Effect of nutritional level of omega-3 fatty acids on growth and health related parameters of Atlantic salmon rared in fresh water.

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ABSTRACT

Fish are the main supplier, in the human food basket, of the health beneficial n-3 highly unsaturated fatty acids (HUFA). Fish oil (FO) has traditionally been used as the dominating lipid component in fish feed. However, limited availability and fluctuation in price of this commodity force the aquaculture industry to use alternative vegetable oil (VO) sources, which reduced the n-3 HUFA level in the fillet of farmed fish.

One strategy to maintain a healthy omega-3 level in the fish fillet may be through selective breeding of fish with high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Another strategy may be to optimize the dietary ratio of fatty acids in order to facilitate the production of EPA and DHA from 18:3n-3 in the fish.

The n-3 HUFA level is a highly heritable trait in Atlantic salmon, genetic selection in combination with diet formulated has been proposed to improve n-3 HUFA content in farmed salmon. And further, genes involve in the HUFA biosynthesis pathway known to regulate by the fatty acid content of the diet.

The aim of this thesis was to study if offsprings of fish selected for high and low desaturase capacities have differences in body EPA and DHA levels and differences in gene expression of lipid regulatory genes. The fish from the two desaturase families were followed at different life stages from start-feeding to 40g. When fed different dietary levels of VO

The two families of Atlantic salmon (*Salmo salar*) were fed diets where the FO was gradually replaced by rapeseed oil (RO) and FO (0%RO, 25%RO, 50%RO, 75%RO, and 100%RO). Samples of whole body and liver were taken at different life-stages from start-feeding until the fish reached a final weight of approximately 40g.

After 182 days of feeding trial when the fish was 40g, the fatty acid compositions of whole body lipid of the two families were clearly affected by the fatty acid compositions of the diets. The high desaturase family had higher EPA and DHA levels in whole body of fish fed the 25%RO, 50%RO and 75%RO inclusion levels, than the low desaturase family.

The gene expression of the Δ6b desaturase in whole body and expression of Δ6 desaturase(Δ6a, Δ6b, Δ6c), Δ5desaturases, elongases(Elovel_2) and ACO in the liver of fish fed different inclusion of RO, showed variation in expression in response to the different inclusion levels of RO fed groups and sampling periods. This variation in expression levels between the two families together with the higher EPA and DHA level in the high desaturase family at final sampling shows the potential of increasing these fatty acids in farmed fish via genetic selection.

ABBREVIATIONS

ACO	Acyl-CoA oxidase
ARA	Arachidonic acid
cDNA	Complementary DNA
CoA	Coenzyme A
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
Elovl	Very long chain fatty acyl elongases
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FO	Fish Oil
GC	Gas-liquid chromatograph
HPLC	High-pressure liquid chromatography
HUFA	Highly unsaturated fatty acids
LA	Linoleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
OA	Oleic acid
PUFA	Polyunsaturated fatty acids
qPCR	Polymerase chain reaction
RNA	Ribonucleic acid
RNase	Ribonuclease
SGR	Specific Growth rate
VO	Vegetable oil

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1. INTRODUCTION

The human nutritional and health benefit obtained by consuming seafood is one of the main reasons for global increase in demand of fish and shellfish (Sena et al. 2010). Fish oil (FO) has been traditionally used as the only source of lipid in commercial diet of fish farming industry (Turchini 2009). Aquaculture is the fastest growing industry, supplying a large proportion of sea food for human consumption. In 2010 the aquaculture industry together with capture fishery supply the world with 148 million tones of fish, among this aquaculture contribute around 60 million tones with an estimated value of US$119 billion. World Aquaculture food production was grown nearly 12 times in the last 3 decades (1980-2010) with an average annual growth of 8.8%. Currently aquaculture supply nearly 50% of the total world sea food production and this growth is expected to increase (*FAO 2012*).

According to FAO (2012) fish and fishery product are the most traded food commodities in the world making around 10% of the world agriculture export and 1% the value of world merchandise. However the growth of marine aquaculture industry at the current situation is highly influenced by fish meal (FM) and FO production from the wild fishery as the lipid source for the production of feed. FO is the major provider of health-beneficial omega-3 Highly Unsaturated Fatty Acid (HUFA). Irrespective of the increased demand of FO by the aquaculture industry, the production of fish meal and fish oil from the wild fishery remains stable since 1980 (FAO 2012,). Due to the prevailing situation, further development of aquaculture is challenged.

In 2006 alone the Aquaculture industry consumed 88% of the total estimated FO produced. The main consumer species at this specific year were farmed salmon, trout, marine finfish, and penaeid shrimp (Tacon et al 2008). Like most carnivores fish salmon farming traditionally used a diet rich in FO and FM. FO and FM are included at 9 to 35% and 20 to 50% respectively in the diet of salmon; this creates an intense pressure in the resources and result in increasing the price of the produces. (Tacon & Metian 2008b).

In spite of worldwide increase demand in FM and FO by the aquaculture industry, there is a decreasing trend in inclusion level of FM and FO in the aqua-feed by using

an alternative source (Tacon et al. 2008). In the future it is expected to decrease consumption of FO especially in species like salmon, trout and eel, this is due to a combination of increase market cost and decreasing of market availability of the resources (Tacon & Metian 2008a). In 2006 an estimated 25.36 million tones of compound aqua feed containing 3.72 million tones of FM and 0.83 million tones of FO was used for a total global production of 23.85 million tones of farmed fish and crustaceans, this results in a fish-in/fish-out ratio of 0.7. (Tacon & Metian 2008).

In order to keep the growth of aquaculture at current pace it is essential for the industry to look for a sustainable alternative source of lipid. Vegetable oil (VO) is the main candidate for replacing the FO, more research is focusing on replacement of aqua feed by increasing inclusion level of VO (Bell et al. 2003; Morais et al. 2012; Tocher et al. 2003). Despite its less composition of n-3 HUFA in comparison to FO many study reported that VO replaced substantial portion of the fish oil without compromising the growth and feeding efficiency while providing specific essential fatty acid (EFA).(Ruyter et al. 2000a; Turchini et al. 2009). A study shows substitution of FO with VO has an effect in reducing the content of tissue EPA and DHA level which in turn is not desirable by consumer (Berge et al. 2009).

Atlantic salmon, (*Salmo salar*) have a long history in Norwegian aquaculture. Family based breeding program was started back in 1975 by AKVAFORSK (Gjedrem & Gjerde 1991). Now at more than 3 decades of aquaculture practice Norway is one of the major producers of Atlantic salmon in the world. According to the Norwegian Seafood Council salmon production in Norway in 2011 alone was 1 006 000 tones. Selective breeding program for traits of great economic importance are becoming increasingly more important as common practice in the aquaculture industry. Further it's becoming a great interest to combine genetic selection for fish with a high efficiency in retaining and/or biosynthesizing of the n-3 HUFAs with optimizing VO inclusion level in fish diets (Morais et al. 2011; Schlechtriem et al. 2007).

FOs are obtained from the tissue of Fatty fish that is rich in omega-3 fatty acid such as eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These oils are usually come from a variety of small pelagic such as anchovies, blue-whiting, herring, mackerel, capelin, menhaden, sardines, sprat and others. These fish species usually don't produce the EPA and DHA themselves instead they accumulate

throughout their life by consuming either microalgae or prey fish that is rich in omega-3 (De Silva et al. 2010).

It is well studied that many fresh water species including salmonids have the capacity to convert 18:3 n-3 from plant origin to the longer chain EPA and DHA. However this conversion is not enough to satisfy the EPA and DHA requirement of Atlantic salmon (Ruyter et al. 2000a). The capacity of converting 18:3n-3 to EPA and DHA in Atlantic salmon is stimulated in the fish fed VO than compared to fish fed diets rich in FO (Kjaer et al. 2008b). In the fresh water stage salmon parr consume a large quantity of invertebrate that contain 18:3n-3 and 18:2n-6 through which the fish convert it to EPA, DHA and ARA, but this capacity is downregulated in the post-smolt stage in the marine environment in which their natural diet is rich in EPA and DHA (Bell et al. 2001).

1.1. Aim of study

The major objective of this thesis is;-

i. To study if the offspring's of brothers and sisters of fish selected for high and low desaturase capacities, have differences in body EPA and DHA levels at different life stages from start-feeding to 50 g
ii. To study how increasing dietary levels of vegetable oil influence the EPA and DHA deposition and gene expression level.

1.2. Background

1.1.1. Lipid and fatty acid

The word lipids are used to describe a chemical heterogeneous group of substances sharing the common property of being insoluble in water but soluble in a range of organic solvents such as chloroform, hydrocarbon or alcohol. Animal lipids can be divided into two main groups; the neutral lipids, which are completely soluble in non-polar solvent like hexane and chloroform and the polar lipids possess a wide range of solvent solubility based on their non-lipid head groups. (Gurr & Harwood, 1991).

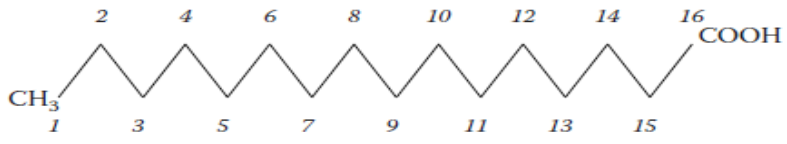
The neutral lipids group includes mainly the triacylglycerols (TAG), sterols, steryl esters, wax esters, and free FA. The polar lipids group includes phosphoglycerides,

sulpholipids, sphingolipids, and glycolipids (Sargent et al. 2002). In general lipid provides cellular building blocks and a good source for energy production.

EFAs are fatty acids that can't be synthesized by the body, and therefore needs to be supplemented in the diets. (Sargent et al. 2002). When VO are used as a the sole lipid source in fish diet, the species need an additional supplement of EFA in order to avoid the risk of nutritional deficiency; normally this can be done by inclusion of the FM (which contain approximately 10% lipid rich in EPA and DHA) in fish diets. As long as the requirement of EFA is fulfilled FO can be replaced by VO sources (Bell et al. 2010; Ruyter et al. 2000a).

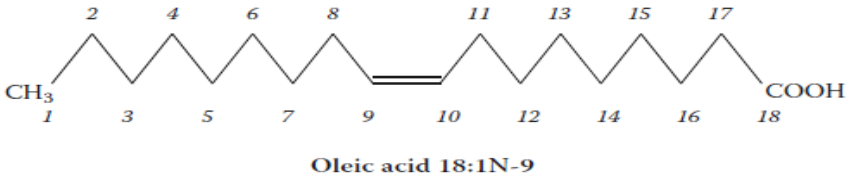
According to the International Union of Physical and Applied Chemists (IUPAC) standard, fatty acids are characterized based on the number of carbon atomsn the fatty acid chain, the location of the double bond and their degree of unsaturation (Sargent et al. 2002). Based on this fatty acids can be categorized in to Saturated Fatty Acids (with no double bounds) (Figure 1a) which found naturally in animal lipid and have a length of between C_{14} to C_{24}. Monounsaturated Fatty Acids (MUFA) which include those group of fatty acid that have a single double bond with chain lengths from C_{14} to $C_{24,}$ this group include one of the major fatty acid found in most of marine and terrestrial product like oleic acid (18:1n-9) (Figure 1b). And the Polyunsaturated Fatty Acids (PUFA) (Figure 1c) include fatty acid group that have a chain length of C_{16} to C_{22} and can contain from 2 to 6 double bond, the most important of the group are n-6 series of the 20:4*n*-6 which synthesized from 18:2*n*-6 (linoleic acid; LA), and the n-3 series of 20:5*n*-3, and 22:6*n*-3 which are synthesized from 18:3 *n*-3 (Bell et al 2010, Sargent et al. 2002). With the exception of cholesterol, all the lipid classes contain fatty acids which esterifies to alcohol groups in glycerides or to amino acid in case of sphingolipids (Christie 2003).

a) Palmitic acid



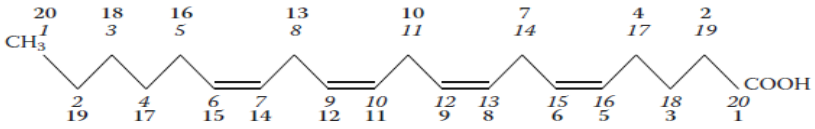
Palmitic acid 16:0

b) Oleic acid

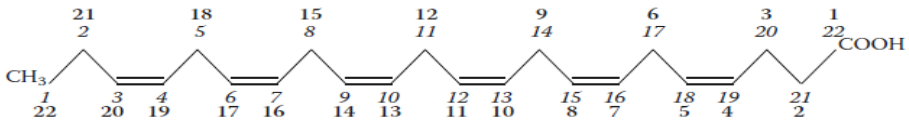


Oleic acid 18:1N-9

c) Arachidonic acid and Docosahexaenoic acid.



Arachidonic acid 20:4n-6
all cis delta-5, 8, 11, 14-eicosatetraenoic acid



Docosahexaenoic acid 22:6n-3
all cis delta-4, 7, 10, 13, 16, 19-docosahexaenoic acid

Figure 1.a). Palmatic acid. b).Oleic acid, c). Arachidonic acid and docosahexaenoic acid showing the *n* (italics) and Δ (bold) carbon-numbering systems. Adapted from Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feed (Turchini et al. 2011)

Lipids are by far the most efficient nutrient as a source of energy which provides approximately around 38.5 kJ g while protein and carbohydrate provides 23.6 kJ g and 17.3 kJ g respectively (Glencross 2009). It is known that all organisms including many fish species required high level of the n-6 and n-3 PUFA, especially those biologically active form of EFA of the C20 and C22 derivative of 18:2n-6 (linoleic acid) and 18:3 n-3 (α-linolenic acid) (Turchini et al. 2009, Bell et al.2001).

Being consumed in large quantity during the growth of farmed fish, fatty acids like 16:0, and 18:1n-9, 20:1n-9 and 22:1n-11 are largely catabolized as a source of metabolic energy especially during the formation of roe by female fish (Henderson *et al.*, 1984a).

Fish fed diets with low levels of the two EFA, 18:2n-6 and 18:3n-3 show a deficiency sign such as low feed efficiency, fatty liver, poor growth, high hepatosomatic index, increased water content in whole body or muscle and high accumulation of 20:3n-9 in the tissue (Glencross 2009; Henderson & Tocher 1987;

Yang et al. 1994). A study with short period dietary deficiency of EFA in Atlantic salmon cause substantial change in the composition of fatty acid in both liver and blood but not having the same effect in carcass lipid. (Ruyter et al. 2000b)

Fatty acid requirement of most fresh water species can be satisfied by dietary inclusion of 18:3n-3 and 18:2 n-6 which can be further elongated and desaturated to the higher level of the DHA and ARA (Henderson & Tocher 1987).

In a natural environment most marine species are well supplied with the EFA in their diet. In the marine environment the alga are rich in 20:5*n*-3 and 22:6*n*-3. Being HUFA more preferable than the 18:3*n*-3 and 18:2*n*-6, most marine fish have little or lost their ability to synthesis HUFA through evolution. Therefore the n-3 EFA requirement of marine fish can only be satisfied by supplying 20:5n-3, 22:6n-3 and 20:4*n*-6 HUFA (Sargent et al. 2002). On the contrary in fresh water environment the primary producer are rich in 18:3*n*-3 instead of 20:5*n*-3 and 22:6*n*-3 as their main HUFA, which make most fresh water fish including anadromous fish such as salmon to have the ability to elongate and desaturate of 18:3*n*-3 to produce 20:5*n*-3 and 22:6*n*-3 (Sargent et al 2002, Voss et al. 1991).

Research show that VO such as rapeseed oil (RO) can be a good replacement of FO in salmon diets, besides having moderate amount of the 18:2n-6 and 18:3n-3 in a ratio of 2:1 and have a sufficient quantity of the 18:1n-9, fish fed diets rich in VO also achieved similar growth and health as fish fed diets rich in FO (Bell et al. 2001). A study show that partially replacement of the fish oil at some stage of the salmon life may not suppress the growth but have a significant effect on the fatty acid composition of the fillet (Berge et al. 2009).

Fish lipids are well known as provider of the n-3 HUFA especially the EPA and DHA, these fatty acids play a vital role in protecting human Cardiac diseases (Harris 1989). In a natural environment salmon, have a higher n-3/n-6 ratio; the balance of this ratio in farmed salmon can be disrupted by feeding diets with a higher inclusion level of VO, which reduces the beneficial value of eating fish as source of n-3 EFA (Sargent et al 2002, Bell et al. 2010).

1.1.2. Fatty acid biosynthesis

The word lipogenesis is used to describe the biosynthesis reactions for the production of endogenous lipid, it starts with acetyl-CoA and builds up the fatty acid by adding two carbon units. In contrast to β- oxidation (which is a catabolism process that occurs in the mitochondria and peroxisomes); lipogenesis occurs in the cytoplasm (Tocher, 2002). The main pathway in lipogenesis is catalized by cytosolic fatty acid synthase(FAS), this is a multienzyme complex which is also identified in fish (Tocher, et al. 1989). FAS give the main product like palmitic acid and stearic acid, which is synthesized by all organisms including fish. It needs a pair of 8 carbon acetyl units for the biosynthesis of palmitic acid, with one acetyl CoA unit used as start material. The remaing seven acetyl units will carboxylated by acetyl-CoA caroxylase to malonyl-CoA, before it is used by FAS in a process that includes sequential condensation steps required NADPH (Henderson & Sargent 1985). It is well known that higher intake of dietary lipid reduce *de novo* fatty acid syntheses, due to the inhibition of the enzymes involved in hepatic lipogenesis (Sargent et al 2002).

Saturated fatty acids such as 16:0 and 18:0 can be biosynthesized *de novo* by all organisms including fish (cook 1996), this SFA can further become desaturated by microsomal fatty acid Δ^9 desaturase to give the MUFA oleic acid(18:1n-9).

1.1.3. Desaturase

A desaturase is an enzyme involved in the addition of double bond in to fatty acyl chain by removing two hydrogen from hydro carbon chain (Shanklin et al.1998).

There are three type of desaturases known in animals; according to their desaturase activity these are Stearoyl CoA desaturases (SCD or Δ 9 desaturases), Δ6 desaturase and Δ 5 desaturase, the first one catalyze the synthesis of MUFA while the last two help in the synthesis of HUFA (Nakamura & Nara 2004). Being important for maintaining cellular function most organisms has a mechanism of preparing unsaturated fatty acid (Magnuson et al.1993,). These desaturase enzymes are a non-heme iron containing enzymes that add double bond in a specific position from the carboxyl end of the fatty acid (Nakamura & Nara 2004).

There are two nomenclatures used to describe the Desaturase, the first one is Δ desaturase which introduce double bond from the carboxylic end of the fatty acid, and the second one is the ω desaturase which introduce double bond from the methyl end of the fatty acid (Bell et al 2010).

The LC-PUFA such as ARA, EPA and DHA are important component of the cell membrane and important precursors for the eicosanoids production, their synthesis require a series of desaturase and elongases.(Meesapyodsuk et al. 2012, Sargent et al. 2002, Hastings et al. 2001).

I .Δ6 desaturase

Δ6 desaturase is a membrane bound acyl-CoA desaturase which catalyze the synthesis of PUFA. It is classified as the 'front-end' desaturase which add double bond between the existing double bond and the carboxyl end of the fatty acid (Sprecher 2000, Pereira et al. 2003). Monroig et al.(2010) clone three Δ6desaturase from salmon(Δ6fad_a, Δ6fad_b and Δ6fad_c) and found the expression level Δ6fad_a is higher in intestine>liver>brain and Δ6fad_b express highly in brain>intestine>gill>liver. They also show that the Δ6fad_c expression level don't regulate nutritionally instead the expression level of Δ6fad_a, and Δ6fad_b significantly increase in intestine and liver respectively when the fish are fed low level of LC-PUFA. Contrary to this finding other study shows that all these three Δ6desaturase are active towards the n-3 fatty acids substrates.(Zheng et al. 2005). This enzyme is active in both C18 and C24 homologues in the PUFA biosynthesis pathway and catalyzes the synthesis of 18:3n-6 from the 18:2n-6; 18:4n-3 from 18:3n-3, 24:5n-6 from 24:4n-6 and 24:6n-3 from 24:5n-3 (Li et al. 2010, Tocher 2010). The expression of Δ6 desaturase and Δ5 desaturase in salmon fed VO is up-regulated than those fish fed FO, affinity for the Δ6 desaturase enzyme increase with level of desaturation and chain length (18:3n-3 > 18:2n-6 > 18:1n-9 > 16:1n-7) (Sargent et al. 1993).

Δ6 desaturase have been cloned from marine fish including gilthead sea bream, turbot and Atlantic cod (Tocher et al. 2006; Zheng, X. et al. 2004). It is believed that the Δ6 desaturase in marine fish is associated with the production of DHA from EPA rather than desaturation of the Alpha-linolenic acid, this may be associated with maintaining membrane DHA level (Tocher 2010). This hypothesis was further

supported by Tocher et al (2006), they found high expression of Δ6 desaturase in the brain of cod, to a slightly lesser extent in liver, kidney and intestine, whereas this gene express highly in the liver and intestine in case of salmon.

II .Δ 5 desaturases

This is another front-end desaturase found in animal Fatty Acid Biosynthesis, which add double bond at Δ 5 position of the C_{20} fatty acid (20:3n-6 and 20:4n-3) after it is been elongated and desaturated by Δ 6 desaturases and elongase (Meesapyodsuk & Qiu 2012). The low potential of marine fish for the synthesis of the C20 and C22 HUFA from their 18:3n-3 is associated with the limited activity of the Δ 5 desaturases. In contrast the presence of Δ 5 and Δ 6 desaturase in Atlantic salmon indicate the potential of this fish in the production of DHA from 18:3n-3 (Tocher 2010). Salmon gene expression of Δ 5 desaturases fed RO was upregulated compared with fish fed FO (Jordal et al. 2005).

III .Δ9 desaturase

MUFA is normally synthesized from saturated fatty acid by Stearoyl CoA (Δ 9) desaturases, this enzyme incorporates the 1st cis-double bond at the 9, 10 position of the fatty acid from the carboxyl end to give palmitoleic acid and oleic acid (Nakamura & Nara 2004). Mammalian Δ9 desaturase , usually referred to as stearoyl-CoA desaturase (SCD), was first purified from rat liver.(Strittma.P et al. 1974).

Δ 9 desaturase is considered as a lipogenic enzyme, not only for it's importance in biosynthesis of MUFA, but also by the way it is requlated by diet and insulin. A study made in rat liver show, the Δ9 desaturase activity was reduced by starvation and diabetes, and it was rapidly induced to high levels during refeeding of high carbohydrate diet (Ntambi 1999). It's known that diet supplimented with PUFA have effect in repressing gene experasion of lipogenic enzyme including Δ 9 desaturase (Clarke & Jump 1993).

1.1.4. Elongases

The biosynthesis pathway of HUFA requires sequential desaturation and elongation of the precursor essential HUFA.(Tocher 2003) The elongase enzymes can be categorized based on its function into two groups as those which involve in the elongation of the

saturated fatty acid and MUFA (ELOVL1, ELOVL3 and ELOVL6) and the other group is those involve in the elongation of PUFA (ELOVL2, ELOVL4 and ELOVL5) (Jakobsson et al. 2006).

A study made in mammals show that the ELOVL5 is involved in the elongation of C-18 and the C-20 PUFA and the ELOVL2 is involved in the elongation of C-20 and C-22.(Leonard et al. 2002). Morais et al (2009) found two ELOVL genes in salmon that are similar in function with that of the mammalian ELOVL2 and ELOVL5.

1.1.5. Monounsaturated fatty acid (MUFA)

The MUFA occurs naturally, with chain lengths from C-14 to C-24 (Sergeant et al. 2002). They are synthesized from saturated fatty acid by Δ 9 desaturases, which insert double bond at the 9, 10 position of the fatty acid from the carboxyl end (Ntambi 1999). Like all animals, desaturation of fatty acid also takes place in the endoplasmic reticulum in fish's through an aerobic process using a CoA linked substrate (Brenner 1974).

The syntheses of MUFA has a great physiological importance due to the lower melting point of the produced fatty acid (16:1n-7 and 18:1n-9) than their precursor saturated fatty acid. Due to this the Δ 9 desaturases play a vital role in the regulation of membrane viscosity by changing the melting point of the fatty acid in the membrane phosphoglycerides (Ntambi 1995). A study performed in carp, show the expression of the gene coding for Δ 9 desaturase is enhanced and further lead to increased production of MUFA in response to cold environment in order to maintain membrane fluidity (Tiku, Gracey et al. 1996).

1.1.6. PUFA biosynthesis

Desaturation of the 16:0 and 18:0 to linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3) is not possible due to the lack of the Δ12 desaturase and Δ15 desaturase in fish, which make these fatty acids essential and must be derived from the diet (Figure2) (Pereira et al. 2003; Tocher 2003). Further desturasion and elongation of the 18:3 n-3 PUFA is needed to give ARA, EPA and DHA. The capacity of a fish to convert the C- 18 PUFA to the C-20 HUFA depends on its ability for fatty acyl desaturation and elongation (Luthria & Sprecher 1997; Sprecher 2000; Tocher 2003)

Traditionally it was accepted that the synthesis of the 22:6n-3 from the 18:3n-3 is achieved by a sequential elongation and desaturation of Δ6, Δ5 and Δ4 desaturase pathway. The synthesis and esterification of the C-22 with their first bond incorporated at the position 4 is a bit complex process than the C-20 acids. The syntheses of DHA form its precursor (22:5n-3) doesn't occur through a simple addition of double bond by the Δ 4 desaturases, instead the 22:5n-3 is chain elongate to 24:5n-3 and convert by Δ 6 desaturases to 24:6n-3 which is then converted by chain shortening reaction to 22:6n-3 in the peroxisomes (Sprecher 2000).

Both the peroxisomes and microsmes are required for the synthesis of the fatty acid having the first double bond at the position 4 and the movement of HUFA between the peroxisomes and endoplasmic reticulum results in partial degradation and re-synthesis cycle (Sprecher 2000; Voss et al. 1991). It is believe that peroxisomes are likely to involve in both synthesis and oxidation of DHA (Nakamura & Nara 2004).

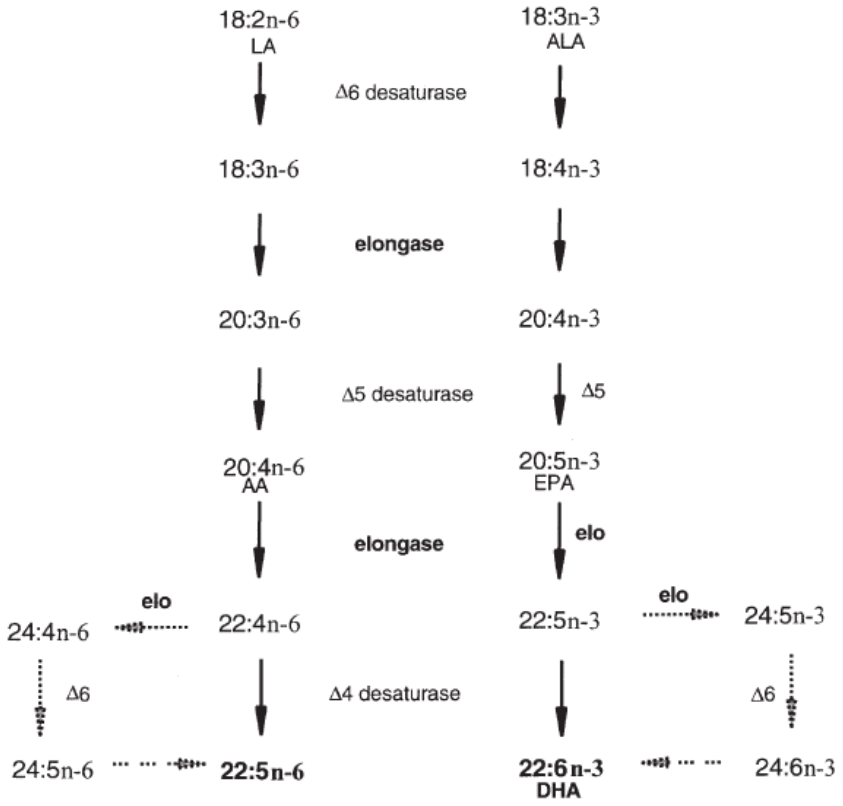


Figure 2 Metabolic pathway of long-chain PUFA. The dashed arrows indicate the "sprecher pathway". Adopted from (Sprecher 2000).

1.1.7. Physiological role of n-3 and n-6

Many studies have shown a selective β-oxidation of fatty acid in fish, normally 16:0, and 18:1n-9, 20:1*n*-9, 22:1*n*-11 and EPA can easily be used in mitochondria β-oxidation. A Study made in Rat show that unlike EPA, DHA is a poor substrate for mitochondrial *β*-oxidation and the catabolism of DHA required peroxmal β-oxidation (Madsen et al.1999). Due to the addition of Δ^4 double bond in 22:6*n*-3 requires a special mechanism and the same for the removal.

The 22:6*n*-3 fatty acid is unique among the other fatty acid by having the 6 cis double bond in the C_{22} fatty acid, this make the 22:6*n*-3 fatty acid very compact. (Applegate & Glomsett, 1986). Many studies show fish respond to the cold environment by restructuring of cellular membrane; this can be done by increasing the ratio of unsaturated fatty acid to that of saturated fatty acid in membrane phospholipids and vice versa is true for the worm environment (Brooks et al. 2002, Sergeant et al. 2002, Tiku, Gracey et al. 1996). Poor growth rate and increased mortality are some of the clinical sign that the fish exhibits when there are deficiency of EFA (Ruyter et al. 2000a; Sargent et al. 1995)

HUFA especially the ARA is a precursor for the production of eicosanoids. Eicosanoids are referred to signaling molecules usually derived from oxidation of the C_{20} essential fatty acid. These hormone-like compounds produced by the cell in the body with a short half-life, and have a wide range of application like inflammatory response, renal function; neural function and the immune response are among the others (Sergeant et al. 2002). Normally both n-3 and n-6 fatty acids are metabolized by the same elongases and desaturases and this create a competition for substrate which affects the nature of HUFA produced and composition of HUFA in cell membrane; that mean the eicosanoida produced largely depend on the ratio of n-3/n-6 in the membrane which largely depend on dietary intake of *n*-6 and *n*-3 PUFA (Bell et al. 1993).

In higher terrestrial mammals ARA is the chief precursor of the eicosanoids. The production of eicosanoids from ARA and EPA is catalyzed by cyclooxygenase and lipoxygenases which produce 2-series prostanoids and 4-series leukotrienes from ARA, and 3-series prostanoids and 5-series leukotrienes from EPA. Eicosanoid derived from ARA are biologically very active and have an inflammatory effect, on

the contrary eicosanoid production from EPA are biologically less active and known by soften the inflammatory effects of ARA and its products (Sargeant et al 2002). Today the western world diet is recognized by having the lower ratio of n-3/n-6 fatty acid which causes major problems for occurrence of inflammatory incidence and cardiovascular disease (Okuyama et al.1997, Bell et al 1993).

It is known that DHA is essential for the function of retina and brain. Study conducted in primate showed that deficiency of this n-3 HUFAs caused in deterioration of visual function (Neuringer et al. 1988).

1.1.8. Selective breeding and the potential to select for higher capacities for EPA and DHA production

Selective breeding in aquaculture holds high potential for the genetic improvement of fish and shellfish. It is becoming common to use selective breeding in aquaculture to enhance traits of commercial importance (Gjedrem & Baranski 2009). Concerning the current fish oil crises, selection for high flesh n-3 HUFA in salmon breeding program would be highly desirable. Karamichou et al. (2006) show the heritable genetic regulating capacity to biosynthesis and/or regulating HUFA deposition in mammals. Leaver et al. (2011) also show the high heritable trait (h^2=0.77±0.14) of flesh n-3 HUFA composition in Atlantic salmon. A study made in Atlantic salmon fed FO or VO found variation in the content of the n-3 HUFA in the flesh of individual salmon regardless of the diet (Schlechtriem et al. 2007).

1.3. Rapeseed Oil (RO)

It is a bright yellow flower which belongs to the family Brassicaceae, and has been cultivated for many years in Asia and now recently its production is extended to Europe. It is one of the few species of oilseed plants that can be cultivated in cold climate (Booth & Gunstone 2004).

Worldwide productions of RO expand from 6.3 million hectares in 1961 to more than 30.2 million hectares in 2007, Asia being the greatest producer of all. Unlike the FO the RO has very few history of fluctuation on the production which makes it a sustainable product and a great candidate as replacement of FO (FAO 2012).

A traditional RO usually contain 23% of oleic acid and 34% of erucic-acid. Low erucic-acid RO contain 60% of oleic acid and 1.2% of erucic-acid and high erucic-acid RO contain 12% oleic acid and more than 50% of erucic-acid, and it is this low erucic-acid RO that studies in fish nutrition which referred as “Canola”(Booth & Gunstone 2004).

MUFA rich vegetable oil has edible and cooking quality and they have been used widely for human consumption. RO is one of the VO rich in the MUFA and currently used in large quantity in aqua feed industry. A study show that VO in a single or mixed form can replace FO completely or in part without showing a noticeable risk of reduction in the growth and or health condition of the fish (Bell et al 2001).

RO and other MUFA rich VOs have ideal physical and chemical texture that suit the extruded aqua feeds; they are liquid at room temperature, not easily oxidized and resistance to thermal treatment. Study show oxidized feed are responsible for peroxidative stress and related health problem (Tocher et al. 2003). Aqua feed made of FO are highly susceptible to oxidation and subsequent rancidity which deteriorate the feed quality and due to this they are usually supplemented with anti oxidant. Addition of MUFA sources to the feed which is naturally rich in antioxidants can reduce the risk of oxidation and reduced the amount of antioxidant added (Turchini et al., 2009).

2. MATERIAL AND METHODS

Table 1: Chemicals and equipment

Chemical and equipment	**Producers**
Hewlett Packard 6890 gas-liquid chromatography (GC)	Avondale, PA, USA
Precellys 24 lysis and homogenization	Bertin Technologies, Montigny-le-Bretonneux, France
NanoDrop 1000 Spectrophotometer	NanoDrop Technologies, DE, USA
Light Cycler 480	Roche Diagnostics Gmbh, Germany
Blender	Dynamics corporation of America, USA
Avanti ^{TM} centrifuge J-301	Beckmann coulter California, U.S.A
GeneAmp^{R}PCR system 9700	California, USA
AffinityScript QPCR cDNA Synthesis Kit	Agilent Technologies, CA, USA
Sodium chloride (NaCl)	Merck, Darmstadt, Germany
PureLink™DNaseTreatment andPurification Kit	California, USA
eppendorf cenyrifuge 5415R	New York, USA
Blender	Dynamic corporation of America, USA
SYBR Green-I Master	Roche Applied Science, Germany
Chloroform	VWR International, PA, USA
Methanol	VWR International, PA, USA
Dimethoxypropane	Sigma, St. Louis, USA
Benzene	Merck, Darmstadt, Germany
Hexane	Merck, Darmstadt, Germany
Sodium chloride (NaCl)	Merck, Darmstadt, Germany
Primers	Invitrogen Ltd, Paisley, UK
DNaseI	Invitrogen, Auckland, NZ
Hydrochloric acid (HCl)	Sigma, St. Louis, USA
DNaseI	Invitrogen, Auckland, NZ
Kontes Pellet Pestle	Daigger, IL, USA
PureLink™ RNA Mini Kit	California, USA

2.1. Fish and experimental design

The experimental fish Atlantic salmon were obtained from the first generation of fish that was selected for high and low desaturase gene expression (Sun 2012). In the previous experiment, 1044 fish were selected from 103 families. Based on the difference found in Δ6fad_b gene expression in liver, 6 families of Atlantic salmon were selected as "high desaturase families" and 6 families were selected as "low desaturase families". The first generations of these families were used to design the current experiment.

The feeding trial was carried out at Nofima research station, Sunndalsøra starting from Jan. 2012 until Aug. 2012. Two families of high and low Δ6fad_b desaturase gene expression were selected for the feeding trial. The fish were hatched between the 4^{th} and 10^{th} of January, and became ready for start-feeding 20.02.12. Around 140$\pm$ 10 Atlantic salmon from each family were allocated randomly to 10 tanks. 5 practical-types commercial extruded feed BioMar brand was further coated with a fish oil (Møllers tran) and rapeseed oil bought from a store with a blender in the Sunndalsøra station. The feeds were formulated to provide different fish oil/ vegetable oil (n-3/n-6) ratio with different inclusion levels of RO and FO (100% RO, 75%RO, 50%RO, 25%RO, and 0% RO). The salmon were fed the different diets for 182 days until they reached 40-50g. Samplings were performed every month (21.03.12, 19.04.12, 23.05.12, and 19.06.12) until the final sampling (22.08.12) when the fish reached 40-50g. At the first two samplings, 10 fish per tank were selected, whereas 15 fish per tank were selected from the last three samplings. The fish were anesthetized in Metacain (MS-222) and weighed. Thereafter, the livers were dissected from another fish and together with the whole body fish frozen in the liquid nitrogen and stored at -80 °C until analyzed. Exception is at the two first samplings, where only whole body was frozen without removing liver due to the small size of the fishes. The average temperature during the experimental period was 11^0C. Feeding was performed 24hours using a belt feeder and the tanks were subjected to a photoperiod regime of 24h light. The fish grew from an average initial weight of 0.179g to a final weight of approximately 39.2g $\pm$ 3.8. The SGR in percentage was calculated among different tanks of fish fed different dietary treatment.

Table 2 Fatty acid composition of diets (% of total fatty acids)

Fatty acid	100%FO	75%FO25%RO	50%FO50%RO	25%FO75%RO	100%RO
Fat(%)	25.0	25.0	24.6	24.3	24.4
C 14:0	5.0	4.6	4.2	3.8	3.5
C 15:0	0.4	0.4	0.4	0.3	0.3
C 16:0	12.8	12.3	11.8	11.4	10.9
C 17:0	0.6	0.5	0.5	0.4	0.4
C 18:0	2.6	2.7	2.6	2.6	2.5
C 20:0	0.2	0.2	0.3	0.4	0.4
C 22:0	0.1	0.1	0.2	0.2	0.2
C 24:0	0.1	0.1	0.1	0.2	0.1
Σ Saturated	21.8	20.9	20.0	19.2	18.2
C 14:1 n-5	0.3	0.3	0.2	0.2	0.2
C 16:1 n-7	7.0	6.1	4.8	4.1	3.3
C 16:1 n-5	0.4	0.4	0.3	0.3	0.2
C 18:1 n-9	17.9	22.1	26.6	31.0	35.1
C 18:1 n-7	0.3	0.2	0.2	0.2	0.1
C 20:1 n-11	2.4	2.1	1.8	1.6	1.3
C 20:1 n-9	7.4	6.3	5.2	4.2	3.3
C 20:1 n-7	0.3	0.3	0.3	0.2	0.2
C 22:1 n-7	0.8	0.7	0.6	0.5	0.4
C 22:1 n-11	6.4	5.9	5.2	4.5	3.9
C 22:1 n-9	0.6	0.5	0.4	0.5	0.5
C 24:1 n-9	0.6	0.6	0.5	0.5	0.5
Σ Monounsaturated	44.4	45.6	46.1	47.6	48.9
C 18:2 n-6	4.5	6.3	8.2	10.0	11.8
C 18:3 n-6	0.2	0.2	0.2	0.2	0.1
C 18:3 n-4	0.1	0.1	0.1	0.2	0.1
C 18:3 n-3	1.3	2.0	2.8	3.5	4.2
C 20:4 n-3	0.4	0.3	0.4	0.4	0.3
C 18:4 n-3	0.1	0.1	0.1	0.1	0.1
C 20:2 n-6	0.3	0.3	0.3	0.2	0.2
C 20:3 n-6	0.1	0.1	0.1	0.1	0.1
C 20:4 n-6	0.5	0.5	0.5	0.4	0.4
C 20:3 n-3	0.1	0.1	0.1	0.1	0.1
C 20:5 n-3	9.2	8.3	7.3	6.3	5.4
C 22:4 n-6	0.0	0.3	0.3	0.3	0.1
C 22:5 n-3	1.4	1.3	1.1	1.0	0.9
C 22:6 n-3	11.5	10.3	8.8	7.4	6.1
Σ Polyunsaturated	29.8	30.2	30.2	30.1	30.0
Sum EPA/DHA	20.7	18.7	16.1	13.8	11.5
Sum n-3	24.0	22.5	20.6	18.8	17.1
Sum n-6	5.7	7.7	9.5	11.2	12.7

2.2. Tissues RNA isolation

PureLink™DNase Treatment and Purification Kit were used for RNA isolation. RNA was isolated from both whole body and/or liver tissue. Prior RNA isolation, salmon whole body was homogenized with dry ice. Liver or whole body samples of around 30mg were transferred into tubes together with ceramic beads and1000µl TRIzol as lysis Buffer.

Then the samples were homogenized in Precellys 24 for 2 × 20 seconds at 5000 rpm with a break of 5 seconds. The samples were centrifuged at 4^0 C and 13000 rpm for 3 minutes. Thereafter 700µl of the supernatant was transferred to the new tube and 700µl of 70% ethanol was added to clear lysate. The samples were mixed well to disperse precipitated RNA. Around 700µl of the mix was transferred into a Spin Cartridge and centrifuged at 12000rpm for 15 seconds to bind RNA to the membranes. The flow trough was discarded and this procedure was repeated with the rest of the samples. 350µl of Wash Buffer I was added to the Spin Cartridge containing the bound RNA in order to wash the column then samples were centrifuged at 12000rpm for 15 seconds at room temperature. The flow-through was discarded; and the Spin Cartridges were placed in a new collection tube. 80µl of Pure Link ^{TM}DNase solution (8µl of 10XDNase I reaction Buffer, 10µl resuspended DNase and 62µl of RNase free water) was added directly on the surface of the Spin Cartridge membrane to remove the genomic DNA. The samples were incubated for 15 minutes at room temperature. Then the samples were washed by adding 350µl Wash Buffer I to the Spin Cartridge to remove protein, genomic DNA, and salts. The Spin Cartridges were centrifuged at 12000rpm for 15 seconds at room temperature and the flow-through was discarded. The samples were washed again by adding 500µl Wash Buffer II with ethanol to the Spin Cartridge and centrifuged at 12000rpm for 15 minutes. The flow-through was discarded and the step was repeated once more.

To dry the membranes the samples were centrifuged at 12000g for 1 minute with the bound RNA and the flow-through was discarded together with the collection tubes. The Spin Cartridges was placed into a recovery tube and the purified total RNA was eluted by adding 50 µl RNase free water. The samples were incubated for 1 minute at room temperature before centrifuged at 12000rpm for 1 minute at room temperature. The eluted RNA was kept in -80 freezer until further analysis.

2.3. RNA measurement

NanoDrop 1000 Spectrophotometer was used to measure the concentration and quality of the RNA harvested. When a sample is exposed to spectrophotometer at 260nm which is a specific light absorption range for nucleic acid it start to absorb light, the more light absorbed by the sample the higher the nucleic acid concentration it have. Unlike nucleic acid protein absorb at 280nm. The 260/280nm is used as a measurement of protein contamination of RNA solution whereas 260nm/230nm ration is used as a secondary measurement of nucleic acid purity. Acceptable values for 260/280nm and 260/230nm ratios were in the range 1.8-2.0 and 1.8-2.3, respectively.

2.4. Precipitation of RNA

Samples with a too low 260/280nm ratio were subjected to purification by precipitation. The RNA samples were added 3X volume of 100% ethanol and 0.1 volume of sodium acetate. Then the samples were incubated at room temperature for 30 minutes and centrifuged at 10000 rpm and 4^0C for 10 minutes. Thereafter the supernatant was removed and the pellet was dried at room-temperature. The RNA was resuspended using 30 µl RNase free water.

2.5. cDNA synthesis

The AffinityScript QPCR cDNA Synthesis Kit was used for the conversion of mRNA to the complimentary strand of DNA (cDNA). The cDNA, was made from 500ng RNA in a reaction volume of 20 µl. 500ng RNA was added to microcentrifuge tube together with RNase free water to make a final volume of 6 µl. Then 10 µl of master mix(contains a buffer that is specifically optimized for QRT-PCR performance, and Deoxynucleotide Triphosphates (dNTPs), MgCl2) was added together with 3 µl of oligo(dT) primer and 1.0 µl of AffinityScript Reverse Transcriptase (RT) enzyme which used to synthesis a complimentary strand of DNA (cDNA). Then the cDNA synthesis was used as a template for exponential amplification using Polymerase Chain Reaction machine (PCR). The PCR machine incubate the reaction at 25°C for 5 minutes (to allow primer annealing), at 42°C for 45 minutes (to allow cDNA synthesis), and finally at 95ºC for 5 minutes (to terminate the cDNA synthesis reaction). The completed first-strand cDNA synthesis reaction was kept at -20°C for later in QPCR.

2.6. QPCR

The mated is based on DNA binding dye (SYBR Green-I). When SYBR Green-I is free in solution display relatively low fluorescence, but when bound to double-stranded DNA, its fluorescence increases by over 1000- fold. The more double stranded DNA that is present, the more binding sites there are for the dye, so fluorescence increases proportionately to DNA concentration. As the targeted DNA is amplify, and simultaneously quantify by the qPCR machine the increasing concentration of double-stranded DNA in the solution can be directly measured by the increase in fluorescence signal.

The 20 µl cDNA reaction mix was diluted 10 times in RNase free water and 4 µl of this dilution was added to the LightCycler®480 multiwell plate 96, Thereafter 0,5µl of the forward primer and 0,5 µl of the reverse primer (final concentration of 0.5 µM) together with 5 µl of SYBR Green-I Master were added. All samples were analyses in parallel and an interplate calibration was included in all plates. The qPCR reaction was run on a LightCycler®480 at the following conditions 95^0C for 5 minutes, 45cycles of 95^0C for 15 seconds and 60^0C for 1 minute. A melting point analysis (95^0C for 5 seconds, 65^0C for 1 minute and heating slowly to 97^0C), was included to confirm the existence of only one PCR product.

Efficiency of the primers was tested on a 2x serial dilution of a cDNA mixture from all samples. Acceptable primer efficiencies were between 1.8 and 2.0. Three genes were evaluated as reference genes; Rpol, Ef1α and Etif by using a program called Bestkeeper. Rpol was identified as the most stable.

2.7. Lipid extraction

Lipid extraction was performed based on the method described by (Folch et al. 1957). The whole body without liver was homogenized on dry ice and was kept in -80 freezer. With the exception of the first two sampling which the entire body was homogenized due to small size of the fish to isolate liver.

Around 2 g of homogenized samples were transferred into Erlenmeyer flask. 6ml of 0.9% NaCl, and 50ml Chloroform: Methanol in ratio of 2:1 was added together with 0.7mg/l Butylert hydroxyl toluen as an antioxidant. The solution was homogenized for 60 seconds with a knife homogenizer. After added 6ml of 0.9% NaCl and homogenized for 5 second

it was separated into two phases, the polar water soluble phase and the organic unpolar chloroform phase. The lower phase is chloroform: Methanol: water in the ratio of 86:14:1 and it contained most of the lipids. The upper phase contains Chloroform: Methanol: water in the ratio of 3: 48: 47 and it contained most of the water soluble components.

The homogenate was allowed to pass through a funnel with a cotton filter to a graded cylinder. The flasks were capped to avoid evaporation and kept in freezer until the next day. The upper water/methanol phase and any protein were removed carefully. The chloroform phase was transferred to a tube for later analyses of fat percentage and fatty acid composition.

2.8. Measurement of fat percentage

20ml of the lower chloroform phase was pipetted to a previously weighed 25ml empty beaker. The Chloroform in the beaker was left to be dried in an electric stove at a temperature of around 60^0C until all the organic solvent was evaporated, then the beakers are transferred to an oven for further drying at 103^0C for 30 minutes.

The total fat weighed was calculated as follow; $\%\,\text{fat} = \dfrac{\text{g fat} \times 100}{\dfrac{\text{I} \cdot \text{U}}{37.5}}$

g fat = evaporated sample in beaker

100 = %

I = weight of the sample in g

U = Pipetted chloroform extract (20ml) in beaker

37.5 = Total volume of solvent (33.3 mL *100/89) = 37.5 mL

(Chloroform in extract solution = 50*2/3 = 33.3 mL)

2.9. Fatty acid composition

The Chloroform phase samples kept in the freezer were used for the Fatty acid composition analysis. The samples were thawed and evaporated at 60^0C with nitrogen over flow. The lipid were trans-esterified to fatty acid methyl esters (FAME) by heating the residual lipid extract with excess of methanol in the presence of acid-catalyst using 2 mL benzene as a solvent, 2 mL metanolic-HCl, and 0.2 mL dimetoxypropan. The tubes

were capped and mixed well, and the samples were kept at room temperature until the next day. 2 ml of Hexan was added as a solvent together with 3ml of 6%$NaHCO_3$ as a neutralizer. The upper organic phase of the samples was transferred to a new tube and evaporated at 60^0C with a nitrogen overflow. The lipid sample was dissolved in 1 ml hexane prior to gas-liquid chromatography (GC) analysis.

2.10. Gas Chromatography (G**C)**

GC is an instrument used to separate chemical in a complex sample. The sample was introduced via a syringe into a heated small chamber, which facilitate the volatilization of the sample that can be carried into the column by the carrier gas such as Helium. Hawlett Packard 6890 Column SGE 60 m *0,25mm ID BPX 70- 0, 25µm, was used for the analysis. The hexane dissolved FAME was introduced by split-injection (50:1) at a temperature of 300^0C. The temperature was programmed to start with 50^0C for 1.2 minutes and further increase to 4^0C/minute to 170^0C, $0{,}5^0$C/minute to 200^0C, and 10^0C/minute to 300^0C. The fatty acid was detected by flame ionization detector (FID). The result appeared in a graph with the y-axis explain the detected chemical respond and the x-axis which tell the retention time. HP Chem Station software was used to analyze the quantity of each fatty acid present by measuring the area of the peak in related to a particular fatty acid. The identification of major pick was performed by using methyl ester standards GLC-85 fatty acid.

2.11. Calculation and Statistical analysis

The specific growth rate (*SGR*) was calculated using the formula $100*(\ln V_1 - \ln V_0)/t$ where V_0 is the initial average weight and V_1 is the final average weight after t days.

The relative quantification of a target gene in comparison to a reference gene was calculated using the mathematical model presented by Roche Diagnostics.

$$R = \frac{(E_{target})^{\Delta CP_{target}\,(control - sample)}}{(E_{ref})^{\Delta CP_{ref}\,(control - sample)}}$$

Where E_{target} is the real-time PCR efficiency of target gene transcript; E_{ref} is the real-time PCR efficiency of a reference gene transcript; ΔCP_{target} is the CP deviation of control –

sample of the target gene transcript; ΔCP_{ref} = CP deviation of control – sample of reference gene transcript. And differences were ranked by Duncan's multiple range tests.

All data, from the fatty acid composition, were subjected to the one-way analysis of variance (ANOVA). For the factor ''diet'', Differences were ranked by Duncan's multiple range tests. We used the Software SAS 9.3, and Minitab® 15.1.30.0.education version. Significance level was set to $P \leq 0.05$.

3. RESULT

3.1. **Fish growth and performance**

The fish grew from an initial weights of 0.179g ± 0.01 to a final weight of 42.0g ± 3.2 and 36.4g ± 1.5 for the high and low desaturase families respectively (Figure 1). There were no major differences in the SGR between the dietary groups within each trial period (Table 1). However there were significant differences in SGR between the two families in the last sampling period (Figure 2). In total mortality was < 12% for all dietary treatment groups in the two families of fish. (Table 2).

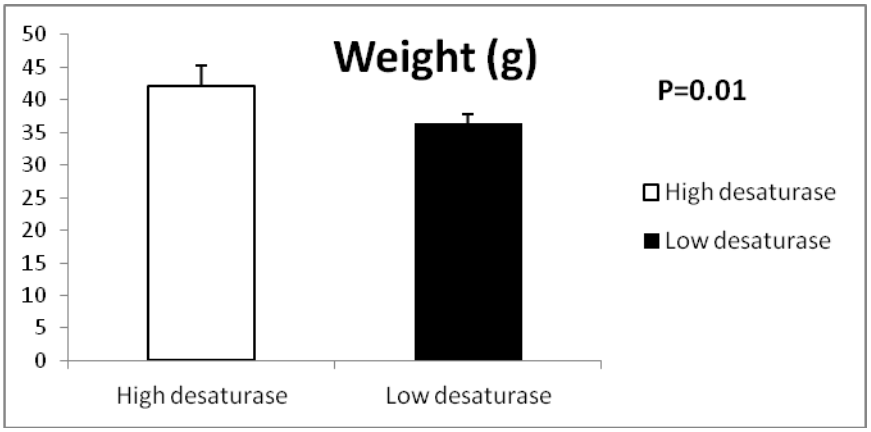


Figure 3. Final weight of the high and low desaturase families at day 182. Data are presented as means of the 5 dietary groups within each family (n = 5) ± SEM.

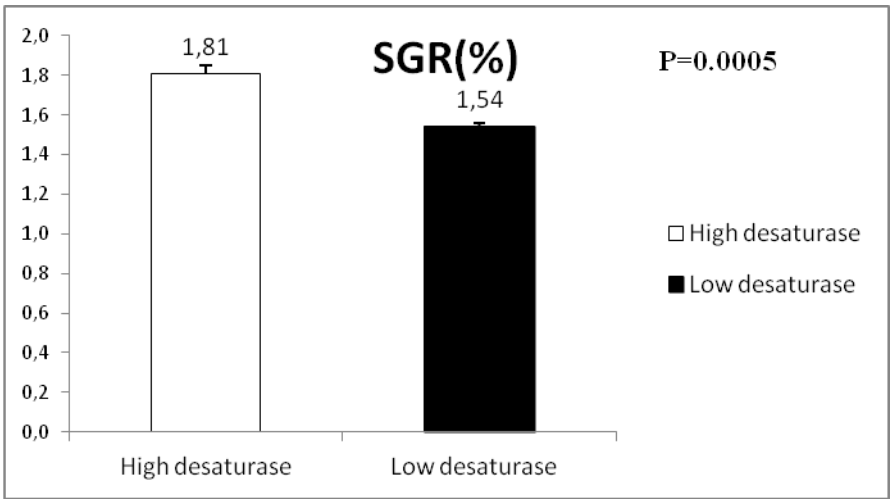


Figure 4. Specific growth rate (SGR %) for the high and low desaturase families during the last period of the trial (118 days – 182 days). Data are presented as means of the 5 dietary groups within each family (n = 5) ± SEM.

Table 3 specific growth rate (SGR %) for the different dietary groups in high and low desaturase families. A regression analyses was performed and revealed no significant differences between the dietary groups within each family

Family	SGR%/day				
High Desaturase	28dg	57dg	91 dg	118 dg	182 dg
100%FO	4.197	4.066	3.273	3.836	1.615
75%FO,25%RO	4.130	4.058	2.857	3.953	1.870
50%FO,50%RO	3.796	4.329	3.067	3.698	1.755
25%FO,75%RO	4.094	4.141	2.882	3.577	1.826
100%RO	3.637	4.231	3.226	3.492	1.758
Low Desaturase					
100%FO	3.411	4.725	2.987	3.672	1.483
75%FO,25%RO	3.398	4.472	3.301	3.677	1.501
50%FO,50%RO	3.936	4.225	3.152	3.802	1.479
25%FO,75%RO	3.285	4.707	3.138	3.678	1.556
100%RO	3.926	4.550	2.960	3.588	1.515

Table 4 mortalities of both the high and low desaturase families of fish fed the experimental diets (means ± SEM).

Family	Mortality			
High desaturase	0-4 weeks	5-8weeks	9-14 weeks	15-26 weeks
100%FO	1.0 ±0.58	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00
75%FO,25%RO	0.0 ±0.00	1.0 ±0.35	3.0 ±0.89	2.0 ±0.58
50%FO,50%RO	5.0 ±0.41	2.0 ±0.58	2.0 ±0.55	2.0 ±0.58
25%FO,75%RO	2.0 ±0.58	0.0 ±0.00	0.0 ±0.00	2.0 ±0.58
100%RO	5.0 ±1.26	0.0 ±0.00	2.0 ±0.55	2.0 ±0.58
Low desaturase				
100%FO	2.0 ±0.58	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00
75%FO,25%RO	3.0 ±1.00	4.0 ±1.53	2.0 ±0.55	2.0 ±0.58
50%FO,50%RO	6.0 ±1.10	1.0 ±0.71	2.0 ±0.55	0.0 ±0.00
25%FO,75%RO	5.0 ±0.96	2.0 ±0.58	0.0 ±0.00	4.0 ±0.84
100%RO	4.0 ±0.52	0.0 ±0.00	2.0 ±0.55	0.0 ±0.00

3.1.1. Fatty acid composition:

The fatty acid composition of Atlantic Salmon following 182 days of feeding experimental diets are shown in Tables 6, 7, 8, 9 and 10. From each family five fish in each dietary group were used for the analysis of whole body fatty acid composition. Fatty acid composition of the body was closely correlated to the fatty acid composition of the diet. As the main component of the RO, the 18:1n-9 fatty acid account for a large part of the sum of monounsaturated fatty acid in the whole body (Figure 5). The 18:1n-9 level in the body increase with increased inclusion level of RO in the feed in both the high and low desaturase families. The same is true for the n-6 fatty acids which increase in whole body lipids with increased inclusion level of RO. A reduction of n-3 PUFA level was observed with increased inclusion of RO in both the high and low desaturase groups.

Two-way ANOVA: C18:1n-9 versus Diet; SAMPLING

Source	DF	P
Diet	4	0,000
Sampling	4	0,000
Interaction	16	0,000

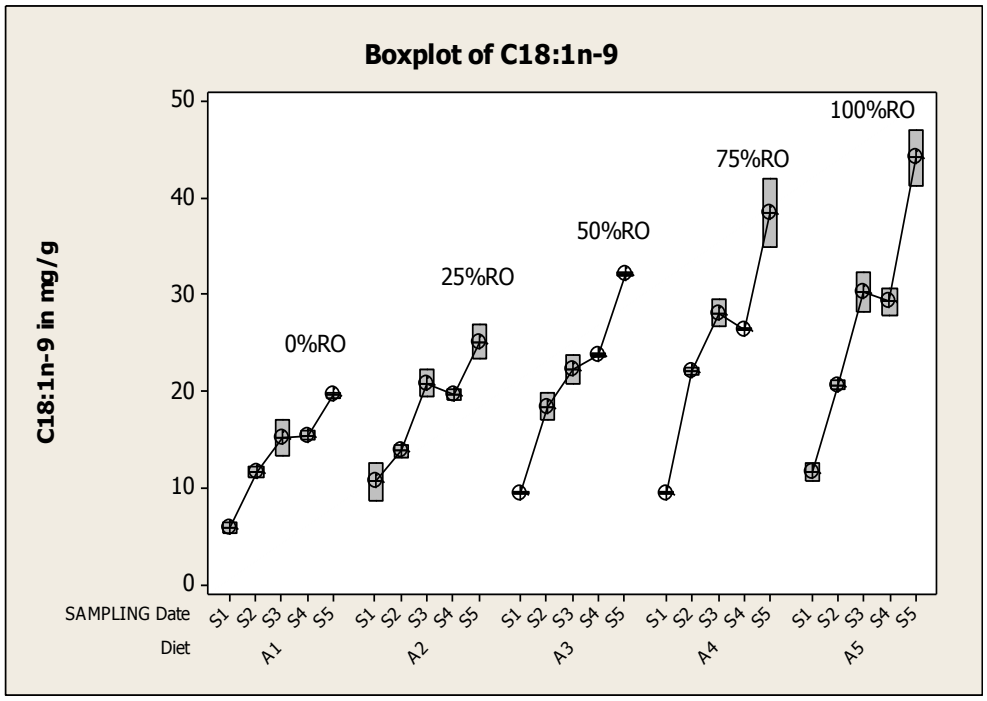
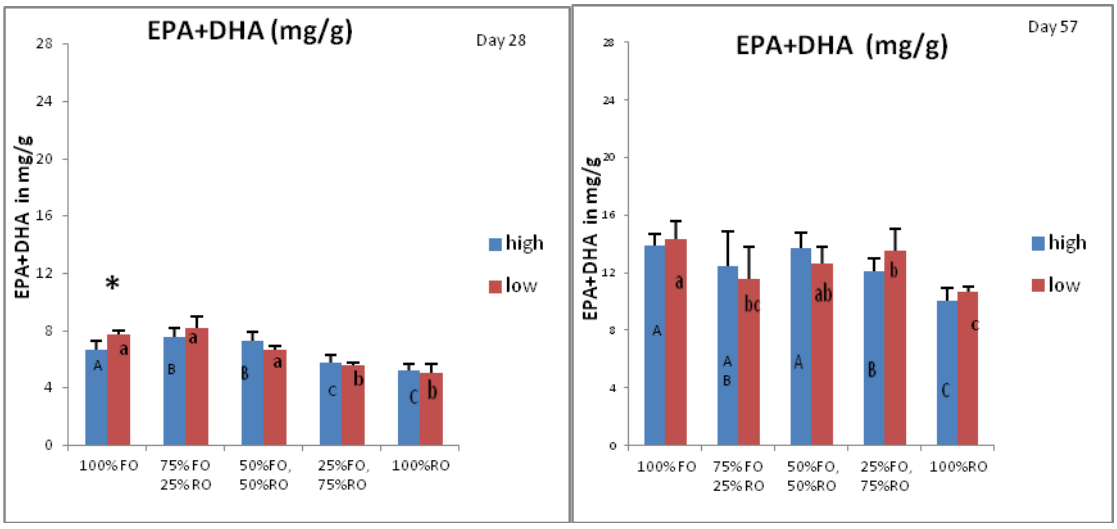


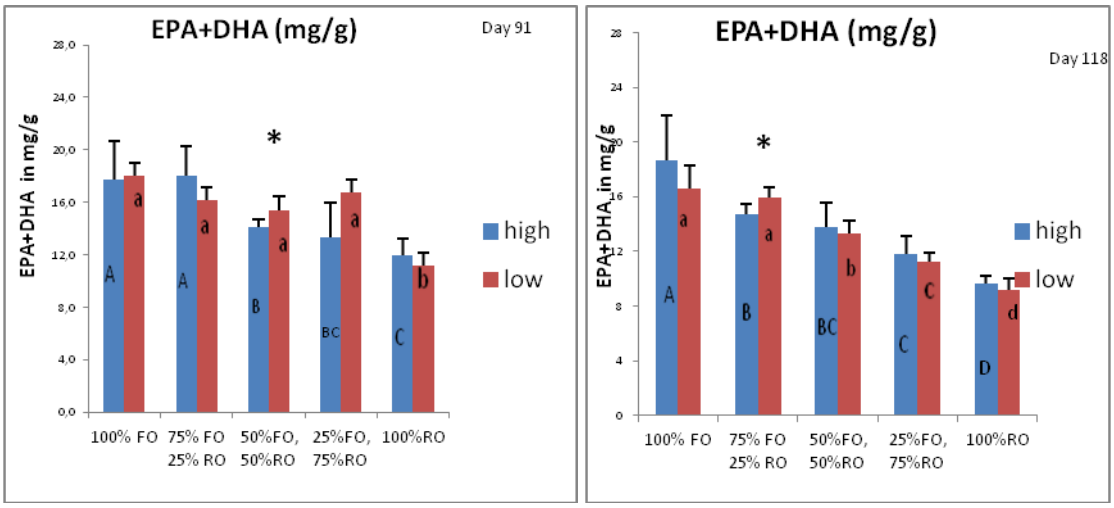
Figure 5 Boxplot of the oleic acid(C 18:1n-9) level in whole body lipids for both high and low desaturase families of fish fed different inclusion level of FO and RO. (S1, S2 S3, S4, and S5 stands for the 28, 57, 91, 118 and 182 day sampling periods) and (A1, A2, A3, A4 and A5 stands for 0%RO, 25%RO, 50%RO, 75%RO and 100%RO respectively). Data are presented as means of the same dietary groups of the two families.

3.1.2. Level of EPA and DHA

The whole body EPA and DHA level were followed at 5 sampling points during the 182 days feeding experiment (Figure 6). The EPA and DHA levels were higher in fish feed 100% FO than in those fed increasing dietary levels of RO.

At the end of the experiment, the high desaturase family fed 75%, 50% and 25% FO had relatively more EPA and DHA than the low desaturase family. And we found relatively higher total fatty acid (mg/g) in these dietary groups of fish in higher desaturase family than the low desaturase family (Table 10).





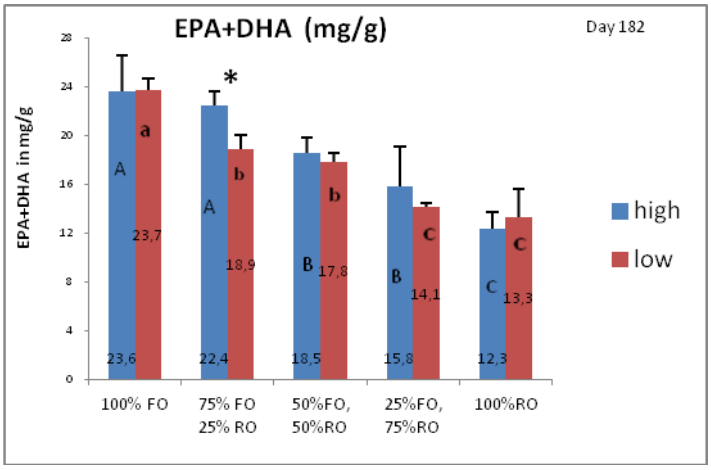


Figure 6 EPA+DHA in mg/g of whole body at 28, 57, 91,118 and 182 sampling days of the "high desaturase families" and the "low desaturase families". Data are means of fish within each tank ± SEM (n=5). Different uppercase and lower case letters indicate significant differences among diets within each family at $p \leq 0.05$. *indicates significant difference between family within diet at $P \leq 0.05$.

Table 5.Fatty acid composition of whole body at day 28 in high desaturase families and low desaturase families. The fatty acid compositions were measured in whole body homogenates of fish fed different inclusion levels of FO and RO diets. The quantity of each fatty acid is given in mg/g. The value given are means± SEM (n=5). Different letter indicated significant differences ($p \leq 0.05$) within diet between the two families.

Family	High	Low	High	Low	High	Low	High	Low	High	Low
Feed type	100FO		75% FO,25% RO		50% FO,50% RO		25% FO,75% RO		100% RO	
Total fatty acid mg/g	28,7 ± 3,38	33,6 ± 1,23	36,7 ± 3,69	49,4 ± 18,74	37,2 ± 1,88	36,4 ± 6,29	33,5 ± 4,16	33,0 ± 1,24	33,4 ± 1,87	36,8 ± 4,13
C 14:0	1,1 ± 0,17	1,3 ± 0,07	1,3 ± 0,14	1,8 ± 0,87	1,2 ± 0,09	1,2 ± 0,23	0,9 ± 0,20	1,0 ± 0,05	0,9 ± 0,07	1,0 ± 0,16
C 16:0	4,8 ± 0,57	5,2 ± 0,12	5,2 ± 0,51	6,6 ± 2,39	5,2 ± 0,30	4,9 ± 0,79	4,4 ± 0,37	4,4 ± 0,16	4,4 ± 0,33	4,5 ± 0,52
C 18:0	1,0 ± $0,11^{b}$	1,1 ± $0,02^{a}$	1,1 ± 0,08	1,5 ± 0,60	1,2 ± 0,07	1,1 ± 0,17	1,0 ± 0,07	1,1 ± 0,04	1,1 ± 0,09	1,1 ± 0,12
Σ Saturated	6,9 ± 0,25	7,6 ± 0,05	7,6 ± 0,23	10,0 ± 0,97	7,5 ± 0,13	7,2 ± 0,34	6,4 ± 0,15	6,5 ± 0,07	6,4 ± 0,15	6,6 ± 0,22
C 16:1 n-7	1,6 ± 0,22	1,7 ± 0,11	2,3 ± 0,30	3,1 ± 1,19	1,5 ± 0,10	1,6 ± 0,26	1,2 ± 0,21	1,3 ± 0,07	1,2 ± 0,04	1,3 ± 0,15
C 18:1 n-9	5,6 ± 0,83	6,4 ± 0,60	8,8 ± 1,20	12,2 ± 5,08	9,6 ± 0,68	10,3 ± 1,87	10,1 ± 1,34	9,4 ± 0,68	10,8 ± $0,52^{b}$	12,5 ± $1,52^{a}$
C 20:1 n-9	2,2 ± 0,42	2,2 ± 0,19	2,5 ± 0,31	3,5 ± 1,35	2,1 ± 0,19	2,4 ± 0,37	1,7 ± 0,21	1,5 ± 0,09	1,3 ± $0,08^{b}$	1,6 ± $0,23^{a}$
C 22:1 n-11	1,5 ± 0,25	1,5 ± 0,08	1,5 ± 0,21	1,9 ± 0,91	1,3 ± 0,09	1,5 ± 0,20	1,0 ± 0,15	1,0 ± 0,08	0,9 ± $0,06^{b}$	1,1 ± $0,13^{a}$
Σ Monounsaturated	11,0 ± 0,28	11,8 ± 0,24	15,0 ± 0,47	20,7 ± 1,98	14,5 ± 0,28	15,8 ± 0,80	14,0 ± 0,58	13,2 ± 0,30	14,2 ± 0,23	16,5 ± 0,68
C 18:2 n-6	1,4 ± 0,22	1,7 ± 0,10	2,3 ± 0,24	3,1 ± 1,07	2,6 ± 0,42	2,8 ± 0,57	3,0 ± 0,47	2,9 ± 0,18	3,3 ± 0,22	3,8 ± 0,50
C 20:5 n-3	1,5 ± $0,14^{b}$	1,8 ± $0,10^{a}$	1,7 ± 0,16	2,4 ± 0,91	1,6 ± 0,06	1,4 ± 0,18	1,2 ± 0,13	1,2 ± 0,07	1,1 ± 0,09	1,0 ± 0,15
C 22:6 n-3	5,2 ± $0,49^{b}$	6,0 ± $0,17^{a}$	5,8 ± 0,48	7,3 ± 2,70	5,7 ± 0,14	5,3 ± 0,73	4,6 ± 0,36	4,4 ± 0,11	4,2 ± 0,36	4,0 ± 0,47
Σ Polyunsaturated	8,0 ± 0,18	9,5 ± 0,04	9,8 ± 0,17	12,8 ± 0,99	10,0 ± 0,19	9,5 ± 0,29	8,8 ± 0,17	8,5 ± 0,06	8,6 ± 0,13	8,9 ± 0,19
Σ n-3	7,6 ± $0,68^{b}$	8,9 ± $0,32^{a}$	8,8 ± 0,70	11,4 ± 4,08	8,8 ± 0,23	8,1 ± 1,14	7,3 ± 0,70	7,1 ± 0,23	6,8 ± 0,53	6,7 ± 0,87
Σ n-6	1,7 ± $0,24^{b}$	2,4 ± $0,13^{a}$	2,9 ± 0,28	3,8 ± 1,32	3,3 ± 0,45	3,4 ± 0,68	3,5 ± 0,52	3,4 ± 0,19	3,8 ± 0,24	4,3 ± 0,45
EPA+DHA	6,6 ± $0,62^{b}$	7,7 ± $0,25^{a}$	7,5 ± 0,62	9,7 ± 3,50	7,3 ± 0,18	6,7 ± 0,91	5,8 ± 0,49	5,6 ± 0,16	5,2 ± 0,45	5,0 ± 0,61

Table 6. Fatty acid composition of whole body at day 57 in high desaturase families and low desaturase families. The fatty acid compositions were measured in whole body homogenates of fish fed different inclusion levels of FO and RO diets. The quantity of each fatty acid is given in mg/g. The value given are means+ SEM (n=5). Different letter indicated significant differences ($p \leq 0.05$) within diet between the two families.

Family	High	Low	High	Low	High	Low	High	Low	High	Low
Feed type	100FO		75% FO,25% RO		50% FO,50% RO		25% FO,75% RO		100% RO	
Total fatty acid mg/g	69,2 ± 6,36	71,2 ± 8,57	66,2 ± 12,84	66,7 ± 6,98	75,2 ± 5,25	72,7 ± 7,62	76,9 ± $5,94^{b}$	83,7 ± $9,22^{a}$	67,4 ± 4,73	70,3 ± 4,90
C 14:0	3,3 ± 0,30	3,5 ± 0,45	3,0 ± 0,52	3,1 ± 0,37	3,2 ± 0,28	3,1 ± 0,33	3,2 ± 0,24	3,6 ± 0,34	2,6 ± 0,27	2,7 ± 0,21
C 16:0	10,6 ± 0,90	11,3 ± 1,20	10,1 ± 2,15	10,2 ± 0,97	11,1 ± 0,93	10,8 ± 1,10	11,4 ± 0,88	11,7 ± 1,08	9,7 ± 0,54	9,7 ± 0,52
C 18:0	2,3 ± 0,30	2,4 ± 0,30	2,1 ± 0,53	2,1 ± 0,20	2,4 ± 0,20	2,3 ± 0,26	2,5 ± 0,20	2,6 ± 0,29	2,2 ± 0,15	2,3 ± 0,25
Σ Saturated	16,1 ± 0,35	17,2 ± 0,48	15,2 ± 0,94	15,3 ± 0,40	16,6 ± 0,40	16,3 ± 0,47	17,1 ± 0,39	17,9 ± 0,44	14,5 ± 0,20	14,6 ± 0,17
C 16:1 n-7	4,1 ± 0,69	4,7 ± 0,59	4,1 ± 0,96	4,1 ± 0,44	3,9 ± 0,47	3,9 ± 0,43	3,9 ± 0,27	3,8 ± 0,51	2,9 ± 0,32	2,6 ± 0,28
C 18:1 n-9	11,9 ± 1,84	11,4 ± 1,40	15,8 ± 3,35	13,2 ± 1,76	20,3 ± 1,94	16,9 ± 1,46	22,2 ± 2,99	23,0 ± 3,11	20,2 ± 1,68	21,7 ± 1,76
C 20:1 n-9	4,3 ± 0,29	4,3 ± 0,52	3,8 ± 0,57	3,7 ± 0,38	3,8 ± 0,38	3,7 ± 0,43	3,1 ± 0,57	3,6 ± 0,65	2,5 ± $0,23^{b}$	3,0 ± $0,32^{a}$
C 22:1 n-11	3,6 ± 0,59	3,4 ± 0,43	2,7 ± 0,54	3,1 ± 0,09	2,5 ± 1,42	3,0 ± 0,35	2,7 ± 0,33	2,8 ± 0,35	2,2 ± 0,17	2,1 ± 0,21
Σ Monounsaturated	23,9 ± 0,68	23,7 ± 0,45	26,5 ± 1,34	24,1 ± 0,74	30,5 ± 0,76	27,5 ± 0,53	31,8 ± 1,30	33,2 ± 1,31	27,8 ± 0,72	29,4 ± 0,74
C 18:2 n-6	3,7 ± 0,30	3,8 ± 0,39	4,2 ± 0,72	4,2 ± 0,41	5,5 ± 0,40	5,3 ± 0,59	6,3 ± 0,51	6,7 ± 0,73	6,1 ± 0,29	6,4 ± 0,49
C 18:3 n-3	1,0 ± 0,08	0,9 ± 0,11	1,2 ± 0,18	1,1 ± 0,11	1,6 ± 0,10	1,5 ± 0,17	1,8 ± 0,14	2,1 ± 0,23	1,8 ± 0,11	2,0 ± 0,18
C 20:5 n-3	3,4 ± 0,43	3,5 ± 0,49	3,1 ± 0,51	2,8 ± 0,57	3,6 ± 1,15	2,9 ± 0,33	2,8 ± $0,14^{b}$	3,3 ± $0,42^{a}$	2,3 ± 0,28	2,5 ± 0,13
C 22:5n-3	1,1 ± 0,19	1,2 ± 0,21	1,0 ± 0,16	1,8 ± 1,18	1,0 ± 0,09	1,0 ± 0,13	0,9 ± 0,04	1,1 ± 0,10	0,6 ± 0,31	0,8 ± 0,05
C 22:6 n-3	10,5 ± 0,60	10,8 ± 0,84	9,4 ± 1,87	8,7 ± 1,73	10,1 ± 0,59	9,7 ± 0,87	9,4 ± 0,72	10,3 ± 1,10	7,8 ± 0,63	8,1 ± 0,34
Σ Polyunsaturated	19,7 ± 0,20	20,2 ± 0,28	18,8 ± 0,70	18,7 ± 0,65	21,9 ± 0,44	20,4 ± 0,31	21,1 ± 0,29	23,4 ± 0,40	18,6 ± 0,19	19,8 ± 0,17
Σ n-3	16,3 ± 1,16	17,0 ± 1,62	14,8 ± 2,72	15,0 ± 1,87	16,9 ± 1,02	15,7 ± 1,49	15,3 ± 1,06	17,0 ± 2,07	12,7 ± 1,41	13,6 ± 0,76
Σ n-6	5,0 ± 0,64	5,2 ± 0,77	5,4 ± 1,03	5,6 ± 0,64	7,1 ± 0,45	6,8 ± 0,75	7,9 ± 0,57	7,8 ± 0,80	7,4 ± 0,52	7,5 ± 0,50
EPA+DHA	13,9 ± 0,74	14,3 ± 1,29	12,5 ± 2,38	11,6 ± 2,25	13,7 ± 1,05	12,6 ± 1,16	12,1 ± 0,84	13,5 ± 1,51	10,1 ± 0,89	10,6 ± 0,44

Table7. Fatty acid composition of whole body at day 91 in high desaturase families and low desaturase families. The fatty acid compositions were measured in whole body homogenates of fish fed different inclusion levels of FO and RO diets. The quantity of each fatty acid is given in mg/g. The value given are means+ SEM (n=5). Different letter indicated significant differences ($p \leq 0.05$) within diet between the two families.

Family	High	Low	High	Low	High	Low	High	Low	High	Low
Feed type	100FO		75% FO,25% RO		50% FO,50% RO		25% FO,75% RO		100% RO	
Total fatty acid mg/g	90,0 ± 18,67	92,9 ± 15,09	104,9± 14,68	93,2 ± 6,85	85,3 ± 4,90	96,3 ± 8,39	95,7 ± 27,75	98,3 ± 12,82	94,7 ± 11,74	90,9 ± 7,28
C 14:0	3,9 ± 0,82	4,1 ± 0,68	4,2 ± 0,61	3,8 ± 0,18	3,3 ± $0,18^{b}$	3,7 ± $0,33^{a}$	3,3 ± 0,91	3,4 ± 0,48	3,0 ± 0,40	3,0 ± 0,19
C 16:0	13,4 ± 2,88	13,9 ± 2,17	14,9 ± 2,04	13,7 ± 0,80	11,8 ± 0,53	13,2 ± 1,29	12, 9± 3,77	13,1 ± 1,70	12,1 ± 1,46	11,7 ± 0,81
C 18:0	2,9 ± 0,79	3,0 ± 0,50	3,5 ± 0,39	3,1 ± 0,36	2,7 ± 0,14	3,1 ± 0,45	3,2 ± 1,28	3,5 ± 0,48	3,1 ± 0,49	3,1 ± 0,24
Σ Saturated	20,2 ± 1,20	21,0 ± 0,92	22,6 ± 0,90	20,5 ± 0,32	17,8 ± 0,21	20,0 ± 0,52	19,4 ± 1,55	20,0 ± 0,70	18,1 ± 0,59	17,8 ± 0,34
C 16:1 n-7	5,8 ± 1,36	6,1 ± 1,08	6,0 ± 0,94	5,7 ± 0,22	3,8 ± $0,27^{b}$	4,3 ± $0,41^{a}$	3,9 ± 1,30	4,3 ± 0,65	2,9 ± 0,33	3,3 ± 0,39
C18:1 n-7	4,5 ± 0,79	4,1 ± 0,67	4,2 ± 0,64	4,1 ± 0,18	2,9 ± 0,15	3,2 ± 0,25	2,9 ± 0,54	4,0 ± 1,09	2,9 ± 0,35	2,1 ± 0,22
C 18:1 n-9	14,7 ± 3,45	16,0 ± 2,79	22,2 ± 3,45	19,6 ± 1,83	20,5 ± $1,17^{b}$	24,3 ± $2,71^{a}$	27,9 ± 9,59	27,3 ± 3,38	30,5 ± 4,24	29,3 ± 2,92
C 20:1 n-9	6,8 ± 1,40	5,6 ± 0,96	5,9 ± 0,88	5,3 ± 0,36	4,2 ± 0,25	4,8 ± 0,39	4,2 ± 1,21	4,8 ± 0,67	3,6 ± 0,48	3,7 ± 0,50
C 20:1 n-11	1,2 ± 0,23	1,4 ± 0,19	1,5 ± 0,21	1,2 ± 0,06	1,2 ± $0,06^{b}$	1,3 ± $0,07^{a}$	1,1 ± 0,28	1,4 ± 0,28	1,1 ± 0,13	1,2 ± 0,22
C 22:1 n-11	4,4 ± 0,98	4,3 ± 0,70	4,6 ± 0,73	4,0 ± 0,24	3,5 ± $0,22^{b}$	4,0 ± $0,31^{a}$	3,6 ± $1,20^{a}$	1,3 ± $1,19^{b}$	3,0 ± 0,37	3,6 ± 0,70
Σ Monounsaturated	37,4 ± 1,11	37,5 ± 0,90	44,4 ± 1,16	40,0 ± 0,67	36,1 ± 0,41	41,9 ± 1,00	43,5 ± 3,57	43,2 ± 1,11	44,1 ± 1,60	43,1 ± 1,04
C 18:2 n-6	4,8 ± 0,96	4,9 ± 0,77	7,0 ± 1,18	5,3 ± 2,19	6,3 ± 1,48	7,8 ± 0,52	8,5 ± 2,16	8,8 ± 1,14	9,9 ± 1,15	9,3 ± 0,80
C 18:3 n-3	1,2 ± 0,20	1,1 ± 0,19	1,7 ± 0,29	1,5 ± 0,08	1,9 ± 0,13	2,2 ± 0,13	2,3 ± 0,67	2,5 ± 0,33	2,8 ± 0,33	2,7 ± 0,23
C 20:5 n-3	3,6 ± 0,60	3,7 ± 0,50	3,7 ± $0,54^{a}$	3,1 ± $0,18^{b}$	3,0 ± 0,19	3,2 ± 0,20	2,5 ± $0,23^{b}$	5,6 ± $2,30^{a}$	2,4 ± $0,31^{a}$	1,6 ± $0,33^{b}$
C 22:5 n-3	1,2 ± 0,22	1,4 ± 0,22	1,4 ± 0,16	1,2 ± 0,09	1,0 ± 0,07	1,1 ± 0,12	0,8 ± 0,07	1,0 ± 0,46	0,8 ± 0,12	0,9 ± 0,30
C 22:6 n-3	14,1 ± 2,36	14,3 ± 2,01	14,4 ± 1,72	13,1 ± 0,68	11,1 ± $0,45^{b}$	12,2 ± $0,96^{a}$	10,8 ± 2,48	11,1 ± 1,19	9,6 ± 0,95	9,6 ± 0,73
Σ Polyunsaturated	24,9 ± 0,89	25,4 ± 0,75	28,1 ± 0,65	24,2 ± 0,90	23,2 ± 0,59	26,6 ± 0,36	24,9 ± 1,12	29,2 ± 0,78	25,4 ± 0,45	24,2 ± 0,27
Σ n-3	20,1 ± 3,38	21,3 ± 3,00	22,0 ± 2,81	19,5 ± 1,05	17,6 ± $0,81^{b}$	19,4 ± $1,40^{a}$	17,0 ± 3,30	20,5 ± 3,88	16,0 ± 1,78	15,0 ± 0,87
Σ n-6	5,7 ± 1,11	5,8 ± 0,94	8,1 ± 1,33	6,3 ± 2,26	7,8 ± 1,86	9,6 ± 0,71	10,2 ± 2,79	10,1 ± 1,30	11,9 ± 1,33	10,4 ± 0,89
EPA+DHA	17,7 ± 2,95	18,0 ± 2,50	18,0 ± 2,20	16,2 ± 0,85	14,1 ± $0,61^{b}$	15,4 ± $1,14^{a}$	13,3 ± 2,68	16,7 ± 3,38	12,0 ± 1,25	11,2 ± 0,55

Table 8. Fatty acid composition of whole body at day 118 in high desaturase families and low desaturase families. The fatty acid compositions were measured in whole body homogenates of fish fed different inclusion levels of FO and RO diets. The quantity of each fatty acid is given in mg/g . The value given are means± SEM (n=5). Different letter indicated significant differences ($p \leq 0.05$) within diet between the two families.

Family	High	Low	High	Low	High	Low	High	Low	High	Low
Feed type	100FO		75% FO,25% RO		50% FO,50% RO		25% FO,75% RO		100% RO	
Total fatty acid mg/g	96.4± 15.80	84.2± 11.2	86.5± 5.21	89.0 ± 4.68	89.3± 14.4	89.9 ± 6.31	87.32± 13.6	86.4 ± 6.92	86.8± 6.81	79.2 ± 8.93
C 14:0	3.9 ± 0.69	3.5 ± 0.47	3.2 ± 0.18	3.3 ± 0.17	3.0 ± 0.5	2.9 ± 0.22	2.6 ± 0.41	2.6 ± 0.17	2.3 ± 0.15	2.1 ± 0.26
C 16:0	14.1 ± 2.53	12.6 ± 1.81	12.3 ± 0.65	12.7 ± 0.44	12.2 ± 2.1	12.2 ± 0.79	11.6 ± 1.94	11.5 ± 0.93	11.0 ± 1.08	10.4 ± 1.28
C 18:0	3.4 ± 0.55	3.0 ± 0.51	3.0 ± 0.18	3.2 ± 0.17	3.1 ± 0.5	3.3 ± 0.28	3.1 ± 0.62	3.1 ± 0.36	3.1 ± 0.42	2.9 ± 0.41
Σ Saturated	21.4 ± 1.11	19.1 ± 0.76	18.5 ± 0.27	19.1 ± 0.16	18.3 ± 0.9	18.4 ± 0.31	17.4 ± 0.83	17.3 ± 0.39	16.4 ± 0.48	15.4 ± 0.55
C 16:1 n-7	6.4 ± 1.10	5.4 ± 0.83	5.0 ± 0.42	4.9 ± 0.12	4.2 ± 1.0	4.4 ± 0.31	3.3 ± 0.45	3.4 ± 0.29	2.6 ± 0.34	2.3 ± 0.38
C18:1 n-7	3.9 ± 0.67	3.3 ± 0.43	3.4 ± 0.17	3.3 ± 0.19	3.1 ± 0.5	3.2 ± 0.24	2.8 ± 0.40	2.9 ± 0.22	2.6 ± 0.17	2.3 ± 0.24
C 18:1 n-9	17.3 ± 3.24	15.0 ± 2.52	19.2 ± 1.58	20.2 ± 1.4	23.6 ± 3.9	24.4 ± 1.96	27.2 ± 5.13	26.8 ± 2.48	30.7 ± 2.98	27.9 ± 3.21
C 20:1 n-9	6.3 ± 1.10	5.3 ± 0.73	5.0 ± 0.33	5.1 ± 0.33	4.3 ± 0.8	4.4 ± 0.34	3.8 ± 0.62	3.6 ± 0.29	3. 2 ± 0.24	2.9 ± 0.33
C 22:1 n-11	4.5 ± 0.80	3.9 ± 0.51	3.5 ± 0.21	3.7 ± 0.26	3.3 ± 0.6	3.2 ± 0.23	2.8 ± 0.42	2.7 ± 0.20	2.3 ± 0.20	2.1 ± 0.26
Σ Monounsaturated	38.4 ± 1.05	33.0 ± 0.86	36.2 ± 0.59	37.2 ± 0.53	38.6 ± 1.5	39.5 ± 0.75	39.9 ± 2.09	39.3 ± 1.00	41.4 ± 1.23	37.5 ± 1.30
C 18:2 n-6	4.6 ± 0.78	4.1 ± 0.48	5.6 ± 0.30	5.8 ± 0.38	7.0 ± 1.1	7.1 ± 0.54	8.1 ± 1.19	7.9 ± 0.74	9.3 ± 0.66	8.4 ± 0.82
C 18:3 n-3	1.1 ± 0.18	1.2 ± 0.14	1.4 ± 0.09	1.4 ± 0.18	1.9 ± 0.3	2.0 ± 0.13	2.2 ± 0.33	2.2 ± 0.22	2.6 ± 0.17	2.3 ± 0.21
C 20:5 n-3	3.9 ± 0.75	3.4 ± 0.25	2.9 ± 0.12^{b}	3.4 ± 0.31^{a}	2.5 ± 0.2	2.5 ± 0.21	2.3 ± 0.28	2.0 ± 0.14	1.5 ± 0.11	1.7 ± 0.16
C 22:5 n-3	1.5 ± 0.29	1.3 ± 0.14	1.1 ± 0.06	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.07	0.8 ± 0.10	0.7 ± 0.08	0.6 ± 0.06	0.5 ± 0.15
C 22:6 n-3	14.7 ± 2.58	13.2 ± 1.44	11.8 ± 0.68	12.5 ± 0.45	11.3 ± 1.6	10.8 ± 0.74	9.4 ± 1.10	9.3 ± 0.45	8.1 ± 0.46	7.5 ± 0.72
Σ Polyunsaturated	25.8 ± 0.97	23.2 ± 0.55	22.8 ± 0.26	24.3 ± 0.14	23.6 ± 0.6	23.3 ± 0.29	22.9 ± 0.51	22.1 ± 0.27	22.2 ± 0.26	20.3 ± 0.33
Σ n-3	22.1 ± 3.94	19.2 ± 1.94	17.9 ± 0.92	19.3 ± 1.03	17.4 ± 2.3	17.0 ± 1.16	15.2 ± 1.88	14.8 ± 0.87	13.3 ± 0.81	12.2 ± 1.20
Σ n-6	5.9 ± 0.82	6.1 ± 0.77	6.4 ± 0.37^{b}	7.4 ± 0.46^{a}	8.8 ± 1.4	8.2 ± 0.58	9.9 ± 1.49	9.2 ± 0.81	11.1 ± 0.72	10.1 ± 1.08
EPA+DHA	18.6 ± 3.33	16.6 ± 1.65	14.7 ± 0.71^{b}	15.9 ± 0.76^{a}	13.8 ± 1.8	13.3 ± 0.92	11.8 ± 1.37	11.3 ± 0.58	9.7 ± 0.56	9.1 ± 0.88

Table 9. Fatty acid composition of whole body at day 182 in high desaturase families and low desaturase families. The fatty acid compositions were measured in whole body homogenates of fish fed different inclusion levels of FO and RO diets. The quantity of each fatty acid is given in mg/g. The value given are means± SEM (n=5). Different letter indicated significant differences ($p \leq 0.05$) within diet between the two families.

Family	High	Low	High	Low	High	Low	High	Low	High	Low
Feed type	100FO		75% FO,25% RO		50% FO,50% RO		25% FO,75% RO		100% RO	
Total fatty acid mg/g	117,1 ± 19,64	114,4 ± 6,75	125,8 ± $7,98^{a}$	104,6 ± $8,17^{b}$	119,8 ± 8,17	116,2 ± 5,82	120,5 ± 25,56	107,7 ± 2,25	112,6 ± 8,32	125,6 ± 19,19
C 14:0	4,9 ± 0,80	4,8 ± 0,18	4,8 ± $0,35^{a}$	3,9 ± $0,27^{b}$	4,1 ± 0,32	3,8 ± 0,17	3,6 ± 0,78	3,2 ± 0,07	2,9 ± 0,16	3,2 ± 0,55
C 16:0	16,5 ± 2,88	16,0 ± 0,75	17,3 ± $1,03^{a}$	14,0 ± $1,12^{b}$	15,6 ± 1,19	14,8 ± 0,88	14,9 ± 3,11	13,1 ± 0,40	13,1 ± 0,93	14,6 ± 2,40
C 18:0	4,0 ± 0,87	3,9 ± 0,37	4,3 ± $0,36^{a}$	3,5 ± $0,32^{b}$	3,9 ± 0,30	3,9 ± 0,30	4,0 ± 0,86	3,4 ± 0,07	3,5 ± 0,36	4,3 ± 0,68
Σ Saturated	25,4 ± 1,18	24,7 ± 0,29	26,3 ± 0,39	21,3 ± 0,47	23,6 ± 0,51	22,6 ± 0,38	22,5 ± 1,32	19,8 ± 0,19	19,5 ± 0,40	22,1 ± 1,03
C 16:1 n-7	7,8 ± 1,55	7,4 ± 0,37	6,8 ± $0,44^{a}$	6,0 ± $0,52^{b}$	5,4 ± 0,24	5,3 ± 0,29	4,2 ± 1,35	3,8 ± 0,14	3,3 ± 0,23	3,2 ± 0,52
C18:1 n-7	4,8 ± 0,77	4,7 ± 0,33	4,8 ± 0,31	4,1 ± 0,35	4,3 ± 0,31	4,2 ± 0,22	4,0 ± 0,85	3,6 ± 0,09	3,5 ± 0,27	3,9 ± 0,59
C 18:1 n-9	19,3 ± 4,56	19,1 ± 1,66	27,7 ± $2,33^{a}$	23,6 ± $2,49^{b}$	32,8 ± 2,41	32,7 ± 1,90	38,6 ± 8,26	34,7 ± 0,76	41,1 ± 3,80	46,4 ± 6,59
C 20:1 n-9	8,2 ± 1,20	8,2 ± 0,38	7,5 ± $0,51^{a}$	6,5 ± $0,58^{b}$	6,2 ± 0,43	6,0 ± 0,29	5,2 ± 1,12	4,7 ± 0,10	4,1 ± 0,31	4,4 ± 0,69
C 20:1 n-11	1,1 ± 0,17	1,1 ± 0,06	1,9 ± 0,12	1,5 ± 0,11	1,5 ± 0,09	1,5 ± 0,05	1,4 ± 0,31	1,2 ± 0,04	1,2 ± 0,03	1,3 ± 0,20
C 22:1 n-11	5,9 ± 1,12	5,7 ± 0,30	5,1 ± $0,39^{a}$	4,3 ± $0,36^{b}$	4,2 ± 0,29	4,1 ± 0,11	3,6 ± 0,85	3,2 ± 0,10	2,8 ± 0,23	2,9 ± 0,51
Σ Monounsaturate	47,2 ± 1,54	46,2 ± 0,57	53,9 ± 0,82	45,9 ± 0,87	54,5 ± 0,88	53,9 ± 0,70	57,1 ± 3,03	51,4 ± 0,27	56,0 ± 1,47	62.2 ± 2,49
C 18:2 n-6	5,4 ± 0,75	5,5 ± 0,27	8,0 ± $0,52^{a}$	7,1 ± $0,63^{b}$	9,7 ± 0,69	9,8 ± 0,39	11,9 ± 2,49	11,0 ± 0,32	13,0 ± 0,89	14,5 ± 2,18
C 18:3 n-3	2,0 ± 0,31	2,0 ± 0,08	2,1 ± 0,14	1,8 ± 0,18	2,8 ± 0,16	2,8 ± 0,12	3,5 ± 0,73	3,2 ± 0,10	3,7 ± 0,27	4,2 ± 0,67
C 20:5 n-3	5,7 ± 0,84	5,8 ± 0,35	5,6 ± $0,38^{a}$	4,5 ± $0,34^{b}$	4,4 ± 0,26	4,2 ± 0,15	3,7 ± 0,76	3,1 ± 0,19	2,7 ± 0,03	2,8 ± 0,46
C 22:6 n-3	17,9 ± 2,33	17,9 ± 0,72	16,9 ± $0,93^{a}$	14,3 ± $0,84^{b}$	14,1 ± 1,04	13,6 ± 0,60	12,2 ± 2,47	11,0 ± 0,24	9,6 ± 0,56	10,5 ± 1,83
Σ Polyunsaturated	31,0 ± 0,88	31,1 ± 0,27	32,6 ± 0,33	27,8 ± 0,29	31,1 ± 0,41	30,4 ± 0,22	31,2 ± 1,01	28,3 ± 0,09	29,1 ± 0,37	32,0 ± 0,84
Σ n-3	27,7 ± 3,74	27,7 ± 1,14	27,6 ± $1,54^{a}$	23,1 ± $1,54^{b}$	23,6 ± 1,59	22,6 ± 1,02	21,1 ± 4,36	18,8 ± 0,53	17,2 ± 1,05	18,7 ± 3,18
Σ n-6	7,3 ± 0,99	7,3 ± 0,40	10,1 ± $0,66^{a}$	9,0 ± $0,73^{b}$	11,9 ± 0,89	11,6 ± 0,49	14,3 ± 2,97	13,1 ± 0,36	15,6 ± 1,07	17,3 ± 2,63
EPA+DHA	23,6 ± 2,99	23,7 ± 1,02	22,4 ± $1,18^{a}$	18,9 ± $1,18^{b}$	18,5 ± 1,29	17,8 ± 0,74	15,8 ± 3,23	14,1 ± 0,36	12,3 ± 0,59	13,3 ± 2,28

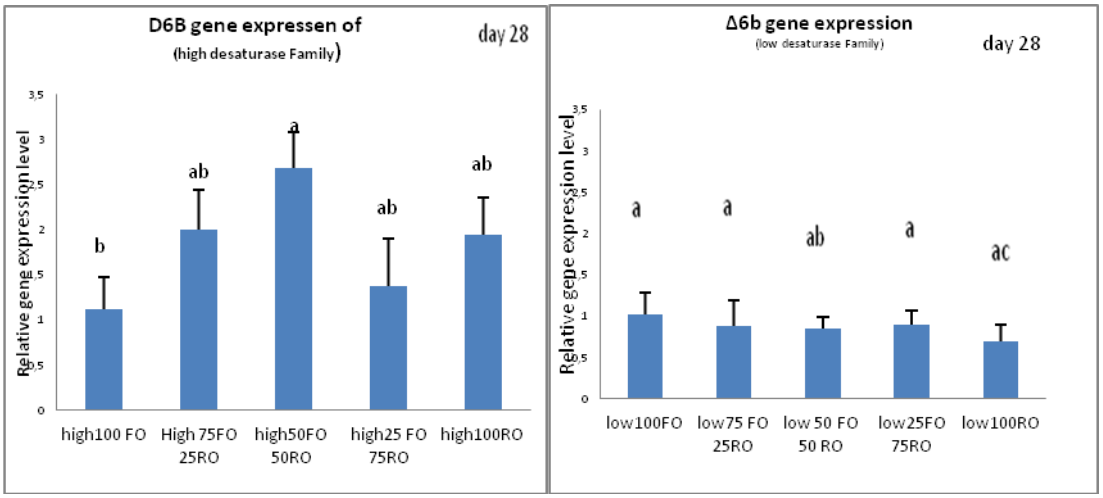
3.2. **Gene expression**

3.2.1. Whole body Δ6b desaturase

Whole body Δ6b desaturase gene expression of fish fed 5 different inclusion levels of FO and RO was analyzed by quantitative PCR. Δ6b desaturase was normalized to the reference gene Rpol. The result showed Δ6b gene expression level change with dietary treatment in both the high and low desaturase families. The expression level of the gene also varies between the two sampling time points (day 28 and day 182). Figure 7A show a significant up-regulation expression level of Δ6b in the high desaturase group at day 28 in the 50% RO dietary group and a trend towards increased in expression level in the 25%RO. In contrary the expression level of this gene at day 28 showed no difference in expression level between the groups in the low desaturase family.

At day 118 (Figure 7B), there were a high fluctuation in the Δ6b desaturase gene expression level between the different dietary groups in the whole body sample. The same fluctuations were seen in both the high and low desaturase families. Due to large fluctuations in gene expression levels at this sampling, it is not possible to draw any conclusion about the dietary effects.

a. After 28 days of sampling period



b. After 118 days of sampling period

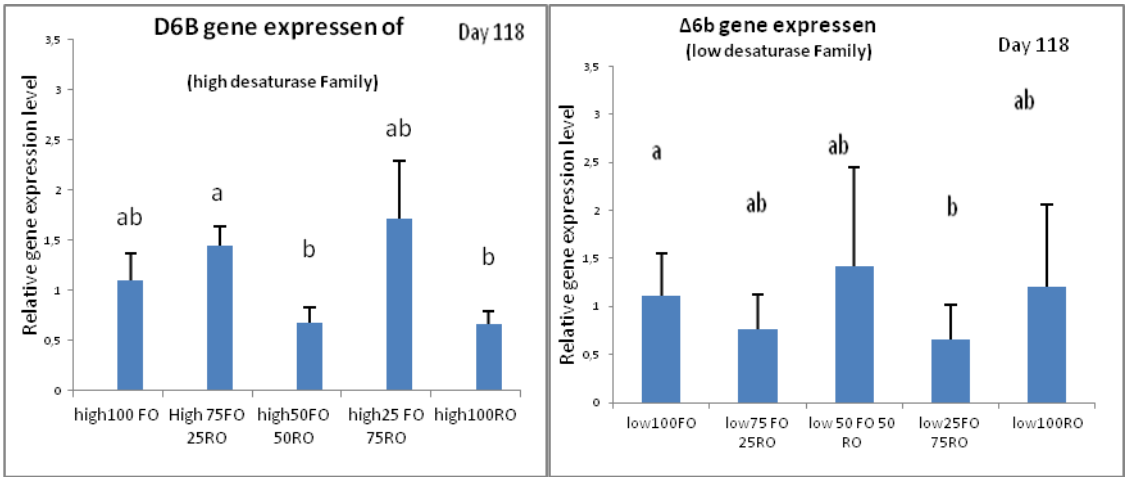
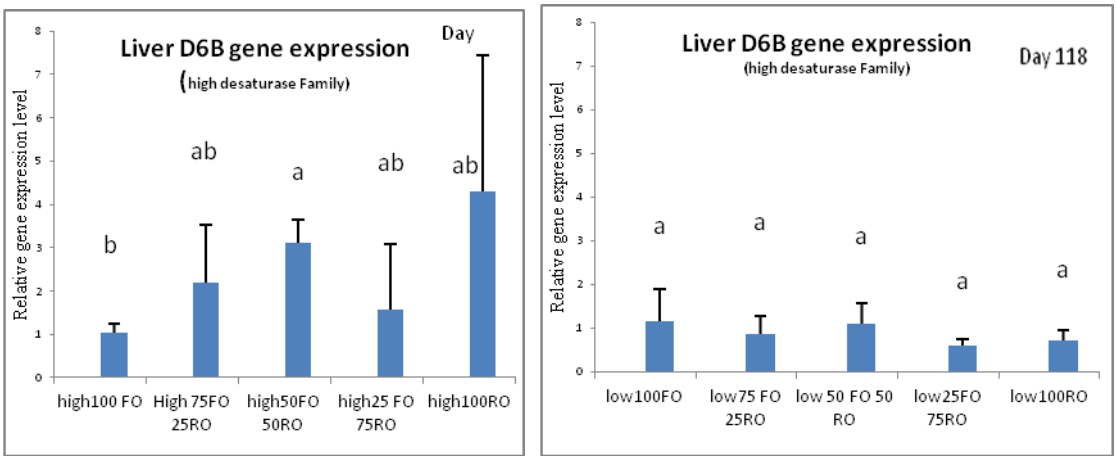


Figure 7 Relative gene expression level of Δ6b in whole body of the high desaturase families and low desaturase families at day 28 sampling (A) and day 118 sampling (B). The gene expression of Δ6b is normalized to the reference gene Rpol. Data are means ± SEM (n=5). The 100FO group is set to 1 and the other groups are calculated relative to this group. Different letters indicate significant differences ($P \leq 0.05$).

3.2.2. Liver gene expression

Due to the small size of the fish to sample the liver at day 28, liver samples was analysed at day 118 and 182. Liver gene expression level at day 118 and day 182 for both high and low desaturase families of fish fed different inclusion level of FO and RO were analyzed by quantitative PCR. All the analyzed genes (Δ6A, Δ6B, Δ6C, Δ5B, ACO and ELOV1_2) showed variation in the expression level in response to the different inclusion levels of FO and RO.

3.2.2.1. Δ6b gene expression



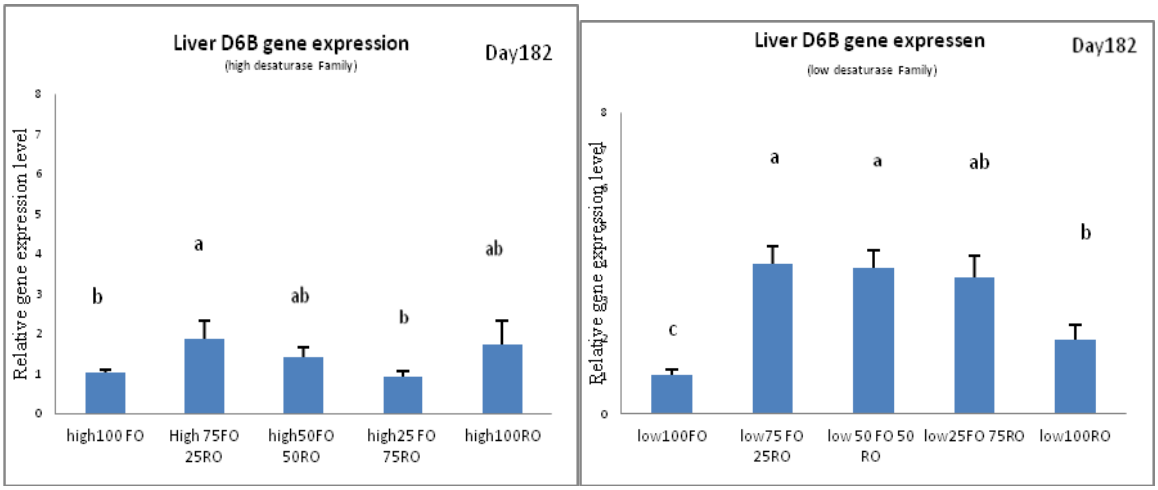
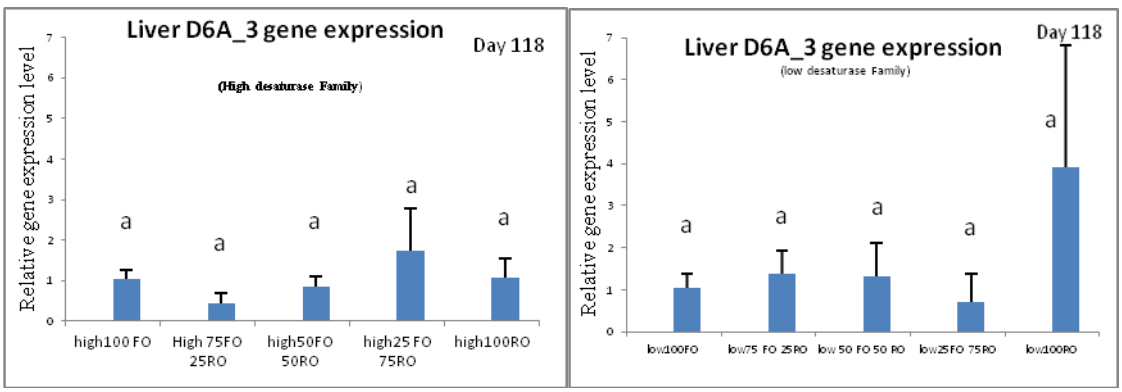


Figure 8 : Relative gene expression level of Δ6b in liver of the high desaturase families and low desaturase families. The gene expression of Δ6b is normalized to the reference gene Rpol. Data are means ± SEM (n=5). The 100FO group is set to 1 and the other groups are calculated relative to this group. Different letters indicate significant differences (P ≤0.05).

There was a tendency to increased expression levels of Δ6b desaturase gene expression level at day 118 in livers of all the RO dietary groups, although it was only significantly different from the 100%FO in the 50%RO fed group in high desaturase family. However the expression level showed no significant differences in the low desaturase family when RO inclusion level increases.

At day 182 the gene showed more or less the same trend in expression level in both high and low desaturase families with only significantly different from the 100%FO in the 25%RO for high desaturase family. Whereas, this gene was significantly different from 100% FO in all dietary groups in low desaturase family.

3.2.2.2. Δ6A gene expression



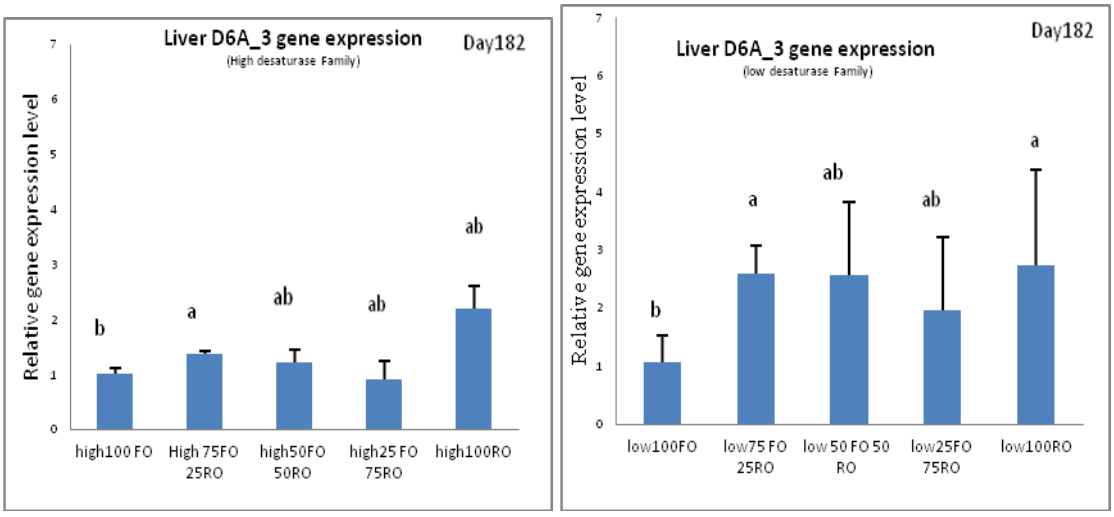
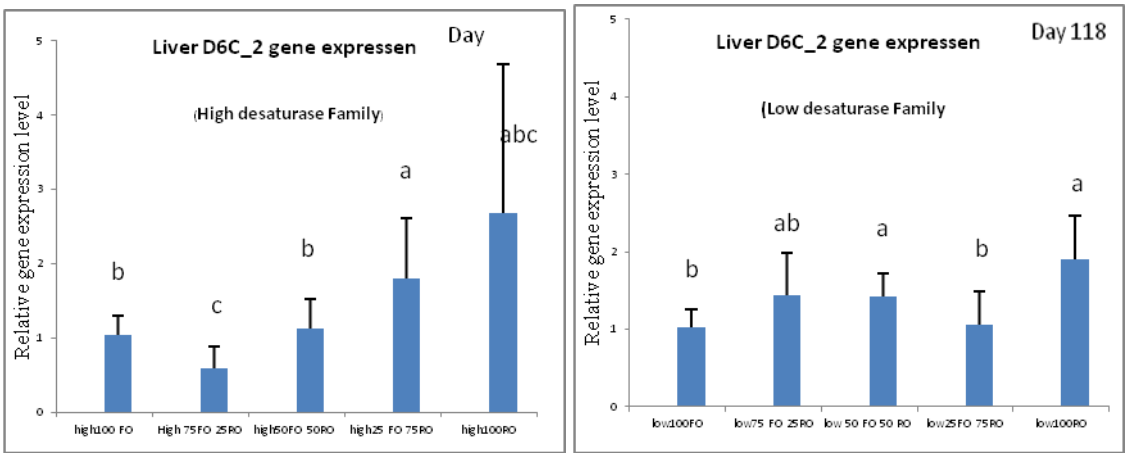


Figure 9 Relative gene expression level of Δ6a in liver of the high desaturase families and low desaturase families. The gene expression of Δ6a is normalized to the reference gene Rpol. Data are means ± SEM (n=5). The 100FO group is set to 1 and the other groups are calculated relative to this group. Different letters indicate significant differences (P ≤0.05).

The expression level of Δ6A_3 shows no significant differences between the dietary groups at day 118 for both high and low desaturase families. However at day 182 the gene was significantly up-regulated from 100%FO in the 25%RO fed group for the high desaturase family as well as at 25%RO and 100%RO fed group for the low desaturase family. With a tendency to decrease in expression level from 25%RO to 75%RO fed fish group in both families.

3.2.2.3. Δ6C gene expression



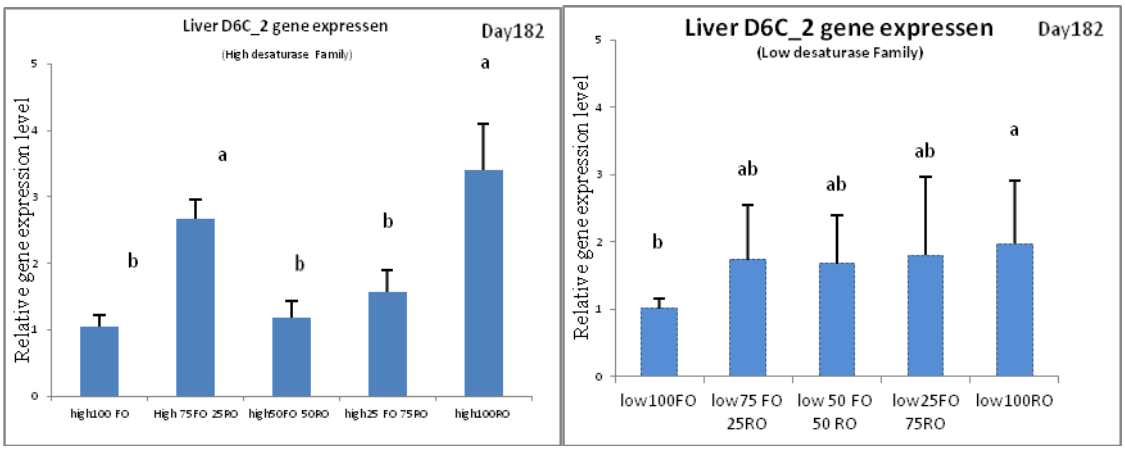
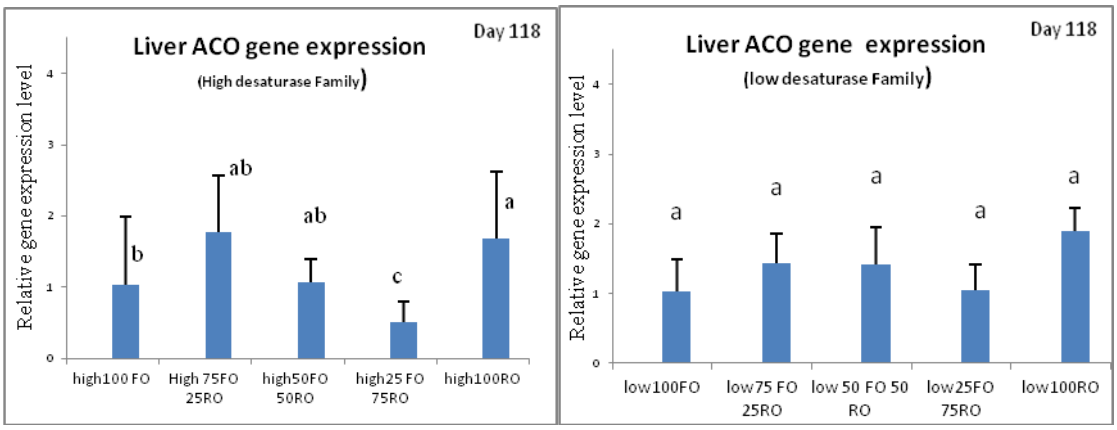


Figure 10 Relative gene expression level of Δ6c in liver of the high desaturase families and low desaturase families. The gene expression of Δ6c is normalized to the reference gene Rpol. Data are means ± SEM (n=5). The 100FO group is set to 1 and the other groups are calculated relative to this group. Different letters indicate significant differences (P ≤0.05).

The Δ6c gene expression at day 118 was significantly up-regulated from 100%FO in the 75%RO fed group in high desaturase family as well as in the 50%RO and 100%RO fed groups for the low desaturase family. With a tendency to increased in expression levels as the RO inclusion level increased in high desaturase family. However this gene showed similar expression levels as the RO level increased for low desaturase family.

At day 182, the gene showed similar expression levels to the day 118 sampling period in both families, with significant differences from 100%FO in the 25%RO and 100%RO for the high desaturase as well as in the 100%RO for the low desaturase.

3.2.2.4. ACO gene expression



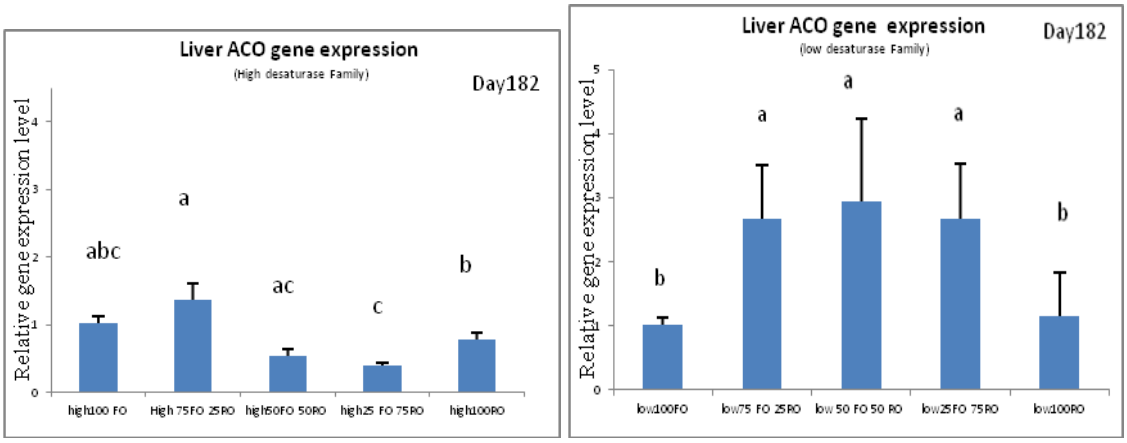
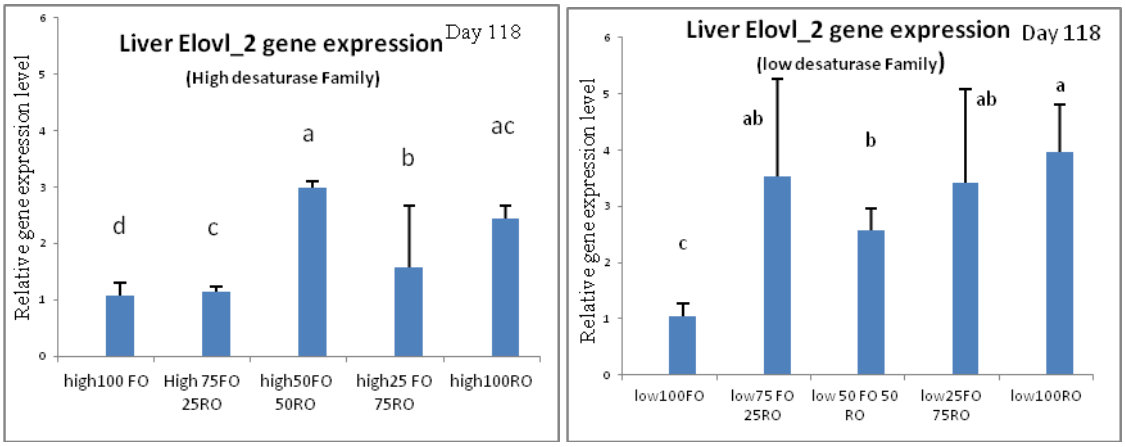


Figure 11 Relative gene expression level of ACO in liver of the high desaturase families and low desaturase families. The gene expression of ACO is normalized to the reference gene Rpol. Data are means ± SEM (n=5). The 100FO group is set to 1 and the other groups are calculated relative to this group. Different letters indicate significant differences (P ≤0.05).

The expression of acyl-CoA oxidase (ACO) at day 118 was significantly up-regulated from 100%FO in the 100%RO fed group for the high desaturase family, with a tendency to reduced in the expression level from 25%RO to 75%RO fed groups as the RO level increased. Mean while the gene showed no expression level in the same sampling day for the low desaturase family.

Despite the above result at day 182 the gene shows significant differences from 100%FO in the 25%RO, 50%RO and 75%RO fed groups for low desaturase family, with similar tendency in expression levels as the RO level increased. Whereas the expression level of this gene showed no significant different in the high desaturase family, with tendency to reduced in expression as RO inclusion level increased.

3.2.2.5. Elovl_2 gene expression



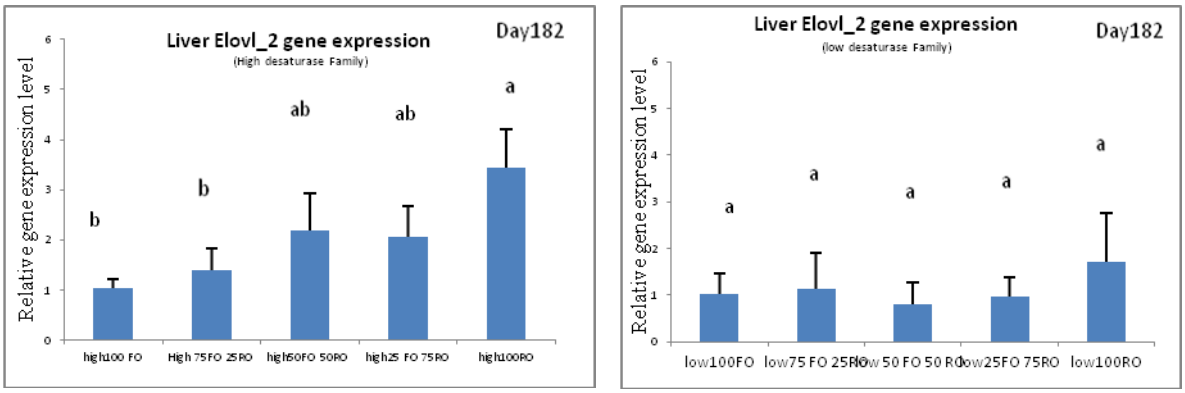
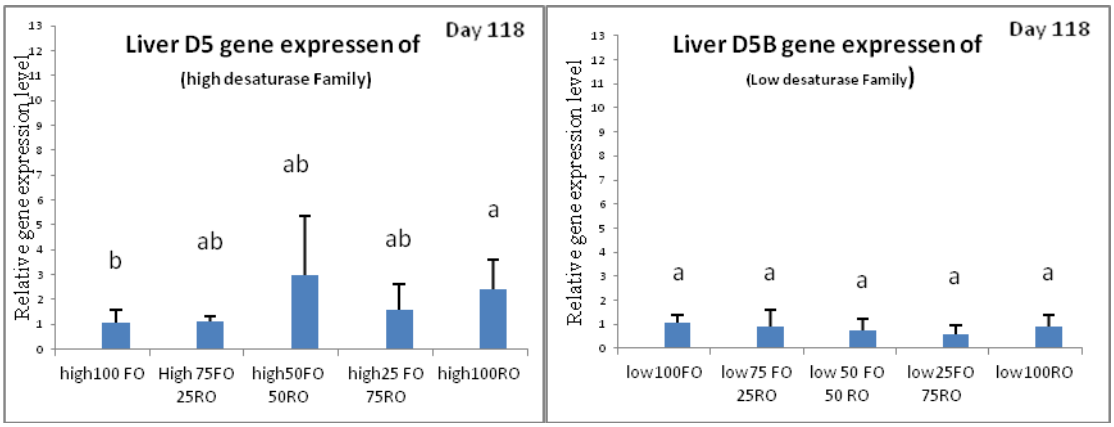


Figure 12 Relative gene expression level of Elovl_2 in liver of the high desaturase families and low desaturase families. The gene expression of Elovl_2 is normalized to the reference gene Rpol. Data are means ± SEM (n=5). The 100FO group is set to 1 and the other groups are calculated relative to this group. Different letters indicate significant differences ($P \leq 0.05$).

The gene expression of Fatty acylelongase (Elovl_2) at day 118 showed significant differences from 100%FO in liver of all the RO dietary groups in both families. With a tendency to increase in expression levels as RO inclusion level increased in the high desaturase family, but the low desaturase family showed a similar expression levels as the RO level increased.

Despite the day 118 the expression level of Elovl_2 showed no significant differences in the low desaturase family at day 182. Whereas this gene was significantly up regulated from 100%FO in the 100%RO fed group for high desaturase family, with tendency to increase as the RO inclusion levels increased.

3.2.2.6. Δ5 gene expression



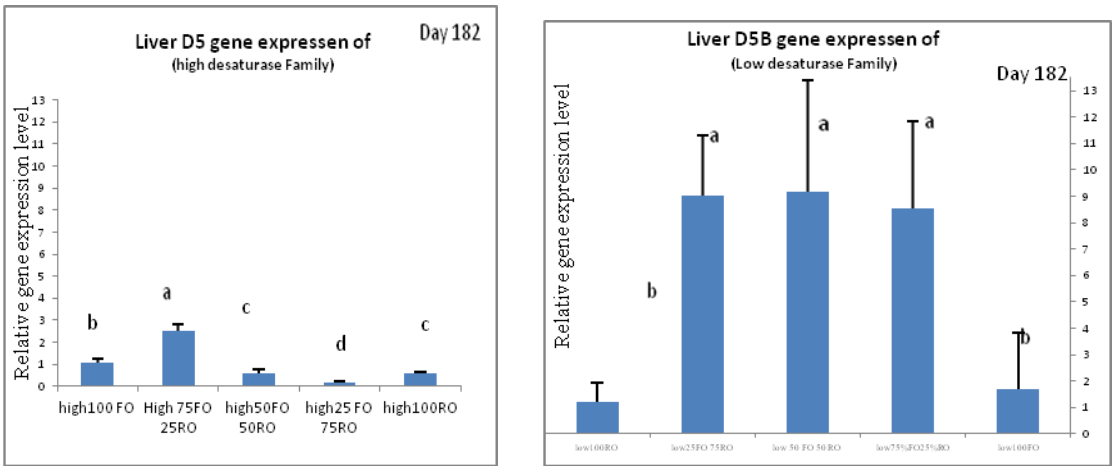


Figure 13: Relative gene expression level of D5 in liver of the high desaturase families and low desaturase families. The gene expression of D5 is normalized to the reference gene Rpol. Data are means ± SEM (n=5). The 100FO group is set to 1 and the other groups are calculated relative to this group. Different letters indicate significant differences (P ≤0.05).

The expression of Δ 5 dersaturase show a significantly different from 100%FO in the 100%RO fed group for high desaturase family, with high fluctuations in gene expression level at day 118. Whereas, the expression level showed no significant different in the low desaturase family at this sampling period.

Despite the above result the gene expression level at day 182 showed significant differences from 100%FO in the 25%RO, 50%RO and 75%RO fed groups for the low desaturase family, with a similar expression level as the RO levels increased. But the gene showed a tendency to reduced in the expression level as the RO level increased for high desaturase family, although it was only significantly different from 100%FO in the 25%RO fed group.

4. DISCUSSION

4.1. Growth

Our study show feeding the two families of Atlantic salmon diets containing increasing inclusion level of RO (0%RO, 25%RO, 50%RO, 75%RO and 100%RO) didn't affect the final weight and the growth rate (SGR). This is in agreement with many other studies where RO was used in salmon feed and no growth difference was observed (Bell et al. 2003; Tocher et al. 2000), and similar result was obtained in Arctic Charr (Pettersson et al. 2009). But at the end of the feeding trial, our result showed a higher growth record for the high desaturase family than for the low desaturase family. We also found significant differences in weight and SGR in the last growth period (day118 - day182) between the two families. Since the parents of the experimental fish were from only two families which again were selected from 103 families, this observed growth differences between the two families may be due to the normal variation in growth potential within the breeding population from SalmoBreed.

4.2. Fatty acid composition

The fatty acid composition of whole body lipid of fish from high and low desaturase families were clearly affected by the fatty acid compositions of their diets, which agree with other previous studies from salmon (Olsen 2011; Torstensen et al. 2000). At the end of our study, the fatty acid Oleic acid (18:1n-9), which is the main component in RO had more than 2-fold higher level in the total body lipid of the fish group fed the 100%RO diet than in fish fed 0%RO in both the high and low desaturase families(Figure 3). This is in agreement with similar study performed in Atlantic salmon fed RO rich diets.(Bell et al. 2003). The total MUFA level increased as the inclusion level of RO increases in both the high and low desturase families; mainly due to the higher concentration of the 18:9n-1 in RO. Meanwhile the 18:3n-3 fatty acid and the 18:2n-6 fatty acid in the whole body also increased with increasing inclusion levels of RO, this are due to the higher content of these fatty acids in RO diet than in the FO diet.

Overall as the dietary inclusion level of RO increased there was a proportional increase in the 18:1n-9, 18:3 n-3 and the n-6 fatty acids; and a decrease in EPA, DHA

and the total polyunsaturated long chain n-3 fatty acids. This was due to the lack of DHA in RO.

Although the fatty acid profiles of the fish follow the fatty acid compositions of the diets, DHA in the flesh is always higher than in the diet (Bell et al. 2001; Kjaer et al. 2008a), this may be due to selective retention of DHA in whole body membranes.

In the present study the EPA and DHA level in whole body showed a significant reduction in both families with increasing inclusion levels of RO (0%RO, 25%RO, 50%RO, 75%RO and 100%RO). The lowest level of these two fatty acids was found in the 100%RO fed group of fish. In the 75%RO, 50%RO and 25%RO fed groups, despite moderate levels of RO in diet showed in favor of an increased EPA and DHA production. The reason for increase in activity in fish fed VO was not known but thought mainly due to the suppression of enzyme activity by EPA and DHA abundance in fish oil (Tocher et al. 2003).

Variation in flesh n-3 HUFA content in fish fed the same diet can be caused by differences in biosynthesis capacities from precursors between the two families. We observed variation in EPA and DHA level between the two families fed the same diet. At the end of the feeding trial (day182), it was strong tendency that the high desaturase family had higher EPA and DHA level in whole body of fish fed the 25%RO, 50%RO and 75%RO inclusion level diets than the low desaturase family. The difference between families was however only significant for the 25%RO group. This difference between the families, indicate difference in a capacities to produce EPA and DHA, in particular with moderate inclusion level of RO in fish diets. This difference was not obvious at the earliest life stages, but was clearer at the latest life stage in our trial. This finding support our hypothesis that the parents of the experimental fish, which were selected for high Δ6 desaturase gene expression and a relatively higher proportion of EPA and DHA in the liver lipids (Sun 2012), transfer a higher capacity for EPA and DHA production to their offspring's. These findings also support the hypothesis that there are potential for selective breeding for the phenotype of higher biosynthesis capacities for EPA and DHA. This assumption is further supported by the studies: a study with salmon following 12 weeks feeding of 100%RO, shows, irrespective of the diet there is a variation in the n-3 HUFA content level between individuals (Schlechtriem et al. 2007). Leaver et al. (2011) reported that the n-3HUFA

is a highly heritable trait in Atlantic salmon and there is a specific mRNA expression pattern in association with the high flesh n-3 HUFA, which is an indication for family dependent deposition in flesh. A number of other studies also observed variation in deposition and retention of the HUFA in different salmon strains (Peng et al. 2003; Pickova et al. 1999).

4.3. Whole body gene expression level of Δ6b desaturase

The present study shows Δ6 desaturase gene expression in the two families varied between the dietary groups and sampling periods. In the high desaturase group we observed increased expression of Δ6 desaturase in the whole body of the fish in the first sampling period as the RO inclusion increased from 0%RO to 50%RO. But in the low desaturase family no differences in gene expression in this sampling day was observed. There were however no significant differences in Δ6 desaturase gene expression between the dietary groups at the later sampling at day 182, showing that the gene expression varies with sampling time. Previous studies performed in Atlantic salmon fed RO, linseed oil or linseed oil supplemented with ARA and FO, reported higher Δ6 desaturation capacity in fish fed FO (Tocher et al. 2000; Tocher et al. 2002), in agreement with our finding from the first sampling in the high desaturase group. Similar results were also found in Atlantic salmon smolts fed diets where FO was replaced by linseed oil in a graded manner (Zheng, X. Z. et al. 2004). It is known that VO diets increase expression of the Δ6 and Δ5 desaturase mainly due to either the high availability of the dietary substrate like α-linolenic acid or the lack of EPA and DHA (Miller et al. 2008).

4.4. Liver gene expression study

All All liver gene expression results were based on samples from day 118 and day182 of the feeding trial for both the high and low desaturase families. The capacity of any species to convert C-18 PUFA to HUFA depends not only on the activity of the different Δ6desaturases, but also Δ5desaturase, different Elovl enzymes (Sargent et al. 2002) and on enzymes in the peroxisomal β-oxidation pathway, including ACO (Sprecher 2000). A study performed with salmon hepatocytes, showed that these genes increased in expression level by feeding RO diet low in EPA and DHA (Moya-Falcon et al., 2005), Which is why we also included analyses of the expression of several of these genes in our study.

Our results showed the liver expression of different Δ6 desaturases (Δ6a, Δ6b and Δ6c) was influenced by different inclusion levels of RO in both families. At day 118 the high desaturase family showed tendency to increased expression level of Δ6b in the liver as the dietary RO inclusion level increased. But the low desaturase family showed no differences in expression level of Δ6b desaturase at the same sampling period, in agreement with the results from whole body gene expression. The response to RO in the high desaturase group was in agreement with Similar results shown by Monroig et al.(2010) who found a higher expression level of the Δ6b in liver of fish fed RO than in the liver of fish fed FO.

The Δ6 desaturase is considered as the main rate-limiting step in the HUFA biosynthetic pathways (Bernert & Sprecher 1975). Many studies show stimulation of this gene in liver of salmon fed RO base diets (Bell et al. 2001; Codabaccus et al. 2011). A similar result was observed in salmon fed other plant oils (Bell et al. 2001; Ruyter & Thomassen 1999) and in case of rat fed diet rich coconut oil(Ulmann et al. 1992).

We also obsarved a fluctuation of Δ6b gene with sampling days. Opposite of what was found in whole body at day118, at day 182 the gene was significantily up-regulated from 100%FO in all dietary treatment groups in the low desaturase family but showed a significant diffence only in the 25%RO fed group for the high desaturase family in the same sampling day. Both the whole body and liver gene expression results showed variation with sampling time points. This can be supported by Zheng et al. (2005) who found a flactuation of Δ6 by the time of sampling.

The Δ6c expression showed a tendency to increase in expression level as the RO level increased in the two sampling points for the high desaturase family (Figure 12), but the low desaturase family showed similar expression level as the RO level increased in both sampling points.

Both in high and low desaturase families, the Δ6c expression showed a tendency to increase in expression level as the RO level increased in the two sampling points. The Δ6c expression did therefore not show the same variation in gene expression at different sampling points as the Δ 6b. To the best of our knowledge, a variation in the expression of this gene with different times and age of the fish has not previously been performed. And we do not know if this gene is more stable expressed than the others.

Our result showed no differences in expression level of Δ6a beween the dietary groups in both families at day 118. However this gene showed increased expression level with RO at day 182 in both families. The results at day 182 are in agreement with results shown by Monroig et al. (2010) who found higher expression level of the Δ6a in liver of fish fed RO and soybean oil than in fish fed FO.

The peroxisomal ACO is an important enzyme in the last steps in production of DHA (Ferdinandusse et al. 2001).The expression level of the ACO gene showed similar expression at the two sampling points in the high desaturase family, with tendency to reduced expression as the RO inclusion level increased from 25%RO to 75%RO. This is in agreement with a previous finding conducted in an *in vivo* study, which shows increased expression levels of ACO when fed high DHA levels (Kjaer et al. 2008a).

However in the low desaturase family, no significant differences in expression level of ACO was found at day 118, while at day 182 the pattern in the low desaturase group was the opposite of what was found in the high desaturase group, the reason why we do not know

Two Elovel have been identified in salmon (Elovel_2 and Elovel_5) (Marais et al., 2009). A study has shown that the Elove_2 is responsible for elongation of 20:4n-6, 20:5n-3 to 22:4n-6 and 22:6n-3 in both rat and human (Jakobsson et al. 2006). The expression of Elovel_2 in the present study showed a tendency to increase in expression level as RO inclusion level increased in both sampling periods for the high desaturase family. An increased expression with increased dietary plant oils in fish diets are in agreement with studies showing the expression level of Elovel_2 in liver of salmon fed VO was increased in fish compared to fish fed FO (Leaver et al. 2008; Morais et al. 2009). Being more active towards C-20 and C-22 fatty acid this increased in expression level of Elovel_2 may be important in salmon aquaculture, which enable salmon to adapt VO diets which are low in the fatty acid greater that C-18.

Being part of the last chain elongation steps in the PUFA biosynthesis, Luthria et la.(1997) show in rat liver this gene can be equally regulatory in the synthesis pathway as is the Δ6 desaturase. Thomassen et al. (2012) suggested that Elovl_2 is significantly important in regulating the conversion of EPA to DHA. Whereas the expression of

Elovel_2 for the low desaturase family showed similar expression level as RO inclusion level increased at day 118 but showed no expression at day 182 for this family.

Our result showed no differences in expression level of Δ5 beween the dietary groups in both families at day 118 and also at day 182 for the high desturase family. However this gene showed increased expression level with RO at day 182 for the low desturase family. This is in agreement with several other studies performed in Atlantic salmon fed diets low in HUFA level (Christiansen et al. 1991; Jordal et al. 2005; Zheng, X. Z. et al. 2004).

In conclusion we observed variation in the gene expression level of the desaturases, elongases and peroxisomal beta oxidation enzyme in the metabolic pathways of PUFA biosynthesis between the two families as a respond to the different inclusion level of RO and FO. This variation in expression level together with the higher EPA and DHA level in the high desaturase family at final sampling shows the potential of increasing HUFA via genetic selection. This approach to increase EPA and DHA level in Atlantic salmon fed diets with very low levels of these important nutrient needs to be further investigated.

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