	P0	P25	P50	P100
Total lipid	37.3 ± 2.6	54.0 ± 19.6	43.2 ± 5.2	38.2 ± 3.5
14:0	2.0 ± 0.3	2.1 ± 0.9	1.6 ± 0.0	1.0 ± 0.1
16:0	23.3 ± 1.6	20.7 ± 1.3	21.2 ± 2.1	21.6 ± 1.6
18:0	6.6 ± 0.5	5.6 ± 0.2	6.3 ± 0.6	6.6 ± 1.2
Total saturated[1]	32.5 ± 1.5	28.8 ± 2.5	29.5 ± 2.8	29.4 ± 1.5
16:1n-7	3.8 ± 1.4	4.6 ± 1.5	3.9 ± 0.1	2.6 ± 0.7
18:1n-9	12.1 ± 3.2 [b]	21.1 ± 2.0 [a,b]	22.0 ± 3.4 [a]	24.6 ± 5.6 [a]
18:1n-7	2.1 ± 0.3 [a,b]	2.4 ± 0.1 [a]	2.0 ± 0.2 [a,b]	1.7 ± 0.2 [b]
20:1n-9	2.6 ± 0.8	3.8 ± 0.8	3.4 ± 0.6	3.9 ± 1.1
22:1[2]	1.0 ± 0.5	1.7 ± 1.2	0.9 ± 0.5	0.4 ± 0.0
24:1n-9	1.0 ± 0.1 [a]	0.9 ± 0.1 [a,b]	0.9 ± 0.1 [a,b]	0.8 ± 0.1 [b]
Total monoenes[3]	22.6 ± 6.2	34.8 ± 3.4	33.2 ± 4.8	34.2 ± 7.4
18:2n-6	2.6 ± 0.3 [b]	4.1 ± 1.6 [a,b]	3.8 ± 0.2 [a,b]	5.5 ± 0.4 [a]
20:2n-6	0.6 ± 0.1 [b]	0.8 ± 0.1 [b]	0.8 ± 0.1 [b]	1.6 ± 0.1 [a]
20:3n-6	0.3 ± 0.0 [b]	0.5 ± 0.2 [b]	0.7 ± 0.3 [b]	1.5 ± 0.2 [a]
20:4n-6	2.1 ± 0.6	1.5 ± 0.5	1.6 ± 0.1	2.3 ± 0.3
Total n-6 PUFA[4]	6.0 ± 0.1 [b]	7.5 ± 1.1 [b]	7.5 ± 0.4 [b]	12.3 ± 0.7 [a]
18:3n-3	0.4 ± 0.0	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.0
18:4n-3	0.3 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	0.0 ± 0.0
20:4n-3	0.5 ± 0.0 [a]	0.3 ± 0.2 [a]	0.2 ± 0.0 [a]	0.0 ± 0.0 [b]
20:5n-3	5.0 ± 1.4 [a]	2.9 ± 0.4 [a,b]	2.3 ± 0.3 [b]	1.0 ± 0.7 [b]
22:5n-3	1.4 ± 0.1 [a]	0.9 ± 0.1 [b]	0.8 ± 0.1 [b,c]	0.4 ± 0.3 [c]
22:6n-3	31.2 ± 3.8	24.0 ± 6.4	26.0 ± 2.2	22.3 ± 5.3
Total n-3 PUFA[5]	38.9 ± 4.6 [a]	28.9 ± 5.7 [a,b]	29.8 ± 1.9 [a,b]	24.0 ± 6.3 [b]
Total PUFA	44.9 ± 4.7	36.4 ± 5.3	37.3 ± 2.2	36.3 ± 6.9
(n-3) / (n-6)	6.4 0.7 [a]	3.9 1.1 [b]	3.9 0.1 [b]	1.9 0.4 [c]

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7;

[4]Includes 18:3n-6, 22:4n-6 & 22:5n-6; [5]Includes 20:3n-3 & 22:4n-3.

Table 3.7. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of gills from rainbow trout fed the experimental diets for 10 weeks

Fatty Acid	P0	P25	P50	P100
Total lipid	70.6 ± 4.4	78.2 ± 24.2	79.2 ± 5.6	72.5 ± 16.1
14:0	4.5 ± 0.3 [a]	3.3 ± 0.1 [b]	3.1 ± 0.1 [b]	1.8 ± 0.1 [c]
16:0	19.2 ± 0.6 [c]	21.8 ± 0.5 [b]	22.3 ± 0.3 [b]	24.7 ± 1.1 [a]
18:0	4.1 ± 0.2	4.1 ± 0.1	3.9 ± 0.0	4.1 ± 0.2
Total saturated[1]	28.8 ± 0.9	30.2 ± 0.9	30.0 ± 0.5	31.1 ± 1.3
16:1n-7	6.6 ± 0.1 [a]	5.2 ± 0.1 [b]	5.0 ± 0.2 [b]	3.1 ± 0.3 [c]
18:1n-9	16.3 ± 0.5 [d]	23.0 ± 0.2 [c]	26.3 ± 0.4 [b]	34.8 ± 1.3 [a]
18:1n-7	3.3 ± 0.1 [a]	2.9 ± 0.1 [b]	2.5 ± 0.2 [b]	2.0 ± 0.1 [c]
20:1n-9	6.0 ± 0.2 [a]	4.7 ± 0.2 [b]	4.6 ± 0.4 [b]	3.6 ± 0.4 [c]
22:1[2]	5.5 ± 0.3 [a]	4.1 ± 0.3 [b]	3.9 ± 0.5 [b]	2.3 ± 0.4 [c]
24:1n-9	0.8 ± 0.1 [a]	0.6 ± 0.1 [b]	0.6 ± 0.0 [b]	0.4 ± 0.0 [c]
Total monoenes[3]	39.4 ± 0.6 [d]	41.3 ± 0.5 [c]	43.6 ± 0.9 [b]	46.9 ± 0.3 [a]
18:2n-6	6.3 ± 0.3 [c]	8.1 ± 0.4 [b]	8.6 ± 0.1 [b]	10.1 ± 0.2 [a]
20:2n-6	0.4 ± 0.0 [b]	0.4 ± 0.0 [b]	0.5 ± 0.0 [b]	0.6 ± 0.0 [a]
20:3n-6	0.2 ± 0.0 [b]	0.2 ± 0.0 [b]	0.2 ± 0.0 [b]	0.4 ± 0.0 [a]
20:4n-6	0.8 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1
Total n-6 PUFA[4]	8.1 ± 0.3 [c]	9.8 ± 0.3 [b]	10.2 ± 0.2 [b]	12.1 ± 0.2 [a]
18:3n-3	1.1 ± 0.0 [a]	1.0 ± 0.2 [a,b]	0.8 ± 0.0 [b,c]	0.6 ± 0.1 [c]
18:4n-3	1.7 ± 0.0 [a]	1.1 ± 0.0 [b]	1.0 ± 0.0 [c]	0.5 ± 0.1 [d]
20:4n-3	1.0 ± 0.0 [a]	0.7 ± 0.1 [b]	0.6 ± 0.0 [b]	0.3 ± 0.1 [c]
20:5n-3	4.9 ± 0.1 [a]	3.4 ± 0.2 [b]	2.8 ± 0.0 [c]	1.4 ± 0.3 [d]
22:5n-3	1.3 ± 0.1 [a]	1.0 ± 0.1 [b]	0.8 ± 0.1 [b]	0.4 ± 0.1 [c]
22:6n-3	13.6 ± 0.6 [a]	11.5 ± 1.1 [a,b]	10.2 ± 0.7 [b]	6.7 ± 1.0 [c]
Total n-3 PUFA[5]	23.7 ± 0.8 [a]	18.8 ± 1.3 [b]	16.2 ± 0.8 [b]	9.9 ± 1.5 [c]
Total PUFA	31.8 ± 1.1 [a]	28.6 ± 1.1 [b]	26.4 ± 1.0 [b]	22.0 ± 1.3 [c]
(n-3) / (n-6)	2.9 ± 0.0 [a]	1.9 ± 0.2 [b]	1.6 ± 0.1 [c]	0.8 ± 0.1 [d]

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7; [4]Includes 18:3n-6, 22:4n-6 & 22:5n-6; [5]Includes 20:3n-3 & 22:4n-3.

Table 3.8. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of heart from rainbow trout fed the experimental diets for 10 weeks

Fatty Acid	P0	P25	P50	P100
Total lipid	29.3 ± 6.3	29.2 ± 7.7	27.5 ± 2.5	34.8 ± 3.2
14:0	2.0 ± 0.7 [a]	1.6 ± 0.3 [a,b]	1.5 ± 0.3 [a,b]	0.8 ± 0.0 [b]
16:0	23.8 ± 0.7	24.0 ± 2.2	24.0 ± 2.7	25.4 ± 0.7
18:0	6.3 ± 0.2	5.7 ± 0.5	5.5 ± 0.5	5.2 ± 0.5
Total saturated[1]	33.8 ± 0.5	31.9 ± 3.2	31.9 ± 3.9	31.9 ± 0.9
16:1n-7	3.5 ± 0.5 [a]	2.8 ± 0.6 [a]	2.5 ± 0.4 [a,b]	1.5 ± 0.1 [b]
18:1n-9	10.5 ± 1.1 [b]	16.7 ± 1.6 [b]	18.0 ± 3.0 [b]	27.1 ± 5.7 [a]
18:1n-7	2.4 ± 0.2	2.2 ± 0.1	2.0 ± 0.2	1.8 ± 0.4
20:1n-9	2.7 ± 0.6 [a]	2.4 ± 0.1 [a,b]	2.0 ± 0.3 [a,b]	1.6 ± 0.3 [b]
22:1[2]	2.5 ± 0.5 [a]	2.0 ± 0.2 [a,b]	1.5 ± 0.3 [b]	0.6 ± 0.0 [c]
24:1n-9	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.3	0.8 ± 0.2
Total monoenes[3]	23.0 ± 2.9 [b]	27.9 ± 2.4 [a,b]	27.5 ± 3.8 [a,b]	34.2 ± 5.2 [a]
18:2n-6	3.7 ± 0.7 [b]	5.3 ± 0.7 [b]	5.6 ± 1.1 [b]	8.8 ± 1.4 [a]
20:2n-6	0.3 ± 0.1 [b]	0.4 ± 0.0 [b]	0.4 ± 0.0 [b]	0.7 ± 0.2 [a]
20:3n-6	0.3 ± 0.0 [b]	0.4 ± 0.1 [b]	0.4 ± 0.0 [b]	0.8 ± 0.1 [a]
20:4n-6	1.4 ± 0.1	1.3 ± 0.3	1.4 ± 0.3	1.6 ± 0.6
Total n-6 PUFA[4]	6.2 ± 0.7 [c]	8.0 ± 0.4 [b,c]	8.5 ± 0.9 [b]	12.7 ± 0.8 [a]
18:3n-3	0.8 ± 0.1 [a]	0.6 ± 0.1 [a,b]	0.6 ± 0.1 [b]	0.4 ± 0.1 [c]
18:4n-3	0.8 ± 0.2 [a]	0.5 ± 0.1 [a,b]	0.4 ± 0.1 [b,c]	0.1 ± 0.0 [c]
20:4n-3	0.7 ± 0.1 [a]	0.6 ± 0.0 [a,b]	0.5 ± 0.1 [b]	0.2 ± 0.0 [c]
20:5n-3	6.3 ± 0.7 [a]	4.4 ± 0.4 [b]	4.2 ± 0.9 [b]	1.5 ± 0.7 [c]
22:5n-3	1.6 ± 0.0 [a]	1.2 ± 0.1 [b]	1.1 ± 0.1 [b]	0.5 ± 0.2 [c]
22:6n-3	26.8 ± 3.0	24.8 ± 4.7	25.3 ± 4.6	18.5 ± 5.4
Total n-3 PUFA[5]	36.9 ± 3.2 [a]	32.2 ± 5.0 [a,b]	32.1 ± 5.3 [a,b]	21.2 ± 6.1 [b]
Total PUFA	43.1 ± 2.5	40.2 ± 4.6	40.6 ± 4.7	33.9 ± 5.4
(n-3) / (n-6)	6.1 ± 1.3 [a]	4.1 ± 0.8 [a,b]	3.9 ± 1.0 [a,b]	1.7 ± 0.6 [b]

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7; [4]Includes 18:3n-6, 22:4n-6 & 22:5n-6; [5]Includes 20:3n-3 & 22:4n-3.

Table 3.9. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of kidney from rainbow trout fed the experimental diets for 10 weeks

Fatty Acid	P0	P25	P50	P100
Total lipid	50.1 ± 11.8	47.8 ± 5.8	40.7 ± 9.7	43.3 ± 1.9
14:0	4.1 ± 0.5 [a]	2.8 ± 0.1 [b]	2.3 ± 0.1 [c]	1.2 ± 0.0 [d]
16:0	22.6 ± 0.9 [b]	24.7 ± 1.2 [b]	24.8 ± 0.8 [a,b]	27.5 ± 1.2 [a]
18:0	4.5 ± 0.3	4.3 ± 0.2	4.3 ± 0.1	4.4 ± 0.2
Total saturated[1]	32.4 ± 1.2	32.6 ± 1.8	32.1 ± 1.4	33.7 ± 1.8
16:1n-7	5.5 ± 0.8 [a]	4.0 ± 0.2 [b]	3.7 ± 0.2 [b]	2.0 ± 0.1 [c]
18:1n-9	14.2 ± 1.0 [c]	20.6 ± 0.9 [b]	22.8 ± 1.4 [b]	31.0 ± 0.8 [a]
18:1n-7	3.1 ± 0.3 [a]	2.6 ± 0.1 [a,b]	2.4 ± 0.2 [b,c]	2.0 ± 0.1 [c]
20:1n-9	3.8 ± 0.6 [a]	3.0 ± 0.2 [a,b]	2.5 ± 0.4 [b,c]	1.8 ± 0.2 [c]
22:1[2]	3.4 ± 0.6 [a]	2.5 ± 0.1 [a,b]	1.9 ± 0.3 [b]	0.7 ± 0.2 [c]
24:1n-9	0.9 ± 0.1 [a]	0.8 ± 0.1 [a]	0.9 ± 0.0 [a]	0.6 ± 0.1 [b]
Total monoenes[3]	31.6 ± 3.1 [b]	34.2 ± 1.4 [a,b]	35.0 ± 1.8 [a,b]	39.0 ± 0.6 [a]
18:2n-6	5.1 ± 0.6 [c]	6.7 ± 0.4 [b]	7.0 ± 0.2 [b]	9.3 ± 0.1 [a]
20:2n-6	0.4 ± 0.0 [b]	0.4 ± 0.0 [b]	0.5 ± 0.0 [b]	0.7 ± 0.1 [a]
20:3n-6	0.2 ± 0.0 [c]	0.3 ± 0.0 [b,c]	0.3 ± 0.0 [b]	0.6 ± 0.1 [a]
20:4n-6	1.3 ± 0.3 [b]	1.3 ± 0.2 [b]	1.4 ± 0.0 [a,b]	1.9 ± 0.1 [a]
Total n-6 PUFA[4]	7.4 ± 0.4 [c]	9.1 ± 0.2 [b]	9.6 ± 0.2 [b]	13.1 ± 0.3 [a]
18:3n-3	0.9 ± 0.1 [a]	0.7 ± 0.0 [b]	0.6 ± 0.0 [b]	0.3 ± 0.0 [c]
18:4n-3	1.2 ± 0.2 [a]	0.7 ± 0.0 [b]	0.6 ± 0.1 [b]	0.2 ± 0.0 [c]
20:4n-3	0.8 ± 0.0 [a]	0.6 ± 0.1 [b]	0.5 ± 0.0 [b]	0.2 ± 0.1 [c]
20:5n-3	6.4 ± 0.8 [a]	4.9 ± 0.3 [b]	4.4 ± 0.5 [b]	1.8 ± 0.5 [c]
22:5n-3	1.3 ± 0.1 [a]	0.9 ± 0.1 [b]	0.8 ± 0.0 [b]	0.3 ± 0.1 [c]
22:6n-3	17.8 ± 2.8 [a]	16.2 ± 0.5 [a]	16.4 ± 1.0 [a]	11.5 ± 1.4 [b]
Total n-3 PUFA[5]	28.5 ± 3.2 [a]	24.1 ± 0.6 [a]	23.3 ± 1.5 [a]	14.2 ± 2.0 [b]
Total PUFA	36.0 ± 3.1 [a]	33.2 ± 0.5 [a]	32.9 ± 1.3 [a]	27.3 ± 1.9 [b]
(n-3) / (n-6)	3.9 ± 0.5 [a]	2.7 ± 0.1 [b]	2.4 ± 0.2 [b]	1.1 ± 0.2 [c]

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7; [4]Includes 18:3n-6, 22:4n-6 & 22:5n-6; [5]Includes 20:3n-3 & 22:4n-3.

3.4 Discussion

The interest from the salmonid culture industry in sustainable alternatives to fish meal and fish oil has been growing rapidly in recent years. Given that, the present study aimed to investigate the effects of the replacement of FO with graded inclusion of PO in diets for rainbow trout, firstly on growth and feed utilization, but also on the FA composition of various tissues.

3.4.1 Growth performance

In terms of the effects on growth and feed utilization, PO has been successfully used in several previous studies, replacing FO in diets of salmonids either alone or in blends with other VO. In Atlantic salmon, Rosenlund et al. (2001) replaced 50%-60% of the dietary FO with PO and other VO with no negative effects on growth. Similarly, no effects on growth or FCR of Atlantic salmon were shown when Bell et al. (2002) used diets containing up to 100% PO of the added oil. Moreover, PO has been successfully used in blends of VO, replacing dietary FO, as reported by Ng et al. (2004). However, fewer studies have been conducted to investigate the effects in rainbow trout. Caballero et al. (2002) fed rainbow trout on diets containing a blend of PO and RO, replacing FO up to 80%, and reported that the growth and FCR were unaffected by the dietary treatments.

In line with the previous studies the results of the present study suggest that replacement of FO with PO, up to 100%, in diets of rainbow trout had no significant effects on the growth of the fish or the feed utilization. Specifically, the weight and the length of the fish, both at the middle and at the end of the trial were, were good and unaffected by the dietary treatments. Similarly, SGR and TGC did not reveal any

negative impacts of the PO dietary inclusion at any stage of the feeding trial. Lastly, feed utilization, as indicated by the FCR, was good, especially for the first 4 weeks of the trial, and unaffected by the dietary inclusion levels of PO. However, the higher FCR for the whole experimental period could be explained, at least partially, by the lower lipid and specific FA (especially saturates) digestibility that PO diets have been reported to have, especially at low culture temperatures, and the fish compensating for any energy loss by increasing their feed intake (Torstensen, et al., 2000; Caballero, et al., 2002; Ng, et al., 2003; Ng, et al., 2004). Nevertheless, the rich content of 16:0 and 18:1n-9 in PO seems to play a role in the overall growth of the fish as these FA have been reported to be preferentially catabolised for energy production (Kiessling and Kiessling, 1993; Henderson, 1996; Caballero, et al., 2002). The results of the present study are in agreement with that hypothesis.

3.4.2 Body composition

The body chemical composition of the fish is of importance as it may affect the quality of the final product or characteristics involved in further processing. However, the results of previous studies regarding the effects of the replacement of FO with VO in salmonids are, to some extent, controversial. For example, previous studies in Atlantic salmon reported that replacement of dietary FO with VO affected the whole body chemical composition (Bell, et al., 2001a; Torstensen, et al., 2004b; Ruyter, et al., 2006), while, other studies have shown no significant effects on body composition due to use of VO (Bendiksen, et al., 2003b). Moreover, different VO, including PO, seem to have different effects on fish body composition (Rosenlund, et al., 2001; Bell, et al., 2002). Specifically, Bell et al. (2002) reported that inclusion of PO resulted in lower body lipid and higher protein contents compared to the fish fed on a FO diet. On the

contrary, Rosenlund et al. (2001) showed that the inclusion of PO had no significant effect on fillet proximate composition of Atlantic salmon when compared to FO fed fish, although there were differences with other VO. In agreement with the latter, the results of the present study suggest that PO had no significant effects on the whole body proximate composition of rainbow trout, although this was a relatively short period feeding trial. The reasons for the differences observed between trials could be down to a number of factors, including the size of fish used, the rate of growth obtained, the temperature of culture, the duration of and the season when the trial was conducted as well as genetic variation in the salmon strains. The effects of PO inclusion in rainbow trout over the whole production cycle should be further investigated.

3.4.3 Tissue fatty acid composition

Several previous studies have shown that the FA compositions of fish tissues reflect that of the dietary FA and are significantly affected by the changes induced by the inclusion of VO. In addition, linear correlations have been reported between individual FA in tissue total lipid and their concentrations in dietary lipid (Bell, et al., 2001a; Bell, et al., 2003a; Tocher, et al., 2003a).

PO is rich in saturated FA and monoenes, largely 16:0 and 18:1n-9, respectively, while it lacks n-3 HUFA. Obviously, such an FA composition differs dramatically from the FA composition of the FO and hence, dietary inclusion of PO at expense of FO will affect the tissue FA composition of the fish. This has been shown in previous studies where PO was included as replacement for FO in diets for rainbow trout (Caballero, et al., 2002; Tocher, et al., 2004) and Atlantic salmon (Torstensen, et al., 2000; Rosenlund, et al., 2001; Ng, et al., 2004). Moreover, Bell et al. (2002) reported that linear correlations existed between the diet total lipid specific FA and the

respective FA in the flesh of Atlantic salmon fed on diets where PO replaced FO up to 100%. The results of the present study are clearly in agreement with these, showing significant differences in the muscle, liver, gills, heart and kidney of the fish due to the inclusion of PO. In general, in the tissues examined, the graded inclusion of PO in the diets, resulted in minor changes in the saturated FA, in a 2-fold or more increase in 18:1n-9, in a 2-fold increase in 18:2n-6, and in decreases in 18:3n-3 and EPA, by half and more than 3-fold, respectively.

However, specific FA were preferably utilized or retained in the tissues, in agreement with previous studies in salmonids fed on PO diets (Rosenlund, et al., 2001; Bell, et al., 2002). In the present study, 16:0 was almost doubled in the diets with increasing PO. However, it was in general unaffected in the fish tissues; slight increases were shown only in muscle, gills and kidney, but not in liver and heart, while these differences were significant only between the P0 and P100 groups. This could be due to the fact that 16:0 is a preferentially utilized for energy, as it is a good substrate for β-oxidation in rainbow trout (Henderson, 1996), but also because only a small amount of it was finally available to the fish due its reduced digestibility (Torstensen, et al., 2000; Caballero, et al., 2002; Ng, et al., 2003; Ng, et al., 2004). The differing incorporation of 16:0 in the tissues will also be related to the relative levels of lipid storage in the individual tissues. Thus, the leaner tissues, such as liver and heart, which contain more polar lipid than TAG, in comparison with gill, muscle and kidney, will tend to store less 16:0 than tissues where adiposity is greater (Sargent, et al., 2002). Moreover, 18:1n-9 was found in lower proportions in the tissues compared to the diets, while 20:1n-9 and 22:1 were found in higher proportions indicating utilization and retention of these FA, respectively, in line with the findings of Bell et al. (2002). The n-6 FA 20:2n-6 and 20:3n-6 were generally increased while ARA was either unaffected

or increased slightly, with increasing dietary PO, in the different tissues although these FA were reduced in the diets. This was probably due to increased desaturation and elongation of 18:2n-6 in fish fed PO and was more noticeable in this study where there was no dietary 18:3n-3 increase. Previous studies have shown higher hepatic desaturation and elongation activities with dietary PO inclusion compared to other vegetable oils (Bell, et al., 2001a; Bell, et al., 2002). This is probably caused by lack of competition between 18:2n-6 and 18:3n-3 for the Δ6-desaturase when PO was used compared to other VO such as rapeseed and linseed oils which both contain appreciable amounts of 18:3n-3 that would tend to inhibit conversion of 18:2n-6 to ARA (Bell, et al., 2001a; Tocher, et al., 2003a). EPA was significantly decreased in all tissues, however not to the same large extent as in the diets. Noticeably, in the muscle it was reduced only by 3-fold, between P0 and P100, while in other tissues the decrease was 5-fold and in the diets 9-fold. Moreover, although DHA was dramatically decreased in the diets contained PO, it was only slightly affected in the tissues, with significant differences shown when replacement was more than 50% of the FO. Hence, preferential retention of these n-3 FA occurred, although increased desaturation and elongation of 18:3n-3 may also have contributed in part to the increased retention.

Moreover, there were some differences in the FA compositions between the different tissues, in terms of the changes shown due to the dietary inclusion of PO and the extent that these occurred. It is known that different tissues are not affected by dietary FA composition to the same degree (Tocher and Harvie, 1988; Brodtkorb, et al., 1997; Rosenlund, et al., 2001). In the present study, 16:0 was increased in muscle, gills and kidney whereas it was unaffected in liver and heart. Moreover, EPA was decreased by approximately 5-fold in liver, heart and kidney but only 3- fold in muscle and gills. Such variability may be induced by the differences in the tissues physiology and role,

their energy requirements and β-oxidation capacity, their ability for desaturation and elongation of specific FA, their composition in terms of the lipid level and the lipid classes that their lipid is constituted of (phospholipids or TAG) and the extent to which lipid deposition occurs in individual tissues.

3.5 Conclusions

The results of the present study, aiming to investigate the effects of graded inclusion of PO, up to complete replacement of FO, in diets of rainbow trout, showed no negative results in growth, feed utilization and proximate composition of the fish. This suggests that PO can be successfully used as a sustainable alternative to FO in rainbow trout nutrition. However, the dietary PO inclusion resulted in significant changes in the FA compositions of the fish tissues, especially at replacement levels of over 50%. These changes, including reductions in n-3 HUFA and the n-3/n-6 ratio, may have a serious impact on the quality of the final product, in terms of its nutritional value to the human consumer, although, it should be noted that the reduction in EPA and especially in DHA were only modest.

Chapter 4. Dietary protein / lipid ratio and rapeseed oil interactions in Atlantic salmon – I

4.1 Introduction

As discussed thoroughly in the "General Introduction", marine FM and FO have, traditionally, been the major protein and oil sources in aquafeeds, especially for salmonids (Sargent and Tacon, 1999; Tacon, 2005). However, given that the resources of wild feed grade fisheries will remain static, while the demand for FM and FO by the aquaculture feed industry will grow significantly in the next decade, the strong dependence of the salmon industry on these commodities could be risky to the long term viability of the sector (Sargent and Tacon, 1999; Tidwell and Allan, 2002; Tacon, 2004).

Vegetable oils (VO) represent sustainable alternatives to FO although, the replacement of FO with VO can be challenging, as VO lack the n-3 highly unsaturated fatty acids (HUFA) which are abundant in FO (discussed in paragraph 1.4.2). The n-3 HUFA, especially EPA and DHA, are not only essential for the optimal growth and development of salmon but they are also accumulated in the fish flesh providing a highly nutritious and health promoting product for the human consumer (De Deckere, et al., 1998; Horrocks and Yeo, 1999; ISSFAL, 2000; Simopoulos, 2003). Hence, any reductions of EPA, DHA and the n-3/n-6 FA ratio in fish tissues, resulting from the replacement of FO with VO, are undesirable (Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2003a). However, as discussed previously in detail (paragraph 1.4.4), RO is considered to be a good substitute for FO both because of its high availability (U.S. Department for Agriculture, 2007) but also because of its nutritional properties for human health (Ackman, 1990) and specifically its FA composition, being rich in 18:1n-9 and moderate in 18:2n-6 (Bell, et al., 2001a).

Moreover, partial reduction of protein from FM could be achieved by the use of high lipid diets, as discussed in paragraph 1.4.6, resulting in less dependence on FM, reduction of the feed cost and also in less waste output from salmon culture and consequently in a lower environmental impact (Halver and Hardy, 2002). Salmon can utilize lipids efficiently, hence the use of such diet formulations could allow protein sparing and even improved growth (Frøyland, et al., 1998; Hillestad, et al., 1998; Bendiksen, et al., 2003b; Torstensen, et al., 2005).

Numerous studies have investigated the replacement of FO with RO, and/or other VO in diets of salmonids (Bell, et al., 2001a; Rosenlund, et al., 2001; Tocher, et al., 2001; Bell, et al., 2002; Bell, et al., 2003a; Bell, et al., 2003b; Bendiksen, et al., 2003b; Ng, et al., 2004; Tocher, et al., 2004; Torstensen, et al., 2004a; Torstensen, et al., 2004b) and also the use of low protein / high lipid diets (Einen and Roem, 1997; Bendiksen, et al., 2003b; Azevedo, et al., 2004a; Solberg, 2004). However, most of these studies were focusing either on the oil source or on the dietary protein/fat level.

In addition, very few were conducted at the very low temperatures encountered in the present study that are common in sites located at extreme latitudes. It is known that temperature plays a significant role in FA metabolism and, in general, in fish nutrition, physiology and growth (Torstensen, et al., 2000; Bendiksen, et al., 2003b; Bendiksen and Jobling, 2003; Guderley, 2004; Ng, et al., 2004; Tocher, et al., 2004; Ruyter, et al., 2006). In particular, the important role of n-3 HUFAs, monounsaturated FA and the ratio of monounsaturated to saturated FA of membrane lipids in low temperature adaptation has been highlighted (Hochachka and Somero, 2002; Sargent, et al., 2002).

The aim of this trial was to elucidate the interactive effects of dietary fat and protein contents and oil source on growth, whole body proximate composition and fatty acid composition of liver and muscle in Atlantic salmon at low water temperatures.

4.2 Materials and methods

4.2.1 Fish and culture conditions

Atlantic salmon (*Salmo salar*) of the NLA strain (03G) with overall mean weight of 1168g were randomly distributed into 18 sea cages of 125 m^3 (5x5x5m) with 137 fish in each cage. Prior to the trial, the fish were stocked in two trial cages and acclimatised for six weeks. The fish were subjected to artificial light (LD24:0) from the middle of December at decreasing ambient temperature (varying between 4-6 $^{\circ}$C). During this holding period the fish were fed commercial pelleted feed (BioOptimal CPK, 9mm, BioMar AS, Norway) in accordance with the manufacturer's recommendations. During the experimental period (February – April 2004) the fish were subjected to artificial light (LD 24:0), provided from sub-merged light (one 400W bulb shared by four cages). The temperature varied from 2.8 $^{\circ}$C to 7.3 $^{\circ}$C with an average temperature of 4.2 ± 0.8 $^{\circ}$C. At the beginning of the trial the temperature was 4.5 $^{\circ}$C whereas at the last sampling it was 6.7 $^{\circ}$C. Salinity was 34.0 ± 0.8 g L^{-1}. Fish were bulk weighed at the start of the trial, after 6 weeks, and at the end of the trial (12 weeks). Mortalities were recorded and dead fish were removed daily.

4.2.2 Experimental diets

Six isoenergetic, practical-type extruded diets (9 mm) were formulated (BioMar TechCentre, Brande, Denmark) to provide either 390 g kg^{-1} protein and 320 g kg^{-1} fat

(high protein (HP) diets) or 340 g kg^{-1} protein and 360 g kg^{-1} fat (low protein (LP) diets). Within each dietary fat and protein level crude RO comprised 0, 30 or 60% (R0, R30, R60) of the total added oil, the remainder of which was FO (Table 4.1). The diets were formulated to meet all the known nutritional requirements of salmonid fish (NRC, 1993). The proximate composition of the experimental diets is shown in Table 4.1 and the FA compositions are shown in Table 4.2. Each of the six experimental feeds was fed daily to satiation by hand to triplicate groups (cages) of fish. When sea temperature was below 5°C the fish were fed to satiation once a day. Above 5°C, two daily meals were provided with a minimum of 4 hours between the meals. In order to facilitate accurate calculations of feed intake and FCR, feed wastage was collected using a lift-up system and calculated on a daily basis.

4.2.3 Sampling procedure

Samples were taken from all diets and stored at -20 °C until analyzed. At the start of the experiment an initial sample of six fish was taken to determine baseline values of whole body proximate composition. At the end of the trial (12th week) three fish per cage were sampled at random from the population in each cage for lipid and FA composition of liver and muscle. Another sample of three fish per cage was used for whole body proximate composition.

Fish were killed with a sharp blow to the head and samples of tissues were dissected and immediately placed in liquid nitrogen as described in the "General Materials and Methods" section. Viscera, liver and heart weights from four fish per cage were recorded for measurement of viscero-somatic index (VSI), hepato-somatic index (HSI) and cardio-somatic index (CSI), respectively. For whole body analysis fish were minced and homogenate sub-samples of each fish were obtained. Initial whole

body samples were pooled in pairs so three samples were finally obtained (n = 3) while 12 week whole body samples were pooled so there was one sample per cage. A muscle sample, representative of the edible portion, was obtained by cutting a steak between the dorsal and ventral fins (NQC). This section was then skinned, de-boned and homogenized. All samples were then stored at -20 °C until analyzed.

4.2.4 Proximate analysis

Proximate analysis was conducted to determine the nutrient composition of diets and whole body samples. Moisture, crude protein, crude fat and ash contents were determined according to AOAC (1995) and modified as described by Bell et al (2001a) and are described in detail in the "General Materials and Methods" section (paragraph 2.5). The diet samples were acid hydrolysed before fat extraction.

4.2.5 Lipid extraction and fatty acid analyses

The methods followed for the lipid extraction and FA analyses of diets and tissues are thoroughly described in paragraph 2.6. Briefly, total lipids of flesh, livers and diet samples were extracted by homogenization according to Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification as described by Christie (1982) and FAME extracted and purified as described by Tocher & Harvie (1988). FAME were separated and quantified by gas-liquid chromatography and individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman, 1980).

4.2.6 Calculations and statistical analysis

Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Thermal Growth Coefficient (TGC), Protein Productive Value (PPV), Condition Factor (K), viscero-somatic index (VSI), hepato-somatic index (HSI) and cardio-somatic index (CSI) were calculated using the formulae presented in paragraph 2.8.

All the data are presented as means ± SD (n = 3) and all statistical analyses were performed using SPSS 13 (SPSS Inc, 2004) as described in paragraph 2.9. The effects of dietary RO, the fat/protein ratio and their interactions on growth and tissue fatty acid compositions were analysed by two-way ANOVA. Differences were regarded as significant when $P < 0.05$ (Zar, 1999).

4.3 Results

4.3.1 Growth performance and feed efficiency

There were no significant differences in initial cage mean weights of the fish (Table 4.3). Following 12 weeks of feeding, the cage mean weight ranged between 1711g and 1784g and the final length of the fish varied from 49.9 to 51.8 cm. No significant effects or interactions of dietary protein level and oil source were identified in final weight and length by two-way ANOVA. However, there was a significant effect of oil source on growth performance (SGR and TGC). Specifically, the inclusion of RO resulted in higher SGR (0.50 vs. 0.55, R0 vs. R60, respectively) and TGC (4.52 vs. 5.00, R0 vs. R60, respectively).

Feed conversion ratios (FCR) were good for all treatments and ranged from 0.81 to 0.87. No significant overall effects and interactions of dietary protein level and oil

source on FCR were identified, although there was a trend (P<0.10) of lower FCR for the fish fed the HP diets compared to LP diets. K ranged from 1.30 to 1.34 and no significant effects and interactions of dietary protein level and oil source were seen. The organ-to-whole-body indices are shown in Table 4.3. VSI varied from 11.7% to 12.6%, HSI from 1.4% to 1.5%, and similar CSI were found for all groups (1.3%). Two-way ANOVA showed no significant effects and interactions of dietary protein level and oil source.

PPV was high for all groups (0.42 - 0.46) and the there was a significant overall effect of dietary protein and fat level (P<0.05), with higher overall PPV for the LP groups compared to the HP groups.

4.3.2 Proximate composition of whole body

The proximate composition of whole body is shown in Table 4.4. Whole body moisture, protein and ash contents were very similar in all groups (approximately 662, 162 and 15 g kg^{-1}, respectively), whereas final lipid content ranged from 145 to 164 g kg^{-1}. Two-way ANOVA did not reveal any significant overall effects or interactions.

4.3.3 Fatty acid composition of diets and tissues

The replacement of increasing proportions of FO with RO in the diets resulted in significant changes in dietary fatty acid compositions (Table 4.2). FO diets had approximately 30% total saturates of which two thirds was 16:0, and about 35% total monoenes. Specifically, 18:1n-9 was around 13% and, 20:1 and 22:1, comprised more than 10% of the diet. The FO diets had 5% n-6PUFA, predominantly 18:2n-6, and approximately 30% n-3PUFA, with over 20% as the n-3HUFA (mainly EPA and

DHA). Graded inclusion of RO resulted in decreased 16:0, 20:1n-9, 20:4n-6, 20:5n-3 and 22:6n-3 (approximately 10%, 2.5%, 0.2%, 3.5% and 4.5% respectively in diets containing 60% RO) and increased 18:1n-9, 18:2n-6 and 18:3n-3 (approximately 43%, 14.5% and 6% respectively in diets containing 60% RO) within both dietary protein levels. The n-3/n-6 ratio decreased from 6.0 in diets containing 100% FO to 1.0 in diets containing 60% RO.

The total lipid content and the fatty acid compositions of muscle and liver are shown in Table 4.5 and Table 4.6. Total lipid content ranged from 92.6 to 117.8 mg lipid g^{-1} tissue and from 49.4 to 81.0 mg lipid g^{-1} tissue for muscle and liver, respectively. RO inclusion increased the total lipid content in liver but not in muscle. RO inclusion significantly affected tissue FA compositions. However, there were no significant interactions between dietary protein level and RO inclusion either in muscle or in liver and in most cases the overall protein level effect was not significant. Specifically, both in muscle and liver a reduction was seen in 16:0, total saturates, 20:1n-9, 22:1, 20:4n-6, 20:5n-3, 22:6n-3, total n-3 PUFAs and n-3/n-6 ratio as RO inclusion increased within both dietary fat and protein levels. Conversely, 18:1n-9, total monoenes, 18:2n-6, total n-6 PUFAs and 18:3n-3 increased in muscle and liver with graded RO inclusion. However, 20:2n-6 did not reflect the dietary content, as it increased both in liver and muscle with increased dietary RO inclusion.

Table 4.1. Feed components and proximate compositions (g kg^{-1}) of the six experimental diets

	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60
Components						
Fishmeal	390	390	390	310	310	310
Oil seed and legume seed meals	225	225	225	235	235	235
Fish oil	280	196	112	318	222	126
Rapeseed oil [a]	0	84	168	0	96	192
Binder	120	120	120	130	130	130
Premixes [b]	18	18	18	23	23	23
Analysed composition [c]						
Moisture	43	47	44	67	58	56
Protein	387	388	391	344	347	342
Lipid	327	323	321	353	347	361
Ash	79	78	78	70	70	69
Gross Energy, kJ g^{-1} [d]	25.2	25.1	25.2	25.3	25.6	25.5
Protein/Energy ratio [e]	15.3	15.5	15.3	13.6	13.4	13.2

[a] Double-low quality rapeseed oil

[b] Vitamin and mineral premixes prepared according to BioMar A/S commercial standards. Includes crystaline amino acids and Carophyl pink to provide 40mg/kg astaxanthin.

[c] Wet weight

[d] Estimated from caloric values of 39.5, 23.6 and 17.2 kJ g^{-1} for fat, protein and carbohydrate, respectively

[e] Calculated g protein kJ^{-1}

Table 4.2. Fatty acid compositions (% by weight of total fatty acids) of the six experimental diets

Fatty Acid	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60
14:0	5.9	3.5	1.8	5.4	3.1	1.9
16:0	20.3	15.2	10.4	19.6	14.3	10.4
18:0	3.6	3.0	2.5	3.5	2.9	2.5
Total saturated[a]	31.1	23.1	15.9	29.9	21.6	16.4
16:1n-7	6.6	4.2	2.3	6.1	4.2	2.2
18:1n-9	13.4	29.0	42.5	13.2	28.0	42.9
18:1n-7	2.5	2.8	3.0	2.5	3.1	2.6
20:1n-9	4.4	3.5	2.6	4.6	3.4	2.5
22:1	6.5	4.8	2.5	6.7	4.3	2.5
24:1n-9	0.8	0.6	0.4	0.9	0.9	0.5
Total monoenes[b]	34.6	45.1	53.4	34.7	44.5	53.4
18:2n-6	3.7	9.4	14.5	3.5	9.4	14.3
20:2n-6	0.2	0.2	0.1	0.3	0.2	0.1
20:4n-6	0.5	0.3	0.2	0.6	0.4	0.2
22:5n-6	0.3	0.2	0.1	0.3	0.2	0.1
Total n-6 PUFA[c]	5.0	10.4	15.1	5.0	10.4	14.9
18:3n-3	1.3	3.7	5.8	1.4	3.9	5.9
18:4n-3	2.9	1.8	1.0	3.0	2.0	1.0
20:4n-3	0.7	0.4	0.2	0.7	0.5	0.3
20:5n-3	10.4	6.7	3.6	11.0	7.4	3.5
22:5n-3	2.0	0.7	0.4	1.2	0.8	0.3
22:6n-3	11.9	8.0	4.6	12.8	8.7	4.3
Total n-3 PUFA[d]	29.3	21.4	15.5	30.3	23.4	15.3
Total PUFA	34.3	31.8	30.7	35.4	33.8	30.2
(n-3) / (n-6)	5.9	2.1	1.0	6.0	2.2	1.0

[a]Includes 15:0, 20:0 & 22:0.

[b]Includes 16:1n-9 & 20:1n-7.

[c]Includes 18:3n-6, 20:3n-6 & 22:4n-6.

[d]Includes 20:3n-3 & 22:4n-3.

Table 4.3. Growth and performance of Atlantic salmon fed the experimental diets for 12 weeks

Parameter	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Initial Weight (g)	1168.4 ± 32.3	1184.6 ± 16.1	1152.4 ± 21.0	1162.8 ± 24.4	1171.9 ± 14.6	1168.4 ± 24.4			
Final Weight (g)	1711.3 ± 79.8	1772.0 ± 36.5	1784.3 ± 48.5	1721.7 ± 47.6	1760.3 ± 27.0	1767.7 ± 57.6	0.812	0.149	0.894
Final Length (cm)	50.5 ± 0.4	50.9 ± 1.1	50.7 ± 1.0	50.7 ± 0.3	49.9 ± 0.6	51.8 ± 0.6	0.829	0.130	0.077
Mortalities[1]	5	1	2	3	0	5			
FCR[2]	0.86 ± 0.01	0.84 ± 0.02	0.81 ± 0.01	0.86 ± 0.02	0.87 ± 0.04	0.85 ± 0.03	0.077	0.160	0.349
SGR[3]	0.49 ± 0.03	0.52 ± 0.01	0.56 ± 0.01	0.50 ± 0.02	0.52 ± 0.01	0.53 ± 0.03	0.723	0.002	0.128
TGC[4]	4.45 ± 0.27	4.75 ± 0.16	5.13 ± 0.11	4.59 ± 0.20	4.78 ± 0.07	4.87 ± 0.28	0.742	0.005	0.227
PPV[5]	0.42 ± 0.03	0.43 ± 0.01	0.44 ± 0.01	0.46 ± 0.02	0.46 ± 0.02	0.46 ± 0.05	0.045	0.799	0.868
K (%)[6]	1.31 ± 0.01	1.30 ± 0.02	1.32 ± 0.04	1.31 ± 0.06	1.30 ± 0.01	1.34 ± 0.01	0.609	0.274	0.941
VSI (%)[7]	12.03 ± 1.00	12.01 ± 0.69	12.41 ± 0.98	12.57 ± 0.27	11.67 ± 0.28	11.90 ± 0.49	0.749	0.515	0.395
HSI (%)[8]	1.34 ± 0.12	1.32 ± 0.02	1.45 ± 0.20	1.36 ± 0.10	1.36 ± 0.07	1.31 ± 0.07	0.646	0.822	0.371
CSI (%)[9]	0.15 ± 0.03	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.01	0.14 ± 0.03	0.12 ± 0.01	0.358	0.556	0.522

All values are mean ± S.D. (n=3).

[1] Total number; [2] Feed Conversion Ratio; [3] Specific Growth Rate; [4] Thermal Growth Coefficient; [5] Protein Productive Value; [6] Condition Factor

[7] Viscero-somatic index; [8] Hepato-somatic index; [9] Cardio-somatic index

Table 4.4. Proximate composition (g kg^{-1} of wet weight) of whole body from Atlantic salmon fed the experimental diets for 12 weeks

	Start [1]	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
								protein	oil	prot x oil
Moisture	666.1 ± 16.5	664.7 ± 12.6	661.7 ± 12.9	665.3 ± 11.5	666.5 ± 6.2	665.8 ± 13.9	650.8 ± 10.9	0.602	0.522	0.350
Protein	176.4 ± 2.2	162.2 ± 4.9	162.1 ± 4.8	162.9 ± 2.5	162.2 ± 2.5	162.2 ± 4.2	162.3 ± 2.0	0.925	0.976	0.983
Lipid	128.9 ± 16.1	150.3 ± 10.8	155.1 ± 6.9	151.9 ± 11.9	145.2 ± 6.7	149.5 ± 13.2	164.5 ± 10.5	0.901	0.250	0.259
Ash	17.5 ± 0.7	14.9 ± 0.3	15.4 ± 1.0	15.0 ± 0.4	16.0 ± 0.7	15.4 ± 1.2	15.1 ± 1.0	0.376	0.672	0.421

All values are mean ± S.D. (n=3).

[1]Values not included in the two-way ANOVA

Table 4.5. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of muscle from Atlantic salmon fed the experimental diets for 12 weeks

	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Total Lipid	117.8 ± 8.5	101.9 ± 6.4	102.8 ± 4.1	92.6 ± 13.9	101.7 ± 17.1	110.0 ± 9.6	0.259	0.748	0.057
Fatty acid									
14:0	4.7 ± 0.4	4.1 ± 0.1	3.4 ± 0.3	5.1 ± 0.4	4.3 ± 0.1	3.6 ± 0.4	0.064	0.000	0.739
16:0	16.0 ± 0.8	15.2 ± 0.9	13.5 ± 1.3	16.2 ± 1.4	14.6 ± 1.1	13.3 ± 1.1	0.649	0.004	0.857
18:0	3.1 ± 0.2	3.1 ± 0.3	3.1 ± 0.4	3.1 ± 0.3	2.9 ± 0.3	2.8 ± 0.3	0.299	0.635	0.825
Total saturated[1]	24.5 ± 1.4	23.2 ± 1.5	21.0 ± 2.5	25.3 ± 2.4	22.7 ± 1.7	20.8 ± 2.1	0.971	0.015	0.874
16:1n-7	6.6 ± 0.1	5.5 ± 0.2	4.4 ± 0.1	6.8 ± 0.0	5.6 ± 0.1	4.6 ± 0.1	0.019	0.000	0.474
18:1n-9	15.7 ± 0.5	23.3 ± 1.1	29.7 ± 0.9	15.9 ± 0.3	22.9 ± 0.4	27.9 ± 1.9	0.197	0.000	0.340
18:1n-7	3.2 ± 0.1	3.4 ± 0.1	3.1 ± 0.1	3.2 ± 0.2	3.3 ± 0.1	3.1 ± 0.3	0.766	0.070	0.613
20:1n-9	7.8 ± 0.5	7.2 ± 0.3	6.7 ± 0.2	8.0 ± 0.2	7.2 ± 0.2	6.8 ± 0.2	0.577	0.000	0.968
22:1	8.3 ± 0.4	7.4 ± 0.5	6.0 ± 0.6	8.5 ± 0.1	7.3 ± 0.2	6.4 ± 0.4	0.573	0.000	0.566
24:1n-9	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.0	0.407	0.002	0.531
Total monoenes[2]	42.9 ± 1.2	48.0 ± 1.4	51.0 ± 0.5	43.8 ± 0.3	47.4 ± 0.2	49.9 ± 1.0	0.426	0.000	0.270
18:2n-6	3.7 ± 0.1	6.3 ± 0.1	8.2 ± 0.9	3.6 ± 0.1	6.3 ± 0.5	8.2 ± 0.6	0.922	0.000	0.942
20:2n-6	0.4 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.179	0.000	0.076
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.870	0.274	0.481
20:4n-6	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.843	0.000	0.756
Total n-6 PUFA[3]	5.1 ± 0.1	7.5 ± 0.2	9.6 ± 0.8	5.0 ± 0.2	7.6 ± 0.6	9.5 ± 0.7	0.817	0.000	0.912
18:3n-3	1.2 ± 0.0	2.2 ± 0.1	3.0 ± 0.4	1.2 ± 0.1	2.2 ± 0.2	3.0 ± 0.3	0.743	0.000	0.968
18:4n-3	1.8 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	1.8 ± 0.1	1.4 ± 0.1	1.1 ± 0.0	0.302	0.000	0.418
20:4n-3	1.5 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	0.936	0.000	0.166
20:5n-3	7.4 ± 0.4	5.4 ± 0.9	4.2 ± 0.4	7.2 ± 0.7	5.7 ± 0.3	4.6 ± 0.2	0.485	0.000	0.679
22:5n-3	2.5 ± 0.1	1.9 ± 0.2	1.6 ± 0.2	2.5 ± 0.3	1.9 ± 0.2	1.7 ± 0.0	0.505	0.000	0.734
22:6n-3	12.9 ± 0.9	9.3 ± 1.2	7.5 ± 0.7	11.9 ± 1.1	9.7 ± 0.5	8.2 ± 0.1	0.898	0.000	0.252
Total n-3 PUFA[4]	27.5 ± 1.1	21.4 ± 2.6	18.4 ± 1.9	26.0 ± 2.3	22.3 ± 1.3	19.8 ± 0.5	0.668	0.000	0.422
Total PUFA	32.6 ± 1.2	28.8 ± 2.8	28.0 ± 2.7	31.0 ± 2.6	29.8 ± 1.9	29.3 ± 1.2	0.766	0.081	0.535
(n-3) / (n-6)	5.4 ± 0.1	2.9 ± 0.3	1.9 ± 0.1	5.2 ± 0.2	2.9 ± 0.1	2.1 ± 0.1	0.557	0.000	0.263

Values are mean ± S.D. (n=3).

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 16:1n-9 & 20:1n-7; [3]Includes 18:3n-6, 20:3n-6 & 22:4n-6; [4]Includes 20:3n-3 & 22:4n-3

Table 4.6. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of liver from Atlantic salmon fed the experimental diets for 12 weeks

	HP-R0	HP-R30	HP-R60	LP-R0	LP-R30	LP-R60	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Total lipid	62.7 ± 16.6	49.4 ± 6.6	81.0 ± 37.0	52.5 ± 16.7	54.9 ± 7.9	69.3 ± 16.2	0.422	0.037	0.519
Fatty acid									
14:0	2.4 ± 0.4	1.8 ± 0.0	1.2 ± 0.1	2.7 ± 0.3	1.8 ± 0.3	1.2 ± 0.1	0.543	0.000	0.714
16:0	15.2 ± 1.7	12.6 ± 1.1	10.0 ± 0.9	14.2 ± 1.0	13.2 ± 1.1	9.1 ± 1.0	0.418	0.000	0.450
18:0	6.1 ± 0.6	5.1 ± 0.3	4.8 ± 0.5	5.5 ± 0.1	4.9 ± 0.5	4.0 ± 0.2	0.022	0.000	0.537
Total saturated[1]	24.4 ± 2.5	20.1 ± 1.4	16.5 ± 1.4	23.2 ± 1.2	20.4 ± 1.2	15.1 ± 1.5	0.359	0.000	0.611
16:1n-7	3.7 ± 0.5	2.6 ± 0.2	1.9 ± 0.2	4.1 ± 0.2	2.6 ± 0.3	1.9 ± 0.2	0.327	0.000	0.268
18:1n-9	15.9 ± 4.0	23.0 ± 3.9	35.7 ± 3.9	16.7 ± 2.0	21.3 ± 2.1	37.1 ± 4.8	0.928	0.000	0.729
18:1n-7	3.1 ± 0.3	2.9 ± 0.2	3.1 ± 0.1	3.4 ± 0.3	3.0 ± 0.3	3.2 ± 0.5	0.234	0.300	0.946
20:1n-9	4.4 ± 0.6	4.2 ± 0.8	5.0 ± 0.2	4.7 ± 0.3	4.0 ± 0.5	5.3 ± 0.7	0.692	0.025	0.615
22:1	1.4 ± 0.2	1.1 ± 0.1	0.9 ± 0.0	2.1 ± 0.4	1.4 ± 0.3	1.0 ± 0.0	0.007	0.000	0.238
24:1n-9	1.0 ± 0.3	0.9 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	0.737	0.011	0.299
Total monoenes[2]	29.8 ± 5.2	35.0 ± 5.1	47.3 ± 4.0	32.1 ± 2.8	33.6 ± 3.4	49.3 ± 6.2	0.661	0.000	0.746
18:2n-6	2.1 ± 0.4	4.9 ± 0.5	8.2 ± 0.4	2.5 ± 0.3	4.9 ± 0.5	9.0 ± 0.5	0.124	0.000	0.396
20:2n-6	0.6 ± 0.1	1.3 ± 0.2	2.1 ± 0.1	0.6 ± 0.1	1.2 ± 0.1	2.1 ± 0.2	0.695	0.000	0.600
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.853	0.001	0.215
20:4n-6	2.2 ± 0.5	1.9 ± 0.4	1.0 ± 0.3	1.9 ± 0.2	2.0 ± 0.3	1.0 ± 0.2	0.524	0.000	0.413
Total n-6 PUFA[3]	5.7 ± 0.1	8.6 ± 0.4	11.9 ± 0.3	5.7 ± 0.3	8.7 ± 0.1	12.5 ± 0.4	0.100	0.000	0.094
18:3n-3	0.7 ± 0.1	1.6 ± 0.2	2.7 ± 0.1	0.9 ± 0.1	1.6 ± 0.2	2.9 ± 0.1	0.047	0.000	0.242
18:4n-3	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.032	0.000	0.879
20:4n-3	1.4 ± 0.2	1.1 ± 0.1	0.8 ± 0.0	1.7 ± 0.1	1.1 ± 0.1	0.8 ± 0.0	0.208	0.000	0.096
20:5n-3	10.4 ± 1.8	8.8 ± 1.3	5.2 ± 0.9	10.3 ± 0.8	9.4 ± 0.8	4.9 ± 1.4	0.921	0.000	0.808
22:5n-3	3.3 ± 0.7	2.9 ± 0.3	1.5 ± 0.2	3.7 ± 0.3	2.5 ± 0.2	1.4 ± 0.3	0.671	0.000	0.370
22:6n-3	23.7 ± 4.6	21.2 ± 3.4	13.4 ± 3.1	21.9 ± 2.1	22.0 ± 2.0	12.1 ± 3.7	0.621	0.000	0.763
Total n-3 PUFA[4]	40.2 ± 5.9	36.3 ± 4.1	24.3 ± 4.2	39.0 ± 3.1	37.3 ± 2.3	23.0 ± 5.2	0.814	0.000	0.877
Total PUFA	45.9 ± 6.0	45.0 ± 3.7	36.2 ± 4.2	44.7 ± 3.1	46.0 ± 2.1	35.5 ± 4.8	0.899	0.002	0.893
(n-3) / (n-6)	7.0 ± 1.0	4.2 ± 0.6	2.1 ± 0.4	6.9 ± 0.7	4.3 ± 0.3	1.8 ± 0.5	0.757	0.000	0.911

Values are mean ± S.D. (n=3).

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 16:1n-9 & 20:1n-7; [3]Includes 18:3n-6, 20:3n-6 & 22:4n-6; [4]Includes 20:3n-3 & 22:4n-3

4.4 Discussion

4.4.1 Growth performance

This study aimed to investigate the effects and interactions of the replacement of FO with RO at two different protein/lipid ratios at low water temperatures. As previously discussed, several studies have shown that replacement of FO with RO, blends of RO and other VO or other VO alone in diets of salmon, has no negative effects on fish growth (Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2002; Bell, et al., 2003a; Bell, et al., 2003b; Bendiksen, et al., 2003b; Ng, et al., 2004; Torstensen, et al., 2004a; Torstensen, et al., 2004b; Torstensen, et al., 2005). Moreover, when low protein feeds were compared to high protein feeds growth was not significantly affected (Azevedo, et al., 2004a; Solberg, 2004), especially at low temperatures (Hillestad, et al., 1998; Bendiksen, et al., 2003b). It is likely that the effect of low temperature masked any potential effects of feed treatment, and that diet-related growth differences observed at the higher temperature were diminished at the lower temperature (Bendiksen, et al., 2003b). In line with the previous studies, the current experiment showed no significant effects due to dietary protein level in final weights, SGR, TGC and FCRs. Nevertheless, oil source had a significant positive effect on SGR and TGC. Graded inclusion of RO, at the expense of FO, resulted in increased SGR and TGC. This is in accordance with other studies and could be a result of improved use of oil for energy, due to superior fatty acid availability from vegetable oils at low water temperatures (Bendiksen, et al., 2003b; Torstensen, et al., 2005). Specifically, it has been shown that the absorption and digestibility of FA in salmonids decreases with increasing saturation and chain length (Olsen and Ringø, 1998; Torstensen, et al., 2000; Caballero, et al., 2002; Ng, et al., 2004). Moreover, the AD of the saturated FA

decreases with decreased temperature while the AD of the monoenes and PUFA is hardly affected (Olsen and Ringø, 1998; Ng, et al., 2004). Hence, the lower levels of saturated FA in the RO increased the lipid AD and the digestible energy content of the RO diets, resulting in improved growth performance of the fish. Although no significant interactions were found, the effect of oil source seemed to differ between LP and HP series which indicates that the most improved growth was shown when the dietary protein and amino acids where in excess (HP feeds).

The protein sparing effect could also have been enhanced by higher dietary oil levels. This is supported by the PPV results, as the overall PPV was significantly improved when the fish were fed the LP diets. Previous studies have also suggested a positive effect of increased dietary lipid content on protein retention and, hence, on protein sparing (Einen and Roem, 1997; Hillestad, et al., 1998; Bendiksen, et al., 2003b).

In the present trial K, VSI, HSI and CSI were not affected by the different dietary treatments. This is in agreement with other studies, as changes in dietary protein / fat ratio in Atlantic salmon have not been associated with changes in K, VSI and HSI (Einen and Roem, 1997; Solberg, 2004) or with the replacement of FO with RO and LO (Rosenlund, et al., 2001; Bendiksen, et al., 2003b). Few reports on diet effects on the CSI are available although a reduced CSI was observed in one study where salmon were fed diets where sunflower oil was used as a FO replacement (Bell, et al., 1991).

4.4.2 Whole body proximate composition

In this study, the different dietary treatments had no influence on the chemical composition of the whole carcass, either due to dietary protein level or due to the oil

source. The moisture, protein and ash content of the carcass were almost constant between the groups and there were only minor differences in the lipid content. This is in agreement with the findings of Hillestad et al. (1998) who reported that the fillet and dressed carcass fat content was not influenced by dietary lipid level, although significant differences in tissue fat content were shown, due to the dietary energy content. Other studies have shown that, when the dietary oil increases, tissue lipid, and usually moisture, increases, while protein decreases (Hillestad and Johnsen, 1994; Einen and Roem, 1997; Einen and Skrede, 1998; Hemre and Sandnes, 1999; Bendiksen, et al., 2003b; Azevedo, et al., 2004a; Solberg, 2004). However, in most of these studies the lipid content of the fillet, carcass or whole body was possibly influenced by the differences in the dietary energy content, as only Azevedo et al. (2004a) and Solberg (2004) used isoenergetic diets. In the present study, no significant differences were observed in the carcass chemical composition due to dietary oil source. This is in agreement with the results reported by Bendiksen et al. (2003b) when FO was replaced with a blend of VO at low water temperatures (2 ^{o}C). By contrast, other studies have shown that when RO replaced FO in diets of Atlantic salmon, the chemical composition of tissues is significantly influenced, to a small extent, although these studies were of longer duration than the present study (Bell, et al., 2001a; Torstensen, et al., 2004b; Ruyter, et al., 2006). However, in the present study there was a significant effect due to the oil source on the total lipid level of the liver; specifically, the inclusion of RO resulted in increased total lipid in the liver, which is consistent with the previous mentioned studies. It has been shown that in Atlantic salmon fat deposition increases as the fish grow larger (Jobling and Johansen, 2003). This was clearly demonstrated in the present study where an increase in lipid content, along with a decrease in crude protein and ash, was observed between the initial and final sampling.

4.4.3 Tissue fatty acid composition

The fatty acid compositions of tissue lipids of Atlantic salmon are known to be highly influenced by dietary fatty acids (Torstensen, et al., 2000; Rosenlund, et al., 2001) and linear correlations exist between individual fatty acids in tissue total lipid and their concentrations in dietary lipid (Bell, et al., 2001a; Bell, et al., 2003a; Tocher, et al., 2003a). In the present study, the differences in the dietary fatty acid compositions resulting from the graded inclusion of RO, at the expense of FO, were not affected by dietary protein level. Hence, diets including similar proportions of RO had very similar fatty acid compositions irrespective of their protein/lipid ratio. As a result the oil source affected significantly, liver and muscle FA compositions while no significant effects on the tissue FA compositions were shown due to the dietary protein level. The results are in line with previous studies showing that dietary fatty acid compositions are reflected in tissue FA compositions (Torstensen, et al., 2000; Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2003a).

However, previous studies have shown that although dietary fatty acids correlated to fatty acids deposited in flesh, specific fatty acids were selectively utilized or retained (Bell, et al., 2001a; 2003a; Torstensen, et al., 2004a). This was also demonstrated in the present study. Dietary 18:1n-9 increased more than 3-fold and 18:2n-6 and 18:3n-3 more than 4-fold in diets containing 60% RO compared to FO diets, whereas in muscle and liver these fatty acids increased only around 2-fold. These data confirm that when certain fatty acids are provided to the fish in high concentrations, they are readily metabolised, largely catabolism by β-oxidation, although they may also be subject to limited desaturation and elongation (Bell, et al., 2003a). On the contrary, n-3 HUFAs were selectively deposited and retained in flesh.

Tissue DHA was reduced only by 30% - 40% and EPA by less than 45% when fish were fed diets containing 60% RO compared to FO groups, whereas in 60% RO diets these HUFAs were only 30% of the concentrations in 0% RO diets. Apart from the selective deposition and retention of these FAs, the moderate reductions in EPA and DHA could also have been affected, even to small extent, by the hepatic desaturation and elongation of dietary α-linolenic acid, which can be increased by inclusion of vegetable oils in the diets (Tocher, et al., 2000; Tocher, et al., 2001; Tocher, et al., 2003a; Tocher, et al., 2003b).

It is well documented that n-3 HUFAs, particularly EPA and DHA and a high n-3 / n-6 ratio, in human diets are beneficial for various aspects of human health including preventive or protective effects in coronary heart disease, rheumatoid arthritis, cancer, neurodevelopmental and mood disorders etc (De Deckere, et al., 1998; Horrocks and Yeo, 1999; Simopoulos, 1999; Hunter and Roberts, 2000; ISSFAL, 2000; Simopoulos, 2003). At present, intensive culture of Atlantic salmon uses marine FO resulting in a highly nutritious and healthy product, as it is rich in n-3 HUFAs and has a high n-3 / n-6 ratio (Bell, et al., 1998). However, in recent times there has been a desire to investigate more sustainable alternatives to fish meal and fish oil for use in aquaculture feeds. Clearly, any changes towards use of vegetable alternatives to marine FO should not be at the expense of the quality and nutritional value of the final product. In this regard the present study showing moderate reduction of EPA and DHA in fish fed diets containing RO, at levels as high as 60%, even at low protein levels, could be significant, although it should be remembered that this was a relatively short trial compared to the whole production cycle for Atlantic salmon.

4.5 Conclusions

The results of this study showed no negative effects on growth and feed conversion, no major detrimental effects on lipid and fatty acid metabolism in Atlantic salmon and an enhanced protein sparing effect, when fish were fed with lower protein feeds where RO replaced FO up to 60% of the total oil. In conclusion, the results of this study suggest that more sustainable, lower protein diets, in which a high proportion of the dietary protein and lipid is of non-marine origin, with moderate/high RO inclusion, can be used successfully in Atlantic salmon culture at low water temperatures.

Chapter 5. Dietary protein / lipid ratio and rapeseed oil interactions in Atlantic salmon – II

5.1 Introduction

The use of VO in fish diets, in order to reduce the use of FO, and consequently the reliance of the salmon industry on marine oils, leading towards sustainability, without compromising the cost effectiveness of modern diets or the quality of the final product, has been discussed previously. It has also been demonstrated that this becomes even more significant for carnivorous species such as Atlantic salmon. Moreover, the need for FM reduction in the diets of Atlantic salmon is also of great importance and thus, high lipid / energy dense diets could play a role towards this goal. However, such feed formulations will potentially increase the requirements of aquaculture for FO; hence the need for sustainable oil alternatives is vitally important.

In the previous chapter the interactive effects of two different fat and protein levels and the inclusion of RO in diets of Atlantic salmon, reared at low water temperatures, were investigated. The results of that study showed that growth (SGR and TGC) was enhanced and a protein sparing effect occurred due to the RO inclusion whereas growth was not negatively affected by low protein levels. These results were in line with previous studies that used RO in diets for Atlantic salmon and other salmonids with generally no detrimental effects on growth and FCR (Bell, et al., 2001a; Caballero, et al., 2002; Bell, et al., 2003a; Ng, et al., 2004; Torstensen, et al., 2004a; Torstensen, et al., 2004b; Torstensen, et al., 2005) or even, in some cases, enhanced growth (Bendiksen, et al., 2003b; Torstensen, et al., 2005). They were also in agreement with studies using low protein / high lipid diets (Einen and Roem, 1997; Hillestad, et al., 1998; Bendiksen, et al., 2003b; Azevedo, et al., 2004b, 2004a; Solberg, 2004).

However, most of the previous studies used diets with relatively high dietary protein contents, ranging from greater than 500 g kg^{-1} to a minimum of approximately

360 g kg^{-1}, and varying lipid levels from as low as 200 g kg^{-1} up to 470 g kg^{-1}, while in the study of Chapter 4 levels of CP/CL of 340/360were successfully used. Hence, how much the dietary protein/lipid level can be further reduced is not clear.

Moreover, as discussed previously, although numerous studies have focused either on the investigation of sustainable alternatives to FM and FO or in the reduction of the dietary protein/lipid level very few have investigated the interactive effects such dietary changes may have on growth and feed utilization (Bendiksen, et al., 2003a; Bendiksen, et al., 2003b). For example, both factors (dietary protein/lipid level and oil source) have been shown to affect the chemical composition of fish tissues (Bell, et al., 2001a; Azevedo, et al., 2004b, 2004a; Solberg, 2004; Torstensen, et al., 2004a; Ruyter, et al., 2006). Similarly, it has been reported previously that the dietary protein and fat level affects the digestibility of nutrients in Atlantic salmon (Einen and Roem, 1997; Azevedo, et al., 2004b). In addition, the dietary FA composition and consequently the oil source also affect the ADC of nutrients and FA (Caballero, et al., 2002; Bendiksen, et al., 2003b; Menoyo, et al., 2003; Ng, et al., 2004). Furthermore, the tissue FA compositions and also the FA metabolism, especially the tissue β-oxidation capacities, are known to be significantly influenced by the diet and especially the dietary FA composition (Torstensen, et al., 2000; Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2003a; Tocher, et al., 2003b; Torstensen and Stubhaug, 2004; Stubhaug, et al., 2005a; Stubhaug, et al., 2007). Nevertheless, although these effects are important for the understanding of the mechanisms of salmon nutrition and influence the growth and the quality of the final product, little is known about how these factors interact.

Furthermore, the results of the study reported in the previous chapter showed that the digestibility of nutrients and especially the ADC of individual FA may play a

key role in the enhanced growth that was shown. However, this hypothesis was not fully confirmed in the previous study due to technical problems in the measurement of the ADC and hence the interactive effects on nutrients and FA ADC should be further elucidated.

Lastly, it is known that temperature plays a significant role in fish nutrition, nutrient and FA metabolism and digestibility largely by affecting the membrane lipid composition (Hazel, 1984; Hazel and Williams, 1990; Bendiksen and Jobling, 2003; Ng, et al., 2004; Ruyter, et al., 2006). The previous trial was carried out at low water temperatures of approximately 4 °C. However, it became clear that further research was needed to assess the impact of protein/lipid ratio, and dietary lipid source, in Atlantic salmon reared at high (summer) water temperatures.

Hence, the aim of this study was to investigate the interactive effects of three different, decreasing, protein / fat ratios and the replacement of FO with RO on growth, feed utilization, nutrient and FA digestibility, whole body chemical composition and tissue FA composition and β-oxidation of large Atlantic salmon grown at high water temperatures.

5.2 Materials and methods

5.2.1 Fish and culture conditions

For the present trial, triplicate groups of Atlantic salmon (*Salmo salar*), were distributed in eighteen sea cages at the Fjord Research Station AS (Helgeland, Dønna, Norway, 66 °N). Prior to the experiment, the fish were acclimatised to the trial cages for 63 days, where the average temperature was 12°C. During this period the fish were fed

a commercial diet from BioMar AS (9 mm; declared composition 360 g kg^{-1} protein and 350 g kg^{-1} fat) according to the producer's recommendations. The sea cages were of 125 m^3 (5x5x5m) with approximately 93 fish, of initial mean weight of 2053g, randomly distributed in each one. The average water temperature during the 10 week experimental period (August – October 2005) was 11.6 ± 1.1 $^{\circ}C$ and the salinity 32.5 ± 0.4 g L^{-1}. During the experimental period the fish were subjected to natural photoperiod. Mortalities were recorded and dead fish were removed daily.

5.2.2 Experimental diets and feeding

The six experimental diets were produced at the BioMar TechCentre (Brande, Denmark) as practical-type extruded pellets (9 mm). The diets were formulated at three different dietary protein/fat levels. Specifically, the diets contained 350 g kg^{-1} / 350 g kg^{-1}, 330 g kg^{-1} / 360 g kg^{-1}, 290 g kg^{-1} / 380 g kg^{-1} of protein / lipid for high protein (HP), medium protein (MP) and low protein (LP) diets, respectively. Within each dietary protein/fat level the oil source was either FO or RO; in the RO diets crude RO comprised 60% of the total added oil, the remainder of which was FO. The diets were isoenergetic with a gross energy content of 25 kJ g^{-1}. The formulations and the proximate compositions of the experimental diets are shown in Table 5.1 and the fatty acid compositions are shown in Table 5.2. The diets were formulated to meet all known nutritional requirements of salmonid fish (NRC, 1993). All diets contained yttrium trioxide (Y_2O_3) as an inert marker to determine the apparent digestibility coefficients (ADC) of nutrients and FA. Each of the six experimental feeds was fed daily to satiation by hand to triplicate groups (cages) of fish. Two daily meals were provided with a minimum of 4 hours between the meals. Uneaten feed was collected using a lift-

up system and calculated on a daily basis in order to facilitate accurate calculations of feed intake and FCR.

5.2.3 Sampling procedure

At the start of the trial the fish were bulk weighed. An initial sample of six fish was taken to determine baseline values of whole body proximate composition. At the end of the trial (10th week) the fish were anaesthetized in MS-222 (metacain, 8mg/L) and individually weighed. Three fish per cage were selected at random from each cage to determine whole body proximate composition. The fish were killed with a sharp blow to the head. Fish were minced and homogenate sub-samples of each fish were obtained. The initial samples were pooled in pairs so three samples were finally obtained (n = 3) whereas the homogenates of the final sampling were pooled providing finally one sample per cage.

At the end of the trial faeces samples were obtained by stripping according to Austreng (1978). The fish were anaesthetized and faeces were collected by gently squeezing the hindgut of the fish. One sample of faeces per cage was finally obtained by pooling faeces from an appropriate, varying number of fish from each cage until a minimum weight of 150 g (wet weight) of faeces was collected. Any water, urine and fish scales were removed and ethoxyquin (ETQ; 400 mg/L, 1 mL/60g wet faeces) was added to each sample which were then stored at -20 °C. The faeces samples were then freeze dried as described in the General Materials and Methods section. The freeze dried samples were also kept in -20 °C until further analysis. Lastly, samples were taken from the six experimental diets and stored at -20°C until analyzed.

5.2.4 Proximate analysis

The nutrient compositions (moisture, crude protein, crude fat, ash and gross energy content) of the six experimental diets and the whole body samples were determined by proximate analysis based on methods described in AOAC (1995) as described in the General Materials and Methods section. Prior to the crude fat extraction the diet samples were acid hydrolysed as previously described. Yttrium trioxide (Y_2O_3) was analysed as described in paragraph 2.5.8.

5.2.5 Lipid extraction and fatty acid analyses

The lipid extraction and FA analyses of diets and tissues were carried as described in paragraph 2.6. Briefly, total lipids of flesh, livers and diet samples were extracted by homogenization according to Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification as described by Christie (1982) and FAME extracted and purified as described by Tocher & Harvie (1988). FAME were separated and quantified by gas-liquid chromatography and individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman, 1980).

5.2.6 Peroxisomal β-oxidation capacity

The peroxisomal β-oxidation capacity of liver, red and white muscle was measured as described in paragraph 2.7. The samples of tissues were taken as described above, immediately frozen in liquid nitrogen and stored in -20 °C until analyzed.

5.2.7 Calculations and statistical analysis

Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Thermal Growth Coefficient (TGC), Protein productive value (PPV) and the ADC (Apparent Digestibility Coefficient) of nutrients and FA were calculated as described in paragraph 2.8.

The effects of the protein/fat ratio (protein level), dietary RO inclusion (oil source) and their interactions on growth, whole body proximate composition and ADC of nutrients and individual FA were analysed by factorial (two-way) ANOVA. When significant interactions of the two factors were observed, the main effects were not discussed further but instead, multiple comparison testing was performed to look at the simple main effects, that is the main effect of one factor at a given level of the other (Zar, 1999, pp. 260-261). The analysis of the simple main effects was done for both factors. Data which were identified as non-homogeneous (Levene's test) were subjected to square root, log or arcsin transformation before analysis. Differences were regarded as significant when $P < 0.05$ (Zar, 1999). All the data are presented as means ± SD (n = 3) and all statistical analyses were performed using SPSS 14.0 (SPSS Inc, 2005).

5.3 Results

5.3.1 Diet proximate and fatty acid composition

The analysed proximate composition of the six experimental diets is shown in Table 5.1. The protein / lipid content of the diets, regardless of the RO inclusion level, was 349 g kg^{-1} / 350 g kg^{-1}, 333 g kg^{-1} / 358 g kg^{-1}, 293 g kg^{-1} / 384 g kg^{-1} for HP, MP and LP, respectively. All diets were isoenergetic with a gross energy value of 25 kJ g^{-1}.

The Digestible Protein / Digestible Energy (DP/DE) ratio was 14.5, 13.5 and 12.3 for the HP, MP and LP diets, respectively. DP and DE were calculated using the ADC values for protein and energy found in this trial (Table 5.4).

The oil source of the diets was either 100% FO or a blend of 40% FO and 60% RO resulting in two different total lipid FA profiles (Table 5.2). The FO diets contained approximately 36% total saturated FA, largely 16:0 (approx. 20%), except for the HP-FO diet which had a slightly higher total saturated FA content (40.4%). The total monoenes were 26%, predominantly 18:1n-9 and 16:1n-7. The total n-6 PUFA were low (4.5%), half of which was 18:2n-6. Lastly, the total n-3 PUFA were as high as 32%, mainly as EPA and DHA (18.5% and 8%, respectively). The 60% inclusion of RO resulted in the reduction of 16:0, and consequently of the total saturated FA, by half compared to the FO diets. The total monoenes almost doubled, largely due to the high amount of 18:1n-9 (38.5%). The total n-6 PUFA in the RO diets increased 3-fold, up to 14%, mainly as 18:2n-6 (13% of total FA). EPA decreased by 70% and DHA by more than half (values were 6% and 3% of FA, respectively) and hence the total n-3 PUFA were reduced by half (17%). The n-3/n-6 PUFA ratio was 7 and 1.2 for the FO and RO diets, respectively.

5.3.2 Growth

The initial mean weight of the fish was 2053 g. After feeding the fish with the experimental diets for 10 weeks their weight ranged from 3340.2 g to 3664.2 g, for HP-RO and MP-RO, respectively (Table 5.3). Two way ANOVA showed a significant effect due to the oil source ($P = 0.032$) with the fish fed the RO diets having a higher final weight compared to the FO diets. The same significant effect due to the oil source favouring the RO group was also observed in both SGR and TGC (0.87 vs. 0.94 and

3.46 vs. 3.81, respectively). The protein level had no significant effects on any of the growth parameters although there was a trend ($P = 0.085$) of lower final weight with decreasing protein level. In addition, no significant interactions between the two factors (protein level and oil source) were observed. Lastly, FCR ranged from 0.99 to 1.10 but was not significantly affected by the dietary treatments.

The PPV ranged from 0.38 to 0.47 (Table 5.3) and the two-way ANOVA showed that the oil source, but not the protein level, had a significant overall effect on PPV ($P = 0.04$); namely, the RO group had a higher PRE compared to the FO group (0.41 vs. 0.45, for FO and RO, respectively). However, the data were non-homogenous so the results of the two-way ANOVA could be misleading. Hence, when the outliers were identified and removed from the data set (PPV^b in Table 5.3) the data –set was fulfilling the assumptions of two-way ANOVA and the results showed a significant effect both due to the protein level and the oil source. Specifically, the LP diets had significantly higher PPV than the other two groups (0.41, 0.43 and 0.47, for HP, MP and LP, respectively). The dietary inclusion of RO resulted in higher PPV compared to the FO groups (0.42 vs. 0.46, for FO and RO, respectively).

5.3.3 Apparent nutrient digestibility

The nutrients ADC are shown in Table 5.4. The DM ADC was relatively low varying from 50.5% – 60.1%. Two-way ANOVA showed a significant interaction of protein level and oil source, therefore the simple main effects were analysed (Figure 5.1a) showing that the LP diets had significantly lower DM ADC compared to HP and MP diets for both oil sources. Regarding the effect of the oil source, RO diets showed a significantly higher DM ADC for the HP diets only.

Energy ADC ranged from 73.2% - 79.2%. Again, a significant interaction of protein level and oil source was observed and, hence, the simple main effects were tested (Figure 5.1b). For the FO diets MP had significantly higher energy ADC than HP and LP, whereas for RO the LP treatment showed a significantly lower energy ADC than HP and MP. Testing the differences between FO and RO at each level of protein showed a significant difference only in the HP group.

The protein ADC varied from 77.3% to 80.4% and only a significant effect of the protein level was shown by the two-way ANOVA; specifically, the increase of protein level resulted in increased protein ADC. As there were no interaction effects, the means are represented as two parallel lines in Figure 5.1c.

Lastly, there was a significant interaction of protein level and oil source for fat ADC (Figure 5.1d). For the FO treatments HP had a significantly lower fat ADC compared to MP and LP, while there were no differences in the RO groups. RO resulted in significantly higher fat ADC at all protein levels.

5.3.4 FA apparent digestibility

The digestibility of each individual FA for each dietary treatment and the effects and interactions of the protein level and oil source are shown in Table 5.5. In general, the ADC of the individual FA decreased with increasing FA chain length. For instance, the ADC of 14:0 varied from 80.0% to 95.0% while the ADC of 22:0 was as low as 30% (30.3% - 68.3%). On the other hand, increasing the number of double bonds increased the FA ADC. The FA with 20 carbons are given as an example: 20:0 varied between 56.2%-78.6%, 20:1n-9 from 92.7% - 97.2%, 20:4n-6 from 97.4% - 98.7% and 20:5n-3 from 97.7% - 99.1%.

For most of the saturated FA, ADC showed significant interactions between the protein level and oil source, hence, the simple main effects of the two factors were tested. For instance, for 16:0 (Figure 5.2a) HP had a significantly lower ADC compared to MP and LP for both oil sources, whereas RO resulted in significantly higher ADC at all protein levels. The total saturates ADC (Figure 5.2b) was significantly lower for the HP group compared to MP and LP for the FO treatments, while no significant differences were shown between the three protein levels for the RO treatments. Furthermore, RO showed a significantly higher total saturates ADC compared to FO, only for the HP groups.

Significant interactions were observed for the medium chain monoenes (16 and 18 carbon atoms) and the total monoenes. The simple main effects of the two factors for 16:1n-7, 18:1n-9 and total monoenes are shown in Figure 5.2 (graphs c, d and e, respectively). In general, the MP diet had a higher ADC for the FO treatments while no significant effect due to the protein level was shown for the RO treatments. Regarding the effects of the oil source, RO resulted in higher ADC at all protein levels, apart from the MP groups for 16:1n-7. The ADC of 20:1n-9, 22:1 and 24:1n-9 was significantly affected both by the protein level and the oil source. Specifically, RO showed significantly higher ADC compared to FO, and LP resulted in lower ADC.

Regarding the ADC of the n-6 and n-3 PUFA, the two factors had a significant effect only on the ADC of 18:2n-6, 18:3n-3 and 18:4n-3. Specifically, the ADC of these FA was significantly affected by both the protein level and the oil source; the MP groups had the highest ADC and the LP groups the lowest, and RO diets had higher ADC than FO, respectively. A significant interaction was shown for the ADC of the total n-6 PUFA; the simple main effects of total saturates ADC are shown in Figure 5.2f

and are very similar to the effects of total monoenes ADC. Only the protein level had a significant effect on EPA; the MP groups had the highest ADC and the LP groups the lowest. DHA was significantly affected only by the oil source resulting in higher ADC for the FO groups compared to the RO. Lastly, no significant effects were shown for the total n-3 PUFA ADC.

5.3.5 Whole body proximate composition

The initial proximate composition of the fish whole body was 63.9% DM, 17.2% crude protein, 16.2% crude fat and 1.8% ash (Table 5.6). At the end of the experimental period moisture varied from 59.3% to 61.3% and was significantly affected both by the protein level and the oil source. Specifically, the LP group had a lower moisture content than the other two treatments. Regarding the oil source the fish fed the FO diets had a higher moisture content compared to the fish fed the RO. Significant interactions between the two factors were shown for the protein content which ranged from 15.8 to 16.6%. The analysis of the simple main effects showed that for the FO groups it was decreased when the dietary protein level decreased while no differences were shown for the RO groups. For the HP groups FO resulted in higher protein content, for the MP there were no differences between FO and RO, while for the LP groups RO had the highest protein content. The fat content was significantly affected by the oil source with RO groups having a higher fat content than the FO groups (21.3% vs. 19.4%) but was not influenced by the dietary protein level. No significant effects were shown on the ash content of the whole body, which varied from 1.6% – 1.8%.

5.3.6 Tissue fatty acid compositions

The total lipid content and the fatty acid composition of muscle and liver from fish fed the six experimental diets for 10 weeks are shown in Table 5.7 and Table 5.8. The muscle total lipid varied from 138.0 to 156.5 mg lipid g^{-1} tissue. The liver lipid content was much lower, compared to that of muscle, ranging from 50.1 to 64.9 mg lipid g^{-1} tissue. Neither the dietary protein level nor the RO inclusion affected the muscle and liver lipid contents and no significant interactions were shown by two-way ANOVA.

Regarding the FA composition of muscle and liver, they were significantly affected by the RO but not by the protein level and no significant interactions between the two factors were shown. Specifically, the inclusion of RO resulted in a significant increase of 18:1n-9, total monoenes, 18:2n-6, 20:2n-6, total n-6 PUFA and 18:3n-3. On the other hand, all saturates, including total saturated FA, 16:1n-7, 22:1, AA, EPA, DHA, total n-3 FA and the n-3/n-6 ratio were significantly reduced when the fish were fed the diets containing RO.

Notably, in muscle EPA was reduced by half (10.4 vs. 5.2%, for FO vs. RO, respectively), while the reduction in DHA was more moderate (7.8 vs. 5.0% for FO vs. RO, respectively). 18:1n-9 increased from 20.9% to 35.1%, 18:2n-6 from 6.6% to 11.6% and 18:3n-3 from 2.0% to 4.6% for FO and RO groups, respectively. Similarly, in liver EPA was reduced from 16.0% to 9.9% and DHA from 16.2% to 13.2% for FO and RO groups, respectively. The increase between FO and RO groups for 18:1n-9, 18:2n-6 and 18:3n-3 was 13.8% vs. 27.9%, 1.9% vs. 7.6% and 0.5% v. 2.9%, respectively.

The differences (Δ) between diet and muscle fatty acid concentrations for the six experimental diets are shown in Table 5.9, where negative Δ values indicate lower values in muscle compared with diet, whereas positive values indicate accumulation in tissues relative to diet. Thus, the saturated FA, ARA, EPA, and the total n-3 PUFA were utilized to a higher extent by the fish fed the FO diets compared to the RO groups. DHA appeared to be slightly utilized in the FO groups but was accumulated in the muscle in the RO groups. On the contrary, 18:1n-9, 18:2n-6 and 18:3n-3 were found in higher concentrations in the muscle in the FO groups but were utilised in the RO groups. Likewise, the differences (Δ) between diet and liver fatty acid concentrations for the six experimental diets are shown in Table 5.10. In liver the 14:0 and 16:0 were utilized in all groups, although lower Δ values were found in the FO groups. Similarly to the muscle, 18:1n-9, 18:2n-6 and 18:3n-3 in liver were much more utilized in the RO groups compared to the FO ones. Lastly, 18:0, AA and DHA were accumulated in liver in all groups.

5.3.7 Peroxisomal β-oxidation capacity

The peroxisomal palmitoyl-CoA oxidation capacity in liver, red and white muscle is shown in Table 5.11. The peroxisomal β-oxidation capacity in liver ranged from 6.6 to 12.5 pmol/min/mg protein, in red muscle from 26.7 to 36.3 pmol/min/mg protein and in white muscle from 1.1 to 1.6 pmol/min/mg protein. In liver and red muscle β-oxidation capacity was significantly affected by the oil source ($P = 0.035$ and $P = 0.034$ for liver and red muscle, respectively). Specifically, RO inclusion resulted in significantly higher β-oxidation in both liver (7.2 vs. 9.7, for FO and RO, respectively) and red muscle (28.2 vs. 33.7, for FO and RO, respectively). However, in white muscle there was a significant interaction of the two factors (protein level and oil source) and

hence, the simple main effects of the two factors were tested, as demonstrated in Figure 5.3. Specifically, the HP group had a significantly higher β-oxidation capacity than MP and LP when FO was the oil source, whereas in contrast, the β-oxidation capacity in HP was lower than the other two groups when RO was included in the diet. Regarding the effects of the oil source on β-oxidation capacity at the three protein levels, the ranking was FO > RO for HP, RO > FO for MP while FO and RO did not significantly differ at LP.

Table 5.1. Feed formulations and proximate compositions (g kg^{-1}) of the experimental diets

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO
Components						
Fishmeal[a]	402	340	268	402	340	268
Oil seed and legume seed meals	181	190	190	181	190	190
Binder	135	130	190	135	130	190
Fish oil	304	330	351	122	132	141
Rapeseed oil[b]	0	0	0	182	198	211
Premixes[c]	9	10	11	9	10	11
Analyzed composition[d]						
Moisture	49	69	69	51	73	67
Dry Matter	951	931	931	949	927	933
Protein	353	338	291	345	328	296
Lipid	350	349	386	351	368	382
Ash	81	75	63	79	73	63
Gross Energy[e]	25.25	25.22	25.32	25.47	25.41	25.36
DP/DE[f]	15.4	14.0	12.3	13.7	13.0	12.3

[a] South-american, Anchoveta oil

[b] European, non-GM, double-low quality rapeseed oil

[c] Vitamin and mineral premixes prepared according to BioMar A/S commercial standards. Includes crystaline amino acids and Carophyl pink to provide 40mg/kg astaxanthin (DSM Roche, Basel, Switzerland)

[d] Wet weight

[e] kJ g^{-1}

[f] Digestible Protein/Digestible Energy ratio was calculated using the ADC values for energy and protein found in the present study and presented in the Results section

Table 5.2. Fatty acid compositions (% by weight of total fatty acids) of the experimental diets

Fatty Acid	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-R0
14:0	8.8	8.5	8.3	3.4	3.0	2.8
16:0	23.2	20.3	20.2	12.1	10.9	10.2
18:0	5.9	4.9	5.0	3.8	4.3	3.5
20:0	0.6	0.5	0.5	0.7	0.8	0.7
22:0	1.3	1.2	1.2	1.9	2.5	1.7
Total saturated[a]	40.4	35.9	35.7	22.0	21.7	19.1
16:1n-7	8.0	8.8	8.7	3.3	3.1	3.0
18:1n-9	9.5	10.9	11.3	37.7	37.7	40.0
18:1n-7	3.2	3.3	3.4	3.7	3.0	3.1
20:1n-9	1.5	1.4	1.3	1.6	1.5	1.5
22:1	1.7	1.7	1.5	1.3	1.1	0.9
24:1n-9	0.5	0.5	0.6	0.4	0.4	0.3
Total monoenes[b]	24.7	26.8	27.0	47.9	47.1	49.1
18:2n-6	2.1	2.4	2.7	12.5	13.0	13.8
20:2n-6	0.1	0.2	0.2	0.1	0.1	0.1
20:4n-6	1.1	1.1	1.1	0.4	0.5	0.4
22:5n-6	0.3	0.3	0.3	0.1	0.1	0.1
Total n-6 PUFA[c]	4.1	4.6	4.8	13.2	14.0	14.8
18:3n-3	0.6	0.7	0.8	6.2	6.3	6.7
18:4n-3	2.1	2.3	2.2	0.8	0.8	0.7
20:4n-3	0.6	0.7	0.6	0.2	0.2	0.2
20:5n-3	17.6	19.0	18.8	5.8	6.0	5.8
22:5n-3	1.9	2.0	2.0	0.7	0.7	0.6
22:6n-3	8.0	8.0	7.9	3.2	3.2	3.0
Total n-3 PUFA[d]	30.8	32.7	32.5	16.9	17.3	17.1
Total PUFA	34.9	37.3	37.3	30.1	31.3	31.9
(n-3) / (n-6)	7.5	7.1	6.8	1.3	1.2	1.2

[a]Includes 15:0

[b]Includes 16:1n-9 & 20:1n-7.

[c]Includes 18:3n-6, 20:3n-6 & 22:4n-6.

[d]Includes 20:3n-3 & 22:4n-3.

Table 5.3. Growth and performance of Atlantic salmon fed the six experimental diets for 10 weeks

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Start Weight, g	2031.7 ± 8.3	2097.0 ± 9.6	2031.7 ± 32.6	2065.3 ± 28.5	2055.7 ± 47.2	2038.3 ± 18.6			
End Weight, g	3340.2 ± 136.0	3491.1 ± 134.2	3352.9 ± 156.2	3591.8 ± 158.7	3664.2 ± 148.0	3405.7 ± 92.1	0.085	0.032	0.483
FCR[1]	1.07 ± 0.06	1.10 ± 0.09	1.06 ± 0.05	0.99 ± 0.05	1.02 ± 0.05	1.09 ± 0.11	0.587	0.262	0.376
SGR[2]	0.86 ± 0.07	0.88 ± 0.07	0.86 ± 0.08	0.95 ± 0.05	0.99 ± 0.03	0.88 ± 0.06	0.262	0.025	0.422
TGC[3]	3.41 ± 0.29	3.54 ± 0.32	3.44 ± 0.34	3.85 ± 0.25	4.04 ± 0.17	3.53 ± 0.26	0.202	0.021	0.414
PPV[4a]	0.40 ± 0.03	0.38 ± 0.05	0.44 ± 0.02	0.43 ± 0.01	0.44 ± 0.02	0.47 ± 0.06	0.113	0.040	0.865
PPV[4b]	0.40 ± 0.03	0.41 ± 0.01	0.44 ± 0.02	0.43 ± 0.01	0.44 ± 0.02	0.51 ± 0.06	0.003	0.003	0.294

All values are mean ± S.D. (n=3)

[1] Feed Conversion Ratio; [2] Specific Growth Rate; [3] Thermal Growth Coefficient; [4] Protein Productive Value ([4a] Including outliers, [4b] Excluding Outliers)

Table 5.4. Apparent nutrient and energy digestibility (%) in Atlantic salmon fed the six experimental diets for 10 weeks

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Dry Matter	56.4 ± 0.6	56.9 ± 0.3	53.8 ± 1.2	60.1 ± 0.5	57.7 ± 1.8	50.5 ± 4.2	0.000	0.692	0.028
Protein	80.4 ± 0.2	79.7 ± 0.2	78.2 ± 1.1	79.8 ± 0.6	78.9 ± 1.7	77.3 ± 1.8	0.002	0.117	0.946
Lipid	82.3 ± 2.0	89.1 ± 0.7	87.0 ± 1.7	92.3 ± 0.7	92.4 ± 0.9	92.0 ± 1.0	0.005	0.000	0.003
Gross Energy	73.2 ± 0.3	76.3 ± 0.2	73.2 ± 1.5	79.2 ± 0.2	78.3 ± 1.3	73.2 ± 1.5	0.001	0.001	0.011

All values are mean ± S.D. (n=3).

Table 5.5. Apparent fatty acid digestibility (%) in Atlantic salmon fed the six experimental diets for ten weeks

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
14:0	80.0 ± 1.5	88.9 ± 0.4	88.4 ± 0.8	90.6 ± 1.9	95.0 ± 0.3	93.6 ± 0.9	0.000	0.000	0.003
16:0	67.1 ± 3.5	82.4 ± 0.8	80.5 ± 1.3	85.5 ± 3.3	91.2 ± 0.1	88.0 ± 1.8	0.000	0.000	0.002
18:0	49.0 ± 6.4	62.1 ± 1.8	60.3 ± 4.2	68.6 ± 12.8	55.1 ± 4.3	48.7 ± 8.8	0.520	0.931	0.006
20:0	56.2 ± 5.8	59.4 ± 1.7	58.0 ± 4.7	78.6 ± 9.7	63.5 ± 3.3	59.3 ± 8.1	0.080	0.008	0.024
22:0	36.2 ± 10.1	32.5 ± 4.8	30.3 ± 8.5	68.3 ± 18.3	44.1 ± 4.6	30.4 ± 15.3	0.019	0.019	0.087
Total saturated[1]	66.2 ± 3.7	79.2 ± 1.0	77.5 ± 1.8	81.8 ± 6.1	78.3 ± 1.4	75.5 ± 4.3	0.070	0.023	0.001
16:1n-7	95.6 ± 0.4	97.7 ± 0.1	95.9 ± 0.8	98.0 ± 0.6	98.0 ± 0.0	97.2 ± 1.2	0.009	0.001	0.043
18:1n-9	95.2 ± 0.1	97.3 ± 0.4	95.3 ± 0.9	98.8 ± 0.4	98.6 ± 0.1	97.7 ± 1.1	0.006	0.000	0.029
18:1n-7	93.4 ± 0.5	95.7 ± 0.0	93.7 ± 0.8	98.1 ± 0.5	97.4 ± 0.3	96.7 ± 1.1	0.011	0.000	0.006
20:1n-9	93.6 ± 0.2	95.2 ± 0.6	92.7 ± 1.1	97.2 ± 0.8	97.1 ± 0.2	95.9 ± 1.7	0.016	0.000	0.319
22:1	92.7 ± 0.5	94.4 ± 0.4	91.6 ± 1.0	95.9 ± 1.1	96.2 ± 0.4	93.9 ± 1.6	0.002	0.000	0.453
24:1n-9	82.1 ± 1.9	87.1 ± 2.9	85.4 ± 0.9	90.9 ± 1.6	92.7 ± 0.7	88.8 ± 3.5	0.039	0.000	0.135
Total monoenes[2]	94.5 ± 0.3	96.7 ± 0.3	94.6 ± 0.9	98.5 ± 0.4	98.3 ± 0.1	97.4 ± 1.2	0.005	0.000	0.022
18:2n-6	95.2 ± 0.3	96.8 ± 0.7	94.8 ± 1.0	98.8 ± 0.4	98.7 ± 0.1	98.1 ± 0.7	0.012	0.000	0.062
20:2n-6	97.0 ± 2.6	99.1 ± 1.6	97.5 ± 2.2	95.9 ± 4.9	98.4 ± 1.4	94.0 ± 4.7	0.273	0.277	0.736
20:4n-6	97.8 ± 0.6	98.7 ± 0.4	97.4 ± 0.8	97.9 ± 0.2	98.1 ± 0.3	97.9 ± 0.8	0.082	0.975	0.180
22:5n-6	97.7 ± 2.0	99.2 ± 1.4	96.5 ± 1.0	98.3 ± 3.0	99.1 ± 1.5	98.7 ± 2.3	0.384	0.350	0.607
Total n-6 PUFA[3]	95.2 ± 0.5	97.2 ± 0.7	95.7 ± 0.7	98.7 ± 0.3	98.5 ± 0.1	98.0 ± 0.8	0.021	0.000	0.023
18:3n-3	97.1 ± 0.3	98.4 ± 0.4	96.9 ± 0.8	99.3 ± 0.3	99.2 ± 0.0	98.7 ± 0.6	0.015	0.000	0.081
18:4n-3	98.2 ± 0.3	99.1 ± 0.1	97.8 ± 0.7	99.1 ± 0.3	99.1 ± 0.1	98.8 ± 0.4	0.013	0.003	0.082
20:4n-3	98.6 ± 1.3	98.9 ± 0.2	97.5 ± 0.7	98.8 ± 1.1	99.5 ± 0.9	98.7 ± 1.2	0.166	0.159	0.702
20:5n-3	98.3 ± 0.5	99.1 ± 0.2	97.7 ± 0.8	98.9 ± 0.3	98.7 ± 0.2	98.6 ± 0.5	0.045	0.091	0.087
22:5n-3	97.7 ± 0.7	98.4 ± 0.3	97.0 ± 0.9	97.9 ± 0.1	97.7 ± 0.1	97.3 ± 0.9	0.058	0.826	0.310
22:6n-3	96.0 ± 1.2	97.1 ± 0.6	95.5 ± 1.0	95.7 ± 1.0	94.4 ± 0.7	94.4 ± 1.8	0.391	0.025	0.207
Total n-3 PUFA[4]	97.6 ± 0.7	98.5 ± 0.3	97.1 ± 0.8	98.4 ± 0.4	98.0 ± 0.2	97.9 ± 0.8	0.098	0.230	0.160
Total PUFA	97.3 ± 0.7	98.3 ± 0.3	96.9 ± 0.8	98.5 ± 0.3	98.3 ± 0.1	97.9 ± 0.8	0.064	0.024	0.168

All values are mean ± S.D. (n=3).

[1]Includes 15:0; [2]Includes 16:1n-9 & 20:1n-7; [3]Includes 18:3n-6, 20:3n-6 & 22:4n-6; [4]Includes 20:3n-3 & 22:4n-3

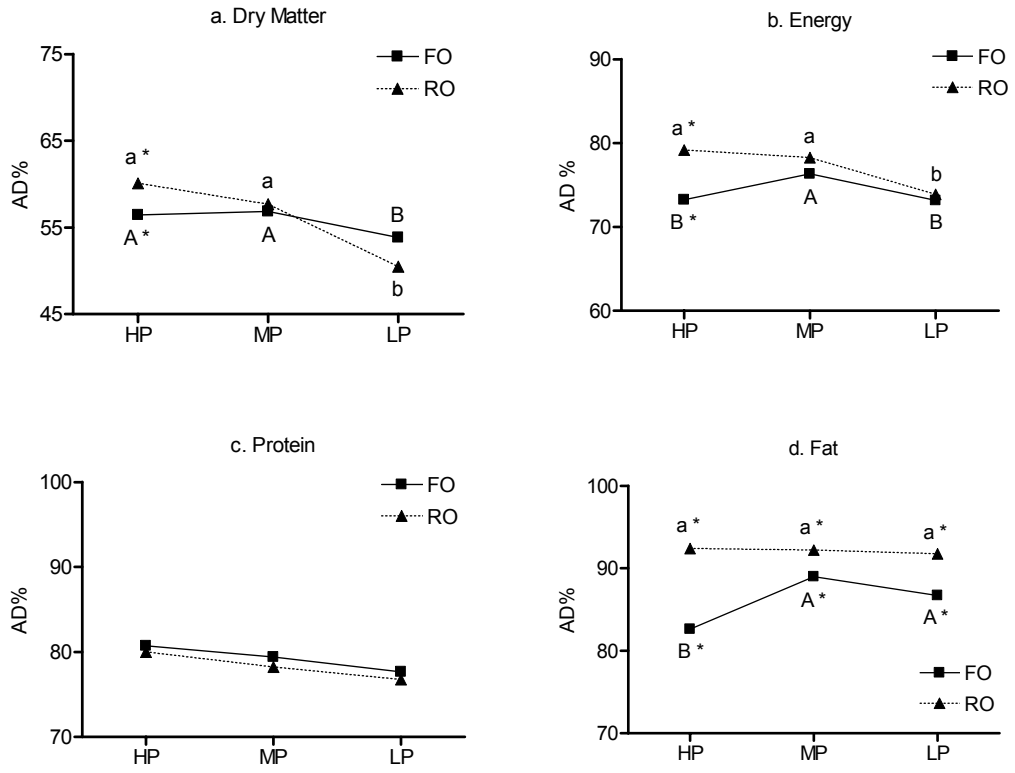

Figure 5.1. Means of the ADC of DM (a), Energy (b), protein (c) and fat (d), for the six experimental diets, in a two-way ANOVA, showing the effects of the two factors and their interaction.

For each oil source, values denoted with different letters are significantly different; uppercase or lowercase letters correspond to FO or RO, respectively. Within each protein level the significant differences between FO and RO values are marked with an asterisk.

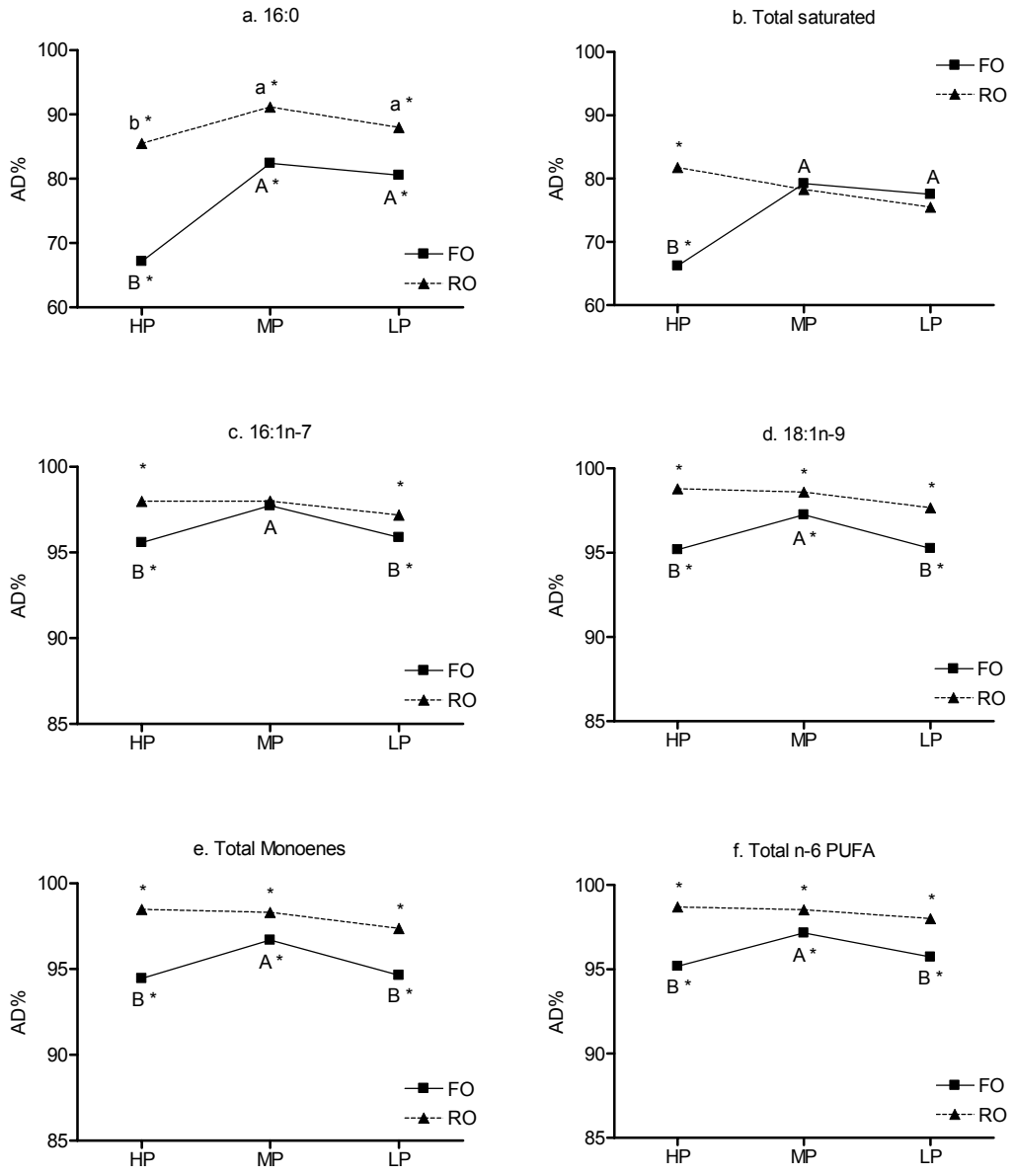

Figure 5.2. Means of the ADC of individual fatty acids, 16:0 (a), total saturated (b), 16:1n-7 (c), 18:1n-9 (d) total monoenes (e), and total n-6 PUFA (e), of the six experimental diets, in a two-way ANOVA, showing the effects of the two factors and their interaction.

For each oil source, values denoted with different letters are significantly different; uppercase or lowercase letters correspond to FO or RO, respectively. Within each protein level the significant differences between FO and RO values are marked with an asterisk.

Table 5.6. Proximate composition (% of wet weight) of whole body from Atlantic salmon fed the experimental diets for 10 weeks

	Start [1]	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
								protein	oil	prot x oil
Moisture	*63.9 ± 0.9*	61.3 ± 0.5	61.4 ± 0.4	60.8 ± 0.3	60.6 ± 1.0	60.7 ± 0.7	59.3 ± 0.5	0.018	0.004	0.468
Protein	*17.2 ± 0.3*	16.6 ± 0.2	16.1 ± 0.3	15.8 ± 0.2	16.2 ± 0.2	16.1 ± 0.3	16.4 ± 0.1	0.092	0.682	0.004
Lipid	*16.2 ± 1.1*	19.1 ± 1.0	19.7 ± 0.1	19.4 ± 0.1	21.5 ± 0.9	20.9 ± 0.7	21.6 ± 0.7	0.808	0.000	0.274
Ash	*1.8 ± 0.2*	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	0.093	0.542	0.428

Table 5.7. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of muscle from Atlantic salmon fed the experimental diets for 10 weeks

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Total Lipid	142.1 ± 14.2	142.2 ± 24.5	138.0 ± 9.7	147.5 ± 13.3	156.5 ± 14.1	143.8 ± 3.8	0.621	0.242	0.843
Fatty acid									
14:0	5.8 ± 1.3	5.6 ± 0.4	5.7 ± 0.3	3.4 ± 0.1	3.2 ± 0.1	3.4 ± 0.3	0.766	0.000	0.988
16:0	17.4 ± 2.9	16.3 ± 1.3	17.0 ± 1.5	12.4 ± 0.4	11.9 ± 0.2	12.3 ± 0.7	0.677	0.000	0.941
18:0	4.0 0.7	3.7 ± 0.4	3.8 ± 0.4	3.1 ± 0.1	3.1 ± 0.0	3.1 ± 0.2	0.695	0.001	0.903
Total saturated[1]	28.6 ± 4.8	26.9 ± 2.7	28.3 ± 3.0	20.5 ± 1.6	19.7 ± 1.1	20.5 ± 2.1	0.705	0.000	0.957
16:1n-7	6.9 ± 0.4	7.3 ± 0.2	7.1 ± 0.3	4.3 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	0.977	0.000	0.106
18:1n-9	21.3 ± 0.6	21.3 ± 0.9	20.2 ± 1.1	33.7 ± 1.5	36.3 ± 1.4	35.3 ± 0.7	0.131	0.000	0.090
18:1n-7	3.6 ± 0.1	3.8 ± 0.3	3.8 ± 0.3	3.4 ± 0.4	3.0 ± 0.2	3.4 ± 0.1	0.427	0.004	0.214
20:1n-9	2.7 ± 0.1	2.8 ± 0.0	2.5 ± 0.2	2.9 ± 0.2	3.0 ± 0.1	2.8 ± 0.1	0.033	0.004	0.632
22:1	2.5 ± 0.2	2.5 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	0.147	0.001	0.489
24:1n-9	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.2	0.571	0.425	0.216
Total monoenes[2]	37.7 ± 0.4	38.6 ± 0.6	36.7 ± 1.7	47.1 ± 1.9	49.0 ± 1.5	48.4 ± 1.0	0.190	0.000	0.336
18:2n-6	6.8 ± 0.6	6.5 ± 0.4	6.6 ± 0.0	11.3 ± 0.1	11.7 ± 0.2	11.8 ± 0.4	0.862	0.000	0.177
20:2n-6	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.194	0.000	0.484
20:3n-6	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.224	0.000	0.308
20:4n-6	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.875	0.000	0.476
Total n-6 PUFA[3]	8.5 ± 0.8	8.4 ± 0.3	8.3 ± 0.1	12.8 ± 0.2	13.2 ± 0.2	13.3 ± 0.5	0.797	0.000	0.379
18:3n-3	2.1 ± 0.3	2.0 ± 0.2	2.0 ± 0.0	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.3	0.924	0.000	0.637
18:4n-3	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.855	0.000	0.475
20:4n-3	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.746	0.000	0.419
20:5n-3	9.9 ± 1.6	10.6 ± 1.2	10.7 ± 0.9	5.7 ± 0.3	5.0 ± 0.1	5.0 ± 0.6	0.996	0.000	0.338
22:5n-3	3.1 ± 0.5	3.3 ± 0.2	3.5 ± 0.2	2.1 ± 0.2	1.8 ± 0.1	1.8 ± 0.2	0.924	0.000	0.184
22:6n-3	7.6 ± 1.5	7.7 ± 1.0	8.0 ± 0.6	5.4 ± 0.5	4.8 ± 0.4	4.7 ± 0.8	0.924	0.000	0.574
Total n-3 PUFA[4]	25.2 ± 3.8	26.1 ± 2.7	26.7 ± 1.9	19.6 ± 1.2	18.1 ± 0.6	17.8 ± 1.9	0.981	0.000	0.451
Total PUFA	33.7 ± 4.4	34.5 ± 2.9	35.0 ± 2.0	32.4 ± 1.4	31.3 ± 0.7	31.1 ± 2.4	0.996	0.039	0.680
(n-3) / (n-6)	2.9 ± 0.3	3.1 ± 0.2	3.2 ± 0.2	1.5 ± 0.1	1.4 ± 0.0	1.3 ± 0.1	0.761	0.000	0.049

Values are mean ± S.D. (n=3).

[1]Includes 15:0, 20:0, 22:0; [2]Includes 16:1n-9 & 20:1n-7; [3]Includes 18:3n-6, 20:3n-6 & 22:4n-6; [4]Includes 20:3n-3 & 22:4n-3

Table 5.8. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of liver from Atlantic salmon fed the experimental diets for 10 weeks

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Total Lipid	61.4 ± 5.1	64.9 ± 7.1	53.8 ± 8.1	60.7 ± 3.3	54.9 ± 11.0	50.1 ± 5.8	0.094	0.182	0.535
Fatty acid									
14:0	2.7 ± 0.8	2.8 ± 0.7	2.7 ± 0.7	1.5 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	0.997	0.000	0.907
16:0	14.2 ± 1.1	14.3 ± 1.8	15.6 ± 1.0	10.5 ± 0.5	11.5 ± 1.3	12.0 ± 1.1	0.144	0.000	0.759
18:0	7.9 ± 0.3	7.6 ± 1.0	7.7 ± 0.9	5.2 ± 0.2	5.6 ± 0.6	5.3 ± 0.5	0.955	0.000	0.705
Total saturated[1]	25.5 ± 1.7	25.9 ± 2.6	26.9 ± 2.2	18.2 ± 0.8	19.6 ± 2.0	20.2 ± 1.6	0.355	0.000	0.899
16:1n-7	4.6 ± 0.6	4.9 ± 0.6	4.4 ± 0.7	2.4 ± 0.0	1.9 ± 0.2	2.0 ± 0.2	0.467	0.000	0.269
18:1n-9	14.8 ± 1.0	14.5 ± 1.4	12.0 ± 1.4	29.8 ± 2.6	26.9 ± 4.5	27.1 ± 4.0	0.038	0.000	0.106
18:1n-7	4.3 ± 0.1	4.4 ± 0.3	3.9 ± 0.2	3.3 ± 0.3	2.7 ± 0.4	3.0 ± 0.2	0.148	0.000	0.103
20:1n-9	2.2 ± 0.1	2.1 ± 0.2	1.6 ± 0.2	3.8 ± 0.2	3.6 ± 0.3	3.1 ± 0.3	0.001	0.000	0.776
22:1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.033	0.000	0.442
24:1n-9	0.6 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.834	0.164	0.739
Total monoenes[2]	27.7 ± 1.3	27.7 ± 2.4	23.5 ± 2.4	40.7 ± 2.8	36.3 ± 5.1	36.3 ± 4.5	0.125	0.000	0.467
18:2n-6	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	7.6 ± 0.3	7.2 ± 0.7	8.2 ± 0.7	0.298	0.000	0.355
20:2n-6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	1.7 ± 0.0	1.9 ± 0.1	1.7 ± 0.2	0.203	0.000	0.299
20:3n-6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.954	0.082	0.598
20:4n-6	2.7 ± 0.2	2.4 ± 0.3	3.0 ± 0.3	1.7 ± 0.3	2.0 ± 0.5	2.1 ± 0.5	0.196	0.001	0.329
Total n-6 PUFA[3]	5.9 ± 0.3	5.7 ± 0.1	6.3 ± 0.1	11.8 ± 0.0	11.8 ± 0.1	12.6 ± 0.3	0.000	0.000	0.162
18:3n-3	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	2.9 ± 0.1	2.7 ± 0.4	3.1 ± 0.3	0.391	0.000	0.352
18:4n-3	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.614	0.000	0.651
20:4n-3	1.3 ± 0.1	1.5 ± 0.2	1.3 ± 0.2	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.897	0.000	0.132
20:5n-3	15.3 ± 1.1	15.6 ± 0.2	17.2 ± 0.9	9.1 ± 0.9	10.5 ± 1.3	10.1 ± 1.1	0.068	0.000	0.236
22:5n-3	6.9 ± 0.4	7.7 ± 1.1	6.6 ± 0.6	3.3 ± 0.1	3.2 ± 0.2	3.0 ± 0.1	0.033	0.000	0.364
22:6n-3	16.3 ± 1.1	15.0 ± 1.2	17.2 ± 1.5	12.3 ± 1.3	14.2 ± 2.2	13.0 ± 2.4	0.738	0.003	0.199
Total n-3 PUFA[4]	40.9 ± 1.6	40.8 ± 0.3	43.3 ± 0.2	29.3 ± 2.1	32.3 ± 3.2	30.9 ± 3.2	0.120	0.000	0.114
Total PUFA	46.8 ± 1.8	46.5 ± 0.2	49.6 ± 0.2	41.1 ± 2.0	44.1 ± 3.1	43.5 ± 3.2	0.141	0.000	0.293
(n-3) / (n-6)	7.0 ± 0.4	7.2 ± 0.1	6.9 ± 0.1	2.5 ± 0.2	2.7 ± 0.3	2.5 ± 0.3	0.155	0.000	0.967

Values are mean ± S.D. (n=3).

[1]Includes 15:0, 20:0, 22:0; [2]Includes 16:1n-9 & 20:1n-7; [3]Includes 18:3n-6, 20:3n-6 & 22:4n-6; [4]Includes 20:3n-3 & 22:4n-3

Table 5.9. Differences (Δ)[1] between diet and muscle fatty acid concentrations[2] for the six experimental treatments

Fatty Acid	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO
14:0	-3.0	-2.9	-2.6	0.0	0.1	0.6
16:0	-5.8	-3.9	-3.2	0.3	1.0	2.1
18:0	-1.9	-1.3	-1.2	-0.6	-1.2	-0.4
Total saturated[3]	-11.8	-9.0	-7.4	-1.4	-1.9	1.4
16:1n-7	-1.1	-1.5	-1.6	1.0	0.9	1.2
18:1n-9	11.7	10.4	8.9	-4.0	-1.4	-4.7
Total monoenes[4]	13.0	11.7	9.7	-0.8	1.9	-0.7
18:2n-6	4.7	4.1	3.9	-1.2	-1.2	-2.0
20:4n-6	-0.4	-0.4	-0.4	0.0	-0.1	0.0
Total n-6 PUFA[5]	4.4	3.8	3.5	-0.4	-0.8	-1.5
18:3n-3	1.5	1.3	1.2	-1.6	-1.6	-2.1
20:5n-3	-7.7	-8.4	-8.1	-0.1	-1.0	-0.8
22:6n-3	-0.4	-0.2	0.1	2.2	1.6	1.8
Total n-3 PUFA[6]	-5.6	-6.6	-5.8	2.7	0.8	0.7

[1] Negative Δ values indicate lower values in muscle compared with diet, whereas positive values indicate accumulation in muscle relative to diet.

[2] FA concentrations are % by weight of total fatty acids in diet and muscle

[3]Includes 15:0, 20:0, 22:0

[4]Includes 16:1n-9, 18:1n-7, 20:1n-9, 20:1n-7,22:1 & 24:1n-9

[5]Includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 & 22:5n-6

[6]Includes 18:4n-3, 20:3n-3, 20:4n-3, 22:4n-3 & 22:5n-3

Table 5.10. Differences (Δ)[1] between diet and liver fatty acid concentrations[2] for the six experimental treatments

Fatty Acid	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO
14:0	-6.2	-5.7	-5.6	-1.9	-1.7	-1.4
16:0	-9.0	-6.0	-4.6	-1.6	0.6	1.7
18:0	2.0	2.7	2.8	1.5	1.3	1.7
Total saturated[3]	-14.9	-10.0	-8.8	-3.7	-2.0	1.1
16:1n-7	-4.1	-3.9	-4.3	-1.1	-1.5	-1.3
18:1n-9	5.3	3.6	0.7	-7.9	-10.8	-13.0
Total monoenes[4]	3.0	0.8	-3.5	-7.2	-10.8	-12.8
18:2n-6	-0.3	-0.5	-0.7	-4.9	-5.8	-5.7
20:4n-6	1.6	1.3	1.9	1.3	1.6	1.7
Total n-6 PUFA[5]	1.8	1.1	1.5	-1.5	-2.2	-2.2
18:3n-3	-0.1	-0.2	-0.3	-3.3	-3.5	-3.6
20:5n-3	-2.2	-3.4	-1.7	3.3	4.5	4.3
22:6n-3	8.3	7.0	9.3	9.1	10.9	10.1
Total n-3 PUFA[6]	10.1	8.1	10.9	12.4	15.0	13.8

[1] Negative Δ values indicate lower values in liver compared with diet, whereas positive values indicate accumulation in liver relative to diet.

[2] FA concentrations are % by weight of total fatty acids in diet and muscle

[3]Includes 15:0, 20:0, 22:0

[4]Includes 16:1n-9, 18:1n-7, 20:1n-9, 20:1n-7,22:1 & 24:1n-9

[5]Includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6 & 22:5n-6

[6]Includes 18:4n-3, 20:3n-3, 20:4n-3, 22:4n-3 & 22:5n-3

Table 5.11. Peroxisomal β-oxidation capacity (pmol/min/mg protein) of liver, red and white muscle from Atlantic salmon fed the experimental diets for 10 weeks

	HP-FO	MP-FO	LP-FO	HP-RO	MP-RO	LP-RO	TWO WAY ANOVA *P*		
							protein	oil	prot x oil
Liver	7.2 ± 2.6	6.6 ± 0.9	7.7 ± 1.8	7.8 ± 1.1	8.7 ± 3.7	12.5 ± 1.9	0.121	0.035	0.288
Red Muscle	30.0 ± 2.6	28.0 ± 2.6	26.7 ± 4.6	36.3 ± 8.2	31.2 ± 4.4	33.6 ± 4.5	0.430	0.034	0.796
White Muscle	1.6 ± 0.1	1.2 ± 0.1	1.3 ± 0.0	1.1 ± 0.1	1.6 ± 0.0	1.4 ± 0.2	0.908	0.719	0.000

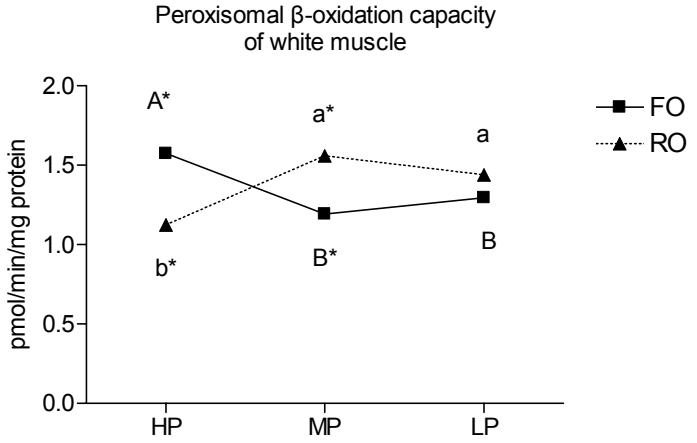

Figure 5.3. Means of the peroxisomal β-oxidation capacity (pmol/min/mg protein) of white muscle from Atlantic salmon fed the six experimental diets, in a two-way ANOVA, showing the effects of the two factors and their interaction.

For each oil source, values denoted with different letters are significantly different; uppercase or lowercase letters correspond to FO or RO, respectively. Within each protein level the significant differences between FO and RO values are marked with an asterisk.

5.4 Discussion

The formulation of aqua feeds with lower protein content, as well as with sustainable ingredients replacing FM and FO, is rising. Hence, the aim of the present study was to elucidate the effects and interactions of various dietary protein/lipid levels and different oil sources (namely FO vs. RO) on the growth, whole body chemical composition and the digestibility of nutrients, including FA, in tissues of Atlantic salmon. Moreover, the changes in FA composition of tissues and the retention of individual FA in specific tissues, as a result of the different dietary treatments were also investigated. In addition, the hypothesis that protein sparing and altered FA catabolism via β-oxidation could occur due to the effect of either of the two factors (protein level and / or oil source) was also tested.

5.4.1 Growth performance

Previous studies have suggested that Atlantic salmon can grow efficiently when fed diets with high dietary fat and low protein content (Einen and Roem, 1997; Hillestad, et al., 1998; Bendiksen, et al., 2003b; Azevedo, et al., 2004b, 2004a; Solberg, 2004). The results of the present study are in agreement with these studies, as no negative effects on growth and FCR occurred when the fish were fed with low protein / high lipid diets. However, most of the previous studies used diets with higher dietary protein and lower lipid contents than the present study. For example, Einen and Roem (1997) used diets containing CP / CL of 425 / 346 g kg^{-1}, which corresponded to a DP / DE ratio of 16.4, with no negative effects on growth and FCR. In the present study the dietary CP/ CL ratio was much lower, ranging from 350/350 to 290/380 g kg^{-1}, or in terms of DP/DE it varied from 15.4 to 12.3. Therefore, it is noteworthy that the protein

level had no significant negative effects on the final weight of the fish, SGR, TGC and FCR, while performance was very good for all treatments, even at protein levels below 300 g kg^{-1}. This suggests that fish can efficiently utilize high amounts of lipid for energy and tolerate relatively low dietary protein levels, which could be of great significance for the Atlantic salmon industry. Such diets could be both more environmentally friendly and economically advantageous compared to "traditional" diets, which contain higher protein levels, without compromising the growth of the fish.

In addition, a significant difference between the dietary treatments was shown on growth due to oil source. Specifically, the replacement of 60% of the dietary FO by RO resulted in higher final weight, SGR and TGC, but not FCR. Numerous earlier studies on Atlantic salmon have concluded that the replacement of FO with RO or other VO has no negative effect on growth and FCR (Bell, et al., 2001a; Rosenlund, et al., 2001; Caballero, et al., 2002; Bell, et al., 2003a; Bell, et al., 2003b; Torstensen, et al., 2004a; Torstensen, et al., 2005). Nevertheless, the positive effect of RO on growth of Atlantic salmon has also been shown previously (Bendiksen, et al., 2003b), including the results of the previous chapter, and a higher growth for larger Atlantic salmon fed with diets with a low level of n-3 FA has also been reported (Menoyo, et al., 2003; Torstensen, et al., 2005). In these previous studies the authors suggested that the increased growth was due to higher digestibility of the RO, and other VO, FA, resulting in better utilization of the dietary oil for energy by the fish. The digestibility results of the present study, discussed below, confirm that theory.

The protein sparing effect could also have been enhanced by both the higher dietary oil levels and the RO inclusion. This is supported by the PPV results, as the overall PPV was significantly improved when the fish were fed the LP diets, although

that is not clear due to statistical ambiguity. Previous studies have also suggested a positive effect of increased dietary lipid content on protein retention and, hence, on protein sparing (Einen and Roem, 1997; Hillestad, et al., 1998; Bendiksen, et al., 2003b). Moreover, the results of the present study indicate undoubtedly a positive effect on PPV due to the dietary inclusion of RO at the expense of FO. The potential effects of dietary VO in protein sparing in fish are largely unknown, however the results of the present study are supported by the increased β-oxidation capacity that was shown in tissues of Atlantic salmon fed with RO diets compared to the FO ones as increased catabolism of FA for energy production may suggest a protein sparing effect. The β-oxidation results are discussed further below.

5.4.2 Nutrient and fatty acid digestibility

The protein ADC was significantly affected by the dietary protein/lipid level. Specifically, the decrease in dietary protein level resulted in lower protein ADC. The results of previous studies are contradictory; Einen and Roem (1997) and Azevedo et al. (2004b) reported no significant differences in protein ADC between diets with varying DP/DE ratios, whereas Bendiksen et al. (2003b) found that protein ADC was decreased when low protein / high fat diets were used. The latter also reported a positive effect of the VO on protein ADC, although such an effect was not shown in the present trial.

Nevertheless, lipid ADC was significantly higher for the RO treatments, compared to the FO, at all dietary protein levels. The lipid digestibility has been correlated to the dietary FA composition and the positive effect of the VO and especially RO has been reported previously (Caballero, et al., 2002; Bendiksen, et al., 2003b; Menoyo, et al., 2003; Ng, et al., 2004). In agreement with previous studies (Einen and Roem, 1997; Azevedo, et al., 2004b) the dietary protein level did not affect

the lipid ADC, at least for the RO diets. However, in the FO treatments the lipid ADC of the HP diet was significantly lower compared to the MP and LP. This was probably due to the higher saturated FA content of this diet, the digestibility of which has been negatively correlated to their dietary level (Caballero, et al., 2002) and is also confirmed in the present study.

The energy ADC was affected by both the dietary protein level and the oil source and significant interactions were shown between the two factors. In line with previous studies (Azevedo, et al., 2004b), the energy ADC of the LP diets was lower for both oil sources, probably because of the respective lower protein ADC. Moreover, the lower lipid ADC of the HP-FO diet apparently reflected on its energy ADC. Lastly, RO inclusion resulted in higher energy ADC only for the HP diets, which could be due to the large difference of the two diets lipid ADC and more specifically in the differences found in the ADC of individual FA, such as 16:0 and 18:1n-9, which are preferentially utilized for energy production by Atlantic salmon (Henderson and Sargent, 1985). In contrast to these results, Ng et al. (2004) found no significant differences in the energy ADC in Atlantic salmon fed diets containing different amounts of palm oil; however, the diets used in that trial did not significantly differ in their levels of 16:0, 18:1n-9 or, generally, in total saturates and monoenes.

Previous studies have reported on the individual FA ADC in diets of salmonids (Torstensen, et al., 2000; Caballero, et al., 2002; Menoyo, et al., 2003; Ng, et al., 2004). In general, they suggested that the digestibility of FA decreases with increasing chain length and saturation. This is in agreement with the results of the present study. For instance, a decrease in ADC of almost 50% was observed for the saturated FA as their chain length increased, while the order of the ADC of FA in terms of their degree of

saturation, was increasing such that 16:0 < 18:1n-9 < 18:2n-6 < 18:3n-3 < 20:5n-3. Moreover, the digestibility of saturated FA, and to a lesser extent of unsaturated FA and especially monoenes, has been negatively correlated largely with their dietary level, but also with dietary n-3 PUFA levels (Torstensen, et al., 2000; Caballero, et al., 2002; Menoyo, et al., 2003; Ng, et al., 2004). These authors suggested that at high levels of saturated FA inclusion, emulsion or micelle formation is impaired, although low levels are easily digested. In the current study, the ADC of 14:0 and 16:0 was significantly higher for the RO treatments compared to FO ones, which may be explained by the level of saturated FA in the RO diets being approximately half that in the FO diets. However, for the higher chain length saturated FA the difference between the oil sources was significant only for the HP treatments. Consistent with the previous studies the digestibility of the monounsaturated FA was also affected by the oil source. In most cases RO diets had significantly higher total monoenes ADC than the FO ones, at all protein levels, suggesting a negative correlation between the dietary saturated FA content and the ADC of the monoenes. The protein level had no significant effect in the RO treatments but resulted in higher monoenes ADC for the MP group of the FO diets. Taking into account that saturated FA and monoenes, largely 16:0 and 18:1n-9, are preferred substrates for β-oxidation the significant increase in their ADC due to RO inclusion apparently influenced positively the energy utilization and consequently enhanced the growth of the fish. It should be noted that RO diets had a much higher (approximately 4-fold) content of 18:1n-9 compared to the FO diets.

The n-6 and n-3 PUFA ADC were affected to a much smaller extent by the dietary treatments compared to the monoenes and saturated FA. The ADC of 18:2n-6, 18:3n-3 and 18:4n-3 were affected by both the protein and the oil source resulting in higher digestibility for the RO diets, while it was lower for the LP groups. Although,

the rest of the n-6 PUFA were not affected by any of the factors, significant interactions were shown for total n-6 PUFA, similar to the monoenes. Again, this is probably because of the saturated FA levels in the diets influencing the digestibility of PUFA (Menoyo, et al., 2003). Lastly, ADC for EPA and DHA appeared more stable than most of the other FA with the former being affected by the protein level (EPA ADC order was MP ≥ HP ≥ LP), while DHA was significantly higher for the FO diets. It should be pointed out that DHA was the only FA for which the ADC was positively affected by the FO content of the diet. These results are in line with previous studies, as EPA and DHA ADC have been shown to be either higher for the FO diets or not affected by dietary VO inclusion (Torstensen, et al., 2000; Caballero, et al., 2002; Ng, et al., 2004). Menoyo et al. (2003) reported that EPA ADC was affected by the dietary saturated FA, while DHA ADC was affected by both the saturated FA and n-3 PUFA dietary levels.

5.4.3 Whole body proximate composition

Although the chemical composition of the fish body is influenced by the dietary composition this effect is in many cases only to a small extent. Previous studies, using either isoenergetic diets (Azevedo, et al., 2004b, 2004a; Solberg, 2004) or diets with a respective increase in the energy content (Hillestad and Johnsen, 1994; Einen and Roem, 1997; Einen and Skrede, 1998; Bendiksen, et al., 2003b), have shown that increasing dietary oil levels results in increased lipid and usually DM content, in the fish body, while the protein content is decreased or in some cases not affected and the ash is largely unaffected. However, in the previous trial (Chapter 4) it was shown that the dietary protein/lipid ratio did not affect the whole body chemical composition. In the present study, the DM content was significantly affected by the protein level, resulting in higher DM content for the LP groups, whereas there was no significant

effect on the lipid and ash of the carcass. However, a significant interaction was shown for the whole body protein, suggesting a decrease with decreasing dietary protein levels.

In agreement with previous studies, the present study demonstrated that the replacement of FO with RO in the diets of Atlantic salmon affected the whole body chemical composition (Bell, et al., 2001a; Torstensen, et al., 2004b; Ruyter, et al., 2006), although no effects on body composition due to use of VO have also been reported (Bendiksen, et al., 2003b), including the results of the previous trial. Specifically, in this study, the DM and lipid content of the fish whole body was increased when the fish were fed with RO, while ash remained unaffected. Regarding the protein content, where a significant interaction of the factors was shown, the analysis of the simple main effects showed that it was higher for the FO groups at the HP level, not affected at the MP level and higher for the RO at the LP level. The latter suggests that RO could have a positive affect in the use of protein for growth when the fish are fed with low protein diets.

5.4.4 Tissue fatty acid composition

As previously discussed the tissue total lipid FA composition reflects the FA composition of the diet, usually following linear correlations between the concentrations of individual FA in the diet and the tissues (Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2003a; Bell, et al., 2003b; Torstensen, et al., 2004a; Torstensen, et al., 2005). That was also shown and discussed in the previous trials of this thesis. The results of the present study are in agreement with the previous studies showing a reduction in saturated FA, 16:1n-7, 20:4n-6, EPA, DHA and n-3/n-6 ratio, respective to the reductions in the dietary FA, with inclusion of RO. Similarly, the increase of 18:1n-9, 18:2n-6 and 18:3n-3 in the diets containing RO, compared to the

FO ones, was reflected in muscle and liver FA. It is clear that, since the FA compositions of the diets were affected by the oil source only, the changes in the muscle and liver FA compositions were also due to the dietary RO inclusion and, as expected, the dietary protein level had no significant effect on the tissue FA composition.

In agreement with the results of the previous trial, the changes in muscle and liver FA indicate selective utilization or retention of individual FA. It has been shown previously that when specific FA are in abundance in the diets they are selectively utilized for energy production, via β-oxidation, and perhaps to a lesser extent for desaturation and elongation, or on the contrary, when FA, and especially n-3 HUFA, are limited in the diet they are retained or deposited in the tissues (Bell, et al., 2001a; Bell, et al., 2003a; Torstensen, et al., 2004a). For example, in the present study 18:1n-9 was increased almost 4-fold, 18:2n-6 more than 5-fold and 18:3n-3 9-fold in the diets when FO was replaced with RO, however the respective increases in muscle were less than or around 2-fold for all of the above FA, while in liver it was approx. 2-fold for 18:1n-9, less than 4-fold for 18:2n-6 and less than 6-fold for 18:3n-3, indicating selective utilization of these FA in the RO groups. On the other hand, although the reduction of EPA and DHA was more than 60% in the RO diets the decrease in muscle was approximately 50% for EPA and 35% for DHA, while in liver the reduction was approximately 35% for EPA and 20% for DHA indicating a selective retention of these FA in the tissues when the dietary supply was reduced. These results are also supported by the Δ values for muscle and liver, that is the differences between diet and tissue fatty acid concentrations. It was shown that, when provided in high concentrations, 18:1n-9, 18:2n-6 and 18:n-3 were highly utilised in both muscle and liver and EPA and DHA were accumulated in the tissues when dietary supply was reduced. In other animals, the

selective retention of essential FA in specific tissues is believed to occur by a mechanism of reacylation of sn-2-monoacylglycerols by hepatic microsomal activity monoacylglycerol acyltransferase (MGAT), during lipolysis (Xia, et al., 1993). However, the moderate reductions of EPA and DHA shown in the present study may also be, partially affected by the enhanced endogenous desaturation and elongation of dietary 18:3n-3 (Tocher, et al., 2001; Tocher, et al., 2003a; Tocher, et al., 2003b).

Furthermore, it is noteworthy that the results of the present trial suggest that the inclusion of RO up to 60%, in expense of FO, in diets of Atlantic salmon at various dietary protein / lipid levels cause only moderate reductions in tissue EPA and DHA. That was also shown in the previous trial where fish were reared at low water temperatures. As previously discussed, reductions in n-3 HUFA affect the quality of the final product, compromise its high nutritional value for the human consumer and should be avoided. Hence, such results, leading to moderate reductions of EPA and DHA, are promising for the use of VO in commercial diets, although the present trial, similarly to the previous one, was conducted over a short time period, which could have masked the real extent of the FA changes that could occur over the whole production cycle of salmon.

5.4.5 Peroxisomal β-oxidation capacity

The peroxisomal β-oxidation activity was measured in liver, red and white muscle. Conducting the assay on-site was not possible and hence the samples of tissues had to be frozen and transferred to the Institute of Aquaculture in Scotland where analyzed. Therefore, the measurement of the total β-oxidation was not possible and the results obtained represent the peroxisomal β-oxidation capacity. Previous studies in Atlantic salmon have shown that different tissues/organs have very different β-

oxidation capacities as a result of their unique and different energy requirements, depending on their functions (Henderson and Tocher, 1987; Frøyland, et al., 2000; Torstensen, et al., 2000; Tocher, et al., 2002; Stubhaug, et al., 2005a; Stubhaug, et al., 2007). In agreement with that, between the three tissues assessed in the present study red muscle had the highest β-oxidation activity and white muscle the lowest. However, it should be noted that the β-oxidation capacities of these tissues, and the consequent ranking, were expressed on a tissue protein content basis. Considering that white muscle accounts for more than 60% of the total body mass of Atlantic salmon, it becomes clear the its role in energy production for the fish is the most significant (Frøyland, et al., 2000; Stubhaug, et al., 2005a).

It is well documented that tissue β-oxidation capacities are affected by various factors, including the diet and especially the dietary FA composition (Torstensen, et al., 2000; Tocher, et al., 2003b; Torstensen and Stubhaug, 2004; Stubhaug, et al., 2005a; Stubhaug, et al., 2007). Specific FA, such as 16:0, 18:1n-9, 22:1n-11 and 20:1n-9 are readily catabolized, although 18:3n-3, 18:2n-6 and even EPA and DHA are also good substrates for β-oxidation, especially when provided at high levels (Henderson and Tocher, 1987; Stubhaug, et al., 2005a; Stubhaug, et al., 2005b). Hence, dietary changes, incorporating VO, could affect the β-oxidation capacities of tissues. However, the results of previous studies are contradictory. Tocher et al. (2003b) showed that β-oxidation capacity was not affected either by the oil content or the oil type in diets of Atlantic salmon. On the contrary, Stubhaug et al. (2005a) reported that dietary RO inclusion had a positive effect on β-oxidation. The results of the present study showed a significant increase in liver and red muscle β-oxidation capacities due to RO inclusion. This could explain, at least partially, the better performance that was shown for the RO groups and the enhanced protein sparing effect. However, in white muscle an

interactive effect of the protein level and the oil source was shown, suggesting a higher β-oxidation capacity for the FO groups than the RO ones at the HP level, whereas RO groups had higher values for the other two protein levels, although the difference was significant only for the MP. The higher β-oxidation capacity of the HP-FO group could be due to the higher content of saturated FA in that diet, although if the hypothesis is correct it remains unclear why such an effect was not reflected in the other two tissues.

5.5 Conclusions

In conclusion, the results of this study suggest that firstly, low protein / high lipid diets can be used with no negative effects on the growth, FCR and chemical composition of Atlantic salmon reared at high water temperatures. Moreover, the replacement of FO with RO can enhance the growth of the fish as well as the nutrient and FA digestibility of the diets, showing an increased protein sparing and β-oxidation. Lastly, RO inclusion significantly affected the tissue FA compositions, as they reflected the FA composition of the diets. However, the reduction in EPA and DHA, resulting from the dietary RO inclusion, was only moderate and hence, the impact on the final product quality, in terms of the nutritional value for the human consumer, was limited.

Chapter 6. Effects of dietary full fat soy bean meal inclusion on Atlantic cod (*Gadus morhua)*

6.1 Introduction

In recent years there has been a continuous decline in cod commercial fisheries, resulting in an increasing interest in cod culture. Specifically, global cod culture production has increased from 169 t in 2000 to 3812 t in 2004 showing a trend for further future increase (FAO, 2006a). Cod require high protein / low oil diets, with high dependence on marine fish meal (FM) and fish oil (FO) (Lie, et al., 1988; Morais, et al., 2001; Lall and Nanton, 2002; Rosenlund, et al., 2004; Karlsen, et al., 2006). Regarding the estimated stable supplies of FM and FO (Pike and Barlow, 2003) and the increased demand and price for these commodities for aquafeeds in the next decade (Tidwell and Allan, 2002; Tacon, 2004) as previously discussed, improvements in feeds that use alternative sustainable protein and lipid sources are vital for the long term future of the cod industry.

Full fat soya bean meal (FFS), along with other soy products, is considered a good potential protein substitute for FM in aquafeeds. These products have been used to replace FM in diets for various fish species such as Atlantic salmon and rainbow trout (Oliva-Teles, et al., 1994; Refstie, et al., 2001; Mundheim, et al., 2004), sea bream and sea bass (Alexis and Nengas, 2001) and Atlantic halibut (Grisdale-Helland, et al., 2002). However, the results of these studies are contradictory as they vary from positive to negative effects on growth and other parameters. These variant results could be explained by the antinutritional factors (ANF) that soybeans contain (Francis, et al., 2001); hence, the effect of the dietary inclusion of these products on the fish depends on the treatment and the processing of the soya beans, the amount and type of ANF in the soya beans, the level of inclusion, the species, the age/size of the fish etc.

In cod, very little is known about the replacement of FM with soya products. A study by Von der Decken and Lie (1993) showed that FFS may replace up to 200 g kg^{-1} of the FM protein with no negative results on growth or feed intake. However, negative results were shown in the same study at the 300 g kg^{-1} level of FFS protein. In a more recent study (Hansen, et al., 2006) plant proteins, including solvent extracted soybean meal, corn gluten meal, and a mix of soy protein concentrate and wheat gluten meal, was used to replace FM at levels up to 440 g kg^{-1} of plant ingredients in the diet. Hansen et al. (2006) concluded that high growth rates and FCR were shown irrespective of the dietary treatment and, although the apparent digestibility coefficients (ADC) of protein and fat were reduced with high inclusions of plant proteins, fish maintained growth by increasing feed intake. These results are in line with a study by Refstie et al. (2006), where extracted soybean meal and a bio-processed soybean meal, with reduced ANF, were used to replace FM in diets for on-grown cod. The authors concluded that cod showed a high tolerance of these soy products, as there were no negative effects on growth and the fish compensated for the reduced ADC of amino acids and lipid by increased feed intake.

Although FFS is included in dietary formulations largely as a protein source, with a crude protein content of ~38%, it also contributes to the dietary fat, containing about 18% lipid (NRC, 1993). More than 50% of the total FAof the soya beans is 18:2n-6, more than 25% is 18:1n-9, less than 10% is 16:0 and around 6% is 18:3n-3, while it does not contain any HUFA such as EPA and DHA (Hertrampf and Piedad-Pascual, 2000; Beare-Rogers, et al., 2001).

Cod, as with other marine fish, require EFA, i.e. arachidonic acid, EPA and DHA for normal growth (Lie, et al., 1992; Lall and Nanton, 2002; Sargent, et al., 2002).

Fish tissue FA compositions largely reflect that of the diet and, as previous studies have shown, cod is no exception to this general principal, although selective utilization or retention of specific FA may occur (Lie, et al., 1986; Lie, et al., 1992; dos Santos, et al., 1993; Kirsch, et al., 1998; Morais, et al., 2001; Morkore, 2006). In cod the main lipid storage tissue is liver. The fat stored in this tissue could be used for the production of cod liver oil and, provided that its FA composition satisfies the basic commercial quality standards, it could be a potentially important by-product of cod culture (Morais, et al., 2001). Cod muscle is lean with very low lipid content (~1% of wet weight), where membrane phospholipids are the dominant lipid class. However, the muscle represents the edible part of the fish and therefore its nutritional value is of importance for the consumer; in particular, high concentrations of HUFA are desirable for promoting human health (Hunter and Roberts, 2000; ISSFAL, 2000; Simopoulos, 2002).

Hence, any inclusion of plant ingredients at the expense of FM and FO in cod diets should ensure that the consequent changes in the dietary FA composition will not be detrimental either to fish growth and health or to the quality of the final product to the consumer. The aim of this study was to investigate the effects of the partial replacement of FM and FO with FFS on growth and tissue FA composition in cod.

6.2 Materials and methods

6.2.1 Fish and culture conditions

The experiment was conducted at the Scottish Association for Marine Science (SAMS) (Ardtoe, Scotland) from February to May 2004. Twelve tanks were each stocked with 30 fish (Atlantic cod, *Gadus morhua*) of initial average weight 220 g. The fish were PIT tagged, allowing individual measurements of biological characteristics to be obtained at each sampling after 6 and 12 weeks. Fish were held in 1600 L volume circular black polypropylene tanks (4 x 3 x 1.6m^2) supplied with UV-treated seawater filtered to 30 μm in a flow-through system at 15 L min^{-1}. The tanks were housed indoors, subjected to a photoperiod regime of 24 h light and the water temperature over the experimental period was 8.3 ± 0.1^oC.

6.2.2 Experimental diets and feeding

The fish were fed four extruded diets, produced by Fiskeriforskning, Norway, for 12 weeks. The control diet did not contain FFS (FFS0) whereas the experimental diets were formulated to contain 120, 240 or 360g of FFS (FFS12, FFS24 and FFS36, respectively) per kg of diet at the expense of FM and FO (Table 6.1). All diets were isoenergetic and isonitrogenous, containing approximately 500 g kg^{-1} protein and 160 g kg^{-1} fat. With regards to amino acids and minerals the diets were balanced for methionine (4.2 g kg^{-1} feed protein), lysine (7 g kg^{-1} feed protein) and digestible phosphorus (7 g kg^{-1} feed protein). In all diets mineral and vitamin premixes were added and the diets were formulated in accordance to all the known nutritional requirements of cold water fish (NRC, 1993; Lall and Nanton, 2002). The analysed proximate composition of the experimental diets is shown in Table 6.1, and the FA

compositions are shown in Table 6.2. The fish were fed to satiation once a day, in the morning. Mortalities and daily feed intake and feed wastage were recorded.

6.2.3 Sampling procedure

Samples were taken from all diets and stored at -20^{o}C until analyzed. After 6 and 12 weeks the fish were weighed individually and SGR, TGC and FCR were determined for each individual. Fish with TGC < 1 for the 0-6 weeks experimental period were not considered representative of the whole population and of the effects of the dietary treatments and, hence, were excluded from all further calculations. The number of fish excluded ranged from 1 to a maximum of 7 fish from each cage. At the end of the trial (12th week) five fish per tank were sampled at random from the population in each cage for lipid and FA composition of liver and muscle. The samples from each tank were pooled to give one sample per tank providing three samples per treatment for each tissue. Sampling was carried out as described in detail in the "General Materials and Methods" section. Specifically, fish were killed with a sharp blow to the head and samples of liver were dissected and frozen immediately in dry ice and then stored in a freezer at -20^{o}C pending analyses. A muscle sample, representative of the edible portion, was obtained by cutting a steak between the dorsal and ventral fins. This section was then skinned, de-boned and homogenized and stored at -20^{o}C until analysed.

6.2.4 Proximate analysis

Moisture, crude protein, crude fat, ash and crude fibre contents of diets and whole body samples were determined as described in the "General Materials and

Methods" section. All methods are based on those described in AOAC (1995) and modified as described by Bell et al (2001a).

6.2.5 Total lipid fatty acid analyses

Total lipids of feed, muscle and liver samples were extracted by the Folch method (Folch, et al., 1957). FAME were prepared from total lipid by acid-catalyzed transesterification as described by Christie (1982) and the extraction and purification of the FAME were carried out as described by Tocher and Harvie (1988). FAME were separated and quantified by gas-liquid chromatography using hydrogen as carrier gas. Individual FAME were identified by comparison to known standards and by reference to published data (Ackman, 1980). All methods have been thoroughly described in the "General Material and Methods" section.

6.2.6 Statistical analysis

Data are presented as means ± SD (n=3). Significant differences between dietary treatments were determined by one-way ANOVA followed by Tukey's post-hoc test to rank the groups (P=0.05). Percentage data and data which were identified as non-homogeneous (Lavene's test) were subjected to square-root, log or arcsine transformation before analysis. Differences were regarded as significant when P<0.05 (Zar, 1999). ANOVA and regression analyses were performed using SPSS 13 (SPSS Inc, 2004).

6.3 Results

6.3.1 Diets

Proximate analysis of the four experimental diets showed that the dietary protein and fat levels were approximately 500 and 160 g kg^{-1}, respectively, and constant between the dietary treatments (Table 6.1). However, there was an increase in dietary fibre with graded inclusion of FFS from 8.4 g kg^{-1} in the FFS0 diet to 16.4 g kg^{-1} in the FFS36 diet.

The increased inclusion of FFS at the expense of FM and FO had a direct effect on the FA compositions of the diets (Table 6.2). In particular, 14:0 decreased, 16:0 remained stable, whereas 18:0 increased resulting in a slight decrease for the total saturates (from 22.5% in FFS0 to 20.9% in FFS36). Total monoenes were reduced from 41.4% in the control group to 33.3% in the FFS36, mainly due to the respective decrease of 16:1n-7, 20:1n-9 and 22:1 (including 22:1n-11 and 22:1n-9) almost by half. However, there was a large increase in 18:1n-9 from 8.5% to 13.9%, for FFS0 and FFS36, respectively. The high content of 18:2n-6 in FFS resulted in an increase of that FA by almost 6-fold and a subsequent increase in total n-6 PUFA, although, the rest of the n-6 PUFA remained stable, totalling less than 1%. 18:3n-3 was increased by more than 2-fold between FFS0 and FFS36. Nevertheless, EPA and DHA were decreased by half. In addition, total n-3 also decreased, from 29.1% to 18.3%, for FFS0 and FFS36, respectively. The n-3/n-6 ratio decreased from 5.4 in FFS0 to 0.7 in FFS36.

6.3.2 Growth performance

All groups showed good performance results regarding weight gain, FCR, SGR and TGC at 6 and 12 weeks (Table 6.3). Fish weights varied from 300 to 350 g and from 467 to 562 g at 6 and 12 weeks, respectively, with the fish fed the control diet having the highest weight gain, although the differences between the groups were not statistically significant. The same trend was shown in SGR (ranging between 0.89 and 1.08) and TGC (varying from 2.49 to 3.09) over the whole experimental period. Fish fed on FFS0 had significantly higher SGR than fish fed on FFS12 and FFS36, and significantly higher TGC than all the other treatments. In both cases, there were no significant differences between the groups fed the FFS. No significant differences were observed in condition factor (K), which varied between 1.28 and 1.38 over the 12 weeks of the trial. With regards to FCR, the range, over the whole experimental period, was from 0.68 for the FFS0 group to 0.84 for the FFS12. The fish fed the FFS0 diets had significantly lower FCR than the fish fed diets containing FFS.

6.3.3 Whole body proximate composition

At the beginning of the feeding trial the proximate composition of the fish whole body was 75.9% moisture, 16.7% crude protein, 3.5% crude fat and 3.1% ash, (Table 6.4). After 12 weeks of feeding on the 4 experimental diets the moisture content ranged from 77.8% to 78.1%, the protein 12.8% to 13.7%, the fat from 5.0% to 6.5% and the ash 2.1% to 2.3%. ANOVA did not reveal any significant differences between the four treatments.

6.3.4 Tissue fatty acid composition

The FA compositions of the cod muscle and liver (Table 6.5 and Table 6.6, respectively) were highly affected by the dietary treatment. The main effect on both tissues was the significant increase in the concentration of linoleic acid, with increasing FFS level. In particular, 18:2n-6 in muscle increased 4-fold, from 3.8% to 15%, for the FFS0 and FFS36 groups, respectively, while, in liver it increased 5-fold, from 4.6% to 22.8%.

In muscle (Table 6.5), further to the increase in 18:2n-6 with graded inclusion of FFS, the concentration of total n-6 PUFA also increased (5.4% - 16.7%, for FFS0-FFS36, respectively). Moreover, 18:3n-3 significantly increased from 0.9 to 1.6% between the control group and FFS36. However, there were significant reductions in 20:1n-9 and 22:1 (including 22:1n-11 and 22:1n-9) (from 3.5% and 1.7 % in FFS0 to 2.0% and 1.1% in FFS36, respectively) and the total monoenes (21% - 16.7% for the FFS0 and FFS36, respectively). The EPA concentration was also decreased from 14.7% in FFS0 to 11.8 in FFS36, although, there were no significant differences between the three groups fed the FFS. The inclusion of FFS resulted in a significant reduction in the n-3/n-6 ratio, from 8.0 to 2.4 for the FFS0 and FFS36 groups, respectively. Finally, no significant differences were shown in the concentrations of 16:0, 18:0, total saturated FA, 18:1n-9, 20:4n-6, DHA and total n-3 PUFA.

Similarly, in liver (Table 6.6), the dietary inclusion of FFS and the consequent major increase in the concentration of 18:2n-6 resulted in a significant increase in total n-6 PUFA (5.5-23.6% for FFS0 and FFS36, respectively). Moreover, 18:1n-9 and 18:3n-3 were also significantly increased from 16.0 and 1.3% to 19.4 and 3.0%, respectively. In contrast, significant reductions were observed between FFS0 and

FFS36, in 16:0 (17.6-13.5%), total saturated (26.1-19.9%), 20:1n-9 (10.7-6.9%), 22:1 (including 22:1n-11 and 22:1n-9) (9.6-4.9%), 24:1n-9 (0.6-0.3%), total monoenes (46.4-39.0), 20:4n-6 (0.3-0.2%), EPA (7.4-5.3%), DHA (9.0-6.4%), total n-3 PUFA (22.0-17.6%) and the n-3/n-6 ratio (4.0-0.7).

The concentrations of specific FA in muscle were plotted against the respective dietary FA concentrations. The plots of muscle FA against dietary FA concentrations are shown in Figure 6.1 and the Correlation coefficients (r), slopes, Y-axis intercepts, F and *P* values from these plots, including the difference (Δ) between diet and muscle FA values for the FFS0 and the FFS36 groups, are shown in Table 6.7. There was a significant linear correlation between dietary and muscle 18:2n-6 and 20:5n-3 (Figure 6.1a and e, respectively; the correlation coefficients were 0.99 and 0.96, respectively). Moreover, a similar trend, although not significant, was shown for 18:3n-3 and 22:1 (Figure 6.1b and d, respectively), with r values of 0.95 and 0.94, respectively. However, the slopes and the intercepts of the linear plots of these FA differed, indicating a different relationship between the dietary and muscle concentrations for each individual FA. This is also supported by the Δ values shown in Table 6.7, where negative Δ values indicate lower values in muscle compared with diet, whereas positive values indicate accumulation in tissues relative to diet. Hence, EPA and DHA were present in higher concentrations in muscle compared to diet both in the FFS0 and FFS36 diets, although at a higher level in the latter. In contrast, the inclusion of FFS in the diet (FFS36) resulted in lower concentrations of 18:2n-6, 18:3n-3 and 18:1n-9 in muscle lipid relative to diet lipid. It is noteworthy that the regression analysis of 18:1n-9 and 22:6n-3 (Figure 6.1c and f, respectively) resulted in almost zero r and slope values and not significant correlations.

The plots of liver FA concentrations against the dietary FA concentrations are shown in Figure 6.2 and the correlation coefficients (r), slopes, Y-axis intercepts, F and P values from these plots, including the difference (Δ) between diet and liver FA values for the FFS0 and the FFS36 groups, are shown in Table 6.8. The regression analysis and the plots of the concentrations of 18:2n-6, 18:3n-3, 18:1n-9, 22:1, 20:5n-3 and 22:6n-3 in the diet against their concentration in the liver, showed significant linear correlations for all of them (correlation coefficients r ranging from 0.98-1.00). Similarly to muscle, the linear relationships between dietary and liver FA concentration differ between the individual FA as the slope values ranged from 0.5 to 0.8 and the intercepts from -1.0 to 10.4. This is also shown by the Δ values shown in Table 6.8. The positive Δ values of 18:1n-9 both for FFS0 and FFS 36 indicate a higher concentration of this FA in the liver compared to the diet. On the contrary, hardly any other FA was at a higher concentration in the liver than in the diet. It should be mentioned, though, that EPA and DHA had higher Δ values in FFS36 than in FFS0 showing a lower utilization and/or increased retention of these FA with the inclusion of FFS.

Table 6.1. Feed ingredients and analysed feed composition (g kg^{-1})

Diet	FFS0	FFS12	FFS24	FFS36
Fish Meal[a]	617	559	501	445
Fish Oil[b]	114	94	75	56
Full Fat Soya[c]	0	120	240	360
Wheat[d]	200	154	106	57
Wheat gluten[e]	50	50	50	50
DL, 99% Met[f]	0	1	2	3
Lys-HCL, 80[g]	0	0.9	1.7	2.4
Di-Ca-phosphate	0	2.5	5	7.5
Premixes[hi]	19	19	19	19
Y_2O_3[j]	0.1	0.1	0.1	0.1
Analysed proximate composition				
Moisture	87.5	86.9	99.7	95.7
Protein	510.3	508.3	505.3	505.0
Fat	162.1	161.1	162.8	165.1
Ash	81.8	81.4	80.2	82.6
Fibre	8.4	12.6	12.9	16.4

[a] LT fish meal, SILFAS, N-5892, Bergen, Norway

[b] NorsalOil, Norsildmel AL, N-5141 Fyllingsdalen, Norway

[c] Full Fat Soya; SOYAXAQUA®, Shouten Industries B.V., 4283 Giessen, The Netherlands

[d] Wheat, Norgesmøllene, N-5853, Bergen, Norway

[e] Wheat gluten, provided by EWOS Innovation, N-4335 Dirdal

[f] DL-Methionine (min 98% Met); Degussa, B-2040 Antwerpen, Belgium

[g] L-Lysine-HCl (min 78% Lysine); Ainomoto Euro-Lysine, 75817 Paris, France

[h] Provided per kg of feed: vitamin D_3, 3000 I.E. ,160 mg; vitamin E, 136 mg; thiamin, 20 mg; riboflavin, 30 mg; pyrodoxine-HCl, 25 mg; vitamin C, 200 mg; calcium pantothenate, 60 mg; biotin. 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B12, 0,05 mg; menadion bisulphite, 20 mg

[i] Provided per kg of feed: magnesium 500 mg; potassium, 400 mg; zinc, 80 mg; iron, 50 mg; manganese, 10 mg; copper, 5 mg.

[j] Yttrium trioxide, included as an inert marker to determine apparent digestibility of nutrients

Table 6.2. Fatty acid compositions (% by weight of total fatty acids) of the experimental diets

Fatty Acid	FFS0	FFS12	FFS24	FFS36
14:0	5.4	4.8	3.8	3.2
16:0	14.6	14.7	14.1	14.0
18:0	2.0	2.4	2.7	3.1
Total saturated[a]	22.5	22.5	21.3	20.9
16:1n-7	5.6	4.9	4.0	3.3
18:1n-9	8.5	10.0	11.8	13.9
18:1n-7	2.0	2.0	1.9	1.9
20:1n-9	9.9	8.4	6.7	5.4
22:1	13.8	12.0	9.6	7.6
24:1n-9	0.8	0.6	0.5	0.5
Total monoenes[b]	41.5	38.6	35.3	33.3
18:2n-6	4.4	12.0	19.4	26.1
20:2n-6	0.2	0.2	0.2	0.2
20:4n-6	0.4	0.4	0.2	0.2
22:5n-6	0.1	0.1	0.1	0.0
Total n-6 PUFA[c]	5.3	13.0	20.1	26.6
18:3n-3	1.5	2.2	3.0	3.6
18:4n-3	4.3	3.5	2.9	2.3
20:4n-3	0.6	0.5	0.4	0.3
20:5n-3	10.2	8.4	7.1	5.4
22:5n-3	0.8	0.5	0.5	0.4
22:6n-3	11.6	9.5	8.3	6.2
Total n-3 PUFA[d]	29.1	24.6	22.3	18.3
Total PUFA	36.0	38.9	43.4	45.8
(n-3) / (n-6)	5.4	1.9	1.1	0.7

[a]Includes 15:0, 20:0 & 22:0.

[b]Includes 16:1n-9 & 20:1n-7.

[c]Includes 18:3n-6, 20:3n-6 & 22:4n-6.

[d]Includes 20:3n-3 & 22:4n-3.

Table 6.3. Growth and performance of cod fed the experimental diets for 12 weeks

	FFS0	FFS12	FFS24	FFS36
Length (cm)				
Start	276.1 ± 6.1	273.5 ± 2.6	270.8 ± 11.5	269.8 ± 7.3
6 weeks	305.6 ± 6.1	298.8 ± 3.4	298.4 ± 11.7	295.4 ± 7.4
12 weeks	343.0 ± 8.4	330.5 ± 3.7	333.1 ± 12.1	364.7 ± 55.8
Weight (g)				
Start	226.4 ± 6.9	229.6 ± 9.9	219.1 ± 30.2	212.5 ± 17.0
6 weeks	349.9 ± 11.6	327.0 ± 16.8	324.3 ± 42.5	300.3 ± 20.1
12 weeks	562.2 ± 35.9	489.2 ± 23.9	488.5 ± 51.2	466.7 ± 23.6
SGR				
0-6 weeks	1.03 ± 0.01 [a]	0.85 ± 0.01 [b,c]	0.94 ± 0.03 [b]	0.82 ± 0.05 [c]
6-12 weeks	1.12 ± 0.08	0.94 ± 0.10	0.97 ± 0.08	1.05 ± 0.07
Overall	1.08 ± 0.04 [a]	0.89 ± 0.05 [b]	0.95 ± 0.04 [a,b]	0.94 ± 0.06 [b]
TGC				
0-6 weeks	2.98 ± 0.02 [a]	2.42 ± 0.11 [b,c]	2.65 ± 0.13 [b]	2.28 ± 0.09 [c]
6-12 weeks	3.09 ± 0.26 [a]	2.49 ± 0.22 [b]	2.53 ± 0.13 [a,b]	2.70 ± 0.17 [a,b]
Overall	3.09 ± 0.14 [a]	2.49 ± 0.18 [b]	2.63 ± 0.04 [b]	2.52 ± 0.13 [b]
FCR				
0-6 weeks	0.71 ± 0.03	0.88 ± 0.10	0.77 ± 0.02	0.92 ± 0.13
6-12 weeks	0.66 ± 0.01 [b]	0.83 ± 0.03 [a]	0.77 ± 0.04 [a]	0.77 ± 0.03 [a]
Overall	0.68 ± 0.02 [b]	0.84 ± 0.05 [a]	0.77 ± 0.01 [a,b]	0.82 ± 0.01 [a]
K				
Start	1.07 ± 0.04	1.12 ± 0.03	1.09 ± 0.02	1.07 ± 0.05
6 weeks	1.21 ± 0.04	1.22 ± 0.04	1.21 ± 0.03	1.15 ± 0.03
12 weeks	1.38 ± 0.02	1.34 ± 0.03	1.33 ± 0.05	1.28 ± 0.03
Mortalities	0	1	0	0

Values are mean ± S.D. Values within a row with a different superscript letter are significantly different ($P<0.05$)

Table 6.4. Proximate composition (% of wet weight) of whole body from Atlantic cod fed the experimental diets for 12 weeks

	Start [1]	FFS0	FFS12	FFS24	FFS36
Moisture	75.9 ± 0.5	77.8 ± 0.2	78.0 ± 0.7	78.1 ± 0.5	77.8 ± 0.5
Protein	16.7 ± 0.3	12.8 ± 0.9	13.1 ± 0.6	13.7 ± 0.6	13.6 ± 0.3
Lipid	3.5 ± 0.3	6.5 ± 1.1	6.2 ± 0.3	5.0 ± 0.6	5.7 ± 0.8
Ash	3.1 ± 0.0	2.1 ± 0.3	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.2

Values are mean ± S.D. Values within a row with a different superscript letter are significantly different ($P<0.05$)
[1]Values not included in the between means comparison

Table 6.5. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of muscle from cod fed the experimental diets for 12 weeks

	FFS0	FFS12	FFS24	FFS36
Total Lipid	9.6 ± 1.6	8.7 ± 1.3	9.9 ± 1.8	10.2 ± 0.5
Fatty acid				
14:0	1.9 ± 0.3 [a]	1.6 ± 0.1 [a]	1.2 ± 0.1 [b]	0.8 ± 0.1 [b]
16:0	22.4 ± 2.3	23.3 ± 1.8	20.8 ± 1.5	19.7 ± 2.5
18:0	4.9 ± 1.2	4.4 ± 0.4	4.5 ± 0.8	5.0 ± 0.7
Total saturated[1]	30.1 ± 4.7	30.4 ± 2.9	27.2 ± 2.6	26.2 ± 4.0
16:1n-7	2.7 ± 0.0 [a]	2.4 ± 0.3 [a,b]	2.0 ± 0.2 [b,c]	1.5 ± 0.1 [c]
18:1n-9	9.8 ± 0.3	9.8 ± 1.0	10.6 ± 0.4	9.5 ± 0.9
18:1n-7	2.3 ± 0.1 [a]	2.2 ± 0.2 [a,b]	2.3 ± 0.1 [a,b]	1.9 ± 0.1 [b]
20:1n-9	3.5 ± 0.4 [a]	3.2 ± 0.4 [a]	2.6 ± 0.2 [a,b]	2.0 ± 0.3 [b]
22:1[2]	1.7 ± 0.0 [a]	1.8 ± 0.0 [a]	1.4 ± 0.1 [b]	1.1 ± 0.1 [c]
24:1n-9	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.2
Total monoenes[3]	21.0 ± 1.1 [a]	20.0 ± 2.0 [a,b]	19.7 ± 1.0 [a,b]	16.7 ± 1.4 [b]
18:2n-6	3.8 ± 0.1 [c]	8.3 ± 0.6 [b]	13.4 ± 0.2 [a]	15.0 ± 1.0 [a]
20:2n-6	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.3 ± 0.3
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
20:4n-6	0.9 ± 0.1	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
Total n-6 PUFA[4]	5.4 ± 0.1 [d]	10.0 ± 0.7 [c]	15.0 ± 0.2 [b]	16.7 ± 0.8 [a]
18:3n-3	0.9 ± 0.0 [b]	1.2 ± 0.1 [b]	1.6 ± 0.1 [a]	1.6 ± 0.2 [a]
18:4n-3	1.5 ± 0.1 [a]	1.3 ± 0.1 [a,b]	1.2 ± 0.1 [b]	0.9 ± 0.1 [c]
20:4n-3	0.6 ± 0.0 [a]	0.5 ± 0.0 [b]	0.5 ± 0.0 [b]	0.4 ± 0.0 [c]
20:5n-3	14.7 ± 0.7 [a]	12.9 ± 0.5 [b]	12.5 ± 0.6 [b]	11.8 ± 0.7 [b]
22:5n-3	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.0	1.1 ± 0.1
22:6n-3	24.6 ± 2.9	22.5 ± 1.2	21.3 ± 0.7	24.8 ± 2.6
Total n-3 PUFA[5]	43.5 ± 3.5	39.6 ± 1.8	38.1 ± 1.5	40.5 ± 3.2
Total PUFA	49.0 ± 3.6 [b]	49.7 ± 1.7 [a,b]	53.1 ± 1.6 [a]	57.2 ± 2.9 [a]
(n-3) / (n-6)	8.0 ± 0.4 [a]	4.0 ± 0.4 [b]	2.5 ± 0.1 [c]	2.4 ± 0.3 [c]

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7; [4]Includes 18:3n-6, 20:3n-6 & 22:4n-6; [5]Includes 20:3n-3 & 22:4n-3.

Table 6.6. Total lipid (mg lipid g^{-1} tissue) and fatty acid compositions (% by weight of total fatty acids) of liver from cod fed the experimental diets for 12 weeks

	FFS0		FFS12		FFS24		FFS36	
Total Lipid	575.7 55.4		604.0 53.2		592.9 24.2		529.3 33.0	
Fatty acid								
14:0	3.7 ± 0.2	a	3.2 ± 0.2	a	2.7 ± 0.2	b	2.3 ± 0.1	b
16:0	17.6 ± 0.9	a	16.9 ± 0.5	a	15.7 ± 0.5	a	13.5 ± 0.8	b
18:0	4.3 ± 0.5		4.4 ± 0.4		4.7 ± 0.1		3.8 ± 0.4	
Total saturated[1]	26.1 ± 1.8	a	25.2 ± 0.9	a	23.8 ± 1.0	a	19.9 ± 1.3	b
16:1n-7	5.6 ± 0.1	a	5.1 ± 0.1	b	4.3 ± 0.2	c	3.9 ± 0.1	d
18:1n-9	16.0 ± 0.9	b	16.7 ± 0.7	b	18.6 ± 0.1	a	19.4 ± 0.4	a
18:1n-7	3.4 ± 0.3		3.3 ± 0.2		3.0 ± 0.3		3.1 ± 0.1	
20:1n-9	10.7 ± 0.2	a	9.6 ± 0.3	b	7.8 ± 0.2	c	6.9 ± 0.4	d
22:1	9.6 ± 0.3	a	8.3 ± 0.2	b	6.4 ± 0.2	c	4.9 ± 0.1	d
24:1n-9	0.6 ± 0.0	a	0.5 ± 0.0	a,b	0.4 ± 0.0	b,c	0.3 ± 0.1	c
Total monoenes[2]	46.4 ± 1.0	a	43.9 ± 1.3	b	40.8 ± 0.1	c	39.0 ± 0.4	c
18:2n-6	4.6 ± 0.1	d	10.2 ± 0.5	c	16.2 ± 0.3	b	22.8 ± 0.5	a
20:2n-6	0.3 ± 0.0	a	0.3 ± 0.0	b	0.4 ± 0.0	c	0.4 ± 0.0	d
20:3n-6	0.1 ± 0.0		0.0 ± 0.0		0.1 ± 0.0		0.0 ± 0.0	
20:4n-6	0.3 ± 0.0	a	0.3 ± 0.0	a,b	0.2 ± 0.0	b	0.2 ± 0.0	b
Total n-6 PUFA[3]	5.5 ± 0.1	d	11.1 ± 0.5	c	17.0 ± 0.3	b	23.6 ± 0.5	a
18:3n-3	1.3 ± 0.1	d	1.7 ± 0.1	c	2.3 ± 0.0	b	3.0 ± 0.1	a
18:4n-3	3.0 ± 0.3	a	2.6 ± 0.2	a,b	2.3 ± 0.0	b,c	1.9 ± 0.1	c
20:4n-3	0.5 ± 0.1		0.4 ± 0.0		0.4 ± 0.0		0.4 ± 0.1	
20:5n-3	7.4 ± 0.9	a	6.5 ± 0.6	a,b	5.8 ± 0.2	b	5.3 ± 0.3	b
22:5n-3	0.7 ± 0.1	a	0.6 ± 0.0	a,b	0.5 ± 0.0	a,b	0.5 ± 0.1	b
22:6n-3	9.0 ± 1.1	a	7.9 ± 0.7	a,b	7.0 ± 0.4	b	6.4 ± 0.5	b
Total n-3 PUFA[4]	22.0 ± 2.6	a	19.9 ± 1.8	a,b	18.4 ± 0.7	a,b	17.6 ± 1.0	b
Total PUFA	27.5 ± 2.7	c	30.9 ± 2.3	b,c	35.4 ± 1.0	a,b	41.1 ± 0.9	a
(n-3) / (n-6)	4.0 ± 0.4	a	1.8 ± 0.1	b	1.1 ± 0.0	c	0.7 ± 0.1	c

Values are mean ± S.D. (n=3). Values within a row with a different superscript letter are significantly different ($P<0.05$)

[1]Includes 15:0, 20:0 & 22:0; [2]Includes 22:1n-11 and 22:1n-9; [3]Includes 16:1n-9 & 20:1n-7; [4]Includes 18:3n-6, 20:3n-6 & 22:4n-6; [5]Includes 20:3n-3 & 22:4n-3.

Table 6.7. Correlation coefficients (r), slopes, Y-axis intercepts, F and P values from plots of dietary fatty acid concentrations vs. muscle fatty acid concentrations for cod fed the four experimental diets, including the difference (Δ)[1] between diet and muscle fatty acid values for the FFS0 or the FFS36 groups[2]

Fatty acid	Correlation coefficient (r)	Slope	Y-axis intercept	F	*P*	Δ FFS0	Δ FFS36
18:2n-6	0.99	0.534	1.860	70.563	0.014	-0.6	-11.1
18:3n-3	0.95	0.383	0.332	18.088	0.051	-0.7	-2.0
18:1n-9	0.03	-0.006	10.011	0.002	0.969	1.3	-4.4
22:1 [3]	0.94	0.103	0.405	15.768	0.058	-12.1	-6.6
20:5n-3	0.96	0.617	8.164	24.605	0.038	4.6	6.3
22:6n-3	0.04	0.028	23.043	0.003	0.962	13.0	18.6

[1] Negative Δ values indicate lower values in muscle compared with diet, whereas positive values indicate accumulation in muscle relative to diet.

[2] FA concentrations are % by weight of total fatty acids in diet and muscle

[3] Includes 22:1n-11 and 22:1n-9

Table 6.8. Correlation coefficients (r), slopes, Y-axis intercepts, F and P values from plots of dietary fatty acid concentrations vs. liver fatty acid concentrations for cod fed the four experimental diets, including the difference (Δ)[1] between diet and liver fatty acid values for the FFS0 or the FFS36 groups[2]

Fatty acid	Correlation coefficient (r)	Slope	Y-axis intercept	F	*P*	Δ FFS0	Δ FFS36
18:2n-6	1.00	0.831	0.566	482.934	0.002	0.2	-3.4
18:3n-3	1.00	0.801	-0.014	270.867	0.004	-0.3	-0.7
18:1n-9	0.98	0.657	10.407	63.421	0.015	7.5	5.5
22:1 [3]	1.00	0.773	-1.021	5662.108	0.000	-4.2	-2.8
20:5n-3	0.99	0.454	2.706	132.046	0.007	-2.8	-0.2
22:6n-3	0.99	0.489	3.248	82.646	0.012	-2.6	0.2

[1] Negative Δ values indicate lower values in liver compared with diet, whereas positive values indicate accumulation in liver relative to diet.

[2] FA concentrations are % by weight of total fatty acids in diet and liver

[3] Includes 22:1n-11 and 22:1n-9

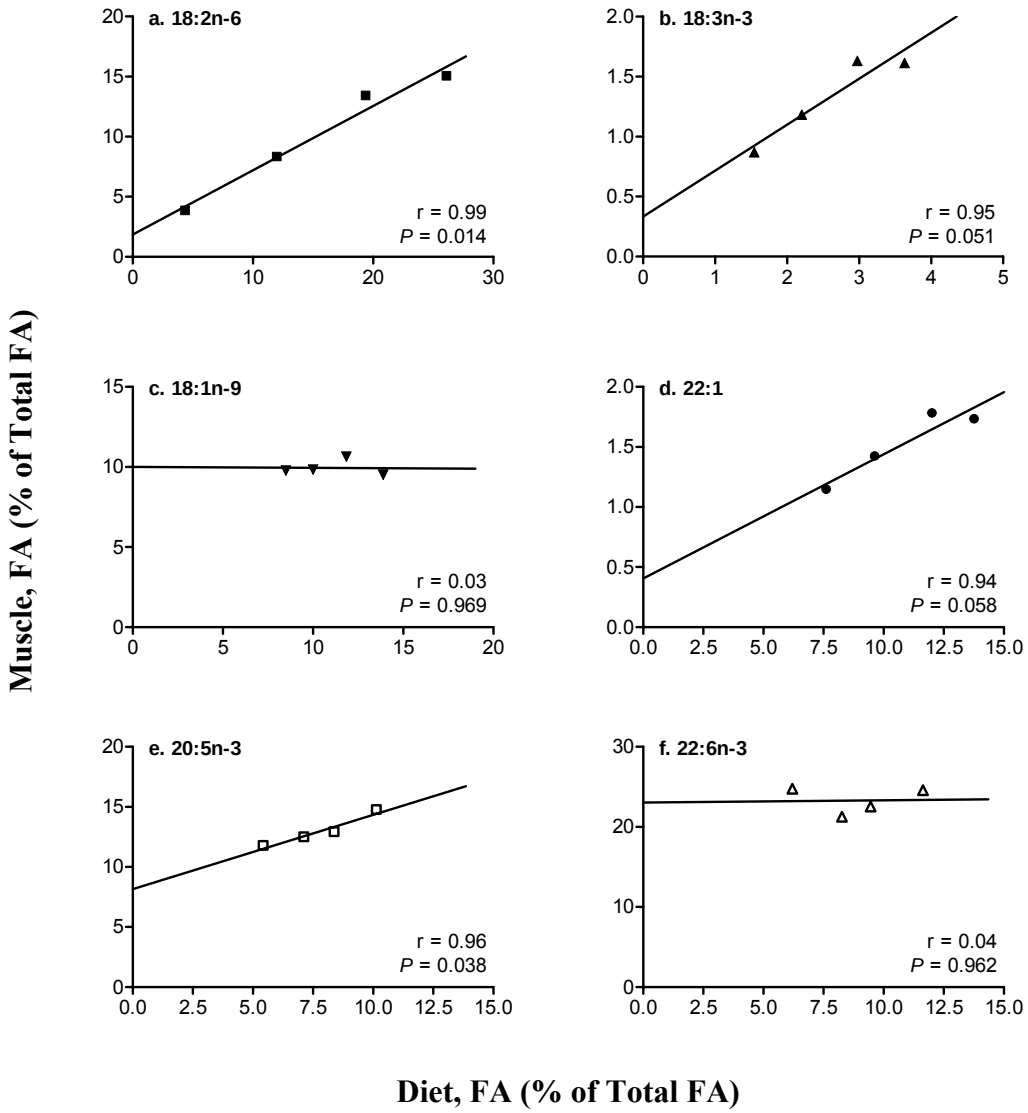

Figure 6.1. Relationship between dietary fatty acids concentrations and muscle fatty acids concentrations of 18:2n-6 (a), 18:3n-3 (b), 18:1n-9 (c), 22:1 (d), 20:5n-3 (e), 22:6n-3 (f) in total lipids of cod fed diets containing increasing levels of full fat soybean meal (FFS)

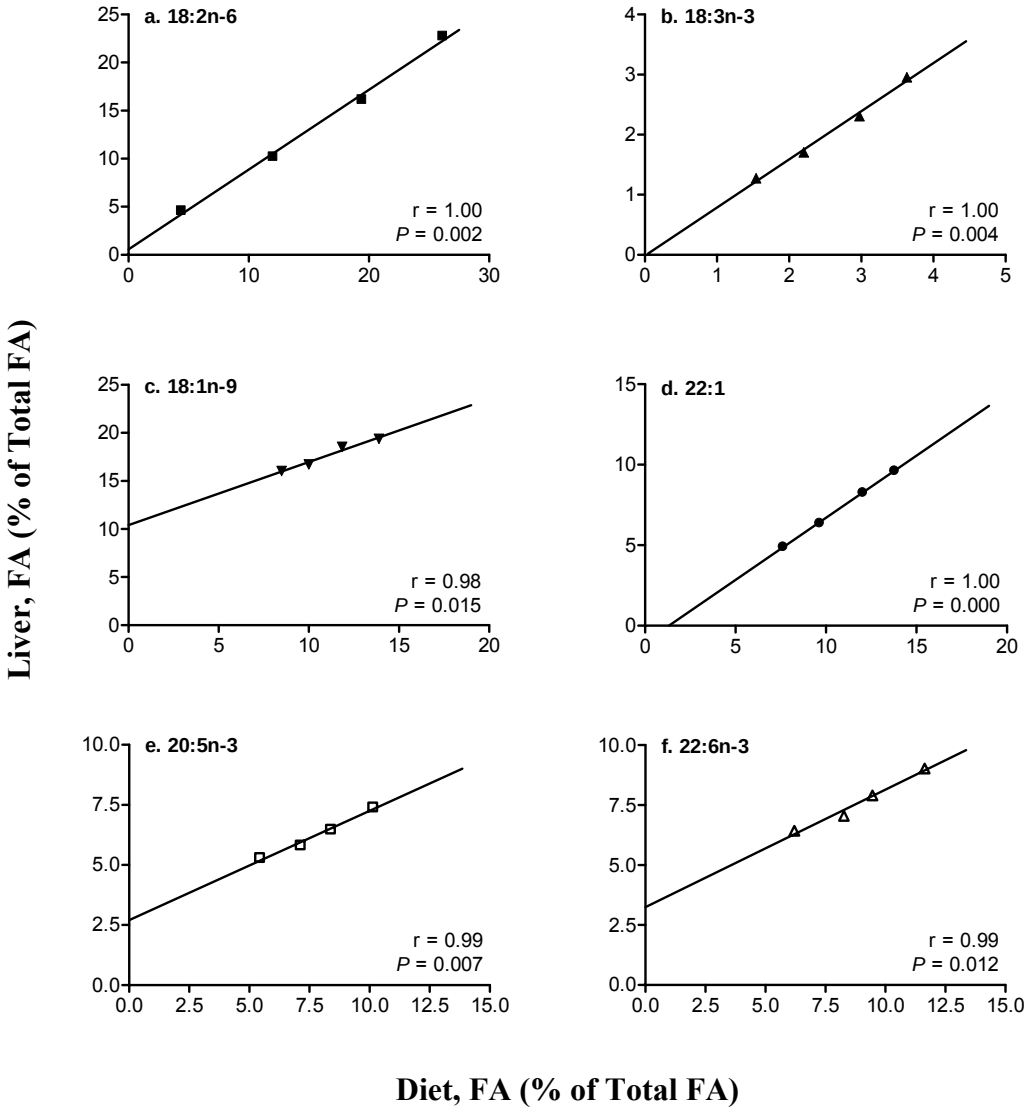

Figure 6.2. Relationship between dietary fatty acids concentrations and liver fatty acids concentrations of 18:2n-6 (a), 18:3n-3 (b), 18:1n-9 (c), 22:1 (d), 20:5n-3 (e), 22:6n-3 (f) in total lipids of cod fed diets containing increasing levels of full fat soybean meal (FFS)

6.4 Discussion

6.4.1 Growth performance

All groups showed good performance, for cod of this size, with regards to weight gain, FCR, SGR and TGC at 6 and 12 weeks. However, in this study a negative effect of the FFS inclusion was clearly shown. In particular, the fish fed the FFS diets showed poorer growth (expressed as SGR and TGC) compared to the control group (FFS0). Both SGR and TGC were significantly lower between the FFS0 and the rest of the groups in the first period of the trial (0-6 weeks) whereas in the second period (6-12 weeks) no significant differences were shown in SGR and only FFS0 and FFS12 differ significantly with regards to TGC. Nevertheless, the differences of the first period probably affected the overall growth of the fish and significant differences between the fish fed the control diet and the fish fed the FFS diets were observed.

These results are in agreement with previous studies in Atlantic cod fed diets containing soya bean meal (Von der Decken and Lied, 1993) and could be due to the presence of anti-nutritional factors in FFS (Francis, et al., 2001). However, Von der Decken and Lied (1993) concluded that only inclusion of FFS above 300 g kg^{-1} negatively affected the growth of the fish. In the present study, the negative effects were shown even with 120 g kg^{-1} inclusion. It is noteworthy that there were very few significant differences between the FFS diets and only in the first experimental period. This indicates that the negative effect was unrelated to the inclusion level and was probably related to a general effect of the FFS on the physiology and consequently the growth of the fish. This is likely due, at least in part, to a change in diet that would probably affect the palatability and therefore the consumption rate by the fish in the initial few weeks. As growth was not different in the 6-12 week period this suggests an

acclimation to the diet and had the experiment been conducted for an extended period an overall growth reduction may not have been seen. Fish, especially cod, are often very sensitive to a dietary change and this could have occurred here although changes in digestibility related to ANFs etc may also have been a factor. It has been shown that the inclusion of plant products, including soya products, in the diets of cod reduces nutrient digestibility (Hansen, et al., 2006; Refstie, et al., 2006). However, in these studies, the fish compensated for the reduced digestibility with increased feed consumption and no negative effects were shown in growth. In the present study, feed consumption was not affected in the first period of the trial and was only slightly, and not significantly, increased in the second period. If it is hypothesized that the digestibility in the present trial was reduced, then the this slight increase in feed intake would not be sufficient for the fish to make up for the reduced digestibility, during the initial 6 week period, and could have resulted in the reduced growth over the 12 week study period.

6.4.2 Whole body proximate composition

The results of the present study suggest that the inclusion of FFS, even to concentrations as high as 36%, did not affect the proximate composition of the cod carcass. This is in agreement with previous studies where the dietary changes had small effects on the chemical composition of either the whole fish, or of specific tissues, such as the muscle or the liver. However, differences between these tissues regarding the extent of compositional changes, to which they may be affected by dietary changes, have also been reported (Lie, et al., 1988; dos Santos, et al., 1993; Hemre, et al., 2004). However, in agreement with the findings of studies in Atlantic salmon (Jobling and Johansen, 2003), size probably plays a more significant role in influencing the whole body composition, given the differences between the start and end protein, lipid and ash

contents, that were shown in the present study. Specifically, the protein and ash were reduced, while the lipid was increased between the start and end samplings.

6.4.3 Tissue fatty acid compositions

Previous studies in cod have demonstrated that tissue fatty acid compositions reflect the dietary fatty acid composition (Lie, et al., 1986; Lie, et al., 1992; dos Santos, et al., 1993; Kirsch, et al., 1998; Morais, et al., 2001; Hemre, et al., 2004; Morkore, 2006). This was clearly shown in the present study, both in muscle and liver. Specifically, in muscle, linear relationships between dietary and tissue FA concentrations were shown for 18:2n-6 and 20:5n-3 and a similar tendency for 18:3n-3 and 22:1. In liver, 18:2n-6, 18:3n-3, 18:1n-9, 22:1, 20:5n-3 and 22:6n-3 were linearly related to dietary FA. Linear relationships between dietary and tissue FA compositions have been previously demonstrated in salmonids (Bell, et al., 2001a; Bell, et al., 2003b; Bell, et al., 2004). In agreement with these studies, the linear correlations obtained in the present trial revealed differences between the relationships of dietary and tissue FA for each individual FA, as slopes and intercepts and also Δ values differed between FA. In particular, EPA and DHA were present in higher concentrations in muscle compared to diet both in the FFS0 and FFS36 diets, although at a higher level in the latter, whilst in liver the concentrations were higher than in the diet only in the FFS36 group. In line with previous studies, these results suggest that when these FA were provided to the fish in low concentrations they were selectively retained in the tissues (Bell, et al., 2003b; Bell, et al., 2004). The differences in retention and utilisation of specific FA in muscle and liver are related to the different functions of FA in the two tissues. Liver is the primary lipid storage organ in cod and thus most of the FA are stored as triacylglycerols while the lean muscle tissue has most of its FA contained in membrane

phospholipids. Since HUFA, especially EPA and DHA, are vital for the function of cell membranes the retention of these HUFA in phospholipids is generally more efficient than in triacylglycerols (Sargent, et al., 2002).

It is important to point out that no significant difference was shown between treatments in muscle DHA, while EPA was only moderately decreased. This is important from the perspective of the nutritional quality of the product for the human consumer, as long-chain HUFA play an important role with regard to human health (Hunter and Roberts, 2000; ISSFAL, 2000; Simopoulos, 2002, 2003). However, although lipid levels in muscle are low the high levels of HUFA present in phospholipids of lean fish are an important source of FA for the consumer. Nevertheless, in liver, EPA and DHA were significantly reduced between fish fed the FFS0 and the FFS36 diets. Noticeably, the reductions were still moderate and are less important as cod liver is less frequently consumed by humans directly although any cod liver oil by-product would be lower in EPA and DHA, and higher in 18:2n-6, than in wild cod products.

In muscle and liver, 18:2n-6 and 18:3n-3 were found in much lower concentrations than in the diet when the fish were fed the FFS diets, indicating that when these FA were abundant in the diet they were selectively utilized for metabolism, probably for energy production (Henderson and Sargent, 1985; Stubhaug, et al., 2006). However, these FA are the precursors of HUFA, through Δ6 desaturation and elongation (Sargent, et al., 2002) and numerous studies in salmonids have shown that the replacement of FO with vegetable oils significantly enhances these pathways (Tocher, et al., 2002; Tocher, et al., 2003a). Hence, the utilization by the fish of these FA demonstrated in the present study could also be for the synthesis of HUFA, in

addition to their oxidation for energy production. However, the desaturation and elongation activities in Atlantic cod, even when fed high levels of vegetable oils, are very low (Bell, et al., 2006) and, as was observed in rainbow trout, more than 90% of dietary 18:3n-3 is used for energy production (Bell, et al., 2001b).

Similarly, 22:1 was also utilized in all treatments; however the inclusion of FFS resulted in lower dietary concentrations of this FA and a subsequent lower utilization compared to the FFS0 group (Henderson and Sargent, 1985; Stubhaug, et al., 2006). Interestingly, the concentration of 18:1n-9 in liver was higher than in the diet, suggesting a preferential retention, whereas in muscle presented a negative Δ value for the fish fed the FFS36 diet. The selective retention of 18:1n-9 in Atlantic cod, rather than mobilization for catabolism, may reflect the structural function of this fatty acid in membrane phospholipids where it is often found in the *sn*-1 position of phospholipids with HUFA being favoured in the *sn*-2 position (Bell and Dick, 1991). Since no significant differences were shown by ANOVA in muscle 18:1n-9, it is clear that only the excess of this FA provided by the FFS diets was used by the fish for metabolism while a specific amount was constantly retained in muscle. These results suggest that in cod 22:1, 18:2n-6 and 18:3n-3 are preferred substrates for oxidation, in agreement with studies in Atlantic salmon (Bell, et al., 2003b).

It is worth mentioning that the effects of the dietary FA composition on tissue FA composition were more clear in liver than in muscle; for instance in muscle the regression analysis of 18:1n-9 and 22:6n-3 resulted in non significant correlations. This is obviously due to the different lipid class composition of these two tissues; in cod, and gadoid fish in general, muscle is mainly constituted of polar lipids located largely in cell membranes, whilst the major lipid class of the liver is TAG (Sargent, et al., 2002).

6.5 Conclusions

The results of this study showed that FFS can be used to partially replace FM and FO in diets for cod with only moderate reductions on growth, no negative effects on whole body proximate and no major detrimental effects on tissue fatty acid compositions, although specific FA of muscle and liver are significantly affected at high FFS inclusion levels. However, further research on the effects of the inclusion of FFS in diets for Atlantic cod on the nutrient and FA digestibility is needed.

Chapter 7. General Discussion, Conclusions and Future Perspectives

7.1 General

In the present study, the effects of the replacement of FM and FO with two VO and an oilseed meal on the growth performance, feed utilization, nutrient and FA digestibility and tissue FA composition and metabolism were investigated in three commercially important European fish species. Specifically, in Experiment I (Chapter 3) PO was used to replace FO in diets for rainbow trout. In Experiments II and III (Chapter 4 and 5, respectively) FO was replaced with RO at various dietary protein/lipid levels aiming at further reductions of FM by using low protein (high lipid) diet formulations which would enhance a protein sparing effect. In Experiments II and III the fish were reared at low and high water temperatures, respectively, in order to elucidate, also, the potential effects of the temperature. Lastly, the effects of the replacement of FM with FFS in Atlantic cod were investigated in Experiment IV (Chapter 6).

7.2 Growth, feed utilization and nutrient and fatty acid digestibility

In general, the results of the present thesis showed no negative effects in growth performance of salmonid fish when FO was replaced with VO, while in the case of RO growth was improved. In Experiment I, PO was included up to 100% of the added oil (complete replacement of FO) in diets for rainbow trout, with no negative results in final weight, SGR, TGC or FCR. The results of this study were in line with previous studies in salmonids which suggested that PO can be used as replacement for FO without affecting the growth of the fish or the feed utilization (Rosenlund, et al., 2001; Bell, et al., 2002; Caballero, et al., 2002; Ng, et al., 2003; Ng, et al., 2004).

Interestingly, studies on the digestibility of PO diets have suggested that lipid ADC and FA ADC are reduced with increasing levels of PO inclusion, although this could potentially have an effect on the performance of the fish, no respective effects on growth have been shown (Torstensen, et al., 2000; Caballero, et al., 2002; Ng, et al., 2003; Ng, et al., 2004). A possible explanation for that could be given by the higher feed consumption by the fish in order to compensate for the energy loss (Caballero, et al., 2002). Moreover the FA composition of the PO, supplying the fish with FA that are readily catabolised for energy production, could be another mechanism contributing to the sufficient growth of the fish (Torstensen, et al., 2000; Caballero, et al., 2002). The results of Experiment I are in line with these hypotheses, however further investigation of the exact mechanisms involved may be required as well as investigating the use of PO in rainbow trout over a longer part of the production cycle.

The important role of the FA compositions of the VO, which are selected as potential replacements for FO, and the consequent differences in the nutrient and FA digestibilities play on the growth performance of the fish was emphasized in Experiments II and III (Chapters 5 and 6, respectively). Specifically, RO was included in the diets of large Atlantic salmon, at various protein / lipid dietary ratios, at expense of FO, comprising 0%, 30% and 60% of the added oil, in Experiment II, and 0% or 60% of the added oil in Experiment III. Interestingly, the inclusion of RO resulted in improved growth (mainly SGR and TGC) in both experiments. Although, several previous studies have shown that replacement of FO with VO, including RO, does not have negative effects on growth of salmonids (Bell, et al., 2001a; Rosenlund, et al., 2001; Caballero, et al., 2002; Bell, et al., 2003a; Bell, et al., 2003b; Torstensen, et al., 2004a; Torstensen, et al., 2005), very few have shown positive effects on growth due to such replacements (Bendiksen, et al., 2003b). The nutrient and FA digestibility results

of Experiment III showed higher ADC of lipid and also of specific FA, such as 14:0, 16:0 and monoenes for the RO groups compared to the FO. Given that these FA (mainly 16:0 and 18:1n-9) are preferentially utilized by the fish for energy production, via β-oxidation, and also that the RO diets had a significantly higher content of 18:1n-9 compared to the FO diets, the improved growth could be explained by better utilization of the dietary lipid (and its constituent FA) by the fish.

The effects of the reduction of the dietary protein level by using high lipid diet formulations were also investigated in the two aforementioned experiments. The use of such diets could reduce the use of FM in salmon diets, and thus the reliance on this commodity, but also the environmental impact of salmon culture activities which are mainly due to feeding practices. In Experiment II, the experimental diets were formulated at two protein/lipid ratios, namely 390 g kg^{-1} protein / 320 g kg^{-1} fat (high protein diets) or 340 g kg^{-1} protein / 360 g kg^{-1} fat (low protein diets). In Experiment III, the diets were formulated at three protein / lipid ratios, specifically 350 g kg^{-1} / 350 g kg^{-1}, 330 g kg^{-1} / 360 g kg^{-1}, 290 g kg^{-1} / 380 g kg^{-1} of protein / lipid for high protein, medium protein and low protein diets, respectively. It should be noted that the experimental diets in both trials were isoenergetic. The results of both studies suggest that the growth performance of the fish, including final weights, SGR and TGC, and the feed utilization were unaffected by the protein/lipid level in the diets. These findings are in line with several previous studies investigating the use of high lipid and/or low protein content in diets for Atlantic salmon (Einen and Roem, 1997; Hillestad, et al., 1998; Bendiksen, et al., 2003b; Azevedo, et al., 2004b, 2004a; Solberg, 2004). However, it should be noted that in the experiments presented in this thesis the protein/lipid levels were much lower than in previous studies, suggesting good growth even at protein levels as low as 290 g kg^{-1}. However, how much more the protein level

in the diets of salmon could be reduced and/or what is the upper limit in the dietary lipid level is largely unknown and hence further research in this area is required.

Furthermore, the potential effect of dietary VO inclusion in protein sparing in fish is largely unknown. The PPV of Experiment III suggest that the protein sparing effect could have been enhanced not only by the higher dietary oil levels, in agreement with the results of Experiment II and also previous studies (Einen and Roem, 1997; Hillestad, et al., 1998; Bendiksen, et al., 2003b), but also by the RO inclusion. In the same experiment, the tissues of the fish fed on RO diets had higher β-oxidation capacity compared to tissues from fish fed FO which indicates increased catabolism of FA for energy production and may support the hypothesis of an enhanced protein sparing effect.

Lastly, it is noteworthy that no significant interactions were seen between the protein/lipid levels and the inclusion of RO on the growth or feed utilization of the fish, in Experiments II and III. This suggests that low protein / high lipid diets, with alternative sustainable oil sources, can be successfully used in Atlantic salmon nutrition. Hence, the reduction in the use of both FM and FO could be achieved with no negative results in the growth or feed utilization. Nevertheless, significant interactions were shown in the nutrient and FA ADC in Experiment III, indicating different effects of RO at different dietary protein/lipid levels and vice versa, which may warrant further investigation.

In contrast to the promising results of FO replacement with VO in the diets of salmonids, as demonstrated in Experiments I, II and III, the replacement of FM with FFS in diets for Atlantic cod had negative results on the growth of the fish. Specifically, in Experiment IV, FFS was gradually included in the diets (12%, 24% and 36% of diet)

at the expense of FM and FO, resulting in lower SGR and TGC compared to the control group (0% FFS). Although, this effect was clear only in the first 6 week period of the feeding trial, it affected the overall growth of the fish at the end of the trial. Previous studies in Atlantic cod have also showed poorer growth due to dietary inclusion of soya bean meal (Von der Decken and Lied, 1993). The presence of anti-nutritional factors in FFS could explain, at least partially, these results (Francis, et al., 2001). Nevertheless, as the effect in the growth was not correlated to the inclusion level of FFS other factors, such changes in the palatability, may also have contributed to these results. Lastly, the nutrient digestibility is known to be affected by dietary inclusion of plant products, including soya products, in the diets of cod (Hansen, et al., 2006; Refstie, et al., 2006). The level up to which FFS can replace FM in cod diets and the exact mechanisms that are involved in such dietary changes, including the effects of ANF and nutrient digestibility, require further research.

7.3 Whole body proximate composition

The results of the present thesis, suggest that the proximate composition of the fish whole body was largely unaffected by the dietary changes employed. This conclusion arose from the results of Experiments I and II where FO was gradually replaced by PO in diets of rainbow trout and RO at various dietary protein/lipid levels in diets of Atlantic salmon, respectively, but also from the Experiment IV, where FM and FO where replaced by FFS. These findings are in agreement with previous studies (Lie, et al., 1988; dos Santos, et al., 1993; Bendiksen, et al., 2003b; Hemre, et al., 2004), and are of importance as the chemical composition of the fish carcass affects the final quality of the product, and its processing properties. However, significant changes due to similar dietary changes have also been reported both due to inclusions of VO

(Bell, et al., 2001a; Torstensen, et al., 2004b; Ruyter, et al., 2006) and also due to varying dietary protein/lipid levels (Hillestad and Johnsen, 1994; Einen and Roem, 1997; Azevedo, et al., 2004a; Solberg, 2004). In agreement with the results of the latter studies, the results of Experiment III reported significant differences in the fish body composition due to the interactive effects of FO replacement with RO and various dietary protein/lipid levels.

7.4 Tissue fatty acid composition

It is clear that changes in dietary formulations, such as the replacement of FO and FM with plant sources, affect the dietary FA compositions. This may have serious implications not only for fish metabolism and growth but also for the quality of the final product, since the fish tissue total lipid FA composition reflects the FA composition of the diet, especially in oily fish like salmonids. The relation between the concentrations of individual FA in the diet and the tissues, which often follows linear correlations, has been demonstrated in several previous studies both in salmonids (Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2003a; Bell, et al., 2003b; Torstensen, et al., 2004a; Torstensen, et al., 2005) and in Atlantic cod (Lie, et al., 1986; Lie, et al., 1992; dos Santos, et al., 1993; Kirsch, et al., 1998; Morais, et al., 2001; Hemre, et al., 2004; Morkore, 2006) and was also confirmed by the results of the four Experiments of this thesis. Specifically, in all four Experiments the FA composition of the tissues was clearly affected by the dietary FA composition, reflecting the changes that were induced by the inclusion of PO in Experiment I, RO in Experiments II and III and FFS in Experiment IV. Noticeably, linear correlations between dietary and tissue FA were shown for several FA in muscle and liver of Atlantic cod in Experiment IV. Lastly, it should be noted that in Experiments II and III the various dietary protein/lipid ratios, to

which the experimental diets were formulated, did not affect significantly the tissue FA composition.

Although, in general, the increases or reductions of FA in the tissues follow the respective changes in the dietary FA composition, the results of the present thesis suggest that specific FA were selectively utilized or retained in the tissues by the fish. This is in line with previous studies reporting specific utilization or accumulation in the tissues of specific FA and suggesting that when specific FA are in abundance in the diets they are selectively utilized for energy production, via β-oxidation, or in addition, but perhaps to a lesser extent they may be desaturated and elongated, whereas on the contrary, when FA, and especially n-3 HUFA, are limited in the diet they are retained or deposited in the tissues (Bell, et al., 2001a; Rosenlund, et al., 2001; Bell, et al., 2002; Bell, et al., 2003a; Torstensen, et al., 2004a).

Hence, 18:1n-9 was selectively utilized, probably for energy production via β-oxidation, in Experiments I, II and III, where it comprised a large proportion of the FA compositions of the PO and RO diets. Moreover, 18:2n-6 and 18:3n-3 were also selectively utilized when in abundance in the diets, as was demonstrated in Experiments II, III and IV. Interestingly, in Experiment I, where the PO diets lack 18:3n-3, 18:2n-6 was probably utilized by the fish, including elongation and desaturation, as was indicated by the significantly increased proportions of 20:2n-6, 20:3n-6 and ARA in the rainbow trout tissues. On the other hand, the n-3 HUFA were decreased in the tissues of the fish when they were fed on the PO, RO or FFS diets. However, in agreement with previous studies suggesting selective accumulation of n-3 HUFA when provided in low dietary concentrations, EPA and especially DHA were, in most cases throughout this thesis, selectively retained in the fish tissues (Bell, et al., 2001a; Bell, et al., 2003a;

Torstensen, et al., 2004b). Specifically, although the dietary decreases in these FA were dramatic, when FO was replaced with plant ingredients, the reductions in the tissues were in most cases only moderate. However, this may have been due to the relatively short duration of these studies and longer trials would need to be conducted to ensure no major deterioration in product quality occurred in the marketed product.

The FA composition of the fish tissues, and especially of the muscle which represents the edible part of the fish, influences the nutritional and quality characteristics of the final product. Fish have traditionally been unique sources of n-3 HUFA for human consumers and the beneficial role of the n-3 HUFA on the prevention of or protection from various human diseases has been well documented (De Deckere, et al., 1998; Horrocks and Yeo, 1999; Simopoulos, 1999; Hunter and Roberts, 2000; ISSFAL, 2000; Simopoulos, 2003). Thus, any negative effects on the FA composition of the fish flesh, resulting from dietary changes such as the replacement of marine oils and meals with vegetable commodities, would be undesirable. In this regard, the moderate reductions of EPA and DHA that were shown in the present thesis, although the feeding trials were of relatively short periods, could be significant for the quality of the final product and its acceptance by the consumer.

7.5 General conclusions

The results of Experiment I, suggest that the dietary PO inclusion, up to complete replacement of FO, in diets of rainbow trout has:

- No negative results in growth and feed utilization
- No negative results in proximate composition of the fish

- Significant changes in the FA compositions of the fish tissues, especially at replacement levels of over 50% although, the reduction in EPA and especially in DHA were only modest.

The results of Experiment II investigating the use of the interactive effects of dietary protein and lipid contents and oil source, namely RO, in Atlantic salmon reared at low water temperatures, showed:

- No negative effects on growth and feed conversion either by the dietary protein/lipid level or by the oil source
- No significant changes in the fish whole body proximate composition
- Significant changes in muscle and liver FA compositions due to the oil source. The tissue FA compositions reflected largely the dietary FA compositions although specific FA were selectively utilized or retained
- An enhanced protein sparing effect, when fish were fed with lower protein feeds where RO replaced FO up to 60% of the total oil

The results of Experiment III, aiming to elucidate the interactive effects of the dietary protein and lipid levels and the replacement of FO with RO in Atlantic salmon reared at high water temperatures, suggest that:

- Low protein / high lipid diets can be used with no negative effects on the growth, FCR and chemical composition of Atlantic salmon
- The replacement of FO with RO can enhance the growth of the fish as well as the nutrient and FA digestibility of the diets, showing an increased protein sparing and β-oxidation.

- RO inclusion significantly affected the tissue FA compositions, as they reflected the FA composition of the diets. However, the reduction in EPA and DHA, resulting from the dietary RO inclusion, was only moderate and hence, the impact on the final product quality, in terms of the nutritional value for the human consumer, was limited.

The results of Experiment IV, investigating the replacement of FM with FFS in diets of Atlantic cod, showed:

- moderate reductions on growth when FFS replaced FM, which noticeably were irrespective of the inclusion level FFS
- no negative effects on whole body proximate composition
- no major detrimental effects on tissue fatty acid compositions, although specific FA of muscle and liver are significantly affected at high FFS inclusion levels
- linear relationships between various tissue and dietary FA, suggesting that specific FA were selectively utilized or retained in the tissues

7.6 Future perspectives

Some of the areas needing further research are mentioned below:

Dietary trials over a longer part of the production cycle, at varying water temperatures, may be required to clarify the effects of, and the mechanisms involved in, the use of PO to replace FO in diets of rainbow trout, on the growth and feed efficiency of the fish and the quality of the final product in terms of its FA composition.

The extent to which the dietary protein /lipid level can be reduced without affecting the growth, the health and the welfare of Atlantic salmon is largely unknown

and should be further investigated. Also the potential positive effects of RO on the growth of Atlantic salmon should be examined over longer periods of the production cycle, ensuring though that the quality of the final product is not compromised by changes in the flesh FA composition. Lastly, future studies may be required to elucidate the interactive effects between the dietary protein/lipid level and the oil source on nutrient and FA digestibility and the protein sparing effects at different water temperatures.

FM could be replaced by FFS in diets for Atlantic cod, however, the maximum levels of FFS inclusion and the exact mechanisms that are involved in such dietary changes, including the effects of ANF and nutrient digestibility, are not yet clear and require further research. Since it was clearly shown by the results of the present study that such dietary changes affect the tissue FA composition, future studies should examine these effects, especially at high FM and FO replacement levels, aiming towards a nutritious final product.

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Appendix. Publications and presentations from the project

Ia. Publications in peer-reviewed journals

- Fonseca-Madrigal, J., Karalazos, V., Campbell, P.J., Bell, J.G., Tocher, D.R., 2005. Influence of dietary palm oil on growth, tissue fatty acid compositions, and fatty acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition 11, 241-250.
- Karalazos, V., Bendiksen, E.Å., Dick, J.R., Bell, J.G., 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.
- Karalazos V., J.G. Bell, J. Treasurer, C.J. Cutts, R. Alderson, T.F. Galloway, S. Albrektsen, J. Arnason, N. MacDonald, I. Pike, 2007. Effects of fish meal replacement with full fat soya meal on growth and tissue fatty acid composition in Atlantic cod (*Gadus morhua*). Journal of Agricultural and Food Chemistry. In Press.

Ib. In progress (publications in peer-reviewed journals)

- Karalazos, V., Bendiksen, E.Å., Bell, J.G., 2007. Interactive effects of dietary protein / lipid level and oil source on growth, feed utilisation and nutrient and fatty acid digestibility of Atlantic salmon. *To be submitted for publication*
- Karalazos, V., Bendiksen, E.Å., Bell, J.G., 2007. Dietary protein / lipid level and oil source interactions on fatty acid metabolism of Atlantic salmon. *To be submitted for publication*
- Karalazos V., et al., 2007. Effects of dietary full fat soya meal on Atlantic cod (*Gadus morhua*). *In progress*
- Karalazos, V., et al., 2007. Dietary palm oil inclusion effects on fatty acid compositions of various tissues in rainbow trout (*Oncorhynchus mykiss*). *In progress*

II. Presentations in peer-reviewed conferences

- Karalazos V., J.G. Bell and E.Å. Bendiksen. 2005. Effects of dietary fat and protein level and rapeseed oil on growth and fatty acid metabolism in Atlantic salmon (*Salmo salar* L.) reared at low water temperatures. World Aquaculture Society Conference 2005 (WAS 05), 9-13 May 2005, Bali, Indonesia. Oral presentation.
- Karalazos V., J.G. Bell, J. Treasurer, C.J. Cutts, R. Alderson, T.F. Galloway, S. Albrektsen, J. Arnason, N. MacDonald, and I. Pike, 2005. Effects of fish meal replacement with full fat soya meal on growth and tissue fatty acid composition in Atlantic cod (*Gadus morhua*). European Aquaculture Society Conference 2005 (EAS 05), 5-9 August 2005, Trondheim, Norway. Poster presentation.
- Karalazos V., E.Å. Bendiksen, and J.G. Bell, 2005. Protein and fat level and rapeseed oil inclusion in diets for Atlantic salmon (*Salmo salar* L.): effects on growth and fatty acid metabolism at low water temperatures. European Aquaculture Society Conference 2005 (EAS 05), 5-9 August 2005, Trondheim, Norway. Oral presentation.
- Karalazos V., J.G. Bell and E.Å. Bendiksen,. 2006. Effects of dietary protein and oil source on nutrient digestibility, growth and fatty acid composition of Atlantic salmon (*Salmo salar* L.). XII International Symposium on Fish Nutrition & Feeding 28 May - 1 June 2006, Biarritz, France. Poster presentation
- Bell J.G., V. Karalazos, J. Treasurer, C.J. Cutts, R. Alderson, T.F. Galloway, S. Albrektsen, J. Arnason, N. MacDonald, and I. Pike,, 2006. Growth and tissue fatty acid composition in Atlantic cod (*Gadus morhua*) fed full fat soya meal to replace fish meal. XII International Symposium on Fish Nutrition & Feeding 28 May - 1 June 2006, Biarritz, France. Oral presentation.
- Karalazos V., J.G. Bell and E.Å. Bendiksen. 2007. Apparent digestibility of nutrients and fatty acids and growth performance in large Atlantic salmon fed on diets with varying protein/lipid ratios and oil source. European Aquaculture Society Conference 2007 (EAS 07), 24-27 October 2007, Istanbul, Turkey. (Oral presentation)

III. Presentations in non peer-reviewed meetings

- Karalazos, V., Bendiksen, E.Å., Dick, J.R., Bell, J.G., 2005. Dietary protein and fat level and rapeseed oil in Atlantic salmon (*Salmo salar* L.): effects on growth and fatty acid metabolism. 37th Scottish Lipids Discussion Group Meeting (Aberdeen), 21 September 2005
- Karalazos V., J.G. Bell, J. Treasurer, C.J. Cutts, R. Alderson, T.F. Galloway, S. Albrektsen, J. Arnason, N. MacDonald, and I. Pike. Effects of fish meal replacement with full fat soya meal on growth and tissue fatty acid composition in Atlantic cod (*Gadus morhua*). British Marine Finfish Association Workshop 2005. Oban (Scotland)