Differences in omega-3 long chain polyunsaturated fatty acids composition among Atlantic salmon (Salmo salar L.) families.

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Master Thesis 30 credits 2013

To my parents

Acknowledgments

This thesis was carried out at the Department of Animal and Aquaculture Sciences (UMB) at Ås, Norway during last year.

I would like to express my gratitude to my supervisor Dr. Magny Thomassen for her generous support during the process. Thank you so much for all your valuable advices and useful discussions. It was privilege to work with you.

I truly wish to thank to Nina Åsper for assistance with laboratory work. Further wish to thank to my close friend Neda Nemati for helping me with statistical analyses.

I would like to express my deepest appreciation to my parents and my brother who were supporting me during these two years and giving me this great chance. Extended thank to my grandmother, uncles and aunts who continually encouraged me to keep my spirits up.

Ås, Norway 15.08.2013
Delaram Mobaraki

Abstract

Fish and seafood are considered as the main sources of highly unsaturated omega-3 fatty acids in the human diet and has lots of health beneficial advantages. However, due to the shortage of fish oil in the aqua-feed and replacement of fish oil with plant oil in fish diet, the n-3 content has been decreased in farmed fish. Nowadays, by improving diet formulations and also genetic selection of fish with high content of both eicosapentaenoic acid and docosahexaenoic acid, the industry tries to improve n-3 content in Atlantic salmon.

The genes which are involved in the biosynthesis of the very long chain fatty acids in Atlantic salmon are regulated by fatty acids in the diet. And omega-3 composition in salmon has in a previous study been found out to be a heritable trait. The aim of this study was to find out if variation exists in the composition of omega-3 fatty acids in different salmon families from a Norwegian breeding company.

Salmon from 10 families, fed the same diet with high concentration of vegetable oil, were selected in October 2012, 10 fish from each family, and the muscle fatty acid composition analyzed. The mean percentage of eicosapentaenoic acid (20:5 n-3, EPA) varied from 2,7 to 3,3 %, the mean percentage of docosahexaenoic acid (22:6 n-3, DHA) varied from 3,9 to 4,9 %, and statistically significant differences were observed among the families. Hardly any significant differences were found between gender, and the content of total fat was not statistically different between families.

Abbreviations

ARA	Arachidonic acid
ALA	Alpha linoleic acid
COA	Coenzyme A
EFA	Essential fatty acid
ELOVL	Very long chain fatty acyl elongases
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
FO	Fish oil
VO	Vegetable oil
GC	Gas-liquid chromatography
HUFA	Highly unsaturated fatty acids
PUFA	Poly unsaturated fatty acids
FO	Fish oil
VO	Vegetable oil

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1. Introduction

Atlantic salmon (*salmo salar*) belongs to a family of fish called salmonidae. Atlantic salmon is a fatty fish, known as the "king of fish" who lives in cold water temperature and therefore west coast in Norway is potentially suitable for breeding salmon. Salmons are born in fresh water and spend 2-3 years in fresh water before becoming smolts. They then migrate to the marine water (normally March-June) to feed and grow. After becoming mature, they return back to fresh water for reproducing (spawn). This pattern is called anadromous fish migration. Salmon is an efficient livestock and have special features. For instance salmon do not need to maintain a body temperature of 37°C and also do not waste energy standing upright. Due to the fact that fish are almost "weightless" in water salmon have the ability to convert almost all what they eat to energy for growth.

In Norway it is possible to farm several types of fish such as Atlantic salmon and Rainbow trout in huge quantities. Cod and halibut are farmed but not as much as the others. Salmon production has increased tremendously in the last two decades with 944,000 tones production in 2010 (Fiskeridirektoratet, 2010). Norway has a high rank in producing and exporting Atlantic salmon all over the world and is expected to become even more globalized in the future. Farmed salmon industry plays an important role especially in terms of economic growth and employment in Norway (Alsos *et al.*, 2007). In the beginning, in Norway there were small local farms and it was more a family business, but by the time it has grown from small to more modern industry (Alsos *et al.*, 2007). New technology and new species are being introduced to increase the production in the industry (Ottersen *et al.*, 2011).

Nowadays salmon industry in Norway is becoming better organized and it has been tried hard to make it more efficient economically and less labour-intensive in comparison with other countries for the fact that labour is expensive in Norway. Despite this tremendous growth in production, the Salmon aquacultures are facing problems such as pollution, negative genetic impacts, biodiversity concerns and disease (Liu *et al.*, 2008). Suppliers are trying to reduce the amounts of pollution like dioxin by controlling the environments of fish farming. Moreover reforming, reorganizing modern technology and also setting up new regulations for using chemical material to reduce the amount of toxin in the fish farming. Selective breeding in aquaculture play an important role for the genetic improvement of fish. Moreover selective breeding in aquaculturehelps to improve traits of commercial importance (Gjedrem *et al.*,

2009). Due to the shortage of fish oil, selection of fish flesh with high level of unsaturated n-3 fatty acids in salmon breeding programs would be highly desirable and also a study by Leaver *et al.*, (2011) indicated that n-3 composition in salmon flesh is a highly heritable trait.

Salmonids have the capacity to convert C18:3 ω3 alpha-linoleic acid (ALA) from vegetable oils to the longer chain C20:5ω3 eicosapentaenoic acid (EPA) and C22:6ω3 docosahexaenoic acid (DHA). However, according Ruyter *et al.*, (2000) the capacity of conversion is too low to cover the requirement of EPA and DHA in Atlantic salmon. The capacity of converting ALA to EPA and DHA is stimulated in the fish fed vegetable oil as compared to fish fed fish oil (Kjaer *et al.*, 2008).

Aim of study

The major objectives of this thesis were:

i. To study if there is any difference in the percentage of omega-3 in muscle from different salmon families.
ii. To check if any such differences is explained by body weight or total fat in muscle.
iii. To study if the gender of salmon effects the percentage of omega-3 in muscle.

2. Background

2.1 Some advantages of fish farming

Salmon are overfished according to marine conservation society (Marine Conservation Society, 2011). Therefore to maintain salmon for future generation, farmed salmon could be a good system for breeding salmon and should be increased in order to meet increasing demands due to the fact that more people are including fish into their diets. Fish farming has already produced 50% of the fish used for human consumption (Deckers, 2010).

Farmed fish is a complete nutrition source which provides high quality in terms of having high protein concentration and long chain polyunsaturated fatty acid. Moreover fish is a rich source of minerals and vitamins including selenium, magnesium, calcium, choline and vitamin A, B12, E and D (Harris *et al.*, 2011). According to the food and agriculture organization FAO, (1997) it also provides 16% of the animal protein consumed by the world's population. Despite the claim that farmed fish should be banned, compelling evidence shows that it has positive effects on human health, energy consumption and also is reliable food source.

In fish farming system there is ability to control fish diets and also improve it to some extent to get high levels of omega-3 from the fish which is beneficial for human health to prevent several disease such as cardiovascular disease, cancer, asthma etc. Farmed fish recirculating aquaculture system (RAS) is a land-based aquatic system and uses water and energy efficiently. RAS helps to re-cycle water which can be re-used to save energy consumption (Martins *et al.*, 2011). RAS improves hygiene and disease management and also disinfects water which prevents pathogens from accumulating in fish farmed (Summerfelt *et al.*, 2009). RAS can provide better environmental conditions and could be more suitable for species that are sensitive to the water quality. Farmed fish can be set up almost everywhere. It needs water and irrigation system. Fish farming with irrigation system decrease costs for aquaculture, agriculture, local industries and also creates jobs and provides food and water for the people.

Farmed salmon is also efficient compared to wild salmon. Wild salmon consumes 10 kg of fish in order to grow one kg whilst farmed salmon consumes 1.1 fish feed to grow 1 kg. Fish farming has been increasing all over the world, still further research is needed in order to understand the mechanisms and processes to make farmed fish even more important part of human diets.

2.2 Fatty acids of the omega-3 and omega-6 families

Fatty acids are organic compounds consisting of a hydrocarbon chain and a carboxylic group. Fatty acid can be saturated, or mono or polyunsaturated Omega-3 and omega-6 FA's are polyunsaturated fatty acids (PUFAs) which mean that they contain more than one double bond. They are called omega-3 when the first double bond from the methyl end of the fatty acid is placed at the third carbon atom. Moreover, in omega-6 the first double bond is 6 carbons away from the non-acid end of the molecule. Salmon is known as an oily fish and contains long chain omega-3 poly unsaturated fatty acid (PUFA). The two main omega-3 poly-unsaturated fatty acids are C20:5ω3 eicosapentaenoic acid (EPA) and C22:6ω3 docosahexaenoic acid (DHA) (Hull *et al.,* 2011).

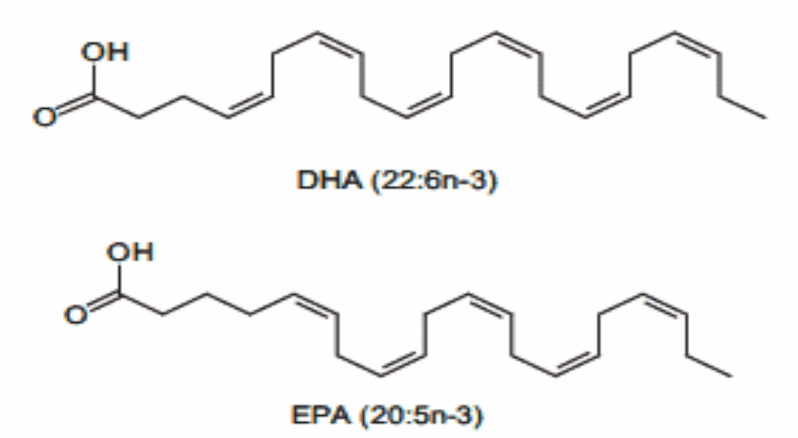

Figure 1- structure of DHA and EPA adapted from Huber et al. (2001)

ALA is an essential fatty acid in vegetable oils. ALA, EPA and DHA are grouped together named omega-3 PUFA (Poudyal *et al.,* 2011). The desaturation and elongation reactions steps lead to convert LA to AA and ALA to EPA and DHA in the liver through desaturation and elongation enzymes called (fad) and (ELOVL) (Cook *et al.,* 1991). According to

Sprecher et al. (2002) Δ6 desaturase enzymes is responsible for production of DPA from AA and DHA from EPA. The elongase enzymes called ELOVL 5 and ELOVL 2 are involved in biosynthesis of polyunsaturated fatty acids. For instance the ELOVL 5 act in the elongation of C18–C20 PUFAs and ELOVL 2 involved in C20–22, but not C18 PUFAs (Leonard, 2002).

DPA is produced by elongation of EPA by ELOVL 5 and ELOVL 2 *in vivo*. The conversion of DPA to DHA requires both elongation and desaturation reactions. The elongation to 24:5*n*−3 and desaturation to 24:6*n*3 is before beta-oxidation (Linderborg *et al.,* 2013). The beta oxidation step conversion of 24:*6n*3 to 22:6*n*3, happens in liver peroxisomes (Harris *et al.,* 2008). The gene sequences for similar elongase enzymes have been cloned from Atlantic salmon (Morais *et al.,* 2009).

The main n-6 fatty acids are linoleic acid and arachidonic acids, linoleic acid is found in cell membrane and abundant in vegetable oils such as corn, safflower, sunflower and rapeseed oils. The linoleic acids are converted to arachidonic acid but the capacity is very limited and must be provided by dietary sources.

Essential fatty acids (EFA) are necessary for numerous processes such as growth, reproduction, vision and also brain development. It was reported that eicosapentaenoic acid (EPA) is primarily oxidized by mitochondria, while docosahexaenoic acid (DHA) appears to be oxidized by the peroxisomes and to a lower degree than EPA (Madsen *et al.,* 1999). Oxygen is considered as an enemy to fatty acids especially unsaturated fatty acids since it creates free radicals and cause oxidation or rancidity.

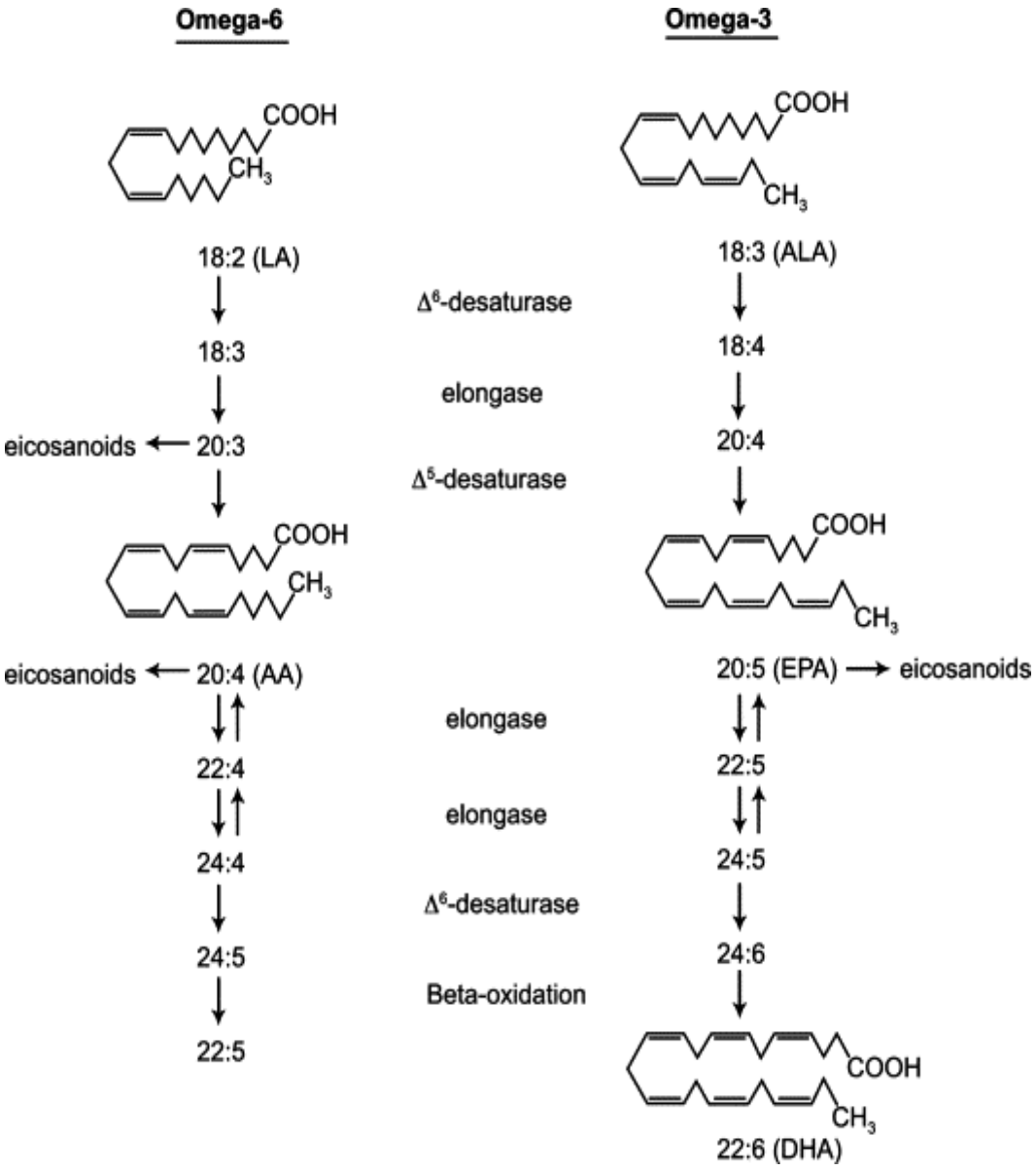

Figure 2- Synthesis of omega-6 and omega-3 fatty acids adapted from Harris et al. (2008).

2.3 Fatty acids oxidation

The fatty acids oxidation starts with removal of two carbon units from β-carbon which is called β-oxidation process. According to Tocher *et al.*, (2003) the β-oxidation process of fatty acids takes place in both the mitochondria and the peroxisomes (Moya-Falcón *et al.*, 2006). The oxidation of fatty acids depends on their chain length. For instance , long chain fatty acids are oxidized in peroxisomes and short and medium chain of fatty acids are oxidized in

mitochondria (Tran *et al.*, 2001). The most important organelles that are involved with β-oxidation especially in the fish are liver, heart and red muscle (Henderson *et al.*, 1987). Studies carried out by Frøyland et al. (2000) illustrated that the mitochondrial β-oxidation is considered as an important origin of energy for liver, heart and red muscles. Moreover, in the mitochondrial β-oxidation the acetyl-CoA enters the tricarboxylic acid cycle to give oxaloacetate, malate and CO_2 (Tran *et al.*, 2001). It has been assumed that the major product of β-oxidation in peroxisome is acetate (Tran *et al.*, 2001). According to Crockett et al. (1993) the activity of peroxisomal β-oxidation is high in fish that adapts to cold temperature.

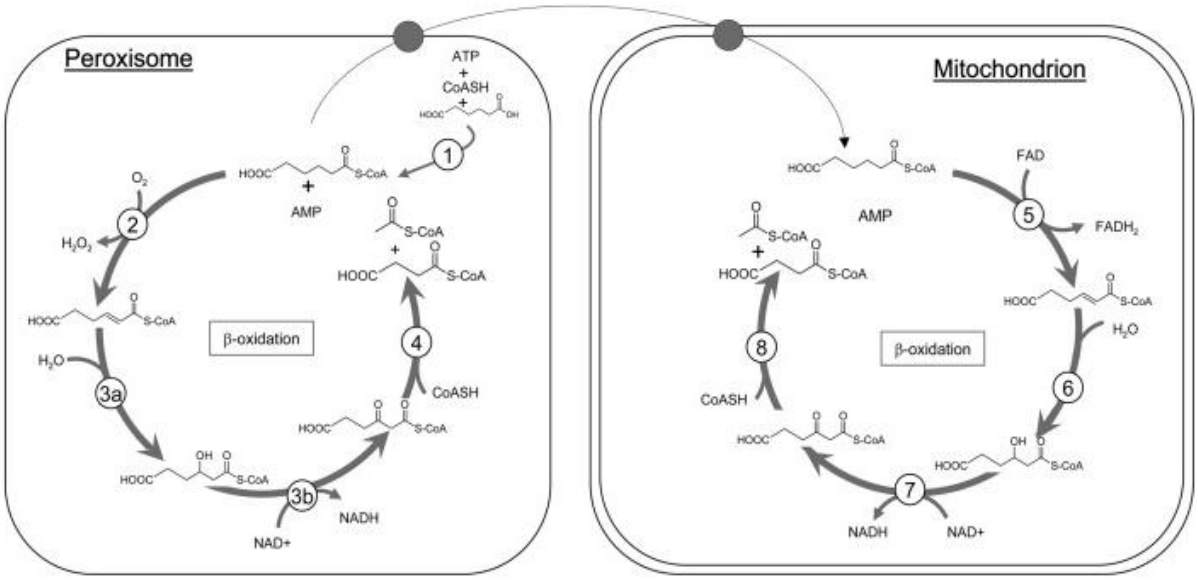

Figure 3: *β*-oxidation in mitochondria and the peroxisomes adapted from Veiga et al. (2012). (1) acyl-CoA ligase; (2) ACO and peroxisomal acyl-CoA dehydrogenase; (3a) enoyl-CoA hydratase activity of multifunctional enzyme; (3b) 3-hydroxacyl: CoA dehydrogenase activity of multifunctional enzyme; (4) peroxisomal 3-keto-acyl-CoA thiolase; (5) mitochondrial acyl-CoA dehydrogenase; (6) enoyl: CoA hydratase; (7) 3-hydroxyacyl: CoAdehydrogenase; (8) mitochondrial3-ketoacyl-CoAthiolase.

2.3.1 Desaturase and desaturation process

Desaturases are the enzymes that introduce unsaturated bonds to the fatty acid chain by removing two hydrogen atoms from hydrocarbon chain (Shanklin *et al.*, 1998). Desaturase enzymes play an important role in the biosynthesis of polyunsaturated fatty acids. Moreover,

the desaturase enzymes are working in specific location, number and stereochemistry of double bonds which exist in fatty acid (Pereira *et al.*, 2003).

According to Pereira et al. (2003) "Desaturation reaction catalyzed by the fatty acid desaturases is an aerobic process utilizing molecular oxygen and reducing equivalents (electrons) obtained from an electron transport chain". Moreover, desaturation reaction takes place in the endoplasmic reticulum of cells (Brenner, 1974). The desaturase enzymes are divided into three types called acyl-CoA, acyl-lipid, and acyl-ACP desaturase and each on them has individual activities. The first types called acyl-CoA are desaturating fatty acids esterified to coenzyme A (CoA). The second type acyl-ACP is desaturating fatty acids which is bound to acyl carrier protein and are present in soluble form. The third are the acyl-lipid desaturases which introduce unsaturated bonds to the lipid-bound fatty acids.

I. Δ6 desaturase and Δ 5 desaturase:

Δ6 desaturase is one of the enzymes that catalyze the synthesis of polyunsaturated fatty acids. The desaturase adds double bond at the Δ6 position of the unsaturated fatty acid. Δ6fad has a preference for both the longer chains and the higher unsaturation fatty acids (Sargent *et al.*, 1993). There is three Δ6desaturase in salmon Δ6fad_a, Δ6fad_b and Δ6fad_c (Monroig *et al.*, 2010).

The first one which is called Δ6fad_a is higher in intestine>liver>brain respectively. The second one Δ6fad_b is higher in brain>intestine>gill>liver. According to Tocher, (2010) this enzyme 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. Δ5 desaturases add double bonds at Δ5 position of long fatty acids like C20 fatty acids and has limited activity. Salmon has both Δ 5 desaturases and Δ6 desaturases which produce DHA from ALA (Tocher, 2010).

2.3.2 Elongases and elongation reaction

The elongase enzymes also has functional activity and divided into two groups (ELOVL1, ELOVL3 and ELOVL6) which are involved in the elongation of the saturated fatty acid and mono-unsaturated fatty acids and the second group are (ELOVL2, ELOVL4 and ELOVL5) which are involved in the elongation of polyunsaturated fatty acids (Jakobsson *et al.*, 2006).

2.4 Health benefits of Omega-3

The human health benefits obtained by consuming seafood is considered as the main reason for global increase of fish and shellfish production (Sena S. De Silva *et al.,* 2010). Regarding the health benefits, several reliable studies have illustrated that omega-3 consumption helps to prevent some diseases including mental disorders, asthma, high blood pressure, and some common cancers such as breast, colon, and prostate (Rose *et al.,* 1999) and it can also protect against cardiovascular disease (CVD) (Dewailly *et al.,* 2007). Moreover meta-analysis indicated that by increasing every 20g/day fish intake the (CVD) risk decreased as much as 7% (He *et al.*, 2004).

According to Whelton et al. (2004) meta-analysis with 228,864 participants, the result illustrated that fish consumption reduced about 20% coronary heart disease. International Society for the Study of Fatty Acids and Lipids suggested that consuming 500 mg of DHA+ EPA/day or 3.5 g/week can provide good cardiovascular health in adult humans (ISSFL, 2004). Studied by (Brenna, 2002) conversion of alpha-linolenic acid (ALA) to docosahexaenoic acid (DHA) is less than 5% in humans, and it highly depends on the concentration of n-6 fatty acids in their dietary sources. American heart association (AHA) suggested that a twice a week fish intake should be included in the diet of general population (Dewailly *et al.,* 2007). Studied by Mozaffarian et al. (2011) indicated that omega-3 consumption declines resting heart rate (HR) and diastolic blood pressure. It has anti-thrombotic influence by increasing the bleeding time, moreover anti-thrombotic effects which have positive impacts on decreasing cardiovascular disease and also anti-arrhythmic effects on human. Moreover, shortage of omega-3 has negative effects on hearing and to some extent damages hearing, especially the cerebral response.

It should be noted that both EPA and DHA are unique components and have individual impacts as well as overlapping actions. For instance DHA has the ability to promote brain function itself and affect platelet function and inflammatory processes (Harris *et al.,* 2007), however, they both have an influence on decreasing blood pressure (Kaur *et al.,* 2011). Infants have low capacity to convert long chain poly unsaturated fatty acids, and therefore highly depend on long chain poly unsaturated fatty acids from breast milk or infant formula (Meldrum *et al.*, 2012). Bourre, (2007) suggested that by taking 200 mg DHA supplement

per day in pregnant women diet plasma DHA increased for fetus. Former studies showed there are a direct relationship between ALA and DHA in the tissues of both mother and fetus. In adult men the conversion of ALA to EPA is approximately 1-5% and also to DHA is less than 0.1%. In women conversion to DHA seems to be better (Harris *et al.*, 2008).

2.5 Chemical composition of fish

Fish species are significantly different in terms of chemical composition. Chemical composition depends on important factors such as fish species; age, size and maturity. Environment and swimming activity also affects the chemical composition of the fish body (Dunajski *et al.*, 1979). The main fish flesh components are proteins, water, carbohydrates, vitamins, minerals and also non-protein-nitrogen (Lynum, 1997). According to Murray et al. (2001) the salmon fillet contains 16-21% protein, 0.2-25% lipid, less than 0.5% carbohydrates and 1.2-1.5% ash.

2.5.1 Fat in salmon body

The fat amounts distributed in the salmon are not equal and decreases from head to the tail (Lie, 2008). Fat contents varies between families from 0.1% to more than 20% (Lie, 2008). The muscle color in most fish species is white to off-white but in the salmonids the flesh is pinkish to red because of carotenoids such as astaxanthin and cantaxanthin which is used in their diets. In farmed salmon astaxanthin and cantaxanthin are obtained from formulated feeds which are supplemented with 50−100 mg/kg synthetic carotenoid (Smith *et al.*, 1992). These synthetic pigments are very expensive and are responsible for 15% of the total feed cost (Prendergast, 1994). According to Storebakken and No, (1992) less than 20% carotenoids which are applied in the feed are retained in the fish flesh.

Major fat storage in salmon is in muscle tissues. In the fatty fish fat is mainly in the muscle but in the lean fish the fat is stored in the liver (Lie, 2008). Liver and intestine are the major sites of lipid synthesis in salmon, muscle biosynthesis being negligible (Tocher *et al.*, 2003). Several studies have indicated that omega-3 long chain polyunsaturated fatty acids biosynthesis in liver and intestine is induced in salmon fed diets with low levels of these nutrients (Zheng, 2005, Leaver, 2008, Bell, 1997 and Bell, 2002), and this is due to

transcriptional activation of genes for the biosynthetic enzymes (Zheng *et al.*, 2004, Leaver *et al.*, 2008 and Morais *et al.*, 2009).

Salmon is known as an oily fish and fat is stored primarily in the muscle and it is also deposited in visceral cavity, white muscle, red muscle, myosepta and belly flap. According to Nanton et al. (2007) who fed salmon fish oil and vegetable oil the result from the study illustrated that the muscle tissues (red and white) contained a significantly lower percentage of fat than myosepta, belly flap and visceral fat tissues and also showed that muscle tissues contained higher levels of omega-3 PUFA.

Fatty acid composition in salmon tissues (muscle and liver) is significantly influenced by the dietary FAs composition (Torstensen *et al.*, 2004). It was reported that fat content in salmon fillets is essential for the texture, flavor and color (Madsen *et al.*, 1998). Studies by Mørkøre and Rørvik (2001) illustrated that salmon has the ability to accumulate fat during the autumn while the amount of fillet fat decreases by approximately 1.5% in the winter. Studies by Mørkøre and Rørvik (2001) showed that by increasing chain length the rates of fatty acid digestibility decreased, and also there was direct relationship between digestibility and the unsaturation of the fatty acids.

2.5.2 Uptake and bioavailability of long-chain omega-3 fatty acids

The lipid digestion is done in the entire digestive tract of fish, including the stomach, pyloric caeca and proximal and distal intestines (Glencross, 2009). Pancreatic lipase hydrolyzes triglyceride ester bonds in the small intestine. This enzyme breaks down FA from the *sn*−1/3 positions to form 2-monoacylglycerols and small amounts of diacylglycerols. The main products of lipid digestion in fish are free fatty acids and glycerol. Moreover studies by (Tocher, 2010) indicated that the most phosphoglycerides are digested to form 1-acyl lysophosphoglyceride. The digestion products are solubilized or emulsified in bile salt micelles and further absorbed. (Smith *et al.*, 1983). Fatty acids of different chain length are absorbed at different locations in the gastrointestinal tract in salmon (Røsjø *et al.*, 2000). For instance long chain fatty acids are mainly absorbed in the mid intestine otherwise medium chain fatty acids are mainly absorbed in the pyloric ceca (Denstadli *et al.*, 2004). Free fatty acids activated by coenzyme a (CoA) form fatty acyl COA (Glencross, 2009). After that fatty

acids COA are re-esterified into triacylglycerols and phosphoglycerides in the intestinal cells (Sargent *et al.*, 1989).

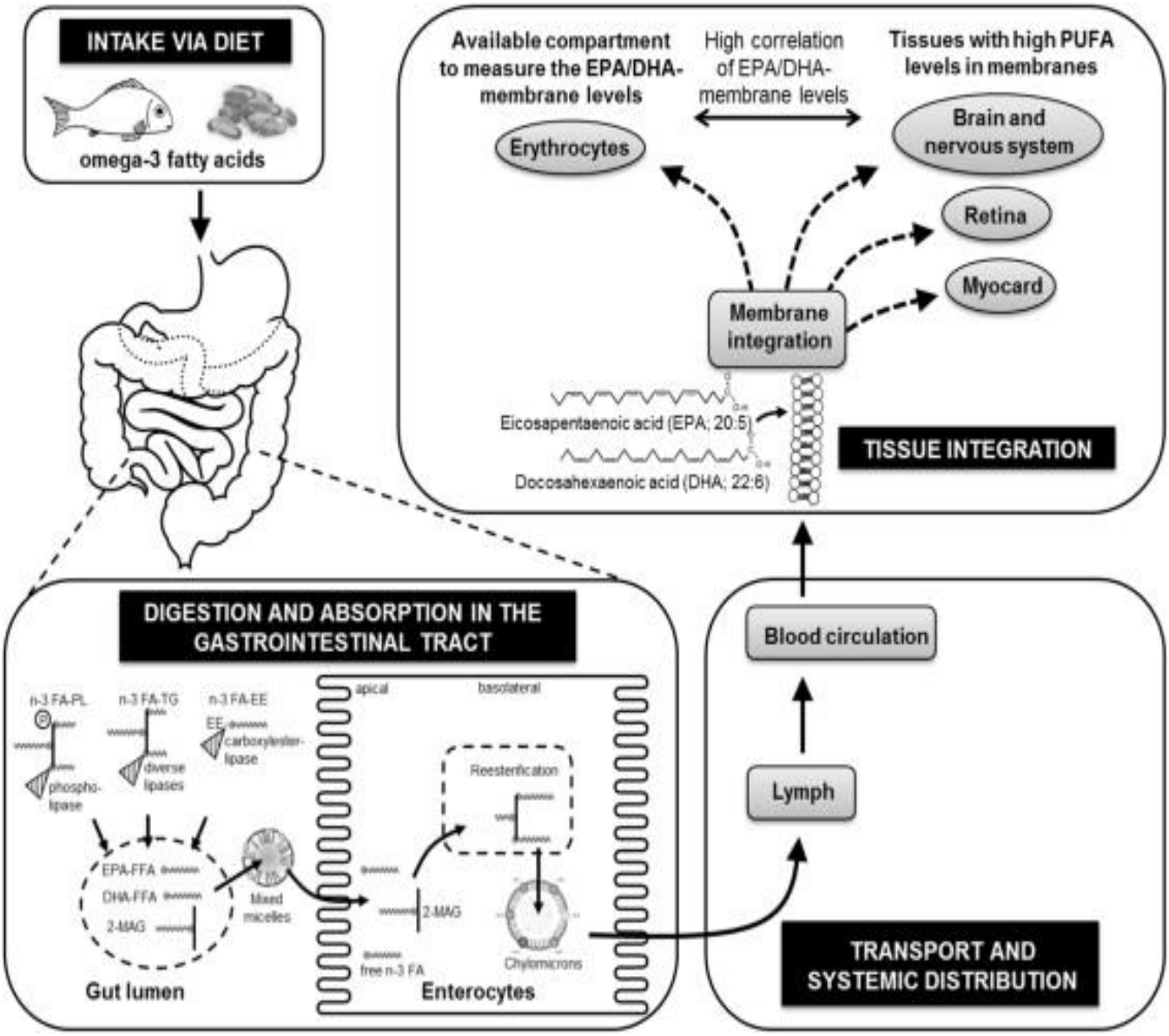

Figure 4- Absorption of long chain of long-chain ω3 from food to tissue. Adapted from Schuchardt and Hahn, (2013).

2.5.3 Protein in salmon muscle

Proteins in fish muscles can be divided into functional, sarcoplasmic and connective tissue. Protein is important for building new tissues (Wilson, 1989; Pickering and Black, 1998) Functional proteins are such as actin and myosin which able the muscle to contract, sarcoplasmic proteins such as globulin and myoalbumin. Moreover, the third one is the connective tissue which is increasing from head to the tail (Sikorski, 1994).
Connective tissue consists of collagen which is less in fish muscle compared to mammals. The protein content in salmon muscle depends on some factors such as season and fish size. For instance according to Belitz *et al.,* (2009) wild salmon has higher protein especially in the feeding season and less in spawning season.

2.5.4 Glycogen, water and dry matter

Approximately 0.3% of fish body weight consists of carbohydrate. According to Lynum, (1997) carbohydrates accumulated as glycogen in the liver and the muscle. Carbohydrates in the body are upon slaughter broken down into lactic acid, and as the result pH will drop in the muscles. Studies by Dunajski et al. (1979) illustrated that the fillet and water binding capacity are affected by muscle pH. Lipid and water together compose approximately 80% of the fish body (Jobling, 2001). Studies by Jobling. (2001) showed that dry matter in salmon body is 25-40% and it mainly depends on the muscle and viscera.

2.6 Diet and feed effect on body composition:

The amount of EPA and DHA are not the same in all types of fish, it depends on several factors such as species, sex, size, diet, water temperature and season (Abbas *et al.,* 2009) and among these factors diet plays an important role.

Food intake and efficiency have been improved in farmed salmon by 40% and 20%, respectively (Thodesen *et al.,* 2001). Farmed fish cannot catch their feed and therefore feed composition depends on the feed offered by fish farmer. One of the advantages of farmed fish over wild fish is the capability to control the environment to some extent which is good for getting high amount of EPA and DHA in feed.

Salmon have the enzymes to elongate the shorter chain of omega-3 to the longer chain but the capacity is limited (Miller *et al.*, 2007) thus they should introduce very long chain omega-3 into their diets. Nowadays companies producing salmon feeds use energy dense diets which consist of low protein and high lipid ratios (Hemre and Sandnes, 1999).

Salmon is carnivorous which means that they do not tolerate high concentration of carbohydrates in their diets. The feed should cover all the fish requirements to provide a healthy fish with fast growing performance and also low mortality.
Low levels of the two essential fatty acids such as 18:2n-6 and 18:3n-3 in fish diets shows deficiency problems. According to Glencross, (2009) for instance low feed efficiency, fatty liver, poor growth, and high hepatosomatic index, increased water content in whole body or muscle and high accumulation of 20:3n-9 in the tissue. Another study in salmon showed that a short period of dietary deficiency of EFA leads to substantial change in the fatty acid composition of liver and blood but not the carcass lipid (Ruyter *et al.*, 2000).

2.6.1 Fish meal and fish oil as a feed for Salmon

It has been more than 15 to 20 years of changes of the composition and formulation of salmonid feeds. During this period one have seen an increase in lipid levels (from 8–40%) and energy contents (>20 MJ/kg) and also a remarkable reduction in carbohydrate levels (from 40-10%) (Hardy, 2002).

Nowadays, significant changes are seen in the marine commodities earlier used in salmon diets. According to the Tacon and Metian, (2008) there are several reasons for finding substitution for these commodities. The initial reason is the shortage of sustainable fish meal (FM) and fish oil (FO), the cost of these commodities are increasing despite the fact that the production remains constant (Tacon and Metian, 2008). In addition they may contain contaminants and also organic pollutants, like dioxins and also PCBs.

The fish meal production was 4.83 million tons worldwide according to IFFO, (2011) and also some by-products in fish meal production has been increased and reached 22% of Norwegian production (Chamberlain, 2011). Fish meal substituted by plant protein in some causes caused reduction in growth or also feed conversion, which may be due to the presence

of anti-nutritional factors that mostly influenced feed intake and gut function (Kaushik *et al.*, 1995, de Francesco *et al.*, 2004, de Francesco *et al.*, 2004 and Espe *et al.*, 2006). Substitution of fish meal with soybean protein concentrate up to 80% or 100% for halibut (Berge *et al.*, 1999) and rainbow trout Oncorhynchus mykiss (Kaushik *et al.*, 1995) showed no negative on growth performance or nutrient utilization. However, according to Gomes et al. (1995) and Espe *et al.*, 2006) studies on rainbow trout and also salmon showed that the total alteration of fish meal with plant protein has influence on growth performance.

According to Kaushik et al. (2004) and Espe et al. (2006) lipid retention declines by applying high levels of plant proteins in salmon feed. Soybean is considered as a good alternative dietary source for fish meal due to the fact that both dehulled and solvent-extracted soybean meal contain high protein and sufficient balance of essential amino acids (Carter *et al.*, 2000). Fish oil is the optimal oil for salmon due to the fact that it contains high levels of the very long chain omega-3 fatty acids. Fish oil production was 1.6 million tons in 1990 but has been decreasing since 2005 (Silva, 2010). At present salmon industry uses about 60% fish oil in the salmon diet (Nasopoulou and Zabetakis, 2012).

2.6.2 Vegetable oil as a feed for Salmon

Vegetable oil is a cheap and abundant source in comparison to fish oil. The production has increased significantly and has been evaluated to be used as an alternative source for fish oil Vegetable oil consists of a mixture of saturated, monounsaturated and polyunsaturated fatty acids (Bell *et al.*, 2002). Vegetable oil contains more shorter chain fatty acids in comparison to fish oil, for instance linoleic (18:2 ω−6) and oleic acid (18:1 ω−9), and are devoid of the very long chain (Caballero *et al.*, 2002).To compensate for this companies are trying to produce gene modified (GM) rapeseed oil which is high in the very long chain omega-3.
According to Tartibian et al. (2010) it has been suggested that approximately 40-50% vegetable oil can be used in salmon diet, but studies by Torstensen et al. (2000), Bell et al. (2001) and Bell et al. (2002) indicated that when vegetable oil inclusion level reached to 50% and above, there was a significant accumulation of 18:2n-6 and reduction of 20:5n-3 and 22:6n-3 in the fish flesh.

There are different kinds of vegetable oil such as rapeseed oil, soybean oil, palm oil and also olive oil. Soybean oil is the cheapest vegetable oil with 211 million tons of production in 2009 (Soystats, 2010). Salmon fed soy bean oil has higher slaughter yields in comparison to the ones fed fish oil (Regost *et al.*, 2004). Rapeseed oil, which is particularly rich in 18:1*n*−9, has become an attractive substitute for fish oil in salmon diets. By diluting or washing out the vegetable oil derived fatty acids and also using fish oil finishing diet omega-3 long chain polyunsaturated can be restored (Bell *et al.*, 2004 and Bell *et al.*, 2005 Torstensen *et al.*, 2004 and Torstensen *et al.*, 2005).

Olive oil as monounsaturated fatty acid can be consumed partially as substituted for fish oil in salmon (Torstensen *et al.*, 2004). Olive oil used partially as substitution for fish in European sea bass culture, during growth out phase (Mourente *et al.*, 2005). Studies by Torstensen et al. (2008) showed that when salmon were fed vegetable oil at low temperature the growth and protein utilization were improved in salmon. The ratio of *n*-3/*n*-6 in vegetable oils is 0–0.3:1 and in fish oils is 5–6:1(Linderborg *et al.*, 2013).

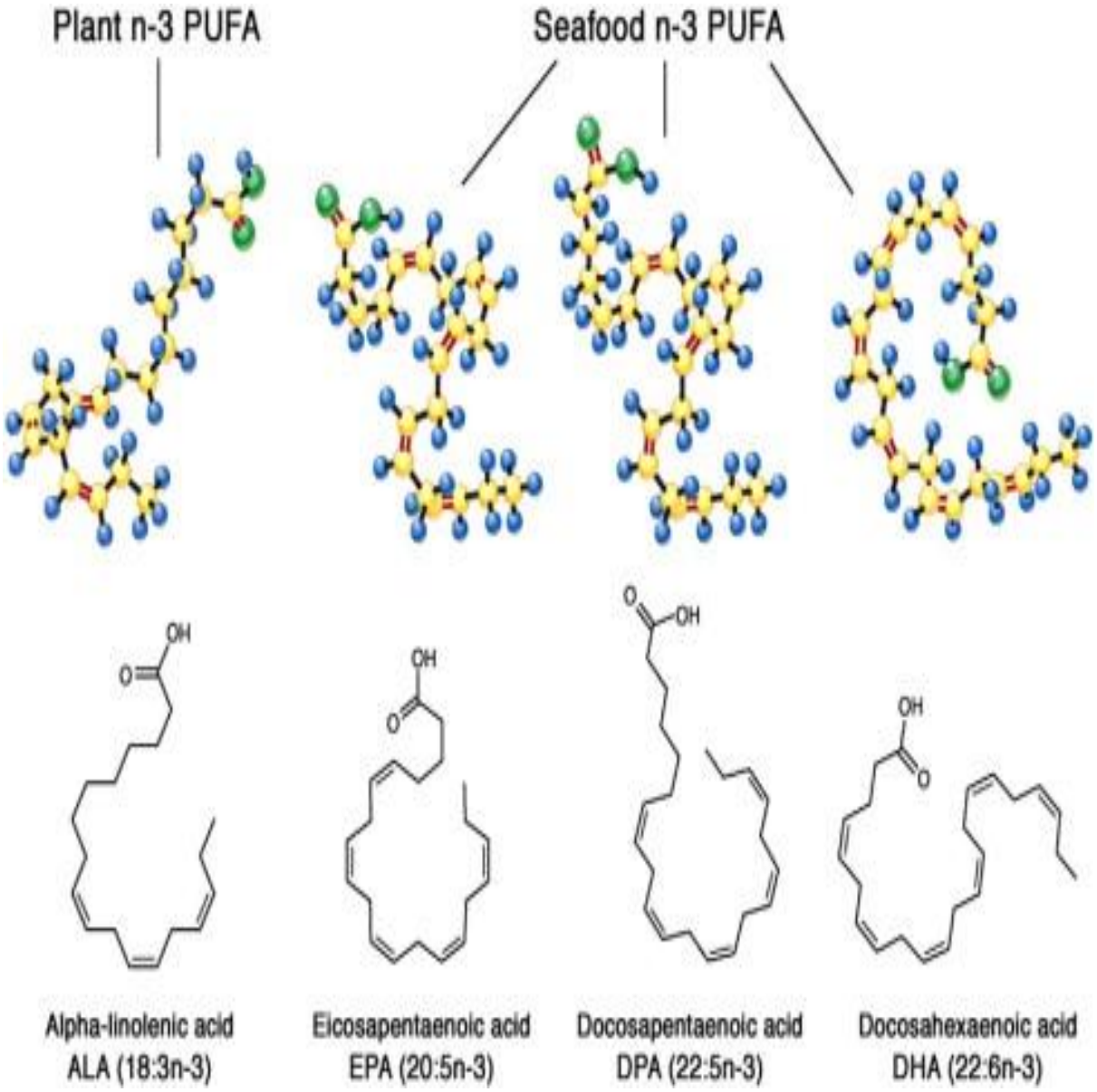

Figure 5- Structure of Alpha-linolenic acid (ALA) derived from plant sources and also Long-chain n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) adapted from Mozaffarian et al. (2011)

3. Material and method

3.1 Fish and experimental design

The salmon families (L11) were produced in autumn 2010 at AquaGens breeding station at Tingvoll, at the west coast of Norway. The fish were kept at Hemne during the fresh water period before transferring to two sea cages at Nofima Marine Sea Water Research station at Averøy in October 2011 and slaughtered in April 2013. This material consisted of a total of about 6000 pit-tagged fish belonging to 100 different families, and the fish were fed a regular commercial salmon diet.

My experiment started in October 2012. A total of 100 fish from one of the two sea cages, and belonging to 10 different families, were randomly sampled. The average body weight of the fish at this time was about 2kg.

Photo by Delaram Mobaraki

3.2 Sampling and recordings

All fish from one of the two sea cages were collected in batches, sedated by using MS222 and identified by reading the pit-tag labels. The 10 families to be used in my study were chosen beforehand, and about 10 fish belonging to each of these families were then sampled and killed by a blow on the head. The rest of the fish were returned to the cage.

For each of the sampled fish the round weight, gutted weight and length was recorded. The fish were then filleted, and the Norwegian Quality Cuts (See figure 6) taken from the left fillet were packed on ice in styroform boxes and sent by car to Nofima Institute at Ås. Here the samples were stored in coolers for 5 days.

After 5 days the samples were taken out for measuring texture at the Nofima fish laboratory, using the TA-XT2 After that the samples were cut into 8 pieces, (as indicated in figure 6) and frozen and stored at -20C until further analysis.

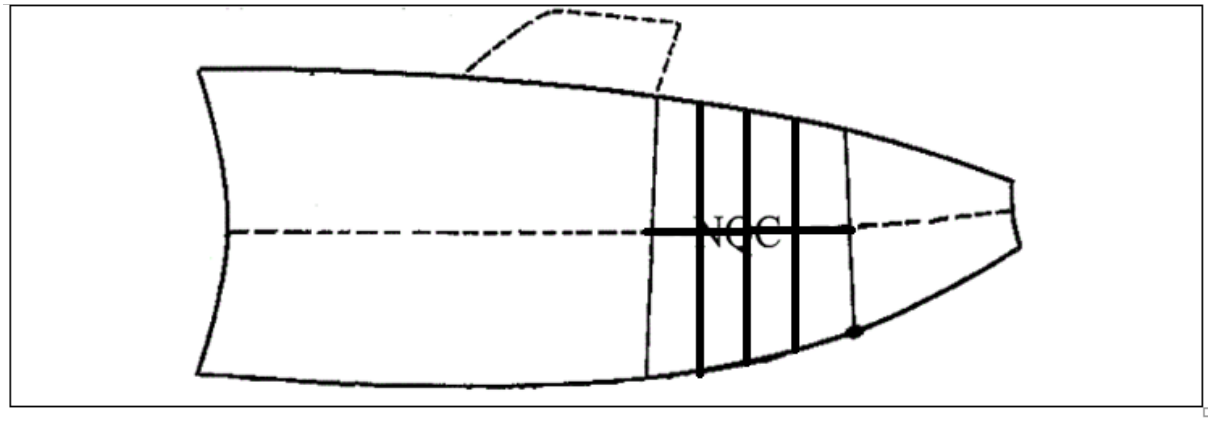

Figure 6- fish flesh the Norwegian quality cut (NQC) adapted from Einen and Thomassen. (1998)

3.3 Total lipid analysis

Pieces from the NQC were used for extraction of total lipids by the Folch method (Folch *et al.,* 1957). About 1.5 g of muscle was homogenized in 30 ml chloroform/ methanol by Ultra-Turax knife homogenizer. After that all the samples were placed in refrigerator for 24 hours. The solution was filtered and then 0, 9% sodium chloride (NaCl) solution was added. The solutions started to separate into two phases. The upper layer was H_2O and methanol (water phase) and the bottom layer consisted of a combination of lipid and chloroform (lipid phase), the samples were placed in refrigerator overnight to be thoroughly separated. The following day the water phase was removed. The amounts of lipid were found out by weighing out the tubes after that lipid the phase had been transferred into 3 tubes, two tubes were labeled "a" and "b" for measuring the amount of fat and the third one for the methylation. The "a" and "b" tubes were placed in water bath and the chloroform evaporated by N_2 gas. Then the amount of fatty acids that were left in tubes had to be weighed out again to get the total fat content by calculating the differences between full tubes and empty tubes.

After evaporation, 25 ml heptane was added to the third tube, the tube were mixed for ten seconds and remixed again after 30 min. Then the tubes were placed in water-bath at 40-50 °C, 0.4 ml metanolic-HCl -3N was added and put in water-bath again for 15 min at 85 °C, then cooling the tubes. After this the contents in the tubes used for methylation were ready to transfer to small tubes which are special for gas chromatography for analyzing the fatty acid composition of each sample. Gas chromatography is the technic to separate the samples into components. The components must have sufficient volatility and thermal stability. The result from GC was explained in a graph with the y-axis and x-axis. The y-axis detected chemical respond and the x-axis show the retention time.

3.4 Analysis of feed

Fish feed pellets (9mm) were mixed with dry-ice (which is carbon dioxide in a solid form). Dry ice was applied as a cooling agent. After mixing the feed with dry ice the sample was ready for grinding. The grinding process helps to homogenize the sample and makes optimum particle size for future analysis. The Folch method which was used for analysis of muscle fat was applied also for analysis of fatty acid composition of feed.

3.5 Statistical analysis

The data acquired from the experiments will be analyzed by analysis of variance ANOVA by using general linear model called GLM statement of the statistical analysis software (SAS). Muscle fat, body weight and fatty acids denote the dependent variables. Gender and diet are used as class variables.

4. Results

4.1 Body weight (round weight and gutted weight), length and condition factor

The results illustrated that both the mean round weight (RW) and gutted weight (GW) were significantly different among the ten families (P = 0.0001) and (P = 0.001), respectively. As shown in table 1 family number 55 had significantly lower RW and GW, while family number 403 had significantly higher RW and GW in comparison with the other families. According to Duncan's test the RW were significantly different in the family number 55. Moreover GW was significantly different across the families number 55, 77,109 and 374. No significant difference between genders was observed in RW and GW.

Significant differences in length and CF were observed (P = 0.0004) and (P = 0.0033). The length was significantly different among the family number 55, 109 and 403. Moreover family number 433 had the higher CF average (1.2 ± 0.04). Across all the families there were significantly differences among number 55, 77, 78, 109, 112, 374 and 433.

Table 1- Round weight (RW), gutted weight (GW), fork length and condition factor (CF) of farmed Atlantic salmon (*Salmo salar* L.) fed one commercial diet

Family	**RW (gr)**	**GW (gr)**	**Length (cm)**	**CF**
55	$1166.4^{d} \pm 169.70$	$1032.1^{a} \pm 149.87$	$44.7^{c} \pm 1.91$	$1.12^{bc} \pm 0.08$
77	$1784.8^{bc} \pm 126.46$	$1595.4^{bc} \pm 116.50$	$51.6^{ba} \pm 1.35$	$1.14^{bc} \pm 0.02$
78	$2139.2^{bac} \pm 156.46$	$1912.7^{bac} \pm 141.13$	$52.9^{ba} \pm 1.40$	$1.25^{a} \pm 0.04$
109	$1652.5^{c} \pm 112.34$	$1468.9^{c} \pm 101.25$	$50.6^{b} \pm 1.00$	$1.11^{c} \pm 0.03$
112	$1927.1^{bac} \pm 112.38$	$1744.5^{bac} \pm 101.57$	$51.3^{ba} \pm 0.78$	$1.28^{a} \pm 0.04$
335	$1975.4^{bac} \pm 158.53$	$1768.5^{bac} \pm 145.18$	$52.2^{ba} \pm 1.63$	$1.19^{bac} \pm 0.02$
370	$2190.8^{ba} \pm 144.46$	$1970.5^{ba} \pm 130.01$	$54.8^{ba} \pm 1.34$	$1.17^{bac} \pm 0.02$
374	$1789.2^{bc} \pm 278.82$	$1582.8^{bc} \pm 248.50$	$51.4^{ba} \pm 2.49$	$1.17^{bc} \pm 0.03$
403	$2390.7^{a} \pm 131.80$	$2130.4^{a} \pm 108.75$	$55.7^{a} \pm 1.07$	$1.19^{bac} \pm 0.03$
433	$2181.6^{ba} \pm 179.40$	$2018.6^{ba} \pm 175.58$	$54.2^{ba} \pm 1.66$	$1.23^{ba} \pm 0.04$

4.2 Muscle texture:

The breaking force (BF) is considered to be the best measure of the hardness of the salmon muscle, and significant differences in mean BF were observed on the fillets from the different families (P = 0.0013). As shown in table 2 family number 109 had significantly lower BF average (6.1 ± 0.39 N) and family number 433 had significantly higher BF average (9.4 ± 0.35N).

Table 2- Breaking force (BF) of the muscle of Atlantic salmon (*Salmo salar* L.)

Family	55	77	78	109	112	335	370	374	403	433
BF	7.3^{bc}± 0.46	7.9^{ba}± 0.58	8.3^{ba}± 0.48	6.1^{c}± 0.39	8.7^{ba}± 0.40	8.6^{ba}± 0.39	8.6^{ba}± 0.50	8.3^{ba}± 0.64	8.0^{ba}± 0.39	9.4^{a}± 0.35

As presented in fig7 and 8 slight positive correlation was seen between the mean weights of the salmon families, and the braking force observed.

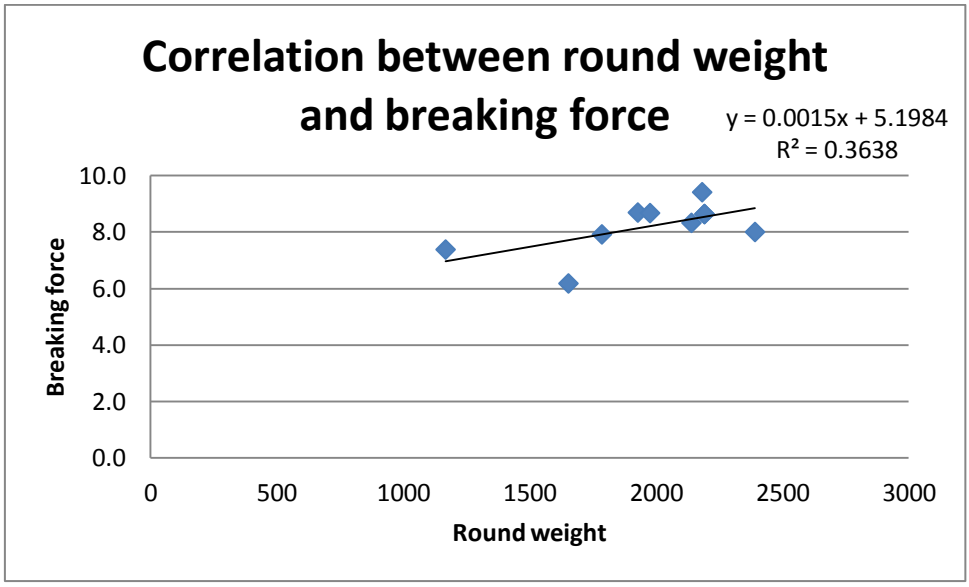

Figure 7- The correlation between the mean round weight of the salmon in the different families and the muscle texture (BF)

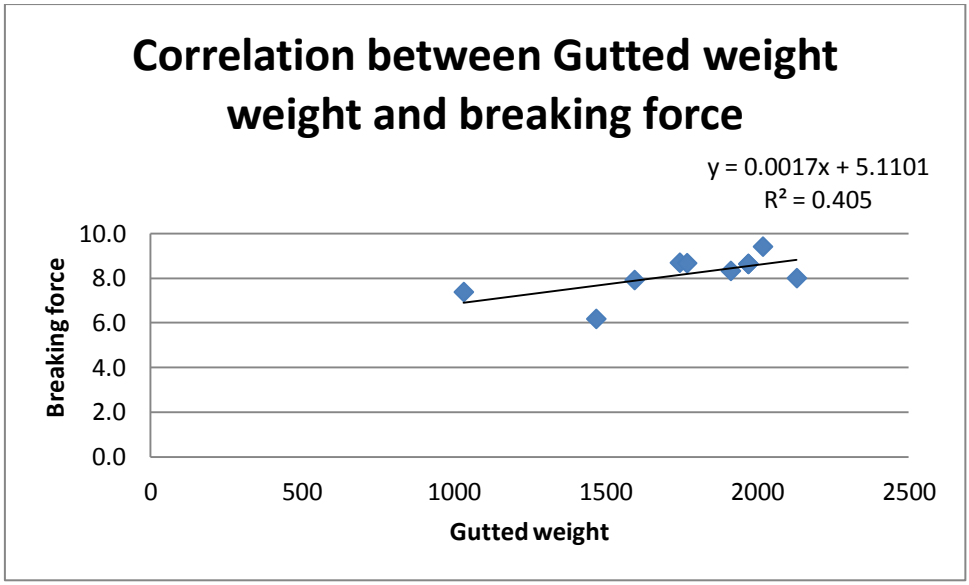

Figure 8- The correlation between the mean gutted weight of the salmon in the different families and the muscle texture (BF)

4.3 Total muscle fat in different families

Total lipid percentages in the muscle varied across all the families between 7.7% and 10.2%. As shown in table 3 family number 355 seemed to have the highest muscle fat and number 374 the lowest in comparison with the other families, but no significant differences were detected (P = 0.522).

Table 3- total muscle fat in different Atlantic salmon families (*Salmo salar* L.)

Family	55	77	78	109	112	335	370	374	403	433
Total fat	8,07± 4,24	9,86± 2,74	10,06± 2,90	8,16± 2,11	9,2± 3,20	10,42± 2,33	8,93± 3,53	7,71± 4,03	8,36± 2,81	9,70± 3,52

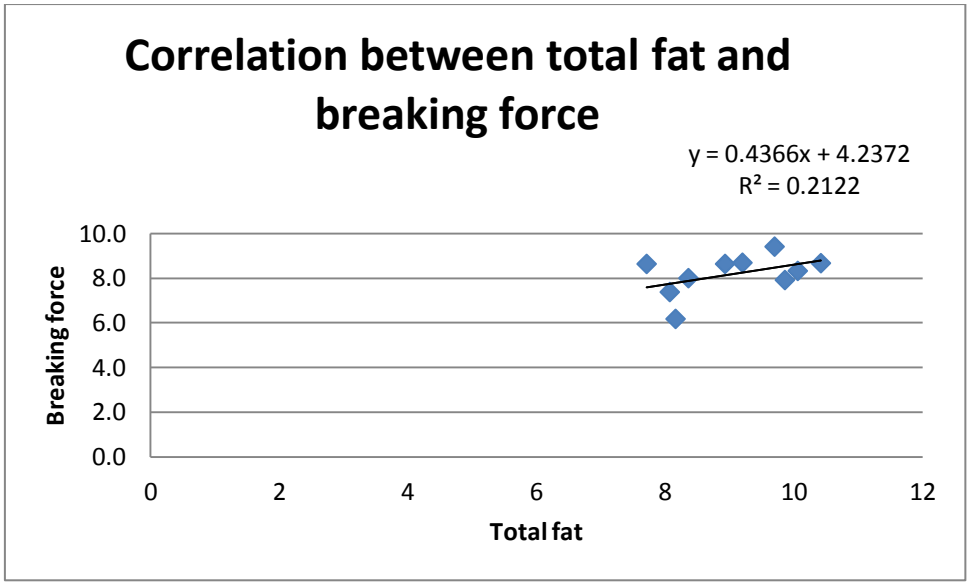

Figure 9- The correlation between the mean total muscle fat of the salmon in the different families and the muscle texture (BF)

As shown in figure 9 there were a slight positive correlation between total lipid content and breaking force in the salmon muscles.

4.4 Fatty acids composition in feed and muscle

The results from the fatty acid analysis are shown in table 4 and 5 Only acids with percentages higher than 0,1% are include. There were significant differences in docosapentaenoic acid (DPA, 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) with (P = 0.0048) and (P = 0.0487), respectively. According to the results DPA percentage in the family number 370 was significantly different from the other families except for family number 112. Moreover the Duncan's test showed that there were significant differences in the DHA percentage in the family number 55 from all the other families except number 370 and 374. There were strong tendency for the saturated stearic acid 18:0 and also Alpha linolenic acid 18:3 n-3, both (p =0.05).

Table 4- Fatty acids composition of omega-3 and omega-6 in muscle of Atlantic salmon in 10 different families

Family No.	C18:2n6c	C18:3n6	C18:3n3	C20:3n6	C20:3n3	C20:4n6	C20:5n3	C22:5n3	C22:6n3
55	14.3^{ba}±0.57	0.1^{a}±0.02	7.7^{b}±0.35	0.2^{a}±0.04	0.5^{b}±0.05	0.3^{a}±0.09	2.7^{c}±0.37	0.8^{c}±0.10	4.9^{a}±1.44
77	14.3^{ba}±0.34	0.1^{b}±0.03	7.9^{ba}±0.28	0.1^{b}±0.03	0.6^{ba}±0.09	0.2^{a}±0.05	2.9^{bac}±0.27	0.9^{bc}±0.06	4.1^{c}±0.49
78	14.4^{ba}±0.54	0.1^{b}±0.02	8.1^{a}±0.36	0.2^{ba}±0.04	0.6^{ba}±0.05	0.2^{a}±0.04	2.8^{bc}±0.27	0.9^{b}±0.07	3.9^{c}±0.43
109	14.3^{ba}±0.27	0.1^{b}±0.02	7.9^{ba}±0.19	0.2^{ba}±0.03	0.6^{ba}±0.05	0.2^{a}±0.03	2.9^{bac}±0.25	0.9^{bc}±0.08	4.2^{bc}±0.47
112	14.3^{a}± 0.25	0.1^{b}±0.01	8.1^{a}± 0.16	0.1^{b}± 0.02	0.6^{ba}±0.04	0.2^{a}±0.03	2.9^{bac}±0.12	0.9^{ba}±0.06	4.1^{c}± 0.23
335	14.3^{a}±0.31	0.1^{b}±0.03	8.1^{a}±0.29	0.2^{b}±0.03	0.6^{ba}±0.10	0.2^{a}±0.04	2.9^{bac}±0.42	0.8^{bc}±0.08	3.9^{c}±0.59
370	14.0^{b}±0.47	0.1^{b}±0.03	7.93^{ba}±0.28	0.2^{ba}±0.03	0.6^{ba}±0.06	0.3^{a}±0.06	3.3^{a}±0.31	1.0^{a}±0.07	4.5^{bac}±0.78
374	14.2^{ba}±0.45	0.1^{b}±0.03	8^{ba}±0.31	0.2^{ba}±0.03	0.6^{b}±0.09	0.3^{a}±0.05	3.1^{ba}±0.61	0.9^{bc}±0.07	4.9^{ba}±1.08
403	14.3^{ba}±0.38	0.1^{b}±0.02	8.1^{a}±0.19	0.1^{b}± 0.01	0.6^{a}±0.09	0.2^{a}±0.04	2.9^{bac}±0.36	0.9^{bc}±0.07	4.3^{bc}±0.94
433	14.2^{ba}±0.32	0.1^{b}±0.02	8.1^{a}±0.33	0.1^{ba}±0.03	0.6^{a}±0.06	0.3^{a}±0.03	2.9^{bac}±0.22	0.9^{b}±0.04	4.1^{bc}±0.44

Table 5- Fatty acids composition in 10 different Atlantic salmon

Family No.	C14:0	C16:0	C16:1n7	C18:0	18:1n9c	18:1n7c	C20:1X	C20:2	C22:1n9
55	2.9^{b}±0.23	10.1^{b}±0.55	2.5^{a}±0.21	2.0^{b}±0.21	42.0^{ba}±1.37	1.6^{ba}±0.16	0.9^{a}±0.08	0.6^{c}±0.07	1.0^{a}±0.19
77	3.0^{a}±0.23	10.6^{ba}±0.51	2.5^{a}±0.19	2.0^{b}±0.15	41.5^{ba}±1.46	1.9^{a}±0.98	0.7^{b}±0.09	0.6^{bc}±0.07	1.0^{a}±0.26
78	2.9^{ba}±0.16	10.3^{ba}±0.62	2.5^{a}±0.11	2.1^{ba}±0.12	41.9^{ba}±1.37	1.6^{ba}±0.06	0.7^{cb}±0.07	0.6^{bac}±0.05	1.1^{a}±0.14
109	2.9^{ba}±0.09	10.4^{ba}±0.29	2.5^{a}±0.09	2.0^{b}±0.09	42.3^{ba}±0.51	1.6^{ba}±0.06	0.7^{cb}±0.04	0.6^{bac}±0.06	1.1^{a}±0.15
112	3.0^{ba}±0.07	10.3^{ba}±0.36	2.5^{a}±0.06	2.0^{ba}±0.10	42.6^{a}±0.63	1.6^{ba}±0.06	0.7^{cb}±0.03	0.7^{bac}±0.07	1.0^{a}±0.08
335	2.9^{ba}±0.12	10.3^{ba}±0.41	2.5^{a}±0.14	2.0^{b}±0.15	42.3^{ba}±0.73	1.6^{ba}±0.08	0.7^{b}±0.11	0.7^{ba}±0.09	1.1^{a}±0.26
370	2.9^{ba}±0.09	10.8a±0.58	2.4^{a}±0.08	2.2^{a}±0.11	41.5^{ba}±1.05	1.5^{b}±0.14	0.7^{cb}±0.07	0.6^{bac}±0.05	1.1^{a}±0.17
374	2.9^{ba}±0.25	10.8^{a}±0.82	2.5^{a}±0.15	2.1^{ba}±0.17	41.4^{ba}±1.07	1.5^{b}±0.11	0.7^{cb}±0.06	0.6^{bac}±0.09	1.0^{a}±0.25
403	2.9^{ba}±0.11	10.6^{ba}±0.62	2.4^{a}±0.07	2.1^{ba}±0.11	41.8^{ba}±0.84	1.6^{ba}±0.08	0.6^{cb}±0.06	0.7^{a}±0.06	1.1^{a}±0.17
433	2.9^{ba}±0.12	10.7^{ba}±0.59	2.5^{a}±0.07	2.1^{ba}±0.06	42.0^{ba}±0.50	1.6^{ba}±0.07	0.7^{c}±0.02	0.6^{bac}±0.07	1.2^{a}±0.13

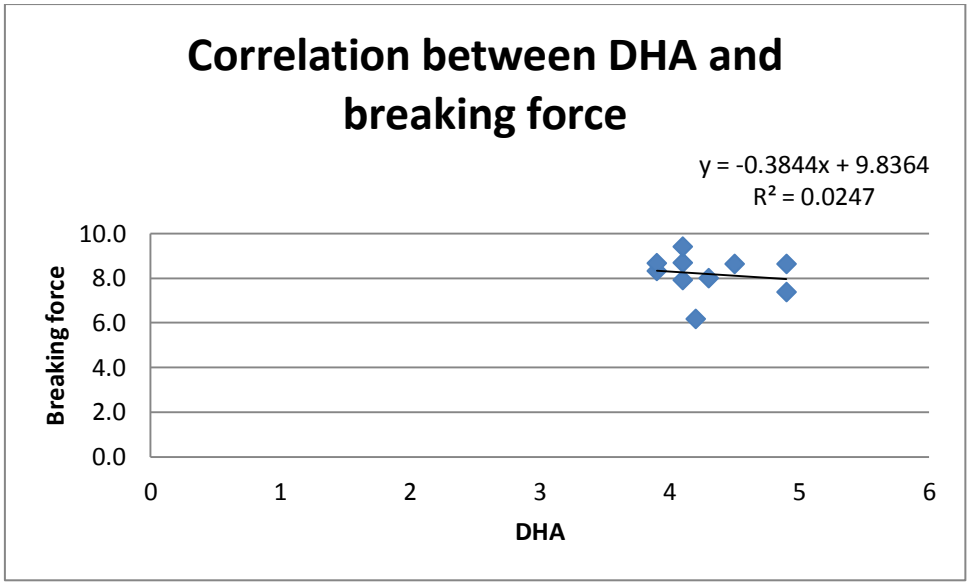

Figure 10- There were not significant differences between breaking force and DHA

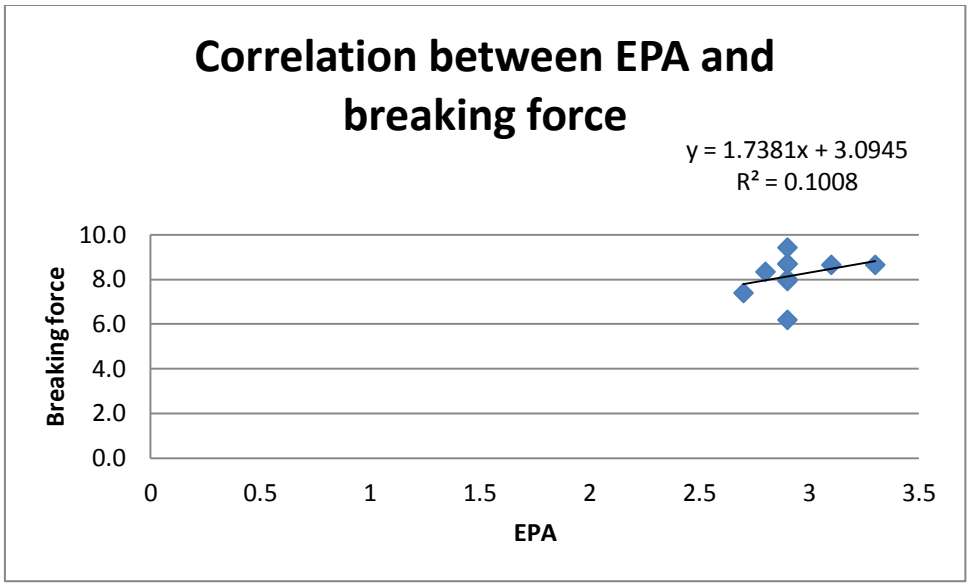

Figure 11- There were slight correlation between breaking force and EPA

4.4.1 The relationship between n-3 fatty acids composition in feed and fish

A strong relation was seen between the fatty acid composition in the feed and in the muscle. This is shown for the omega-3 fatty acids in table 6. As illustrated in figure 10, however, the results indicated that the mean percentage of alpha-linolenic acid (ALA, 18:3 n-3) and EPA were lower, and DPA and DHA higher in muscle than in the feed.

Table 6- Unsaturated fatty acids of the omega-3 family in feed and in10 different Atlantic salmon families

Omega-3	C18:3n3	C20:5n3	C22:5n3	C22:6n3
Fish	8.0 ± 0.13	3.2 ± 0.14	0.9 ± 0.04	4.9 ± 0.35
FEED	7.8 ± 0.06	4.4 ± 0.04	0.4 ± 0.01	2.8 ± 0.04

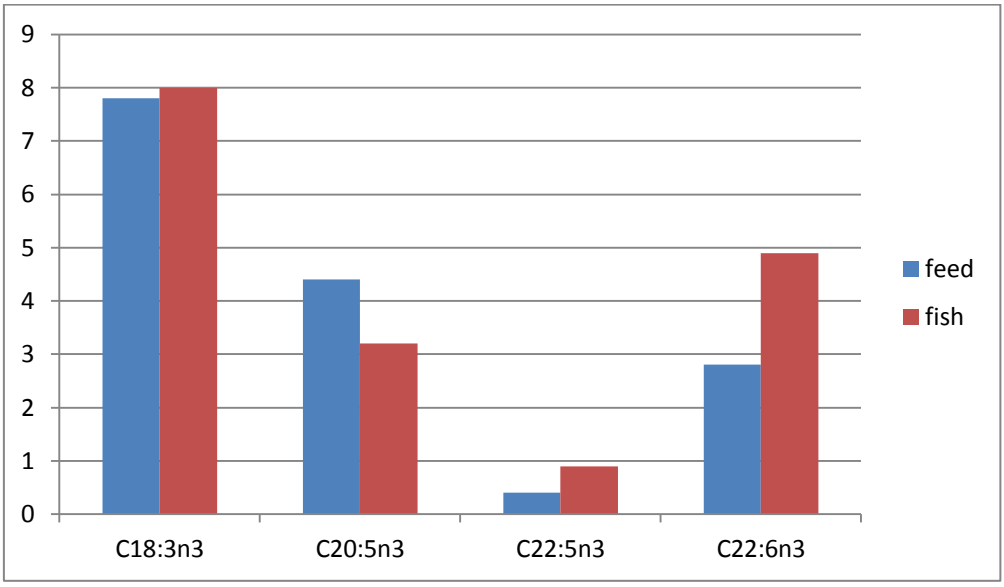

Figure 12- The correlation between n-3 fatty acids composition in feed and fish

4.5 Sex differences in fatty acid composition

Across all the fatty acids there were observed significant differences only in dihomo-gamma-linolenic acid, 20:3 n-6 (P = 0.04) between genders. Moreover, according to the table 7 the 20:3 n-6 was higher in family number 55 in both female and male (0,27 ± 0,03) and (0,68 ± 0,03) respectively.

Table 7- Sex differences in dihomo-gamma-linolenic acid, 20:3 n-6 in 10 different families

Family	55	77	78	109	112	335	370	374	403	433
N6203(F)	0,27± 0,03	0,20± 0,00	0,20± 0,00	0,22± 0,02	0,20± 0,00	0,21± 0,01	0,23 ± 0,02	0,23± 0,03	0,20± 0,00	0,22± 0,02
N6203(M)	0,63± 0,03	0,18± 0,02	0,22± 0,02	0,23± 0,03	0,20± 0,00	0,20± 0,00	0,20± 0,00	0,20± 0,00	0,20± 0,00	0,20± 0,00

5. Discussion

5.1 Body weight, length and condition factor

Comparison of the means of the 10 fish from the 10 different families of Atlantic salmon indicated that there may be significant differences in the growth rates of these families, fitting with a general knowledge from salmon breeding that growth rate has a strong genetic background. To have information about body weight is important since the study by Olsen and Skjervold. (1995) indicated that in addition to age and fat content, body weight is another reason which can cause differences in n-3 fatty acids in farmed salmon. Their study, which was done on two year-groups of salmon indicated that for the youngest group some fatty acids, such as 18:0 and 22:5 n-3, were more influenced by the body weight than the lipid content, whereas a second group of fatty acids, including 16:0,18:2 n-6; 18:3 n-3, 20:4 n-6, and 20:5 n-3 were hardly influenced by the body weight. Moreover, for the important fatty acids such as DHA, 55-75% of the variance was combined impact of variable lipid content and fish weight.

The condition factor ranging from 1.11 to 1.28 was significantly different across the families. The levels were quite normal. Studies by Einen and Thomassen. (1998) illustrated that the variation of condition factor in salmonids is from 0.7 to 1.9.

5.2 Muscle texture

Significant differences among families were observed in the muscle texture as measured as breaking force. And this also agrees with other former study on salmon done by Bahuaud et al. (2011).

5.3 Total fat content

The total fat content of the muscle ranged from 7.7% to 10.4%, but showed no significant differences among all the families. According to Leaver et al. (2011), by increasing lipid content in the salmon the content of individual fatty acid (n-3 polyunsaturated fatty acids)

increased. It was further found in that study that the percentage of total fatty acids in muscle seemed to be a highly heritable trait. The percentage of muscle omega-3 long chain PUFA has on the other hand an inverse relation both with total flesh lipid and also final weight. According to Leaver et al. (2011) the difference in lipid deposition between families could be due to increased triacylglycerol from dietary lipids than an increase in tissue phospholipids. Nevertheless, there was still a large variation in flesh omega-3 long chain (PUFA) content across families, even among families showing the same total flesh lipid content, indicating changes in the triacylglycerol composition of stored fat.

5.4 Fatty acids composition:

Former studies have illustrated that fatty acid compositions of fish tissues is determined by the type of dietary lipid and also the capability of fish to modify the dietary fatty acids through desaturation and elongation reaction (Bell *et al.*, 1993). This clear effect of diet was seen also in our study, and this also agrees with other former studies from salmon (Olsen, 2011; Torstensen *et al.*, 2000).

Salmon fed diets that include plant oils, which are consequently rich in ALA but low in EPA and DHA, cause significant reduction in total omega-3 and also omega-3 long chain PUFA, in particular DHA and EPA (Polvi and Ackman. 1992, Bell *et al.*, 2003 and Bell *et al.*, 2004). According to Ruyter et al. (2006) and Kjær et al. (2008), high levels of plant oils in the diet for Atlantic salmon lead to higher accumulation of fat in the liver than compared to the livers of fish fed a fish oil diet, resulting in decreased relative deposition of EPA and DHA in the liver.

The result from our study showed that there were significant differences in the percentages of DPA and DHA among the 10 families. These 10 families were fed one type of diet with the same dietary lipid but still there are some variations in the percentages of n-3 between the families. This variation might be due to differences in desaturation and elongation capacities in their biosynthesis pathway between the different families. According to Leaver et al. (2011) flesh n-3composition seemed to be a highly heritable trait (h^2=0.77±0.14), which means that contents of n-3 HUFA vary between families.

5.4.1 Docosahexaenoic acid (22:6n-3)

According to the result presented in table 4 the percentage of DHA ranged from 3.9% to 4.9%, and significant differences were seen across families The results showed that the muscle percentage of DHA was increased compared to the diet, which agree which previous studies done by Bell et al. (2001) and Kjaer et al. (2008) As mentioned above, this may be due to the elongation and desaturation activity in the salmon, but it can also be due to selective retention of DHA in the body The studies by (Caballero *et al.*, 2002; Regost *et al.*, 2003; Torstensen *et al.*, 2004) showed that by reducing the level of fatty acids in salmon diet the retention of DHA increased in muscle. And also the studies by Thomassen et al. (2012) reported that salmon fed rapeseed oil converted more EPA to DHA through elongase and desaturase activity. Moreover the same study by Thomassen et al. (2012) showed that in the salmon which fed RO and RO + EPA conversion of the EPA to DHA was higher than the one that fed high levels of DHA included (FO and RO + EPA + DHA), indicating a regulation of these enzymes by product inhibition.

The difference between the families might be due to the variation in biosynthesis capacities to produce EPA and DHA which is in agreement with the study of Leaver et al. (2011) indicating that n-3 HUFA seemed to be a highly heritable trait in Atlantic salmon. It is still not known which enzymes are responsible for the difference between the families but might be due to differences in Δ6fad activity.

6. Conclusion

In conclusion the differences observed in the present study also may indicate that the genetic background can have a significant impact on the metabolism of the long chain omega 3 fatty acids. So selective breeding programs selecting those families that have higher EPA and DHA level can help the industry to get more long chain omega-3 fatty acids, even when vegetable oils are used in the diet. The results obtained did not suggest any major differences in gender.

7. References

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