Regulation of Genes related to Lipid Metabolism in Atlantic salmon (*Salmo salar* L.)

In Vitro and *In Vivo* Studies

AnnaLotta Schiller Vestergren
Faculty of Natural Resources and Agricultural Sciences
Department of Food Science
Uppsala

Licentiate Thesis
Swedish University of Agricultural Sciences
Uppsala 2012

Cover: “Kokanee: In the Moment”, Shelley Hocknell Zentner, 2010
Printed with permission of the artist (Watercolour and Pastel on paper)
(www.shelleyhocknell.com)

ISSN 110-5411
ISBN 978-91-576-9112-5

Print: SLU Service/Repro, Uppsala 2012

Regulation of Genes related to Lipid Metabolism in Atlantic salmon (*Salmo salar* L.). *In Vitro* and *In Vivo* Studies

Abstract

Fish is a vital source of highly valuable omega-3 (n-3) fatty acids (FA) in the human diet. With declining commercial fisheries, aquaculture fish constitute a growing proportion in human consumption. Sustainable development of aquaculture dictates that the fish feed used not solely is based on fish oil (FO) but also contain increasing levels of vegetable oil (VO). The replacement of FO with VO influences FA composition in fish tissues with the decrease of the n-3 highly unsaturated fatty acids (HUFAs), as main consequence rendering a fish less beneficial for human health. Accordingly the last decade of salmonid research has been focusing on increasing the amount of HUFAs in fish fed VO diets. Part of this focus has been on the addition of bioactive compounds to VO diets. In this thesis 2 studies are presented trying to shed more light on the potential positive effects of bioactive compounds.

Paper I examine *in vivo* the effects of sesamin inclusion on Atlantic salmon (*Salmo salar* L.) fed VO-based diets with different n-6/n-3 FA ratios. Fish were fed for 4 months. In Paper II *in vitro* effects of bioactive compounds (genistein, lipoic acid, sesamin/episesamin and sesamin) were investigated in Atlantic salmon primary hepatocytes. Analyses were made 12h and 48h after addition of bioactive compounds, with most effects seen after 48h. In both studies, the FA composition and expression of genes involved in transcription, lipid uptake, desaturation, elongation and β-oxidation were examined.

Genes involved in transcription regulation, β-oxidation, elongation and desaturation were affected by addition of bioactive compounds in both *in vivo* and *in vitro* experiments. Effects on the FA composition were found, but no clear affect on the DHA content. High level of sesamin supplementation had negative effect on growth rate and live weight.

Keywords: β-oxidation, elongation, desaturation, gene expression, sesamin, episesamin, lipoic acid, genistein, DHA, n-6/n-3 fatty acid ratio

Author's address: AnnaLotta Schiller Vestergren, SLU, Department of Food Science, Uppsala BioCentrum, P.O. Box 7051, 750 07 Uppsala, Sweden
E-mail: annalotta.schiller.vestergren@ slu.se

Dedication

To the strong Ladies that have been and to the strong Ladies that will come…

Sadly missed along life's way, quietly remembered every day...No longer in our life to share, but in our hearts, you're always there.

Anonymous

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Schiller Vestergren, A., Wagner, L., Pickova, J., Rosenlund, G., Kamal-Eldin, A., Trattner, S. (2012). Sesamin modulates gene expression without corresponding effect on fatty acids in Atlantic salmon (*Salmo salar* L.). *Lipids* 47(9), 897-911.

II Schiller Vestergren, A., Trattner, S., Mráz, J. Ruyter, B., Pickova, J. (2011). Fatty acids and gene expression responses to bioactive compounds in Atlantic salmon (*Salmo salar* L.) hepatocytes. *Neuroendocrinology Letters* 32(Suppl. 2), 41-50.

The contribution of AnnaLotta Schiller Vestergren to the papers included in this thesis was as follows:

I Participated in the planning of gene expression studies and experimental work together with supervisors. Performed the laboratory work as well as the evaluation and analysis of the gene expression data. Mainly responsible for preparation of the manuscript.

II Participated in the planning of gene expression studies and experimental work together with supervisors. Performed the laboratory work as well as the evaluation and analysis of the gene expression data. Was together with the co-authors responsible for preparation of the manuscript.

Abbreviations

ARA	Arachidonic acid (20:4 n-6)
ACO	Acyl-CoA oxidase
ALA	α-linolenic acid (18:3n-3)
bp	Base pair
CD36	Cluster of differentiation 36
CPT1	Carnitine palmitoyltransferase 1
Δ5FAD	Delta 5 fatty acid desaturase
Δ6FAD	Delta 6 fatty acid desaturase
DHA	Docosahexaenoic acid (22:6 n-3)
DPA	Docosapentaenoic acid (22:5n-3)
DTA	Dodecylthioacetic acid
ELOVL	Elongase of very long chain fatty acids (four different transcripts)
EPA	Eicosapentaenoic acid (20:5n-3)
EF1α	Elongation factor 1a
ETiF	Eukaryotic translation initiation factor 3
ER	Endoplasmic reticulum
ES	Episesamin
FA	Fatty acid
FO	Fish oil
G	Genistein
HUFA	Highly unsaturated fatty acid, here in this thesis defined as PUFAs constituting of 20 or more carbon molecules with at least three double bonds (equivalent to LCPUFA)
LA	Linoleic acid (18:2n-6)
LPA	Lipoic acid
LCPUFA	Long chain polyunsaturated fatty acids
LDL	Low-density lipoprotein
LXRα	Liver X receptor α
MUFA	Monounsaturated fatty acids

n-3	Omega-3
n-6	Omega-6
n-6/n-3	n-6/n-3 PUFA
n.s.	Not significant
NUOR	NADH-ubiquinone oxidoreductase
PPAR	Peroxisome proliferators-activated receptor
PPRE	Peroxisome proliferator response element
Pre-mRNA	Precursor mRNA
PL	Phospholipids
PUFA	Polyunsaturated fatty acid
RPL2	RNA polymerase II polypeptide
RXR	Retinoid-X-receptor
SAFA	Saturated fatty acid
S	Sesamin
SAFA	Saturated fatty acids
SD	Standard deviation
SR-B1	Scavenger receptor class BI
SRE	Sterol regulatory element
SREBP	Sterol regulatory element-binding protein
TAG	Triacylglycerols
UTR	Untranslated regions
VO	Vegetable oil

1 Introduction

In recent years, there has been increased focus on the role of dietary fatty acids (FA) on human health and disease.

To simplify basically, two families of FA are needed to be present in adequate and balanced quantities within the human body for man to stay healthy. These two groups of FA are the omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA).

Meeting the dietary demands of a growing human population for balanced PUFA intake, and at levels required for normal health and development, is a major challenge. One way to meet this demand is through aquaculture of fish, such as salmon, tuna, sardines, cod, mackerel, and trout that all contain high levels of n-3 long chain polyunsaturated fatty acids (LCPUFA) and are low in n-6 FAs. Both these factors are clearly beneficial for human health (Simopoulos, 2002; Horrocks & Yeo, 1999; Simopoulos, 1999a; Kyle & Arterburn, 1998).

However, aquaculture is a sensitive to both economical as well as environmental influences (Tacon & Metian, 2009). To understand how such influences affect the outcome of aquaculture including physical condition of the fish and beneficial effects for human health is therefore of importance. One way to achieve a better understanding of the regulation of the PUFA metabolism is to monitor the underlying internal molecular mechanisms.

1.1 Lipids & Fatty acids

Lipids are a diverse group of compounds that are classified together on the basis of their insolubility in water. Roughly we can talk about two classes of lipids – neutral and polar lipids. Neutral lipids primarily include triacylglycerols (TAG), diacylglycerols, monoacylglycerols and sterols, which mostly serve as storage and sources of energy. Neutral lipid composition, particularly TAG, reflect changes made in the dietary FA composition (Olsen

et al., 1991). Polar lipids are mainly phospholipids (PL) that predominantly are incorporated into membrane structures. PL also reflect the PUFA composition of the diet, but shorter PUFA such as ALA and LA are normally extensively elongated and desaturated prior to incorporation into PL (Olsen *et al.*, 1991).

Fatty acids with one double bond are called monounsaturated fatty acids (MUFA), while fatty acids containing two or more double bonds are called PUFA.

1.1.1 Polyunsaturated fatty acids

In PUFA, the position of the first double bond in relation to the methyl end of the carbon chain is of importance for the nomenclature. If the first double bond is present next to the third carbon atom from the methyl end, the FA is classified as an n-3 FA, while if it is on the sixth carbon atom the FA is termed n-6 FA.

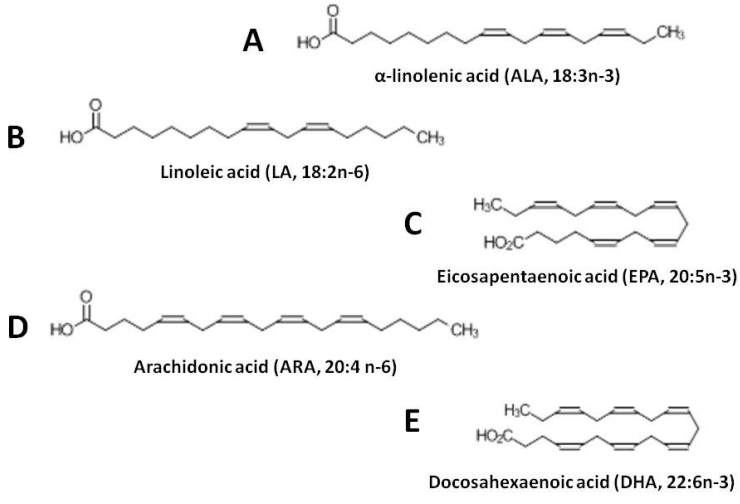

Figure 1 Chemical structure of polyunsaturated fatty acids – A. α-linolenic acid (18:3n-3) and B. Linoleic acid (18:2n-6) C. Eicosapentaenoic acid (20:5n-3); D. Arachidonic acid (20:4n-6) and E. Docosahexaenoic acid (22:6n-3)

Of the n-6 and n-3 FAs, are two classified as essential, since they cannot be synthesized in higher vertebrates including humans and are vital to human health - α-linolenic acid (ALA, 18:3n-3), the key essential n-3 FA, and linoleic acid (LA, 18:2n-6), the corresponding essential n-6 FA (*Figure 1A & 1B*).

These essential FA can be found in many different sources of food, especially in nuts, various plant seeds and oils.

In the body, LA can be metabolized to its longer chain derivate, arachidonic acid (ARA, 20:4 n-6) (*Figure 1D*), and ALA can be metabolized to eicosapentaenoic acid (EPA, 20:5n-3) and further on to docosahexaenoic acid (DHA, 22:6 n-3) (*Figure 1C & 1E*).

However, in most vertebrates, the ALA conversion to EPA or DHA is very limited. Fish and shellfish being naturally high in EPA and DHA are without doubt the most important source for the highly unsaturated n-3 fatty acids (HUFA). Furthermore, fish is naturally low in n-6 FA and consequently therefore generate a low n-6/n-3 ratio, which is known to be beneficial for human health (Simopoulos, 2002; Horrocks & Yeo, 1999; Simopoulos, 1999a; Kyle & Arterburn, 1998).

1.2 Health effects of n-3 fatty acids

The dietary balance of n-6 and n-3 FA is important for homeostasis and normal development in humans (Simopoulos, 2000). Simopoulos (1999b) together with Kyle & Arterburn (1998) stressed towards the end of the 1990ties, the need to increase the n-3 FA within the human diet. By doing so man would return to pre-industrial period intake levels and thereby reduce the risk of many diseases, such as arteriosclerosis, coronary heart disease, inflammatory diseases and possibly behavioural disorders (Connor, 2000; Horrocks & Yeo, 1999).

Besides the role as preventer of many of our most common causes of death, the essential FAs, LA and ALA, plus especially their longer chain derivates (EPA and DHA), are important components of cell membranes (De Wilde *et al.*, 2002; Stauffer, 1996). The functionality of cell membranes specifically the fluidity, is vital. The membrane fluidity is dependent on the ratio of different FAs, in particular the n-3 and n-6 PUFA. In turn the ratio of these n-3 and n-6 PUFA is determined by dietary intake (Cartwright *et al.*, 1985).

Changes to the dietary n-6/n-3 ratio effectively modify the fluidity of phospholipid membranes (Jumpsen *et al*., 1997). As can be seen from *Figure 1C* and *1E* n-3 PUFAs such as EPA and DHA have a natural curved shape, allowing gaps between molecules when incorporated into cell membranes. These gaps increase the fluidity of the membrane allowing ion channels to undergo conformational change and subsequent membrane fusion to occur, enabling cell-to-cell communication (Singer & Nicolson, 1972). In contrast, n-6 PUFAs (*Figure 1D*) are straighter and narrower and are therefore reducing the number of gaps present in the membrane leading to a decrease in the

fluidity. As a result here of, the membranes are less flexible and can potentially cause signal conductance problems (Lapillonne *et al.*, 2003).

In particular, high concentration of DHA is important in neuronal membranes. DHA constitutes more than 30% of the total phospholipid composition of plasma membranes in the brain and is taken up by the brain in preference to other FA (De Wilde *et al*., 2002). DHA is crucial for maintaining membrane integrity and, consequently, neuronal excitability, synaptic function and cognitive abilities by making plasma membrane more fluid at synaptic regions. Besides DHA, both EPA and ARA are important for normal brain and nerve development (Gómez-Pinilla, 2008).

The n-6 and n-3 FA are metabolically and functionally distinct and have opposite physiological effects. The three PUFAs play an important role as precursors for eicosanoids - highly biologically active paracrine hormones (Sargent *et al.*, 1999). Generally, eicosanoids are produced in response to different kinds of stress. The n-6 PUFAs and their conversion products favor immune and inflammatory reactions. The major precursor of eicosanoids is ARA whereas those formed from EPA are less biologically active. EPA competitively inhibits the formation and actions of eicosanoids formed from ARA. The n-3 PUFA remove metabolic fuel from storage towards oxidation and are involved in anti-inflammatory reactions (Price *et al.*, 2000).

1.3 Aquaculture

1.3.1 From global fisheries to aquaculture

Meeting the dietary demands of a growing global population for a correct dietary balance of PUFAs is a major challenge in which a sustainable aquaculture plays an important role.

Global production of fish from aquaculture has grown substantially with ~10% per year over the past decades (Sargent, 2001), reaching 52.5 million tonnes in 2008, compared with 32.4 million tonnes in 2000. Roughly 50% of fish used for human consumption are now farmed and, with global fisheries generally in decline (FAO, 2010) and the continues growth of the global human population, this proportion is increasing.

In developed countries, aquaculture largely focuses on carnivorous species (e.g., salmon, bluefin tuna and sea bass). Norway and Chile are the world's leading aquaculture producers of salmonids, accounting for 36.4 and 28.0 percent of world production (1.5 million tonnes), respectively (FAO, 2010). The growth in aquaculture was made possible by the development of formulated fish feed including amino acids, FAs, minerals and vitamins fulfilling all essential requirements. The diets have traditionally been relaying

on fish meal and fish oil (FO) rich in n-3 HUFAs from natural fisheries to generate a diet high in both lipids and proteins promoting a cost-efficient fish growth. The amount of FO consumed in the aquaculture sector has grown threefold since 1992 and today around 90% of all FO produced goes to aquafeeds (Tacon, 2005). Accordingly, the development of aquaculture has been heavily depending on the availability of FO supply as the sole lipid source for aquafeeds. FO is made primarily from small pelagic fish such as sardines and anchovies, naturally high in both EPA and DHA (Regost *et al.*, 2004). However the landings from global fisheries of pelagic fish cannot keep up with this increasing demand from the industrial aquaculture. It is therefore of importance to find a sustainable alternative to FO as lipid source in fish feed. Consequently, this has been one of the main concerns for the aquaculture industry during the last decade.

1.3.2 Reduction of fish oil in fish feeds

Mainly three different actions have been undertaken to reduce the amount of FO used in fish feed and still render satisfactory levels of EPA and DHA. First of all, FO has fully or partly been exchanged with VOs naturally high in ALA or stearoidonic acid (18:4n-3) and low in LA. Secondly, bioactive compounds have been used to promote the ability of fish to convert ALA to EPA and DHA (Trattner *et al.*, 2008a; Kennedy *et al.*, 2007a; Kleveland *et al.*, 2006a). Finally, selective breeding for heritable traits associated with EPA and DHA composition has been evaluated (Leaver *et al.*, 2011; Olesen *et al.*, 2003; Gjedrem, 1997). This thesis will focus on the use of bioactive compounds as a supplement to VO diets to counteract the decrease in n-3 LCPUFA seen in the fish tissues.

Vegetable oils in fish feed

In parallel to the growing aquaculture production, the global production of fishmeal and FO remains stable or even decline. Due to this shortage of FO, an increase in aquaculture production, and the need for alternative sustainable oil resources in fish feeds, FO has increasingly been replaced by vegetable oils (VO) (Powell, 2003; Thomassen & Røsjø, 1989). In 1997 the acute lack of fish-based raw materials, as a result of the weather phenomenon, El Niño in the tropical parts of the Pacific Ocean, escalated the search for alternatives to FO. At this point the industry started to replace some of the FO in salmon feed with VO on a more permanent basis (FAO, 2008; Tacon, 2005). Research has as a result of this been focusing on how the replacement of the FO in aquafeeds with increasing amounts of VO has influenced the growth and health of the cultivated fish.

Studies have shown that VO to a great extent can replace FO in fish feed without compromising growth, flesh astaxanthin levels or mortality rate (Sanden *et al.*, 2011; Torstensen *et al.*, 2005; Bell *et al.*, 2003; Rosenlund, 2001; Ruyter & Thomassen, 1999). The taste of salmon is known to vary, depending on the composition of the salmon feed. However, sensory analyses have show that fillets from salmon fed a mixture of VO have roughly the same taste and aroma as fillets from salmon whose feed has included FO, but with a somewhat less characteristic marine taste and aroma (Sanden *et al.*, 2011; Torstensen *et al.*, 2005).

However, the tissue FA composition has been shown to be highly subjective to differences in diet lipid composition (Torstensen *et al.*, 2000; Tocher & Dick, 1990). Some VO, such as linseed oil, has a naturally high content of n-3 ALA. These VO can provide a potentially good substrate for the production of EPA and DHA. In order to ensure that the level of saturated, MUFAs and PUFAs in VO based feed is at roughly the same level as in FO-based feed, a mixture of LO, rape seed oil and palm oil can be used (instead of a single VO) (Torstensen *et al.*, 2005). By introducing a finishing diet period immediately preceding slaughter, the EPA and DHA content in the muscle are increased (Mráz, 2012; Rosenlund, 2001).

To some degree, carnivorous fish species, such as salmon, are able to convert ALA into EPA and DHA. However, this conversion will most probably only try to meet the demands of the fish itself, leaving less EPA and DHA to meet the needs of human consumption. Consequently, if FO is substituted with VO significant decreases in the content of n-3 LCPUFA in the fish tissues will be observed (Sanden *et al.*, 2011; Pettersson *et al.*, 2009; Torstensen *et al.*, 2005; Bell *et al.*, 2001). This has also been seen in sea bass (*Dicentrarchus labrax* L.), where the flesh FA profile is impoverished in n-3 PUFAs when fish were fed VO diets (Mourente *et al.*, 2005), with a concomitant decrease of nutritional value.

In terms of the consumer's health, it would be beneficial to maintain as high as possible amounts of n-3 LCPUFA in fish muscle (Ackman, 1996). The inclusion of bioactive compounds to aqua feeds may serve to improve the FA profile of farmed fish.

1.3.3 Bioactive compounds

Bioactive compounds are naturally occurring constituents presented in small amounts in plant products and lipid rich foods providing health benefits beyond the basic nutritional value of the product (Kris-Etherton *et al.*, 2002). Many of these substances affect the lipid metabolism and/or exhibit antioxidative properties. Low level of dietary antioxidants has been suggested to increase the

level of n-3 LCPUFAs in Atlantic salmon and salmon eggs (Bell *et al.*, 2000; Pickova *et al.*, 1998). A number of bioactive substances e.g. sesamin (S), episesamin (ES), tetradecylthioacetic acid (TTA) and lipoic acid (LPA) have been reported to affect lipid metabolism and/or FA composition in rainbow trout, Atlantic salmon and pacu (*Piaractus mesopotamicus*) (Trattner *et al.*, 2008a; Moya-Falcón *et al.*, 2004).

Sesamin

Sesamin is an oil soluble lignan found in the sesame seed and oil. During the refining process of the sesame oil, ES is formed from S. Sesame lignans are well studied in mammals with significant effects on lipid metabolism. It has been shown to increase β-oxidation (Jeng & Hou, 2005; Ashakumary *et al.*, 1999) and affect elongation and desaturation of FAs in rats (Fujiyama-Fujiwara et al., 1995) and to lower serum levels of triacylglycerols and cholesterol in rats and humans (Jeng & Hou, 2005; Kushiro *et al.*, 2002; Kamal-Eldin *et al.*, 2000). Enzymes involved in both the desaturation and β-oxidation of FAs are affected by S, both at the level of enzymatic activity and mRNA levels (e.g. acyl-CoA oxidase (ACO) and carnitine palmitoyl transferase 1 (CPT 1)) (Jeng & Hou, 2005; Kiso *et al.*, 2005; Kushiro *et al.*, 2002). Ide *et al.* (2001) also showed that S decreased the hepatic activity and mRNA expression of enzymes involved in FA synthesis.

Sesamin was shown to reduce Δ5 fatty acid desaturase (Δ5FAD) enzymatic activity in *Mortierella alpina* fungus and rat hepatocytes (Shimizu *et al.*, 1991) but no similar effects could be detected on Δ5FAD mRNA levels in rats (Umeda-Sawada *et al.*, 2003). The lipid modulating effects are possibly via the activation of peroxisome proliferator-activated receptors (PPARs) and inhibition of sterol regulatory element-binding protein-1 (SREBP-1) (Ide *et al.*, 2004; Ide *et al.*, 2003; Ashakumary *et al.*, 1999).

Kushiro *et al.* (2002) showed that episesamin is more effective than sesamin in increasing the activity and gene expression of FA oxidation enzymes. In rats, S and ES are absorbed via the lymph and metabolized by the liver, and S has been reported to be metabolized faster than ES. Yasuda *et al*. (2012) showed a clear difference in metabolism between sesamin and episesamin in human liver microsomes by P450 (CYP2C9 and CYP1A2), UDP-glucuronosyltransferase, and catechol-O-methyltransferase, resulting in different biological effects.

Lipoic acid

Another bioactive compound interesting for fish feed is lipoic acid (LPA), a naturally occurring thiol-compound with two sulfur atoms next to each other

connected through a disulfide bond. It is a potent antioxidant with one lipophilic and one lipofobic part, shown to have vitamin C and vitamin E sparing effects in mammals (Kozlov *et al.*, 1999; Lykkesfeldt *et al.*, 1998). LPA is synthesized in both mammals and plants within the mitochondria by lipoic acid synthase as a part of the *de novo* synthesis of FA (Hiltunen *et al.*, 2010b; Morikawa *et al.*, 2001; Wada *et al.*, 1997). LPA has also been shown to be active in the cellular energy metabolism playing part in the citric acid cycle (Bast & Haenen, 2003) as a covalently attached cofactor required for the activity of mitochondrial enzyme complexes (Wollin & Jones, 2003; Reed, 1998).

Huong and Ide (2008) were able to show that LPA decreased the PL and TAG concentrations in serum and liver of rodents. LPA was also capable of lowering the cholesterol concentration in mice serum (Yi & Maeda, 2006) and rat liver (Huong & Ide, 2008). A dose-dependent decrease of both the gene expression and activity of the lipogenic enzymes - fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase, malic enzyme and pyruvate kinase was observed. Similar effect was also observed by the same group on the mRNA levels of other modulators of the fatty acid synthesis - spot 14, adiponutrin, stearoyl-CoA desaturase 1, and members of the elongation and desaturation cascade - Δ5FAD and Δ6FAD (Huong & Ide, 2008).

LPA has been shown to affect the FA composition of fish muscle, towards higher levels of EPA (Trattner *et al.*, 2007).

Genistein

Genistein (G) is a phytoestrogen, i.e., plant-derived compounds that possess estrogen-like biological activity. G is formed after hydrolysis of the isofavone genistin, which is found abundantly in soybean. G is known to exhibit antioxidative and hormone like effects (Yuan *et al.*, 2007) and has therefore has been the subject of numerous studies for its possible beneficial and adverse health effects in humans and other mammals. G has been found to inhibit the oxidation of low-density lipoprotein (LDL) in human blood (Safari & Sheikh, 2003) and enhance the expression of gene involved in the lipid catabolism through activation of carnitine palmitoyltransferase (CPT1) and PPARα (Kim *et al.*, 2004). Studies on mice have shown that hepatic fatty acid synthase, β-oxidation and CPT1 activities were all significantly lower in groups given genistein supplement (Ae Park *et al.*, 2006).

In vitro experiments have also shown that G binds and activates the three zebra fish estrogen receptors ERα, ERβ-A and ERβ-B and turn on the estrogen pathway (Sassi-Messai *et al.*, 2009). G has also shown to inhibit hepatic and renal estrogen metabolism in rainbow trout (*Oncorhyncus mykiss*), Atlantic

salmon (*Salmo salar* L.), and lake trout (*Salvelinus namaycush*) (Ng *et al.*, 2006) and thereby increase the bioavailability of estradiol-17β.

However, both *in vitro* and *in vivo* studies have shown that higher treatments with $0.25x10^{-4}$ M genistein had toxic effects on zebra fish embryos (Kim *et al.*, 2009; Sassi-Messai *et al.*, 2009) with retarded hatching times, malformations and increased mortality in a dose-dependent manner.

1.4 Gene expression

Gene expression as the word states deals with the difference in expression of genes as a response to external and/or internal stimuli of which daily environmental influences play a crucial role.

The regulation of all processes from production through activation and deactivation to degradation of proteins involves at one point or another regulation of gene expression directly or indirectly.

Simplified, the genetic information contained in the DNA is transcribed into a primary transcript (Precursor mRNA or pre-mRNA), which is further processed to produce a mature mRNA molecule within the nucleus (*Figure 2*) (Moore, 2005; Levine & Tjian, 2003).

The pre-mRNA consists of a copy of the whole gene sequence, including both the exons, which code for the protein and introns, which are sections of non-coding DNA between the exons. The transformation into a mature RNA involves a splicing event, a 5'capping process and polyadenylation where introns are removed, the 5'-end is modified and adenine residues added to the 3'-end of the mRNA. Hereafter, the mature mRNA is transported from the nucleus to the cytoplasm where it is translated to produce a protein according to the amino acid sequence encoded by first the gene and then the mRNA (Pandya-Jones, 2011; Moore, 2005).

All mRNA contain both a coding sequence, which are translated to produce the appropriate protein, and untranslated sequences. These untranslated sequences/regions (UTRs) are present at both the 5'- and 3'-ends of the coding region. UTRs play a critical role in controlling gene expression through regulation of the polyadenylation, translation, stability and localization of the mRNA. The regulation occurs through the interaction of specific often rather short sequences that form secondary structures, such as bulges and stem-loops within the UTR with specific proteins (Barrett *et al.*, 2012).

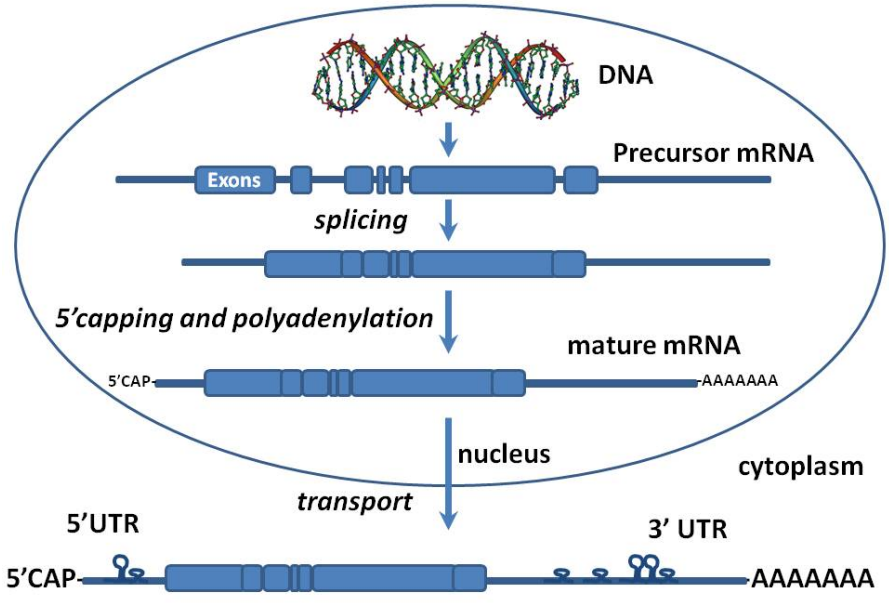

Figure 2 Schematic diagram over the critical stages from the transcription of a gene composed of 7 exons, a 5'-untranslated region (5'UTR) and a 3'-untranslated region (3'UTR) until the mature mRNA is transported out of the nucleus to the cytoplasm.

Theoretically, there are three possible outcomes for fully processed mRNA in the cytoplasm: they may be translated to produce protein, they may be immobilized or inactivated, and/or they may be degraded.

In eukaryotes, the regulation of gene expression can occur at any step ranging from DNA–RNA transcription to post-translational modification of protein (Moore, 2005).

The first regulation possibility occurs during the transcription where the chromatin arrangement and changes in DNA structure (epigenetic process including CpG methylation) influences accessibility of promoter sequences and the differential activity of transcription factors, determine whether or not genes are transcribed (Gräff *et al.*, 2011; Schneider & Grosschedl, 2007).

Additionally a verity of post-transcriptional regulation mechanisms such as the modulation of the activity of RNA binding proteins, alternative splicing, and presence of small non-coding RNAs triggering RNA interference are involved in modifying the stability and distribution of the mRNA ultimately affecting the outcome of the gene expression machinery (Moore, 2005; Bartel, 2004; Ambros, 2001).

1.5 Molecular aspects of lipid metabolism in salmonids

Lipids and FAs are together with proteins the major macronutrients in the diet of Atlantic salmon, constituting more than 30% of the diet (Leaver *et al.*, 2008a). Given the change in FA composition of salmonid feed from FO towards VO that is in progress, is it important to study the consequences on the underlying molecular mechanisms in both tissues and cells. Through oxidation the FAs can be used as energy source, or stored and deposited in adipose tissues (Tocher, 2003). All steps in the lipid metabolism from uptake to the conversion of LA and ALA in fish to ARA, EPA, and DHA as well as to the β-oxidation are under nutritional regulation. In turn the dietary FAs act as regulators of gene transcription and consequently steer enzyme activity (Jump & Clarke, 1999; Hesketh *et al.*, 1998).

As a result hereof, the regulation of lipid homeostasis in Atlantic salmon is a complex balance between e.g. lipid uptake, transport, storage and biosynthesis. Each single one of these processes needs to be controlled independently as well as in co-junction with the other processes on both a tissue specific as well as whole body level (Tocher, 2003). As shown schematically in *Figure 3* this can be reduced to two inter-linked processes; the influence of FA intake on gene expression and protein synthesis, and the influence of gene expression on FA requirements.

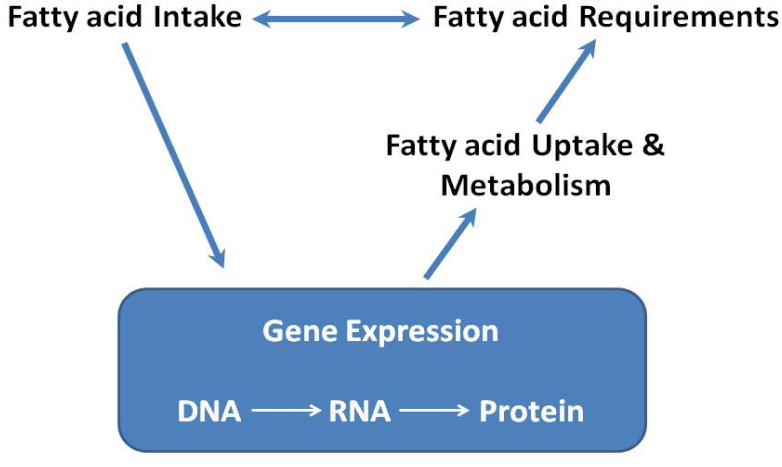

Figure 3 Inter-relationship between fatty acid homeostasis and gene expression.

FAs may alter the amount of functional protein expressed by a specific gene through a range of transcriptional, post-transcriptional and post-translational mechanisms. Dietary FAs are well known to have profound effect on gene

expression regulation. FAs and their metabolites can either indirectly influence the gene expression regulation by activating different transcription factors in the cytoplasm or directly by entering the nucleus by themselves or in association with ligand-activated transcription factors.

1.5.1 Genomic background in salmonids

Fish are the oldest and most diverse members of the vertebrates starting their evolution 540 million years ago (Powers, 1991). The salmonids genome is complex due to a fairly recent additional genome duplication that is believed to have occurred between 25 and 120 million years ago (Ohno, 1999; Allendorf & Utter, 1976).

Tetraploidizations or genome duplications are important evolutionary events which most probably are responsible for the large increases in genome size and diversity early in vertebrate evolution (Ohno *et al*., 1968).

At first, a duplication of a gene merely creates a redundancy. This acquired redundancy might present an advantage. However, if there is no immediate use for the duplicated gene, it will be allowed to drift without restraint or selective pressure. If this free drift does not result in the acquisition of a new, significant function by the duplicated gene, it is expected to degenerate (diploidization) (Ohno, 1999). In salmonids a large percentage of loci (50-75%) have remained as functioning duplicates and the diploidization process has not come to an end (Hordvik, 1998; Young *et al.*, 1998; Allendorf, 1978; Bailey *et al.*, 1978).

Consequently, a specific locus in one species may still have four alleles, while in another species it may be converted to a pair of isoloci (e.g. pair of duplicated loci having gene products with identical constitution and electrophoretic mobility (Waples, 1988)). Accordingly, several gene duplicates have been cloned and described for salmonids (Morash *et al.*, 2010; Evans *et al.*, 2008; Leaver *et al.*, 2007; McKay *et al.*, 2004; Hordvik, 1998; Kavsan *et al.*, 1993; Ohno *et al.*, 1968) making it even harder to shed light over the intriguing gene regulation mechanisms in these species.

1.5.2 Transcription factors

A transcription factor is a protein that binds to specific DNA sequences within the promoter region, and by doing so controlling the transcription of genetic information from DNA to mRNA. This function is performed single-handedly or in a complex with other proteins, by promoting (as an activator), or blocking (as a repressor) the binding of RNA polymerase to specific promoter sequences (Latchman, 1997).

It has been shown that dietary fat influence the gene expression by controlling the activity or abundance of central transcription factors (Jump *et al.*, 2005).

Peroxisome proliferator activated receptors

One nuclear receptor family that influences transcription according to nutritional state is the peroxisome proliferator-activated receptor (PPAR) family. PPARs are activated by ligands and respond to changes in lipid and glucose homeostasis in mammals (Jump *et al.*, 2005). PUFAs are known to be one of these PPAR activating ligands. The ligand binding causes a dimerization of PPAR with the retinoid-X-receptor (RXR) forming a heterodimer (*Figure 4*). The PPAR–RXR complex activates target genes by recognizing promoter regions called peroxisome proliferator response elements (PPREs). The DNA consensus sequence for the PPRE is AGGTCAXAGGTCA, with X being a random nucleotide. If not activated, PPARs remain in the nucleus in a repressed state mediated by nuclear receptor co-repressors (Chinetti-Gbaguidi *et al.*, 2005).

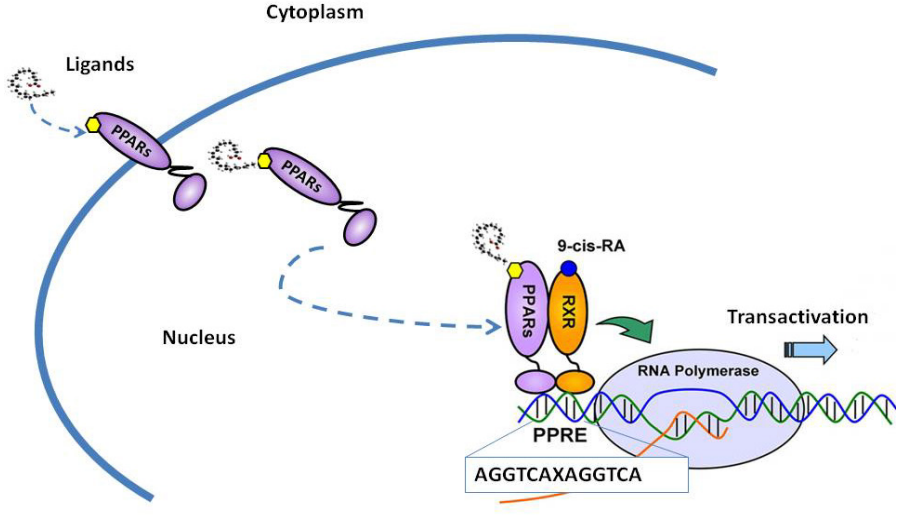

Figure 4 Schematic overview of ligand-induced conformational change peroxisomal proliferator-activated receptor (PPARs), translocation into the nucleus, dimerization with a retinoid-X-receptor (RXR) and following binding to *cis*-regulatory elements in the promotor region of the target gene.

PPARα has been shown to activate the gene coding for the β-oxidation enzyme, carnitine palmitoyltransferase 1 (CPT1) by binding to a peroxisome proliferator response elements upstream in the promoter region of the gene, thereby playing an important role in regulating β-oxidation in rodents (McGarry & Brown, 1997), humans (Varanasi *et al.*, 1996) and fish (Boukouvala *et al.*, 2010). Similarly, PPARα also target genes coding for other β-oxidation enzymes ACO (Varanasi *et al.*, 1996) and hydroxyacyl

dehydrogenase, fatty acid binding proteins and the transmembrane fatty acid transporters such as CD36 and FAT. Both PPARα and PPARγ have been shown to induce the transcription of the transmembrane fatty acid transporter, CD36 and SR-B1 (Burri *et al.*, 2010; Poirier *et al.*, 2001; Motojima *et al.*, 1998).

All different subtypes have been identified in Atlantic salmon. Four genes coding for four different subtypes of PPARβ have been identified in Atlantic salmon. These subtypes were grouped into two families based on differences in exons and exon-flanking regions. Each subtype had a characteristic expression pattern varying between tissues (Leaver *et al.*, 2007). Furthermore have two forms of PPARγ been described in Atlantic salmon liver (Andersen *et al.*, 2000; Ruyter *et al.*, 1997).

Sterol regulatory element-binding proteins

Another group of key regulator of lipid and cholesterol metabolism is the sterol regulatory element-binding proteins (SREBP) that are attached to the nuclear envelope or bound endoplasmic reticulum (ER) (reviewed by Jump *et al.*, 2005). The FA levels, both intracellular and membrane levels are under constant supervision by SREBP and are coordinated with *de novo* lipid biosynthesis (Horton *et al.*, 2002). SREBP belong to a family of transcription factors consisting of SREBP-1a, SREBP-1c, and SREBP-2 proteins that are encoded by two unique genes, Srebp-1 and Srebp-2 (Horton *et al.*, 2002). The three SREBP regulate slightly different target genes and have different activation intensities as well as show different tissue specific expression patterns. SREBP-1c regulates the transcription of genes involved in FA metabolism, such as fatty acid synthase, SREBP-2 regulate both lipid homeostasis and cholesterol biosynthesis (Nakamura *et al.*, 2004).

Upon activation the inactivated membrane-bound SREBP is proteolytic released. When a specific cellular lipid level is low, the SREBP is transported to the Golgi, where it is processed by proteases to its active form. Afterwards SREBP is freed to move through the cytoplasm to the nucleus. In the nucleus, SREBP binds to the sterol regulatory element (SRE) DNA sequence that are found in the control regions of the genes that encode enzymes needed to make lipids. This binding to DNA leads to the increased transcription of the lipogenic target genes (Osborne & Espenshade, 2009).

Taggart *et al.* (2008) and Leaver *et al.* (2008b) showed in Atlantic salmon, that SREBP-2 were upregulated when dietary FO was replaced with VO.

Liver X receptors

Liver X receptors (LXR) are transcription factors whose activity is regulated by sterols. Equally, liver X receptor α (LXRα) and LXRβ have been shown to activate SREBP-1c in rodents (Cruz-Garcia *et al.*, 2009; Zhou *et al.*, 2008). However, high levels of unsaturated FAs activate LXR which in turn mediate a feed-back regulation on SREBP-1c, suppressing SREBP-1c transcription and consequently FA synthesis.

The activated LXR induce cholesterol catabolism and *de novo* FA biosynthesis in liver through SREBP-1c, which has led to the suggestion that LXRs are sensors of the balance between cholesterol and FA metabolism. This has rendered a lot of focus on LXR and the gene coding for LXR has been cloned and characterized in several fish species besides salmonids (Cruz-Garcia *et al.*, 2009).

1.5.3 Uptake & transport

The liver is the crossing point for the exogenous and the endogenous transport of lipids. The most predominant mechanism in which lipids are taken up into the cells is through binding of lipoproteins to cell-surface trans-membrane lipoprotein receptors. Low-density lipoprotein receptor (LDL-R) and high-density lipoprotein receptor scavenger receptor class B, type 1 (SR-B1) are among the most important lipoprotein receptors regulating the cholesterol levels in the plasma.

Scavenger receptor class B, type I

Scavenger receptor class B, type 1 (SR-B1) is a cell-surface, high-density lipoprotein receptor and a member of CD36 receptor family. SR-B1 is expressed in all tissues engaged in cholesterol metabolism (Rhainds & Brissette, 2004). Hepatic SR-BI has been shown to be negatively regulated by 17β-estradiol (Sassi-Messai *et al.*, 2009; Lopez & McLean, 2006).

High levels of SR-B1 has been identified in the gut of Atlantic salmon which can indicate that SR-B1 has an important function in the uptake of lipids from the intestine (Kleveland et al., 2006b). Transcription factor SREBP has been shown to bind to the distal motif in the SR-B1 gene in both human and rodents and thereby inducing SR-B1 transcription (Rhainds & Brissette, 2004). PUFAs have been reported to increase hepatic cholesterol uptake, by activation of PPARα and/or PPARγ. PPARγ/RXR binds to a response element in the SR-BI promoter which in turn induces the expression of SR-B1. Furthermore, hepatocyte nuclear factor 4α (HNF4α) was found to enhance PPARγ-mediated SR-BI transcription in rat hepatocytes (Malerød *et al.*, 2003).

1.5.4 FA synthesis

FAs in fish can arise from two sources. Either FAs can be synthesis *de novo* from non-lipid carbon sources, or directly from dietary lipid (Jump, 2011).

De novo synthesis

Acetyl-CoA derived mainly from protein can be converted to SFAs via the combined action of acetyl-CoA carboxylase and fatty acid synthetase (Hiltunen *et al.*, 2010) in the mitochondria. The rate of FA *de novo* synthesis has been shown to be inversely correlated to the level of dietary lipids (Henderson, 1996).

Desaturation and Elongation

The capacity of marine fish species varies in the ability to convert the vegetable C18 precursors LA and ALA to LCPUFAs. In freshwater species such as Atlantic salmon or rainbow trout, the desaturation/elongation pathway is under nutritional regulation. Thus, when these fish species are fed a diet lacking FO, they might possibly be able to modulate the activity of the enzymes to produce the LCPUFAs.

The biosynthesis of LCPUFAs is similar in salmonids to that of other vertebrates (Cook & McMaster, 2002). The conversion of LA to ARA and ALA to EPA and DHA involves a chain of desaturation and elongation steps (*Figure 5*). The same enzymes catalyze the conversion of both n-6 and n-3 fatty acid precursors into LCPUFAs. The two main enzyme families involved in these conversions are the elongases of very long fatty acids (ELOVL) and the fatty acyl desaturases (FAD)(Ruxton *et al.*, 2005). The synthesis of DHA requires two additional elongation steps and a second Δ6 desaturation followed by a peroxisomal chain shortening (Sprecher, 2000) compared to EPA and ARA formation.

Pawlosky *et al.* (2001) showed the rate-limiting step of the biosynthesis of LCPUFAs in healthy humans lays in the conversion of ALA to EPA (20:5n-3), whereas approximately 63% of all plasma EPA was accessible for production of 22:5n-3, and 37% of 22:5n-3 was available for synthesis of DHA (22:6n-3).

Nakamura & Nara (2004) suggested these enzymes are regulated by a negative feedback loop and that an excessive intake of either LA, ALA, or any other PUFA can lead to a suppression of the PUFA synthesis. If the ratio of n-3 to n-6 is not balanced at dietary intake, the excess of one type FA could suppress the conversion of the other PUFA group, increasing the imbalance even further.

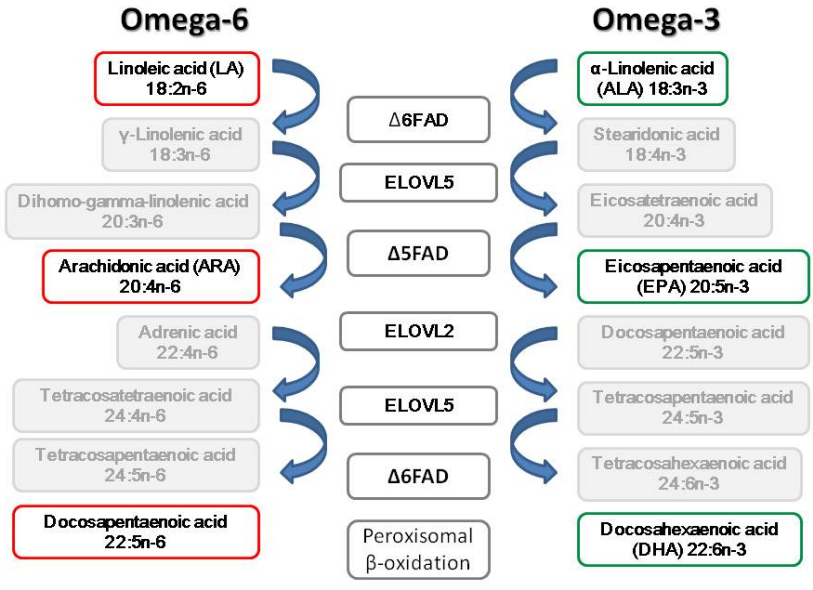

Figure 5 Elongation and desaturation pathway of n-6 and n-3 fatty acids. Adapted from (Voss *et al.*, 1991) and modified after (Trattner, 2009).

Most marine fish are not able to convert PUFA to LCPUFA due to lack of one or more steps in the biosynthetic pathway as a result of relative deficiencies in one of the two enzymes in the desaturation and elongation cascade (Leaver *et al.*, 2008a). However, fish that display a "freshwater pattern" like salmonids possess all the genes necessary for producing active Δ5FAD, Δ6FAD and ELOVLs, as well as the capacity to synthesize both n-3 and n-6 LCPUFAs (Zheng *et al.*, 2009; Henderson, 1996).

Our knowledge of the biomolecular mechanisms behind the LCPUFA biosynthesis in salmonids has increasing under the last two decades. Both Δ5FAD and Δ6FAD have been isolated and characterized in Atlantic salmon.

Several studies have shown that both the genes and the biosynthesis of LCPUFA are upregulated in salmonids after VO feeding (Tocher *et al.*, 2001). Highly conserved binding sites for the transcription factors SREBPs and NF-Y have been identified in the promoter region of the salmon Δ6FAD. In addition to these sites, a site was identified by Zheng *et al.* (2009) in salmon, which showed high similarity to a site that recognized by Sp1 transcription factor, and which was required for full expression of the salmon Δ6FAD gene.

Sesamin/episesamin and TTA have been shown to increase β-oxidation products and the levels of DHA in rainbow trout muscle (Trattner *et al.*, 2008a)

as well affect the expression of Δ5FAD and Δ6FAD, CPT1, PPARα and PPARγ in Atlantic salmon hepatocytes (Trattner *et al.*, 2008b).

Low levels of dietary antioxidants have been suggested to increase the level of long chain n-3 FA in catfish (*Ictalurus punctatus*) (Baker & Davies, 1996) and in Atlantic salmon (Bell *et al.,* 2000) as well as in salmon eggs (Pickova *et al.*, 1998). LPA has been shown to increase the portion of EPA levels in pacu muscle (Trattner *et al.*, 2007).

Recently ELOVL4 has been isolated and characterised in Atlantic salmon. ELOVL4 has been shown to elongate C20 and C22 PUFA and to convert EPA and 22:5n-3 to 24:5n-3, an intermediate substrate for DHA biosynthesis (Carmona-Antoñanzas *et al.*, 2011).

1.5.5 β-oxidation

The β-oxidation of FAs take place in both mitochondria and peroxisomes but the mitochondrial β-oxidation is quantitatively more important and can use a wide range of different FAs as substrate (Henderson, 1996).

There are substrate preferences for SAFAs and MUFAs over PUFAs in the β-oxidation pathway. Both DHA and EPA are relatively spared from β-oxidation when dietary levels of these FAs are low (Torstensen *et al.*, 2004).

Tocher *et al.* (2003) showed that changes in dietary oil type from FO to VO had no significant effect on β-oxidation in Atlantic salmon hepatocytes.

The β-oxidation occurs in peroxisomes, when the FA chains are too long to be processed in the mitochondria but, the oxidation ceases at octanyl-CoA. It is proposed that very long chain (greater than C22) FAs undergo the initial oxidation in peroxisomes followed by final oxidation in mitochondria. ACO is the first and rate-limiting enzyme of peroxisomal β-oxidation (Varanasi *et al.*, 1996).

The most significant difference for β-oxidation in the peroxisomes compared to the oxidation in the mitochondria is that peroxisomal β-oxidation is not coupled to any ATP synthesis. Additionally, peroxisomal β-oxidation requires enzymes specific to the peroxisome and to very long FAs.

β-oxidation in the peroxisome starts with a rate-limiting step requiring the use of a peroxisomal carnitine acyl transferase for transport of the activated acyl group into the peroxisome instead of CPT1 and CPT2 used by the mitochondria (Pagot & Belin, 1996).

2 Objectives

As Atlantic salmon is among the most popular fish species in our Western diet, the content of EPA and DHA within Atlantic salmon fillet as well as factors influencing these amounts are of importance. This thesis focuses on molecular regulation of the lipid metabolism in Atlantic salmon with the major emphasis given to LCPUFA biosynthesis.

The anticipation is that understanding the molecular mechanisms will enable manipulation and optimization of the activity of desaturation and elongation of n-3 LCPUFA pathway to enable efficient and effective use of VO in aquaculture while maintaining the high nutritional quality from the wild catch fish. The long term objective for the research behind this thesis is to contribute to the development of diets adjusted for the individual fish species needs, a sustainable aquaculture as well as to an optimal use of LCPUFA.

Specific objectives were to:

- Study the effect of sesamin supplementation to VO based diets on the expression of genes related to FA metabolism and on the FA composition in Atlantic salmon after *in vivo* trails. (Paper I)
- Study the effect of addition of lipoic acid, genistein, episesamin and sesamin to the culture media of Atlantic salmon hepatocytes *in vitro* on the expression of genes related to FA metabolism and if potential gene expression effects could be related to changes in the FA composition. (Paper II)

3 Material and methods

In the section below a short description is given of the material and methods used in the studies included this thesis. More details on the specific procedures described, see Paper I-II.

3.1 The design of the experimental series

An overview of the material tested, specific methods, software and techniques used in the various studies is presented in *Table 1*.

In Paper I, Atlantic salmon (*Salmo salar* L.) with an average final weight of 554g were fed vegetable oil oil-based diets with different inclusions of sesamin. The diets used differed in n-6/n-3 fatty acid (FA) ratio (0.5 and 1) and sesamin content (high 5.8 g/kg, low 1.16 g/kg and no sesamin). The oils used in the feeds were a mixture of rapeseed, linseed and palm oil. Fish were fed for 4 months. We evaluated the effects of sesamin supplementation on fatty acid composition and expression of hepatic genes involved in transcription, lipid uptake, desaturation, elongation and β-oxidation in liver as well as white muscle (*Table 1*).

In Paper II, hepatocytes were isolated from Atlantic salmon (1300 g) according to the two-step collagenase procedure (Kjær *et al.*, 2008; Dannevig & Berg, 1985; Seglen, 1976). The fish were kept in seawater at 10^{o}C and fed a commercial diet prior to isolation of hepatocytes.

The aim was here to evaluate the effects of bioactive compounds - the mixture of sesamin/episesamin, sesamin, lipoic acid and genistein, known to act as either antioxidants and/or influence lipid homeostasis in mammals. An array of gene expression assays was designed covering transcription factors and genes coding for proteins/enzymes involved in the lipid metabolism. The analyzed genes are listed in *Table 1*. Furthermore, the FA composition in Atlantic salmon hepatocytes was analyzed.

Table 1 *Summary of experimental design and build up for Paper I – II*

Study	**No. I**	**No. II**
Species	Atlantic salmon	Atlantic salmon
Fish size	554g	1300g
Samples [b)]	Liver/White muscle	Hepatocytes
Sample size [b)]	1.7mg	1.7mg
Number of replicates	6[cd)]	6[d)]
Environmental conditions	Seawater at 12°C	Seawater at 10°C
Control diet[e)]	Commercial Fish Feed	Commercial Fish Feed
Treatment	Sesamin/Episesamin	Lipoic acid
	SH = 5.8 g/kg feed	Sesamin/Episesamin Genistein
	SL = 1.16 g/kg feed	
Vegetable oil diet[f)]	V0.5 = 0.5 n-6/n-3 FA	
	V1 = 1.0 n-6/n-3 FA	
Measurements	Lipid analysis	Lipid Analysis
	Gene expression	Gene expression
Target genes	PPARα, PPARβ1A, PPARγ, PGC-1, SREBP-1, SREBP-2, LXR, CD36, SP-B1, ELOVL2, ELOVL5a, ELOVL5b, Δ5FAD, Δ6FAD, ELOVL4, ACO	PPARα, PPARβ1A, PPARγ, CD36, ELOVL2, ELOVL5a, Δ5FAD, Δ6FAD, ACO
Housekeeping gene	NUOR	RPL2

a) Liver, white muscle, red muscle, heart, brain, stomach, gills, intestine and kidney

b) For the gene expression studies only

c) Only liver was tested in gene expression experiments.

d) All tests performed in triplicate

e) All diets contained the recommended levels of vitamins and minerals

f) Rapeseed, linseed and palm oil

3.2 Lipid analysis

The total lipid from diets, tissue, cells and the medium were extracted by using hexane:isopropanol (3:2 by vol.) (Hara & Radin, 1978).

Total lipids of muscle tissue and liver were separated into triacylglycerols (TAG) and phospholipids (PL) on thin-layer-chromatography according to Pickova *et al.* (1997). The total lipids in the diets, and the triacylglycerols and phospholipids were methylated to fatty acid methyl esters following the method described by Appelqvist (1968) and analyzed with gas chromatography according to Trattner *et al.* (2008a) (*Table 2*). The peaks were identified by comparing their retention times with a standard mixture.

Table 2 *Fatty acid composition (%) in experimental diet or media used in Papers I-II*

	Average control	Paper I		Paper II
	Fish oil	Low n-6/n-3	High n-6/n-3	Culture media
LA (18:2n-6)	3.20	14.6	15.3	3.70
ALA (18:3n-3)	2.40	27.5	13.1	1.00
ARA (20:4n-6)	0.40	0.10	0.10	2.40
EPA (20:5n-3)	6.90	1.1	1.3	0.90
DHA (22:6n-3)	9.70	1.6	1.8	1.20
SAFA	24.8	17.5	18.8	40.7
MUFA	41.5	34.0	47.0	22.3
n-3 PUFA	23.3	30.9	16.9	3.60
n-6 PUFA	6.20	15.4	15.9	6.90
n-6/n-3	0.27	0.50	0.94	1.92

SAFA saturated fatty acids (14:0, 16:0, 18:0); *MUFA* monounsaturated fatty acids (16:1n-7, 18:1n-9, 18:1n-7, 20:1, 22:1); *PUFA* polyunsaturated fatty acids

3.3 Gene expression analysis

Gene expression in liver was investigated by quantitative Real-Time PCR using an array of target genes coding for enzymes involved in the lipid homeostasis.

Total RNA was isolated using the spin purification method followed by DNase treatment. The total RNA was quantified and reverse transcription First strand cDNA was synthesized using the High-Capacity cDNA Archive kit.

Real-time PCR analysis of the relative abundance of mRNA was assessed using Power or Fast SYBR®Green chemistry and gene specific primers designed using available Atlantic salmon sequences from the online version of GenBank®(NCBI) (Trattner *et al.*, 2008b) using the Primer Express® software or copied from literature references. Primers for Real-time PCR analysis with corresponding Genbank accession numbers are listed in *Table 3a-c*.

All samples were run simultaneously for each gene in triplicate with a non-template control on each plate. A melt curve analysis was performed after each run to ensure that only a single product was amplified.

Elongation factor 1a (EF1α), NADH-ubiquinone oxidoreductase (NUOR), Eukaryotic translation initiation factor 3 (ETiF) and RNA polymerase II polypeptide (RPL2) were evaluated for their stability across all experimental variables and samples thereafter the most stable reference gene was chosen using the DataAssist software version 2.0. The ΔC_T was calculated by subtracting the C_T for the reference gene from the C_T for the gene of interest. The relative expression was then calculated comparing the ΔC_T values for fish

fed the different experimental diets with fish fed the standard fish oil diet using the term $2^{-\Delta\Delta CT}$ and reported as arbitrary fold change units (Livak & Schmittgen, 2001).

3.4 Statistical analysis

All data in the tables are presented as mean values ± standard deviation (SD). Difference between values were considered as significant when $P \leq 0.05$. FAs were compared using the General Linear Model (GLM) in SAS statistical software. The model included the fixed effect of treatment and random effect of individual. Correlation tests were performed using Minitab 15 statistical software. Relative expression of the different genes, in relation to housekeeping genes were determined and mean values as well as SD were calculated using StepOne™ software version 2.2 and DataAssist software version 2.0. The 95% confidence interval was calculated and used for statistical discrimination evaluation.

Table 3a. *Sequences of primers used to amplify housekeeping genes and equivalent Genbank accession numbers used*

Primer	Forward primer (5'-3')	Reverse primer (5'-3')	GenBank Acc. no
RPL2[a]	TAACGCCTGCCTCTTCACGTTGA	ATGAGGGACCTTGTAGCCAGCAA	CA049789
EF1-α[a]	CACCACCGGCCATCTGATCTACAA	TCAGCAGCCTCCTTCTCGAACTTC	AF321836
NUOR[b]	CAACATAGGGATTGGAGAGCTGTACG	TTCAGAGCCTCATCTTGCCTGCT	DW532752
ETiF[c]	CAGGATGTTGTTGCTGGATGGG	ACCCAACTGGGCAGGTCAAGA	DW542195

Abbreviations: RPL2 = RNA polymerase II polypeptide, EF1-α = Elongation factor 1α, NUOR = NADH-ubiquinone oxidoreductase, ETiF = Eukaryotic translation initiation factor 3. Already designed and validated in a) Jorgensen et al. (2006) b) Bahuaud et al. (2010) c) Castro et al. (2011)

Table 4b. *Sequences of primers used to amplify transcription factors and equivalent Genbank accession numbers used*

Primer	Forward primer (5'-3')	Reverse primer (5'-3')	GenBank Acc. no
PPARα[a]	TCCTGGTGGCCTACGGATC	CGTTGAATTTCATGGCGAACT	DQ294237
PPARβ1A[b]	GAGACGGTCAGGGAGCTCAC	CCAGCAACCCGTCCTTGTT	AJ416953
PPARγ (long/short)	CATTGTCAGCCTGTCCAGAC	ATGTGACATTCCCACAAGCA	AJ292963
PGC-1α	CAACCACCTTGCCACTTCCT	CGGTGATCCCTTGTGGTCAT	FJ710605.1
LXR[e]	GCCGCCGCTATCTGAAATCTG	CAATCCGGCAACCAATCTGTAGG	FJ470290
SREBP-1	GACAAGGTGGTCCAGTTGCT	CACACGTTAGTCCGCATCAC	NM001195818
SREBP-2[h]	TCGCGGCCTCCTGATGATT	AGGGCTAGGTGACTGTTCTGG	NM001195819

Abbreviations: PPAR = Peroxisome proliferator-activated receptor, PGC-1α = Proliferator-activated receptor gamma coactivator 1 alpha, LXR = Liver X receptor α, SREBP = Sterol regulatory element binding protein. Already designed and validated in a)(Jorgensen et al., 2006) b)(Kleveland *et al.*, 2006a) c)(Morais et al., 2009) d)(Trattner *et al.*, 2008d) e)(Cruz-Garcia et al., 2009) f)(Bahuaud et al., 2010) g)(Castro et al., 2011) h)(Minghetti et al., 2011) i)(Carmona-Antoñanzas et al., 2011)

Table 5c. *Sequences of primers and Genbank accession numbers used*

Primer	Forward primer (5'-3')	Reverse primer (5'-3')	GenBank Acc. no
CD36[d]	GGATGAACTCCCTGCATGTGA	TGAGGCCAAAGTACTCGTCGA	AY606034
Δ5FAD [d]	GAGAGCTGGCACCGACAGAG	GAGCTGCATTTTTCCCATGG	AF478472
Δ6FAD [d]	AGAGCGTAGCTGACACAGCG	TCCTCGGTTCTCTCTGCTCC	AY458652
ACO[b]	CCTTCATTGTACCTCTCCGCA	CATTTCAACCTCATCAAAGCCAA	DQ364432
CPT1[d]	GTACCAGCCCCGATGCCTTCAT	TCTCTGTGCGACCCTCTCGGAA	AM230810
SR-B1[b]	AACTCAGTGAAGAGGCCAAACTTG	TGCGGCGGTGATGATG	DQ266043
ELOVL5a[c]	ACAAGACAGGAATCTCTTTCAGATTAA	TCTGGGGTTACTGTGCTATAGTGTAC	AY170327
ELOVL5b[c]	ACAAAAAGCCATGTTTATCTGAAAGA	CACAGCCCCAGAGACCCACTT	DW546112
ELOVL2[c]	CGGGTACAAAATGTGCTGGT	TCTGTTTGCCGATAGCCATT	TC91192
ELOVL4[i]	TTGTCAAATTGGTCCTGTGC	TTAAAAGCCCTTTGGGATGA	HM208347

Abbreviations: CD 36 = cluster of differentiation 36, Δ5FAD = Δ5 desaturase, Δ6FAD = Δ6 desaturase, ACO = acyl-CoA oxidase, CPT1 = carnitine palmitoyl transferase I, SR-B1 = Scavenger receptor class BI, ELOVL = Elongation of very long chain fatty acids gene. Already designed and validated in a)(Jorgensen et al., 2006) b)(Kleveland *et al.*, 2006a) c)(Morais et al., 2009) d)(Trattner *et al.*, 2008d) e)(Cruz-Garcia et al., 2009) f)(Bahuaud et al., 2010) g)(Castro et al., 2011) h)(Minghetti et al., 2011) i)(Carmona-Antoñanzas et al., 2011)

4 Summary of results

4.1 Lipid analysis

In Paper I the fat content in the white muscle was ~1.6% regardless of treatment, whereas the fat content in the liver increased significantly from ~5% to 7-8% in fish with the highest level of sesamin supplementation.

The percentage of SAFA was significantly lower in both liver and white muscle samples from fish fed VO compared to fish fed FO in both the triacylglycerols (TAG) and phospholipids (PL) fractions. The relative amount of SAFA in both TAG and PL fractions of the liver and white muscle of fish fed FO were on average 23% and 26.5% respectively. The supplementation of sesamin to the VO diets decreased the amount of SAFA in the TAG fractions of both white muscle and liver (*Table 4a*) compared to a strait VO diet. In the liver the amount of SAFA was lowered in a dose dependent manner. The decrease was independent of the n-6/n-3 value in the diet fed to the fish. Of the individual SAFA 16:0 was most clearly affected by the addition of sesamin to the feed followed 18:0. The addition of bioactive compounds to the hepatocytes cell media did not affect the content of SAFA.

The amount of MUFA in the PL fraction from white muscle and liver samples from fish fed FO diet are from 14.5% and 32.3%, respectively. The level of MUFA in the PL fraction of the liver was significantly decreased regardless of n-6/n-3 ratio and sesamin supplementation. Contradictorily, the MUFA content in the PL fractions of the white muscle was significantly increased regardless of n-6/n-3 ratio and sesamin supplementation. In the TAG fractions of both white muscle and liver in fish fed the diet with the higher n-6/n-3 ratio, the amount of MUFA was at a significantly higher level compared to the control fish fed FO diets. Sesamin, when added to the feed, did significantly decrease the level of MUFA in the TAG fractions for the higher n-6/n-3 ratio group in both white muscle and liver.

Table 6a *The effect of bioactive compounds on saturated (SAFA) and mono unsaturated fatty acid (MUFA) in the triacylglycerols and phospholipids fractions (% of total identified, mean value ± SD) of white muscle and liver from fish fed vegetable oil diet (Paper I) compared to fish feed fish oil based diet and in hepatocytes after incubation with respective treatment (Paper II)*

		Paper I								Paper II
		Low n-6/n-3				High n-6/n-3				
	Supplement	triacylglycerols		phospholipids		triacylglycerols		phospholipids		Culture media
		white muscle	liver	white muscle	liver	white muscle	liver	white muscle	liver	
SAFA	control[1)]	-7.8±0.8^{c}	-3.9±3.8^{b}	-5.1±0.1^{c}	1.1±1.3	-7.0±0.2^{bc}	-5.7±1.5^{bc}	-3.2±0.5^{b}	-2.5±0.9^{b}	-
	sesamin	-7.7±0.5^{bc}	-6.5±1.8^{bcd}	-3.8±1.3^{bc}	0.2±0.4	-6.9±0.2^{b}	-7.8±1.7^{cd}	-3.5±0.9^{b}	-3.3±0.8^{b}	-0.6±1.2
	high sesamin	-8.0±0.4^{c}	-7.4±1.6^{c}	-4.4±1.0^{b}	0.3±1.4	-7.6±0.3^{bc}	-9.2±1.2^{c}	-2.9±0.8^{c}	0.0±0.6	-
	episesamin									0.1±1.43
	lipoic acid									-0.5±0.43
	low genistein									-0.5±0.75
	high genistein									-0.1±0.79
MUFA	control[1)]	-8.1±0.5^{d}	3.1±3.7	2.4±0.9^{b}	-14.8±0.9^{c}	4.5±0.2^{e}	22.2±1.8^{c}	5.9±0.7^{c}	-12.7±0.8^{c}	-
	sesamin	-7.8±0.3^{d}	1.0±5.2	2.2±1.3^{b}	-14.6±0.7^{d}	3.8±0.2^{bc}	13.9±4.2^{b}	6.1±0.8^{c}	-12.4±0.5^{bc}	1.0±1.39
	high sesamin	-8.2±0.6^{d}	5.2±2.6	2.4±0.8^{b}	-12.9±1.2^{c}	3.6±0.3^{b}	14.8±1.5^{b}	5.9±1.4^{c}	-11.2±1.0^{b}	-
	episesamin									0.6±1.26
	lipoic acid									-1.5±2.88
	low genistein									0.1±2.74
	high genistein									-0.1±2.32

1) Vegetable oil control diet without supplementation of bioactive compounds

The results are rather different in the TAG fraction for fish fed the low n-6/n-3 diet. The amount of MUFA in the liver was the same as in fish fed the FO diet and no effect was seen with the addition of sesamin. This can be compared to the significant increase of MUFA in the corresponding TAG fraction in fish fed the high n-6/n-3 VO feed followed by a significant decrease with the addition of sesamin to the diet.

In white muscle samples from fish fed the low n-6/n-3 diet, the amount of MUFA in the TAG fraction was significantly decreased compared to fish fed FO diet, but no increase or additional decrease was seen when sesamin was added.

The amount of LA as well as ALA increase in TAG as well as PL fractions in both white muscle and liver samples from fish fed VO diets (*Table 4b*) regardless of n-6/n-3 ratio compared to fish fed FO diet. No effects were seen on the LA and ALA composition in Paper II after incubation of any of the bioactive compounds used.

Looking at the changes in EPA and DPA in *Table 4c*, the amount of EPA increases in the liver of fish fed VO diet regardless of n-6/n-3 ratio both in the TAG and PL fractions. When sesamin is added to the feed the amount of EPA is increased significantly even further. In contrast, EPA decrease in the white muscle samples in all fish fed VO and no effect can be seen with addition of sesamin.

Table 7b *The effect of bioactive compounds on the amount of LA (18:2n-6) and ALA (18:3n-3) in the triacylglycerols and phospholipids fractions (% of total identified, mean value ± SD) of white muscle and liver from fish fed vegetable oil diet (Paper I) compared to fish feed fish oil based diet and in hepatocytes after incubation with respective treatment (Paper II).*

		Paper I								Paper II
		Low n-6/n-3				High n-6/n-3				
	Supplement	triacylglycerols		phospholipids		triacylglycerols		phospholipids		Culture media
		white muscle	liver	white muscle	liver	white muscle	liver	white muscle	liver	
LA (18:2n-6)	control[1)]	8.7±0.2^{bcd}	5.3±1.2^{c}	4.7±0.8^{b}	4.3±0.5^{d}	8.4±0.4^{d}	5.1±0.7^{c}	6.6±0.4^{c}	4.7±0.2^{cd}	-
	sesamin	8.5±0.2^{bc}	6.8±1.0^{b}	4.5±0.5^{b}	5.0±0.5^{bc}	8.5±0.2^{cd}	6.1±0.9^{bc}	6.4±0.4^{c}	4.8±0.2^{bc}	0.48±0.8
	high sesamin	8.8±0.2^{bc}	6.1±0.9^{bc}	4.8±0.4^{b}	5.2±0.4^{bc}	9.0±0.3^{b}	6.2±0.8^{bc}	6.5±0.7^{c}	5.3±0.4^{b}	-
	episesamin									0.26±0.7
	lipoic acid									0.29±0.7
	low genistein									0.11±0.8
	high genistein									0.29±0.7
ALA (18:3n-3)	control[1)]	17.4±0.5^{b}	8.52±1.8^{c}	10.9±1.0^{b}	3.52±0.9^{c}	6.0±0.3^{c}	2.71±1.8^{d}	5.3±0.4^{c}	1.27±0.8^{d}	-
	sesamin	17.0±0.6^{b}	11.1±2.3^{b}	10.3±0.9^{b}	4.25±0.7^{b}	5.9±0.1^{c}	2.72±4.2^{d}	5.0±0.4^{c}	1.51±0.5^{d}	0.53±0.6
	high sesamin	17.9±0.8^{b}	9.82±1.4^{bc}	10.3±1.1^{b}	4.58±1.2^{b}	6.4±0.5^{c}	2.87±1.5^{d}	5.1±0.6^{c}	1.69±1.0^{d}	-
	episesamin									0.34±0.5
	lipoic acid									0.36±0.4
	low genistein									0.10±0.5
	high genistein									0.21±0.4

1) Vegetable oil control diet without supplementation of bioactive compounds

Table 8c *The effect of bioactive compounds on the amount of EPA (20:5n-3) and DHA (22:6n-3)in the triacylglycerols and phospholipids fractions (% of total identified, mean value ± SD) of white muscle and liver from fish fed vegetable oil diet (Paper I) compared to fish feed fish oil based diet and in hepatocytes after incubation with respective treatment (Paper II).*

		Paper I								Paper II
		Low n-6/n-3				High n-6/n-3				
	Supplement	triacylglycerols		phospholipids		triacylglycerols		phospholipids		Culture media
		white muscle	liver	white muscle	liver	white muscle	liver	white muscle	liver	
EPA (20:5n-3)	control[1)]	-2.7±0.2^{c}	5.3±1.2^{b}	-0.7±0.2^{b}	4.3±0.5^{d}	-3.1±0.1^{d}	5.1±0.7^{b}	-1.0±0.1^{c}	4.7±0.2^{cd}	-
	sesamin	-2.9±0.2^{cd}	6.8±1.0^{c}	-0.9±0.5^{bc}	5.0±0.5^{bc}	-3.0±0.1^{cd}	6.1±0.9^{bc}	-1.0±0.1^{c}	4.8±0.2^{bc}	0.0±0.12
	high sesamin	-3.1±0.2^{d}	6.1±0.9^{bc}	-1.2±0.4^{cd}	5.2±0.4^{bc}	-3.2±0.1^{d}	6.2±0.8^{bc}	-1.3±0.1^{d}	5.3±0.4^{b}	-
	episesamin									0.03±0.1
	lipoic acid									0.15±0.1
	low genistein									-0.03±0.2
	high genistein									0.05±0.1
DHA (22:6n-3)	control[1)]	-8.3±0.2^{b}	-9.4±0.5^{bc}	-14.7±2.4^{b}	-11.4±2.3^{d}	-8.6±0.2^{b}	-9.3±0.4^{bc}	-15.7±1.0^{b}	-8.6±1.0^{bc}	-
	sesamin	-8.2±0.2^{b}	-9.1±0.6^{b}	-14.1±1.4^{b}	-10.4±0.8^{cd}	-8.5±0.2^{b}	-9.8±0.5^{bc}	-15.1±2.2^{b}	-9.3±0.8^{bc}	-1.30±1.0
	high sesamin	-8.4±0.2^{b}	-10.1±0.2^{c}	-13.1±1.7^{b}	-12.2±1.7^{d}	-8.4±0.2^{b}	-9.9±0.7^{bc}	-14.2±2.0^{b}	-7.9±1.3^{b}	-
	episesamin									-1.00±0.8
	lipoic acid									0.70±2.2
	low genistein									-0.10±1.9
	high genistein									-0.50±2.2

1) Vegetable oil control diet without supplementation of bioactive compounds

4.2 Gene expression

An overview of the relative changes in gene expression from Paper I and II are presented in *Table 5*.

These results are normalized again a housekeeping gene selected on the basis that this gene was unaffected by any of the experimental parameters tested. A second round of normalization was the performed against the control treatment. The end data is presented as relative fold change compared to control. Here shown as series of minus and plus signs depending of if the gene expression was down- or upregulated, respectively. One minus or plus sign symbolizes the change of 1 magnitude compared to the results of the untreated control.

In the *in vivo* experiment described in Paper I no clear significant effect were seen on PPARs, PGC-1 and LXR by sesamin addition. In fish fed the low n-6/n-3 ratio VO diet sesamin increased the expression of both Δ5FAD and Δ6FAD. However when fed the high n-6/n-3 ratio VO diets the transcription of Δ5FAD and Δ6FAD were down-regulated by sesamin. Both SREBP-1 and SREBP-2 expression increased by sesamin supplementation to the low n-6/n-3 ratio VO diet.

The most pronounce effect of treatment with bioactive compounds in Paper II was seen on genes regulating the desaturation and elongation process of ALA and LA to EPA/DHA and ARA respectively.

The effects of the different bioactive compounds were more apparent after 48hr of incubation. In general sesamin, episesamin and lipoic acid increased the expression of Δ5FAD, Δ6FAD, ELOLV2, ELOLV5a, CD36, CPT-1 and ACO. Sesamin increased PPAR1β and γ and episesamin increased PPARα and 1β. Incubation with lipoic acid for 12hr increased the expression of all PPAR, however further incubation for 48hr decreased the expression PPARα and 1β. During the same incubation period, lipoic acid significantly increased PPARγ transcription.

Table 5. *The effect of bioactive compounds on the relative gene expression in liver from fish fed vegetable oil diet (Paper I) compared to fish feed fish oil based diet and in hepatocytes (Paper II) after incubation with respective treatment. Decrease in fold change shown here as minus signs and an increase in fold change shown as plus signs. One minus or plus sign equals to a change in fold change of one magnitude. In the grey areas no gene expression analysis was performed.*

Paper		Supplement	Hours of incubation	Gene Expression																
				PPAR			PGC-1	SREBP		LXR	FAD		ELOVL				CD36	SP-B1	CPT1	ACO
				α	β1	γ		1	2		Δ6	Δ5	2	5a	5b	4				
I	Low n-6/n-3	control[1)]					-	-	-	-		-			++	- -			+	
		sesamin					-	++	+		+++	+++			++	++			+	
		high sesamin			-		-	+		+	+++	+++		+	+	- -				+++
	High n-6/n-3	control[1)]					-				++++	+++			+	- -	-		+	
		sesamin						-			++	++				- -			++	
		high sesamin			-	+										+				-
II	Culture media	sesamin	12		+	+						-	12+							++
			48		++	+++					++	++	+++	++			++		++	+++
		episesamin	12	+									11+							-
			48	+	+						+	+	+	+			+++		+++	++
		lipoic acid	12	++	++	++							10+				+		++	+++
			48	-	-	++++					+	+	++++	+++			+		+	+++
		low genistein	12	++++	++	+						-							+	++
			48	-		-											-			
		high genistein	12														+			
			48	-	+															

1) Vegetable oil control diet without supplementation of bioactive compounds

5 General Discussion

The main objective was to investigate the effect of the added bioactive compounds both *in vivo* and *in vitro* on the expression of genes related to lipid homeostasis in Atlantic salmon. Further we wanted to evaluate if the effect of sesamin supplementation was dependent of the underlying n-6/n-3 ratio of the VO diet.

Our anticipation was to increase our understanding of the regulation of the molecular mechanisms for FA uptake, LCPUFA biosynthesis, mitochondrial and peroxisomal β-oxidation by investigating the effect of bioactive compounds.

5.1 Effects on growth performance

Replacement of FO with VO did not affect growth performance in Paper I, which is in accordance with results on Atlantic salmon (Torstensen *et al.*, 2005; Bell *et al.*, 2001; Torstensen *et al.*, 2000) and rainbow trout (Trattner *et al.*, 2008a). However, did addition of sesamin have a dose-dependent negative effect on growth, but only the highest inclusion resulted in significantly reduced weight of the fish. This is in agreement with results obtained from trails with juvenile barramundi (*Lates calcarifer*), where the growth rate were reduced in fish receiving sesamin regardless of which dietary oil that were used in the feed (Alhazzaa *et al.*, 2012). This is contradictory to results on growth performance in studies where the inclusion of sesamin in the feed was tested on Baltic Atlantic salmon (Trattner *et al.*, 2011), rainbow trout (Trattner *et al.*, 2008a) and carp (Mraz *et al.*, 2010). On the other hand, the reduced growth with the addition of high sesamin content is in agreement with studies of other bioactive compounds e.g. dodecylthioacetic acid and tetradecylthioacetic acid (TTA) (Kleveland *et al.*, 2006a), and Moya-Falcon *et al*. (2004) where these substances significantly lowered the body weight of Atlantic salmon.

5.2 Effects on lipid content

5.2.1 Total lipid content

In the *in vivo* study in Paper I with Atlantic salmon, the white muscle lipid content was not affected by sesamin supplementation. Liver fat content was however significantly increased by addition of the high level of sesamin, confirming previous findings in rats (Ashakumary *et al.*, 1999). This is in contrary to results obtained by Moya-Falcón *et al.* (2004) where the liver lipid content was reported to be unaffected by the addition of TTA to Atlantic salmon diets. Equally did neither the addition of conjugated linoleic acid nor TTA affect the total amount of lipids in liver of Atlantic cod (Kennedy *et al.*, 2007b).

5.2.2 Fatty acid composition

As expected, did the composition of FAs in white muscle and liver to a great extent mirror the FA composition in the different diets. For example was the amount of MUFA in the TAG fractions significantly higher in both white muscle and liver from fish fed the VO diet with the high n-6/n-3 compared to the control fish fed FO diets as a consequence of the significant higher amount of MUFA in the VO feed. Equally did the replacement of FO with VO reduce the proportion of DHA in both white muscle and liver. Increased levels of LA and ALA were also observed as an effect of VO inclusion compared with FO. These results are in agreement with those in the study of Bell *et al*. (2001), who found that replacement of FO with rapeseed oil (100%) lowered the proportion of DHA in both liver and muscle but to somewhat different degrees.

In Paper I did the two different n-6/n-3 ratios have an impact on n-3 FA content in fish, mainly in that the higher ratio resulted in lower amounts of ALA. This decrease was not reflected in higher percentage of DHA and EPA.

No effect of sesamin supplementation was seen on the amount of ALA (18:3n-3) in neither the TAG nor PL fractions of white muscle and liver samples. Equally did sesamin not cause any changes in the levels of ALA (18:3n-3) in the hepatocytes studied. This is conflict with results on studies with sesamin supplementation to juvenile Baltic Atlantic salmon, where a significant decrease of ALA (18:3n-3) in the white muscle PL fraction where seen together with a slight increase of DHA (22:6n-3) (Trattner *et al.*, 2011).

Sesamin did however affected FA involved in the synthesis of DHA, e.g. 20:3n-3 increased, EPA and 22:5n-3 decreased and a slight increase in DHA was detected (non significant). In previous studies a significant increase in DHA was observed on addition of a dietary sesamin/episesamin mixture in

both white muscle of rainbow trout (Trattner *et al.*, 2008a) and in Atlantic salmon hepatocytes (Trattner *et al.*, 2008b).

In Paper II the gene expression results were not supported by the FA composition. Sesamin and episesamin treatments increased the proportion of 18:4n-3 and 20:4n-3. Lipoic acid increased the proportions of 20:3n-3 and 20:4n-3. After 48 h incubation with lipoic acid, episesamin or sesamin, an up regulation of all the genes chosen as markers (ELOVL5a, Δ5FAD, ELOVL2 and Δ6FAD) for the biosynthesis of PUFAs were seen. Equally did the addition of Echium oil, rich in stearoidonic acid (18:4n-3) and γ-linoleic acid (18:3n-6), increase the expression of the Δ6FAD and elongase in barramundi tissues, but this did not lead to a significant accumulation of DHA, but both the amount EPA and ARA were increased (Alhazzaa *et al.*, 2011). The increased levels of intermediate FAs in the PUFA synthesis and the increased expression of involved genes support that these bioactive compounds might have an impact on the lipid metabolism. During the trial salmon hepatocytes incubated with G did not show any significant difference in FA composition compared to control incubations. In general the effects on FA profile were low and not as significant as the gene expression results.

Environmental factors

The difference in response to bioactive compounds compared to previously published results could be explained by several factors. First of all may the physiological response to sesamin vary dependent on fish size or species. Within the same species, variations in the response to sesamin may depend on age, gender and possibly environmental conditions such as temperature and feed composition.

The feeding period can also affect physiological response to sesamin. In Paper I, the experimental diets were fed to fish for four months, which is longer than in previous studies (8-11 weeks) (Mraz *et al.*, 2010; Trattner *et al.*, 2010; Trattner *et al.*, 2008a). Furthermore, seawater fish have a lower capacity to convert ALA to DHA than freshwater fish (Sales, 2010; Zheng *et al.*, 2004; Sargent & Tacon, 1999).

In the *in vivo* study in Paper I, pure sesamin was used, whereas Trattner et al. (2011; 2008a) supplemented the fish diet with an equi-mixture of sesamin/episesamin for rainbow trout and Baltic Atlantic salmon. The same mixture was used in a study on Atlantic salmon hepatocytes (Trattner *et al.*, 2008b). It has previously been shown in mammals that episesamin might be more effective in modulating the activity of enzymes involved in lipid metabolism. Therefore, the presence of episesamin in the diet of fish may be important for modulation of FA composition.

In Paper II, the fish were feed commercial FO based diet before scarification and hepatocytes preparation. Commercial FO based is particularly high in n-3 LCPUFAs. The individual fish used in the experiment were equally well nourished and with a high fat content.

In previous studies episesamin has been shown to be more potent than sesamin. In this study there were no clear difference between them, only in the case of CD36 and CPT1, the expression were more upregulated for episesamin than sesamin. The expression of PPARγ was higher in the sesamin treated cells.

Genetic variation

The amount of dietary n-3 LC-PUFA, EPA and DHA stored in white muscle is a trait that has been shown to be highly heritable in Atlantic salmon (Leaver *et al.*, 2011). Morais *et al.* (2011) showed that it would be possible to identify individual fish as well as groups/families of fish that respond differently to different VO diets depending on their genetic background. In principal does lean fish display a more pronounce effect in response to the substitution of FO with VO compared to fat fish.

Since no data were present in regards of genetic background of the fish tested in Paper I and II, is it difficult to refer to any difference in specific genetic traits compared the tested fish in previously reported results. However, this factor cannot be overlooked as a possible candidate influencing the outcome of these supplementation studies.

5.3 Effects on lipid related gene expression

5.3.1 Transcription factors

With a few exceptions was there very limited response to addition of bioactive compound on all transcription factors tested regardless of the bioactive compound was added to the feed as in Paper I or to the cell media as in Paper II.

Peroxisome proliferator activated receptors

Sesamin showed no effect on PPARα expression which is contradictory to the findings of Trattner *et al.* (2008b) that showed that the expression of PPARα was significantly down regulated in the liver of rainbow trout fed sesamin as a supplement to a mixed oil diet.

In the case of PPARγlong in Paper I, significant upregulation was seen when sesamin was added to higher n-6/n-3 ratio diets, while for PPARβ1A sesamin decreased the mRNA expression level. This could indicate that the effect of

sesamin is not caused by ligand binding to the PPARs. Cloning analysis of the ligand binding regions of PPARα and PPARγ genes in Atlantic salmon have revealed that they contain additional amino acid residuals, which could suggest that the ligand binding properties in salmon PPARs can differ from those seen in rodents (Andersen *et al.*, 2000). This could explain the deviation from findings in rodents (Ashakumary *et al.*, 1999).

After 12h incubation, PPARγ was upregulated in G 0.005mM, LPA and S treated cells. The upregulation of PPARγ remained in the LPA and S treated cells after 48h.

Sterol regulatory element-binding proteins

Expression of SREBP-1 and SREBP-2 was significantly increased by sesamin addition to the low n-6/n-3 ratio diets, and in fish fed these diets the SREBP target genes, desaturases and elongases were also significantly upregulated. It has been shown in liver hepatocytes of rodents that ELOVL5 elongase, Δ6FAD and Δ5FAD desaturase expression are regulated by both PPARα and SREBP-1c (Qin *et al.*, 2009; Matsuzaka *et al.*, 2002). In agreement with this, the present study showed increased expression of SREBP, elongases and desaturases.

Liver X receptors

Fish fed the V0.5SL and V0.5SH diets showed increased expression of LXR compared with fish fed the V0.5S0 diet. This could indicate that S acts on SREBP-1c directly or indirectly by activation of LXR. The increased expression of LXR and SREBP can also be associated, since LXRα and LXRβ have been shown to activate SREBP-1c in rodents (Cruz-Garcia *et al.*, 2009; Zhou *et al.*, 2008).

5.3.2 Uptake of fatty acids

Since both PPARα and PPARγ have been shown to induce the transcription of CD36 and SR-B1 (Burri *et al.*, 2010; Poirier *et al.*, 2001; Motojima *et al.*, 1998), it was not surprising that the effects on these genes were limited. No effects were seen on SR-B1 in Paper I, which is in agreement with observations made by Kleveland *et al* (2006b), where no effect in SR-BI expression was seen in response to either oleic acid or the EPA and DHA.

Only CD36 was significantly downregulated in tissues from fish fed the higher dietary ratio of n-6/n-3 with sesamin added. The gene CD36 is also regulated by PPARγ (Zhou *et al.,* 2008), which are present in two forms, PPARγ long, expressed in liver and involved in the regulation of FA metabolism and PPARγ short suggested to be present in Atlantic salmon

adipocytes and involved in adipocyte differentiation (Todorčević *et al.*, 2008; Vegusdal *et al.*, 2003; Ruyter *et al.*, 1997). The expression of CD36 follows the same expression pattern as PPARγ in all treatments after 48h.

The expression of CD36 was upregulated after 48h of incubation with ES, S and LPA 12 and 48h. The gene, CD36 is involved in the uptake of lipids (Pohl *et al.*, 2005), indicating that these three bioactive compounds possibly can increase the hepatic uptake of FA from the media. The effect of ES was twice as high as for S. This finding is in agreement with Kushiro *et al.* (2002), who reported more potent effects of episesamin than sesamin. The increased expression of CD36 could be triggered by the increased FA metabolism measured in this study, as increased expression of β-oxidation, desaturation and elongation markers or vice versa.

The transcription factor PPARα has been shown to be a regulator of FA metabolism in mice, by inducting genes coding for CD36 (Burri *et al.*, 2010; Poirier *et al.*, 2001; Motojima *et al.*, 1998). Similarly in this study the expression of PPARα was related to the expression of the long chain FA transporter, CD36 in LPA 12h, genistein 0.005mM 48h and episesamin 48h.

5.3.3 Elongation and desaturation

The transcription rate of all elongases except ELOVL5a was increased in the liver of salmon fed VO, irrespective of n-6/n-3 ratio, compared with that of fish fed a FO diet. This is in line with Morais *et al.* (2009) who reported that expression of ELOVL5b and ELOVL2, but not that of ELOVL5a, was significantly increased in both liver and intestine when Atlantic salmon were fed VO instead of FO. ELOVL5b codes for genes involved in the elongation of C18 to C20 PUFA and ELOVL2 for genes involved in the elongation of C20 to C22 (Morais *et al.*, 2009; Hastings *et al.*, 2004). The increase in ELOVL5b and ELOVL2 in fish fed the VO diet (V0.5) with low sesamin addition was accompanied by upregulation of SREBP-1c and a non significant increase in the amount of 20:3n-3 and DHA in both the TAG and the PL fractions.

Expression of SREBP-1 and SREBP-2 was significantly increased by sesamin addition to the low n-6/n-3 ratio diets, and in fish fed these diets the SREBP target genes desaturases and elongases were also significantly upregulated. It has been shown in liver hepatocytes of rodents that ELOVL5 elongase, Δ6FAD and Δ5FAD desaturase expression are regulated by both PPARα and SREBP-1c (Qin *et al.*, 2009; Matsuzaka *et al.*, 2002). In agreement with this, Paper II showed increased expression of SREBP, elongases and desaturases.

Expression of desaturation and elongation genes increased significantly with addition of S. The effect of S seemed to be influenced by the n-6/n-3 ratio

in the feed. For the lower n-6/n-3 ratio S increased desaturation and elongation, while for the higher n-6/n-3 ratio S decreased both desaturation and elongation. This is similar to results reported by Trattner *et al.* (2008b). A possible interaction between feed FA composition and sesamin content could explain the different results between the groups in the present study.

After 12h incubation Δ5FAD showed a significant downregulation in cells incubated G 0.005mM, LPA and S. However, the expression of both Δ6FAD and Δ5FAD, were upregulated by ES, S and LPA after 48h. There were striking and rapid changes in Δ5FAD gene expression from a pronounced downregulation to an upregulation in G 0.005mM, LPA and S. The upregulation of desaturases after 48h incubation are contradictory to results obtained by Trattner *et al.* (2008a), where downregulation of both Δ6FAD and Δ5FAD were detected after incubation with S. However, the downregulation of the desaturases were seen in combination with an increased amount of radio-labeled DHA synthesized from 14C 18:3n-3 (Trattner *et al.,* 2008a). In the present study no significant effects were observed on DHA levels, but on 18:4n-3 and 20:4n-3, possibly due to differences in size of fish. It has been shown that the synthesis of DHA decrease with size/age in Atlantic salmon (Zheng *et al.*, 2004). Another explanation can be the nutritional status of the fish at the time of sampling. Possibly, the desaturase activity, the gene expression and consequently the response to treatment depend on how stout the individual fish was at the time of sampling. In the present study the fish were fatter than in the study by Trattner *et al* (2008a). For further studies a weight and length relation could be interesting and helpful to understand the metabolism of lipids. Furthermore, in the previous study radio-labeled FA was used, whereas in this study total FA were analyzed and some changes could be masked by the endogenous pool of FA.

The expression of ELOVL2 was the most sensitive gene for the bioactive compounds tested in this study, in agreement with Morais *et al*. (2009), who suggested ELOVL2 to be more prominent in salmon in contrast to rats where ELOVL5 is the most sensitive to dietary changes. After 12h of incubation with LPA, ES or S the most pronounce effect was seen for ELOVL2 and were still upregulated after 48 h. ELOVL2 is involved in the elongation of 20 and 22 carbon FA towards longer FA (Monroig *et al.*, 2009).

5.3.4 β-oxidation

Markers for β-oxidation, CPT1 and ACO were upregulated after 48h of incubation with LPA, ES and S. Low concentrations of G also increased mRNA levels for β-oxidation markers after 12h. This is in agreement with our previous study, which shown increased levels of β-oxidation products after

addition of S to Atlantic salmon hepatocytes (Trattner *et al.,* 2008a) and in studies on rodents (Jeng & Hou, 2005; Ashakumary *et al.,* 1999). LPA is also suggested to increase of β-oxidation by increasing adipokine, which increases the amount of the phosphorylated form of adenosine monophosphate kinase (AMPK). Activation of AMPK stimulates phosphorylation of ACO and decreases the enzyme activity, which enhances FA oxidation through decreased hepatic concentration of malonyl-CoA, an inhibitor of CPT1 (Huong & Ide, 2008).

Genistein has been shown to be effective in decreasing the activity of enzymes involved in fatty acid synthesis as well as increasing the activity of enzymes involved in β-oxidation in rodents (Takahashi *et al.*, 2009). Our results with 12h incubation with genistein support previous results showing that genistein act in the same manner as fibrates, known agonists of PPARα both *in vivo* and *in vitro* studies ion rodents (Ricketts *et al.*, 2005) and as a ligand for PPARγ (Dang *et al.*, 2003). Further CPT1 which also is regulated by PPARs and coordinately is regulating hepatic fatty acid oxidation was identified in a genetic screen searching for soy- and isofavone-regulated mRNAs in rats consistently with our results (Iqbal *et al.*, 2002).

Earlier studies in rats (Jeng & Hou, 2005; Ashakumary *et al.*, 1999) and in salmon hepatocytes (Trattner *et al.*, 2008b) found that sesamin positively influenced the activity and gene expression of both ACO and CPT1, which are involved in peroxisomal and mitochondrial β-oxidation, respectively. In the low n-6/n-3 ratio groups we found similar results for ACO but not for CPT1. However, in our study both ACO and CPT1 were significantly downregulated after high sesamin addition in the high n-6/n-3 ratio diet. This might indicate that the n-6/n-3 ratio influenced the response of β-oxidation genes to sesamin.

The transcription factor PPARα has been shown to be a regulator of FA metabolism in mice, by inducting genes coding CPT1 (Burri *et al.*, 2010; Poirier *et al.*, 2001; Motojima *et al.*, 1998). In the hepatocyte study in Paper II, PPARα and CPT1 were significantly upregulated in G 0.005mM 12h, LPA 12h and ES 48h, indicating that PPARα trigger the expression of its target gene for mitochondrial β-oxidation. The expression of PPARα was related to the expression of the long chain FA transporter, CD36 in LPA 12h, G 0.005mM 48h and ES 48h. After 12h incubation, PPARγ was upregulated in G 0.005mM, LPA and S treated cells. The upregulation of PPARγ remained in the LPA and S treated cells after 48h.

5.4 Lack of correlation between changes in lipid related gene expression and lipid content

5.4.1 Genetic variation

In view of the whole genome duplication event that occurred in salmonids (Allendorf, 1978), transcriptomic and gene expression studies are often very tricky to analyze as well as to compare due to the presence of duplicated and highly similar genes whose transcripts might be differentially regulated.

The amount of dietary n-3 LC-PUFA, EPA and DHA stored in white muscle is a trait that has been shown to be highly heritable in Atlantic salmon (Leaver *et al.*, 2011). Morais *et al.* (2011) showed that it would be possible to identify individual fish as well as groups/families of fish that respond differently to different VO diets depending on their genetic background. In principal does lean fish display a more pronounce effect in response to the substitution of FO with VO compared to fat fish. The shift in diet mainly affects the expression of lipid and carbohydrate metabolism genes through their signaling pathways. In lean fish both PPARα and PPARβ were downregulated in response to the VO diet. This could not be seen in fat salmon. However, for another transcription factor, SREBP-1, the gene expression was clearly up-regulated in the case of fat fish but not in that of the lean salmon. When dietary FO was exchanged with VO, the LCPUFA biosynthesis was up-regulate in a genotype specific manner. In lean fish compared to fat fish Δ5FAD, Δ6FAD and ELOVL2 were significantly up-regulated, which was reflected in liver FA composition.

5.4.2 Negative feedback regulation

The unlimited excess of n-3 HUFA in salmonid feed prior to the shift in dietary oils can possibly be the cause of the reduced capacity in salmonids to swiftly increase the synthesis of n-3 HUFA, when the oil in feed is changed from FO to VO. It is possible that the breaking point from a salmonid perspective where the amount of polyunsaturated n-3 FA is too low and there is an urgent need for *de novo* synthesis is not reached in the natural lifespan of the fish investigated so far.

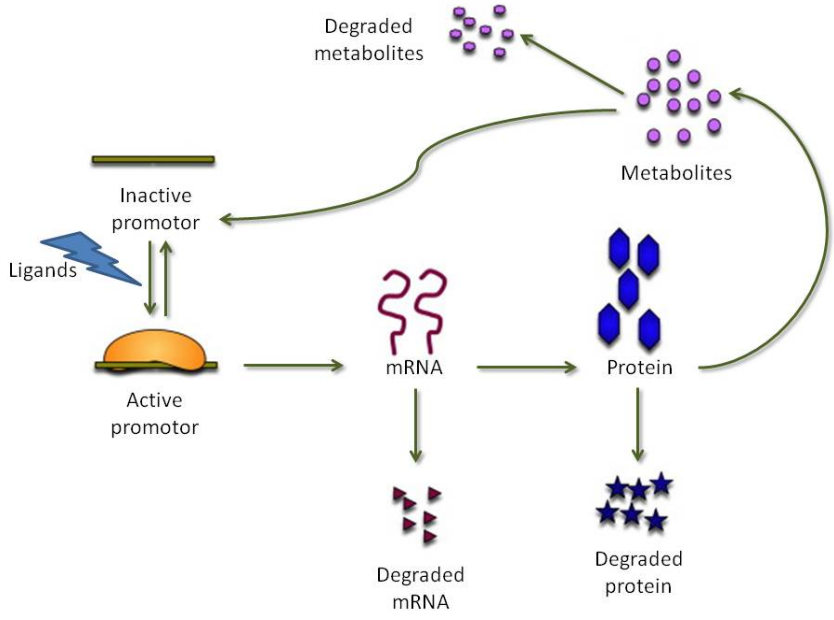

Figure 6 Schematic representation of a gene expression system subject to negative feedback regulation. Modified after (Zeron & Santillán, 2010)

Negative feedback regulation at the transcriptional level (*Figure 6*) is one of the most common motifs in gene regulatory networks (Zeron & Santillán, 2010). Nakamura & Nara (2004) suggested Δ5FAD and Δ6FAD are regulated by a negative feedback loop and that an excessive intake of either LA, ALA, or any other type of PUFA can be a problem, leading to a suppression of the PUFA metabolic pathway. So if the ratio of n-3 to n-6 is not balanced at dietary intake, the excess of one type FA could suppress the conversion of the other fatty acid type, increasing the imbalance of the dietary ratio even further.

In Paper II, the fish were feed commercial FO based diet before scarification and hepatocytes preparation. Commercial FO based is particularly high in n-3 LCPUFAs. The individual fish used in the experiment were equally well nourished and with a high fat content. Even though the amount of EPA and DHA was low in the culture media of the hepatocytes, it is likely that the feeding conditions prior to slaughter with high levels of both EPA and DHA could influence the potential positive effect of bioactive compounds on the process of desaturation, elongation and β-oxidation to DHA.

In general, in Paper II our results suggest a time dependent response regardless of bioactive compounds added to the medium, with more

pronounced effects after 48h. However, the individual bioactive compounds generated different effects. One can speculate that the mRNA turnover is extremely sensitive towards dietary changes resulting in a constant fluctuation of mRNA levels. Such a fluctuation could indicate that mRNA analysis as the only measurement without regarding potential post-transcriptional regulation mechanisms, protein levels or enzyme activity measurements is difficult to evaluate and relate to biochemical responses.

This is supported by the findings of Henderson & Sargent (1984) who showed that the peroxisomal β-oxidation is increased only when there was an imbalance between the amount of the MUFA 22:1 to PUFAs, a situation which is very unlikely to occur in natural fish diets as well as in standard FO diet. Furthermore did Tocher *et al.* (2003) prove that there is also a significant correlation between the activity of the LCPUFA biosynthetic pathway and dietary n-3 LCPUFA levels.

PUFA suppress the expressions of lipogenetic genes, and induce expression of PPARs which simultaneously induce the transcription of genes encoding proteins of lipid oxidation and thermogenesis (Price *et al.*, 2000).

Thomassen *et al.* (2012) showed that when both EPA and DHA were added to the rapeseed oil diet, the total process of desaturation, elongations and β-oxidation to DHA was significantly reduced (to about 50%) in Atlantic salmon. *In vitro* studies by the same group indicate that the inhibition is triggered by DHA and not by EPA accumulation. The inhibition occurred mainly at the Δ6 desaturation step from 24:5n-3 to 24:6n-3, and at the second elongation step (ELOVL2) from 22:5n-3 to 24:5n-3. Gene expression measurements showed equally, a significant inhibition of Δ5FAD and Δ6FAD genes coupled with a slight inhibition of the gene coding for ELOVL2 by both FO diet as well as rape seed oil feed supplemented with both EPA and DHA.

Moreover several studies have demonstrated that the expression of Δ6FAD mRNA was lower in liver of salmon fed FO compared to fish fed VO (Thomassen *et al.*, 2012b; Moya-Falcón *et al.*, 2005; Torstensen *et al.*, 2004). By this one can postulate that FO actually suppresses the desaturation and elongation of LCPUFAs.

I have in *Figure 7* tried to summarize the different possibilities of regulation in the desaturation and elongation process of LCPUFAs. I would like to emphasis that most likely not only one but several regulation pathways are involved in the desaturation and elongation cascade. The first and traditional alternative for feedback inhibition (*Figure 7 I.*) is where high levels of the metabolite in this case DHA directly inhibit the transcription of DNA to mRNA with the decrease of mRNA expression as end result. If this mode of action was dominating the current situation we would expect a decrease in the

gene expression of either Δ5FAD, Δ6FAD, ELOVL5 or ELOVL2, followed later by a decrease in enzyme activity and finally a decrease in amount of DHA.

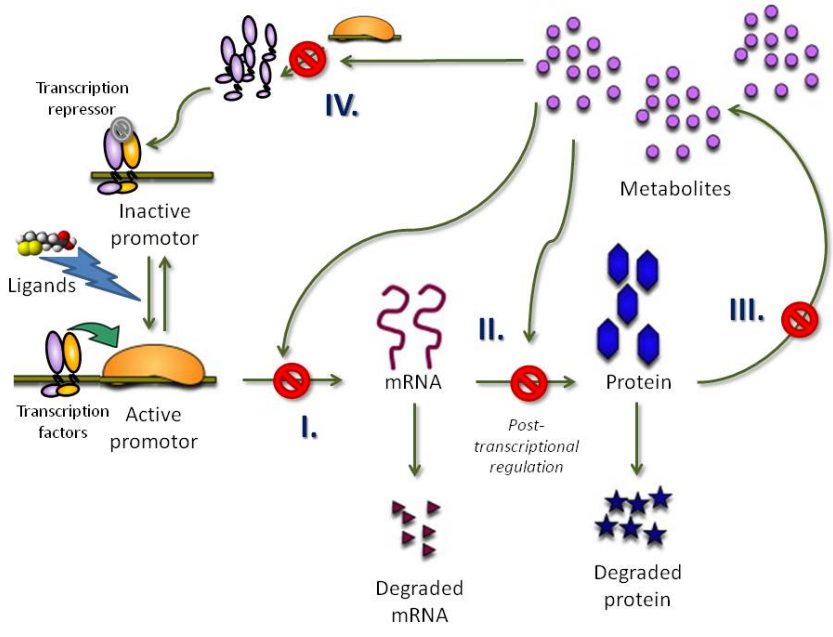

Figure 7 Schematic representation of different possible negative feedback regulation mechanisms in fish rich in EPA and/or DHA.

The second alternative is the post-transcriptional regulation or RNA silencing (*Figure 7 II.*). Here no inhibition can be seen on the mRNA level ultimately decreasing the amount of active enzymes. In some cases it can even be possible to see an increase in the mRNA expression. In step III (*Figure 7 III.*) the feedback mechanisms somehow inactivate either the Δ5FAD or Δ6FAD desaturase and/or ELOVL5 or ELOVL2 elongase generating no end product (DHA). Finally the feedback mechanism could target other target genes or genes coding for transcription factors with effects on that particular gene expression as primary effect e.g. decreases in the expression of PPAR or SREBP.

5.4.3 Post-transcriptional regulation

Post-transcriptional regulation is the control of gene expression at the RNA level after the transcription and before the translation of the gene. After being produced, the stability and distribution of the different transcripts is regulated

(post-transcriptional regulation) by means of RNA binding protein that controls the various steps and rates of the transcripts. These steps involve alternative splicing, RNA degradation, addition of poly(A) tail, processing, RNA editing and exportation from nucleus to the cytoplasm, removal of the 5-prime cap from mRNA and finally the regulation of the actual translation.

During the last decades the picture of gene regulation has become still more complex with the discovery of and focus on epigenetic regulation. Four major components of epigenetic regulation are promoter methylation, histone modification, chromatin conformation changes, and altered expression of non-coding RNAs, especially microRNAs (miRNAs).

Andreassen *et al.* (2009) was to my knowledge first to indicate that the *S. salar* genome contained conserved 7-mers in the 3'UTRs identical to miRNA target sequences suggesting that miRNA and RNA silencing also play a role in controlling protein expression in *S. salar*.

The divergences between increases in gene expression of target genes in the desaturation and elongation cascade and the actual lack of response in FA content of EPA and DHA are most likely a result of a combination of feedback regulation and post-transcriptional regulation such as RNA silencing. This still needs to be verified.

The lack of correlation between desaturase expression and LCPUFA biosynthesis may also indicate that the effect of sesamin was mediated through other mechanisms not yet understood.

Several studies have shown that the enzymes and the genes involved in the biosynthesis of highly unsaturated FA are upregulated after VO feeding (Zheng *et al.*, 2005; Tocher *et al.*, 2001a). In our study, even though the results were not consistent, gene expression of the desaturases tended to be upregulated for fish fed the high n-6/n-3 VO diet, but the supplementation of sesamin decreased this upregulation somewhat even though the net gene expression was increased compared to fish fed FO. This is in agreement with findings reported for Atlantic salmon hepatocytes (Trattner *et al.*, 2008b). In fish fed the low n-6/n-3 VO diet sesamin significantly increased the gene expression of both Δ5FAD and Δ6FAD. VO feeding decreased the actual amount of DHA in the fish fillet and the sesamin inclusion did not have any clear significant effect.

6 Main findings and conclusions

In conclusion, we could demonstrate that it is possible to interact with the metabolism of lipids by addition of bioactive compounds. Lipoic acid, sesamin and episesamin showed more effects than genistein on gene expression. These three compounds also exerted some changes of FAs, whereas genistein did not. The few changes in FA composition, compared to our previous studies suggest that nutritional background and life stage of the fish interfere with the metabolism of lipids in cell cultures of primary hepatocytes.

7 Future perspectives

The perspective for future studies is to continue research towards sustainable aquaculture with the main goal to find suitable replacements of fish oil in fish diets.

Some specific areas of future interest are:

- To study the ability of different fish families and species to metabolize EPA and DHA.
- To study the relation of gene expression and protein expression after addition of bioactive compounds or changed lipid sources.
- To study the post transcriptional regulation and possible interactions with lipid metabolism.

Acknowledgements

First of all I would like to thank my supervisors. A special warm thanks to my main supervisor, Professor **Jana Pickova**, for teaching me about fish, fish physiology and the wonderful world of PUFAs as well as opening the door to your home, to your friendship and all your good cooking. For introducing me to Ben & Jerry's "Cherry Garcia ice cream" and giving me **Snubben**, my first Airedale terrier. And Dr. **Sofia Trattner**, my wonderful co-supervisor, I would like to thank you for your never ending encouragement and for charging my love of molecular biology.

A special warm thanks to all the people at the Food Science department and fellow Ph.D. students, who gave me the privilege to be a part of their community during these past years. Sincere thanks for your warm welcome and for becoming my friends.

To **Honza** for all intriguing discussions and new possibilities! Thanks for all hours of writing together elevating our ideas into something hopefully understandable.

To **Galia Zamaratskaia**, I will not thank you for all the extra kilos you helped putting around my waist with your fantastic Russian pastries and jams, but I will thank you for introducing me to the challenging world of HPLC and for being such a warm and generous person.

To **Liane** my sweet German roommate and fun travel companion.

To **Björn Ingemarsson**, for being my mentor and role model in the intriguing world of science.

Most of all, I would like to thank those closest to my heart. Without you none of this would have been possible or meaningful.

To **Peter**, the love of my life, my husband and life companion – I would like to thank you for being there, supporting me and cheering me on. For telling me that I am intelligent, beautiful and that I definitely am going to make it even during the very darkest stretches of this journey.

To **Mark**, for being the meaning of my life, and the glitter in my eyes. To **Franz** for giving you to me.

Sara "Sötnos" and **Carl Magnus** "Pannkaka" for welcoming me into your family and letting me be a part of your life. You symbolize the very true meaning of what a "bonus" can be. To **Susanne** for being my friend even if we had all odds against us.

To my brother, **Karl** for never letting me feel sorry for myself and for always giving me wonderful stories and laughs to carry with me. To his wonderful **Veronica** and to their sons, **Viktor** and **Jonathan**.

My Father, **Bernt Schiller**, for never ending encouragement and to **Gill** for being my friend and my father's beloved companion.

To **Xano** for symbolizing everything good in life and for keeping me sane during hours of stress by forcing me to go for good long walks.

To **Anna** for reminding me where I truly belong and for returning me to the mother ship!

To my lovely ladies – **Anna**, **Sussie** and **Solan**, their families and my goddaughters **- Saga**, **Hanna** and **Linn**.

In memory of **Janne**, the friend everybody should have! You should have been here today…

December 2012

References

Ackman, R.G. (1996). DHA: can it benefit salmon marketing? *Journal of Aquatic Food Product Technology* 5(4), 7-26.

Ae Park, S., Choi, M.S., Cho, S.Y., Seo, J.S., Jung, U.J., Kim, M.J., Sung, M.K., Park, Y.B. & Lee, M.K. (2006). Genistein and daidzein modulate hepatic glucose and lipid regulating enzyme activities in C57BL/KsJ-db/db mice. *Life Sciences* 79(12), 1207-1213.

Alhazzaa, R., Bridle, A.R., Carter, C.G. & Nichols, P.D. (2012). Sesamin modulation of lipid class and fatty acid profile in early juvenile teleost, Lates calcarifer, fed different dietary oils. *Food Chemistry* 134(4), 2057-2065.

Alhazzaa, R., Bridle, A.R., Nichols, P.D. & Carter, C.G. (2011). Up-regulated desaturase and elongase gene expression promoted accumulation of polyunsaturated fatty acid (PUFA) but not long-chain PUFA in lates calcarifer, a tropical euryhaline fish, fed a stearidonic acid- and γ-linoleic acid-enriched diet. *Journal of Agricultural and Food Chemistry* 59(15), 8423-8434.

Allendorf, F.W. (1978). Protein polymorphism and the rate of loss of duplicate gene expression. *Nature* 272(5648), 76-78.

Allendorf, F.W. & Utter, F.M. (1976). Gene duplication in the family Salmonidae. III. Linkage between two duplicated loci coding for aspartate aminotransferase in the cutthroat trout (Salmo clarki). *Hereditas* 82(1), 19-24.

Ambros, V. (2001). microRNAs: Tiny regulators with great potential. *Cell* 107(7), 823-826.

Andersen, Ø., Eijsink, V.G.H. & Thomassen, M. (2000). Multiple variants of the peroxisome proliferator-activated receptor (PPAR) [gamma] are expressed in the liver of Atlantic salmon (Salmo salar). *Gene* 255(2), 411-418.

Andreassen, R., Lunner, S. & Høyheim, B. (2009). Characterization of full-length sequenced cDNA inserts (FLIcs) from Atlantic salmon (Salmo salar). *BMC Genomics* 10(502).

Appelqvist, L.-Å. (1968). Rapid methods of lipid extraction and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. *Arkiv För Kemi* 28, 551-570.

Ashakumary, L., Rouyer, I., Takahashi, Y., Ide, T., Fukuda, N., Aoyama, T., Hashimoto, T., Mizugaki, M. & Sugano, M. (1999). Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat. *Metabolism-Clinical and Experimental* 48(10), 1303-1313.

Bahuaud, D., Mørkøre, T., Østbye, T.K., Veiseth-Kent, E., Thomassen, M.S. & Ofstad, R. (2010). Muscle structure responses and lysosomal cathepsins B and L in farmed Atlantic salmon

(Salmo salar L.) pre- and post-rigor fillets exposed to short and long-term crowding stress. *Food Chemistry* 118(3), 602-615.

Bailey, G.S., Poulter, R.T.M. & Stockwell, P.A. (1978). Gene duplication in tetraploid fish: model for gene silencing at unlinked duplicated loci. *Proceedings of the National Academy of Sciences of the United States of America* 75(11), 5575-5579.

Baker, R.T.M. & Davies, S.J. (1996). Increased production of docosahexaenoic acid (22:6 n-3, DHA) in catfish nutritionally stressed by the feeding of oxidized oils and the modulatory effect of dietary α-tocopheryl acetate. *Journal of Fish Biology* 49(4), 748-752.

Barrett, L.W., Fletcher, S. & Wilton, S.D. (2012). Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. *Cellular and Molecular Life Sciences* 69(21), 3613-3634.

Bartel, D.P. (2004). MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* 116(2), 281-297.

Bast, A. & Haenen, G. (2003). Lipoic acid: A multifunctional antioxidant (Reprinted from Thiol Metabolism and Redox Regulation of Cellular Functions). *Biofactors* 17(1-4), 207-213.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J. & Sargent, J.R. (2001). Replacement of fish oil with rapeseed oil in diets of atlantic salmon (Salmo salar) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *Journal of Nutrition* 131(5), 1535-1543.

Bell, J.G., McEvoy, J., Tocher, D.R. & Sargent, J.R. (2000). Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. *Journal of Nutrition* 130(7), 1800-1808.

Bell, J.G., McGhee, F., Campbell, P.J. & Sargent, J.R. (2003). Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil ‘‘wash out’’. *Aquaculture* 218, 515–528.

Boukouvala, E., Leaver, M.J., Favre-Krey, L., Theodoridou, M. & Krey, G. (2010). Molecular characterization of a gilthead sea bream (Sparus aurata) muscle tissue cDNA for carnitine palmitoyltransferase 1B (CPT1B). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 157(2), 189-197.

Burri, L., Thoresen, G.H. & Berge, R.K. (2010). The role of PPARα activation in liver and muscle. *PPAR Research* art. no. 542359.

Carmona-Antoñanzas, G., Monroig, Ó., Dick, J.R., Davie, A. & Tocher, D.R. (2011). Biosynthesis of very long-chain fatty acids (C>24) in Atlantic salmon: Cloning, functional characterisation, and tissue distribution of an Elovl4 elongase. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* 159(2), 122-129.

Cartwright, I.J., Pockley, A.G. & Galloway, J.H. (1985). The effects of dietary ω-3 polyunsaturated fatty acids on erythrocyte membrane phospholipids, erythrocyte deformability and blood viscosity in healthy volunteers. *Atherosclerosis* 55(3), 267-281.

Castro, V., Grisdale-Helland, B., Helland, S.J., Kristensen, T., Jørgensen, S.M., Helgerud, J., Claireaux, G., Farrell, A.P., Krasnov, A. & Takle, H. (2011). Aerobic training stimulates growth and promotes disease resistance in Atlantic salmon (Salmo salar). *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 160(2), 278-290.

Chinetti-Gbaguidi, G., Fruchart, J.C. & Staels, B. (2005). Role of the PPAR family of nuclear receptors in the regulation of metabolic and cardiovascular homeostasis: New approaches to therapy. *Current Opinion in Pharmacology* 5(2 SPEC. ISS.), 177-183.

Connor, W.E. (2000). Importance of n-3 fatty acids in health and disease. *American Journal of Clinical Nutrition* 71(1), 171S-175S.

Cook, H.W. & McMaster, C.R. (2002). Fatty acid desaturation and chain elongation in eukaryotes. In. New Comprehensive Biochemistry pp. 181-204.

Cruz-Garcia, L., Minghetti, M., Navarro, I. & Tocher, D.R. (2009). Molecular cloning, tissue expression and regulation of liver X Receptor (LXR) transcription factors of Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss). *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* 153(1), 81-88.

Dang, Z.C., Audinot, V., Papapoulos, S.E., Boutin, J.A. & Löwik, C.W.G.M. (2003). Peroxisome proliferator-activated receptor γ (PPARγ) as a molecular target for the soy phytoestrogen genistein. *Journal of Biological Chemistry* 278(2), 962-967.

Dannevig, B.H. & Berg, T. (1985). Endocytosis of galactose-terminated glycoproteins by isolated liver cells of the rainbow trout (Salmo gairdneri). *Comparative Biochemistry and Physiology -- Part B: Biochemistry and and Molecular Biology* 82(4), 683-688.

De Wilde, M.C., Farkas, E., Gerrits, M., Kiliaan, A.J. & Luiten, P.G.M. (2002). The effect of n-3 polyunsaturated fatty acid-rich diets on cognitive and cerebrovascular parameters in chronic cerebral hypoperfusion. *Brain Research* 947(2), 166-173.

Evans, H., De Tomaso, T., Quail, M., Rogers, J., Gracey, A.Y., Cossins, A.R. & Berenbrink, M. (2008). Ancient and modern duplication events and the evolution of stearoyl-CoA desaturases in teleost fishes. *Physiological Genomics* 35(1), 18-29.

FAO (2008). FAOSTAT, faostat.fao.org/. (8 January 2011).

FAO (2010). FAO Fisheries and Aquaculture Technical Paper500(1).

Fujiyama-Fujiwara, Y., Umeda-Sawada, R., Kuzuyama, M. & Igarashi, O. (1995). Effects of Sesamin on the Fatty Acid Composition of the Liver of Rats Fed N-6 and N-3 Fatty Acids-Rich Diet. *j.Nutr.Sci.Vitaminol.* 41, 217-225.

Gjedrem, T. (1997). Flesh quality improvement in fish through breeding. *Aquaculture International* 5(3), 197-206.

Gómez-Pinilla, F. (2008). Brain foods: The effects of nutrients on brain function. *Nature Reviews Neuroscience* 9(7), 568-578.

Gräff, J., Kim, D., Dobbin, M.M. & Li-Huei, T. (2011). Epigenetic Regulation of Gene Expression in Physiological and Pathological Brain Processes. *Physiological Reviews* 91(2), 603-649.

Hara, A. & Radin, N.S. (1978). Lipid extraction of tissue with low toxicity solvent. *Analytic Biochemistry* 90, 420-426.

Hastings, N., Agaba, M.K., Tocher, D.R., Zheng, X., Dickson, C.A., Dick, J.R. & Teale, A.J. (2004). Molecular cloning and functional characterization of fatty acyl desaturase and elongase cDNAs involved in the production of eicosapentaenoic and docosahexaenoic acids from α-linolenic acid in Atlantic salmon (*Salmo salar*). *Marine Biotechnology* 6(5), 463-474.

Henderson, R.J. (1996). Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archives of Animal Nutrition/Archiv fur Tierernahrung* 49(1), 5-22.

Henderson, R.J. & Sargent, J.R. (1984). Lipid metabolism in rainbow trout (Salmo gairdnerii) fed diets containing partially hydrogenated fish oil. *Comparative Biochemistry and Physiology -- Part B: Biochemistry and* 78(3), 557-564.

Hesketh, J.E., Vasconcelos, M.H. & Bermano, G. (1998). Regulatory signals in messenger RNA: Determinants of nutrient-gene interaction and metabolic compartmentation. *British Journal of Nutrition* 80(4), 307-321.

Hiltunen, J.K., Autio, K.J., Schonauer, M.S., Kursu, V.A.S., Dieckmann, C.L. & Kastaniotis, A.J. Mitochondrial fatty acid synthesis and respiration. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1797(6-7), 1195-1202.

Hiltunen, J.K., Autio, K.J., Schonauer, M.S., Kursu, V.A.S., Dieckmann, C.L. & Kastaniotis, A.J. (2010a). Mitochondrial fatty acid synthesis and respiration. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1797(6–7), 1195-1202.

Hiltunen, J.K., Chen, Z., Haapalainen, A.M., Wierenga, R.K. & Kastaniotis, A.J. (2010b). Mitochondrial fatty acid synthesis - An adopted set of enzymes making a pathway of major importance for the cellular metabolism. *Progress in Lipid Research* 49(1), 27-45.

Hordvik, I. (1998). The impact of ancestral tetraploidy on antibody heterogeneity in salmonid fishes. *Immunological Reviews* 166, 153-157.

Horrocks, L.A. & Yeo, Y.K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research* 40(3), 211-225.

Horton, J.D., Goldstein, J.L. & Brown, M.S. (2002). SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. *Journal of Clinical Investigation* 109(9), 1125-1131.

Huong, D.T.T. & Ide, T. (2008). Dietary lipoic acid-dependent changes in the activity and mRNA levels of hepatic lipogenic enzymes in rats. *British Journal of Nutrition* 100(1), 79-87.

Ide, T., Ashakumary, L., Takahashi, Y., Kushiro, M., Fukuda, N. & Sugano, M. (2001). Sesamin, a sesame lignan, decreases fatty acid synthesis in rat liver accompanying the down-regulation of sterol regulatory element binding protein-1. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids* 1534(1), 1-13.

Ide, T., Hong, D.D., Ranasinghe, P., Takahashi, Y., Kushiro, M. & Sugano, M. (2004). Interaction of dietary fat types and sesamin on hepatic fatty acid oxidation in rats. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids* 1682(1-3), 80-91.

Ide, T., Kushiro, M., Takahashi, Y., Shinohara, K., Fukuda, N. & Sirato-Yasumoto, S. (2003). Sesamin, a Sesame Lignan, as a Potent Serum Lipid-Lowering Food Component. *Japan Agricultural Research Quarterly (JARQ)* 37(3), 151 – 158.

Iqbal, M.J., Yaegashi, S., Ahsan, R., Lightfoot, D.A. & Banz, W.J. (2002). Differentially abundant mRNAs in rat liver in response to diets containing soy protein isolate. *Physiological Genomics* 11, 219-226.

Jeng, K.C.G. & Hou, R.C.W. (2005). Sesamin and sesamolin: Nature's Therapeutic Lignans. *Current enzyme Inhibition* 1(1), 11-20.

Jorgensen, S.M., Kleveland, E.J., Grimholt, U. & Gjoen, T. (2006). Validation of reference genes for real-time polymerase chain reaction studies in Atlantic salmon. *Marine Biotechnology* 8(4), 398-408.

Jump, D.B. (2011). Fatty acid regulation of hepatic lipid metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care* 14(2), 115-120.

Jump, D.B., Botolin, D., Wang, Y., Xu, J., Christian, B. & Demeure, O. (2005). Fatty Acid Regulation of Hepatic Gene Transcription. *J. Nutr.* 135, 2503–2506.

Jump, D.B. & Clarke, S.D. (1999). Regulation of gene expression by dietary fat. *Annu. Rev. Nutr.* 19, 63-90.

Jumpsen, J., Lien, E.L., Goh, Y.K. & Clandinin, M.T. (1997). Small changes of dietary (n-6) and (n-3)/fatty acid content ratio alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neuronal and glial cells in rats. *Journal of Nutrition* 127(5), 724-731.

Kamal-Eldin, A., Frank, J., Razdan, A., Tengblad, S., Basu, S. & Vessby, B. (2000). Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. *Lipids* 35(4), 427-435.

Kavsan, V., Koval, A., Petrenko, O., Roberts, C.T. & Leroith, D. (1993). Two Insulin Genes Are Present in the Salmon Genome. *Biochemical and Biophysical Research Communications* 191(3), 1373-1378.

Kennedy, S.R., Bickerdike, R., Berge, R.K., Dick, J.R. & Tocher, D.R. (2007a). Influence of conjugated linoleic acid (CLA) or tetradecylthioacetic acid (TTA) on growth, lipid composition, fatty acid metabolism and lipid gene expression of rainbow trout (Oncorhynchus mykiss L.). *Aquaculture* 272(1-4), 489-501.

Kennedy, S.R., Bickerdike, R., Berge, R.K., Porter, A.R. & Tocher, D.R. (2007b). Influence of dietary conjugated linoleic acid (CLA) and tetradecylthioacetic acid (TTA) on growth, lipid composition and key enzymes of fatty acid oxidation in liver and muscle of Atlantic cod (*Gadus morhua* L.). *Aquaculture* 264(1-4), 372-382.

Kim, D.J., Seok, S.H., Baek, M.W., Lee, H.Y., Na, Y.R., Park, S.H., Lee, H.K., Dutta, N.K., Kawakami, K. & Park, J.H. (2009). Developmental toxicity and brain aromatase induction by high genistein concentrations in zebrafish embryos. *Toxicology Mechanisms and Methods* 19(3), 251-256.

Kim, M.S., Park, J.Y., Namkoong, C., Jang, P.G., Ryu, J.W., Song, H.S., Yun, J.Y., Namgoong, I.S., Ha, J., Park, I.S., Lee, I.K., Viollet, B., Youn, J.H., Lee, H.K. & Lee, K.U. (2004). Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nature Medicine* 10(7), 727-733.

Kiso, Y., Tsuruoka, N., Kidokoro, A., Matsumoto, I. & Abe, K. (2005). Sesamin ingestion regulates the transcription levels of hepatic metabolizing enzymes for alcohol and lipids in rats. *Alcoholism: Clinical and Experimental Research* 29(11 SUPPL.).

Kjær, M.A., Vegusdal, A., Gjøen, T., Rustan, A.C., Todorčević, M. & Ruyter, B. (2008). Effect of rapeseed oil and dietary n-3 fatty acids on triacylglycerol synthesis and secretion in Atlantic salmon hepatocytes. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1781(3), 112-122.

Kleveland, E.J., Ruyter, B., Vegusdal, A., Sundvold, H., Berge, R.K. & Gjøen, T. (2006a). Effects of 3-thia fatty acids on expression of some lipid related genes in Atlantic salmon (Salmo salar L.). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 145(2), 239-248.

Kleveland, E.J., Syvertsen, B.L., Ruyter, B., Vegusdal, A., Jørgensen, S.M. & Gjøen, T. (2006b). Characterization of Scavenger Receptor Class B, Type I in Atlantic Salmon (*Salmo salar* L.). *Lipids* 71, 1017–1027.

Kozlov, A.V., Gille, L., Staniek, K. & Nohl, H. (1999). Dihydrolipoic acid maintains ubiquinone in the antioxidant active form by two-electron reduction of ubiquinone and one-electron reduction of ubisemiquinone. *Archives of Biochemistry and Biophysics* 363(1), 148-154.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. & Etherton, T.D. (2002). Bioactive compounds in foods: Their role in the prevention of

cardiovascular disease and cancer. *American Journal of Medicine* 113, PII S0002-9343(01)00995-0.

Kushiro, M., Masaoka, T., Hageshita, S., Takahashi, Y., Ide, T. & Sugano, M. (2002). Comparative effect of sesamin and episesamin on the activity and gene expression of enzymes in fatty acid oxidation and synthesis in rat liver. *The Journal of Nutritional Biochemistry* 13(5), 289-295.

Kyle, D.J. & Arterburn, L.M. (1998). Single Cell Oil Sources of Docosahexaenoic Acid: Clinical Studies. In: Simopoulos, A.P. (Ed.) *The return of n-3 fatty acids in to the food supply 1.Land-based animal food products and their health effects*. pp. 117-131. Basel: Karger; 83).

Lapillonne, A., Clarke, S.D. & Heird, W.C. (2003). Plausible mechanisms for effects of long-chain polyunsaturated fatty acids on growth. *Journal of Pediatrics* 143(4 SUPPL.), S9-S16.

Latchman, D.S. (1997). Transcription factors: An overview. *International Journal of Biochemistry and Cell Biology* 29(12), 1305-1312.

Leaver, M.J., Bautista, J.M., Björnsson, B.T., Jönsson, E., Krey, G., Tocher, D.R. & Torstensen, B.E. (2008a). Towards fish lipid nutrigenomics: Current state and prospects for fin-fish aquaculture. *Reviews in Fisheries Science* 16(SUPPL.1), 71-92.

Leaver, M.J., Ezaz, M.T., Fontagne, S., Tocher, D.R., Boukouvala, E. & Krey, G. (2007). Multiple peroxisome proliferator-activated receptor β subtypes from Atlantic salmon (Salmo salar). *Journal of Molecular Endocrinology* 38(3-4), 391-400.

Leaver, M.J., Taggart, J.B., Villeneuve, L., Bron, J.E., Guy, D.R., Bishop, S.C., Houston, R.D., Matika, O. & Tocher, D.R. (2011). Heritability and mechanisms of n- 3 long chain polyunsaturated fatty acid deposition in the flesh of Atlantic salmon. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics* 6(1), 62-69.

Leaver, M.J., Villeneuve, L.A.N., Obach, A., Jensen, L., Bron, J.E., Tocher, D.R. & Taggart, J.B. (2008b). Functional genomics reveals increases in cholesterol biosynthetic genes and highly unsaturated fatty acid biosynthesis after dietary substitution of fish oil with vegetable oils in Atlantic salmon (*Salmo salar*). *BMC Genomics* 9(299).

Levine, M. & Tjian, R. (2003). Transcription regulation and animal diversity. *Nature* 424(6945), 147-151.

Livak, K.J. & Schmittgen, T.D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* 25(4), 402-408.

Lopez, D. & McLean, M.P. (2006). Estrogen regulation of the scavenger receptor class B gene: Anti-atherogenic or steroidogenic, is there a priority? *Molecular and Cellular Endocrinology* 247(1–2), 22-33.

Lykkesfeldt, J., Hagen, T.M., Vinarsky, V. & Ames, B.N. (1998). Age-associated decline in ascorbic acid concentration, recycling, and biosyntheis in rat hepatocytes - Reversal with (R)-α-lipoic acid supplementation. *FASEB Journal* 12(12), 1183-1189.

Malerød, L., Sporstøl, M., Juvet, L.K., Mousavi, A., Gjøen, T. & Berg, T. (2003). Hepatic scavenger receptor class B, type I is stimulated by peroxisome proliferator-activated receptor γ and hepatocyte nuclear factor 4α. *Biochemical and Biophysical Research Communications* 305(3), 557-565.

Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Yoshikawa, T., Hasty, A.H., Tamura, Y., Osuga, J.I., Okazaki, H., Iizuka, Y., Takahashi, A., Sone, H., Gotoda, T., Ishibashi, S. & Yamada, N. (2002). Dual regulation of mouse Δ5- and Δ6-desaturase gene expression by SREBP-1 and PPARα. *Journal of Lipid Research* 43(1), 107-114.

McGarry, J.D. & Brown, N.F. (1997). The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *European Journal of Biochemistry* 244(1), 1-14.

McKay, S.J., Trautner, J., Smith, M.J., Koop, B.F. & Devlin, R.H. (2004). Evolution of duplicated growth hormone genes in autotetraploid salmonid fishes. *Genome* 47(4), 714-723.

Minghetti, M., Leaver, M.J. & Tocher, D.R. (2011). Transcriptional control mechanisms of genes of lipid and fatty acid metabolism in the Atlantic salmon (Salmo salar L.) established cell line, SHK-1. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1811(3), 194-202.

Monroig, Ó., Rotllant, J., Sánchez, E., Cerdá-Reverter, J.M. & Tocher, D.R. (2009). Expression of long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis genes during zebrafish Danio rerio early embryogenesis. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1791(11), 1093-1101.

Moore, M.J. (2005). From birth to death: The complex lives of eukaryotic mRNAs. *Science* 309(5740), 1514-1518.

Morais, S., Monroig, O., Xiaozhong, Z., Leaver, M.J. & Tocher, D.R. (2009). Highly Unsaturated Fatty Acid Synthesis in Atlantic Salmon: Characterization of ELOVL5-and ELOVL2-like Elongases. *Marine Biotechnology* 11(5), 627-639.

Morais, S., Pratoomyot, J., Taggart, J.B., Bron, J.E., Guy, D.R., Bell, J.G. & Tocher, D.R. (2011). Genotype-specific responses in Atlantic salmon (Salmo salar) subject to dietary fish oil replacement by vegetable oil: A liver transcriptomic analysis. *BMC Genomics* 12.

Morash, A.J., Le Moine, C.M.R. & McClelland, G.B. (2010). Genome duplication events have led to a diversification in the CPT I gene family in fish. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* 299(2), R579-R589.

Morikawa, T., Yasuno, R. & Wada, H. (2001). Do mammalian cells synthesize lipoic acid? Identification of a mouse cDNA encoding a lipoic acid synthase located in mitochondria. *FEBS Letters* 498(1), 16-21.

Motojima, K., Passilly, P., Peters, J.M., Gonzalez, F.J. & Latruffe, N. (1998). Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor α and γ activators in a tissue- and inducer-specific manner. *Journal of Biological Chemistry* 273(27), 16710-16714.

Mourente, G., Dick, J.R., Bell, J.G. & Tocher, D.R. (2005). Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and beta-oxidation of [1-C-14]18:3n-3 (LNA) and [1-C-14] 20:5n-3 (EPA) in hepatocytes and enterocytes of European sea bass (*Dicentrarchus labrax* L.). *Aquaculture* 248, 173-186.

Moya-Falcón, C., Hvattum, E., Dyrøy, E., Skorve, J., Stefansson, S.O., Thomassen, M.S., Jakobsen, J.V., Berge, R.K. & Ruyter, B. (2004). Effects of 3-thia fatty acids on feed intake, growth, tissue fatty acid composition, β-oxidation and Na+,K+-ATPase activity in Atlantic salmon. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* 139(4), 657-668.

Moya-Falcón, C., Thomassen, M.S., Jakobsen, J.V. & Ruyter, B. (2005). Effects of dietary supplementation of rapeseed oil on metabolism of [1-^{14}C]18:1n-9, [1-^{14}C]20:3n-6, and [1-^{14}C]20:4n-3 in Atlantic salmon hepatocytes. *Lipids* 40(7), 709-717.

Mráz, J. (2012). Lipids in common carp (*Cyprinus carpio*) and effects on human health. *Acta Universitatis agriculturae Sueciae* 2012:75.

Mraz, J., Schlechtriem, C., Olohan, L.A., Fang, Y.X., Cossins, A.R., Zlabek, V., Samuelsen, T. & Pickova, J. (2010). Sesamin as a potential modulator of fatty acid composition in common carp (Cyprinus carpio). *Aquaculture Research* 41(11), e851-e861.

Nakamura, M.T., Cheon, Y., Li, Y. & Nara, T.Y. (2004). Mechanisms of regulation of gene expression by fatty acids. *Lipids* 39(11), 1077-1083.

Nakamura, M.T. & Nara, T.Y. (2004). Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. In. pp. 345-376.

Ng, Y., Hanson, S., Malison, J.A., Wentworth, B. & Barry, T.P. (2006). Genistein and other isoflavones found in soybeans inhibit estrogen metabolism in salmonid fish. *Aquaculture* 254(1-4), 658-665.

Ohno, S. (1999). Gene duplication and the uniqueness of vertebrate genomes circa 1970-1999. *Seminars in Cell and Developmental Biology* 10(5), 517-522.

Ohno, S., Wolf, U. & Atkin, N.B. (1968). Evolution from fish to mammals by gene duplication. *Hereditas* 59(1), 169-187.

Olesen, I., Gjedrem, T., Bentsen, H.B., Gjerde, B. & Rye, M. (2003). Breeding programs for sustainable aquaculture. *Journal of Applied Aquaculture* 13(3-4), 179-204.

Olsen, R.E., Henderson, R.J. & Ringø, E. (1991). Lipids of arctic charr, Salvelinus alpinus (L.) I. Dietary induced changes in lipid class and fatty acid composition. *Fish Physiology and Biochemistry* 9(2), 151-164.

Osborne, T.F. & Espenshade, P.J. (2009). Evolutionary conservation and adaptation in the mechanism that regulates SREBP action: What a long, strange tRIP it's been. *Genes and Development* 23(22), 2578-2591.

Pagot, Y. & Belin, J.M. (1996). Involvement of carnitine acyltransferases in peroxisomal fatty acid metabolism by the yeast Pichia guilliermondii. *Applied and Environmental Microbiology* 62(10), 3864-3867.

Pandya-Jones, A. (2011). Pre-mRNA splicing during transcription in the mammalian system. *Wiley interdisciplinary reviews. RNA* 2(5), 700-717.

Pawlosky, R.J., Hibbeln, J.R., Novotny, J.A. & Salem Jr, N. (2001). Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. *Journal of Lipid Research* 42(8), 1257-1265.

Pettersson, A., Johnsson, L., Brannas, E. & Pickova, J. (2009). Effects of rapeseed oil replacement in fish feed on lipid composition and self-selection by rainbow trout (Oncorhynchus mykiss). *Aquaculture Nutrition* 15(6), 577-586.

Pickova, J., Dutta, P.C., Larsson, P.O. & Kiessling, A. (1997). Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (Gadus morhua) stocks. *Canadian Journal of Fisheries and Aquatic Sciences* 54(10), 2410-2416.

Pickova, J., Kiessling, A., Pettersson, A. & Dutta, P.C. (1998). Comparison of fatty acid composition and astaxanthin content in healthy and by M74 affected salmon eggs from three Swedish river stocks. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 120(2), 265-271.

Pohl, J., Ring, A., Korkmaz, Ü., Ehehalt, R. & Stremmel, W. (2005). FAT/CD36-mediated long-chain fatty acid uptake in adipocytes requires plasma membrane rafts. *Molecular Biology of the Cell* 16(1), 24-31.

Poirier, H., Niot, I., Monnot, M.C., Braissant, O., Meunier-Durmort, C., Costet, P., Pineau, T., Wahli, W., Willson, T.M. & Besnard, P. (2001). Differential involvement of peroxisome-

proliferator-activated receptors α and δ in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine. *Biochemical Journal* 355(2), 481-488.

Powell, K. (2003). Fish farming: Eat your veg. *Nature* 426(6965), 378-379.

Powers, D.A. (1991). Evolutionary Genetics of Fish. In. pp. 119-228.

Price, P.T., Nelson, C.M. & Clarke, S.D. (2000). Omega-3 polyunsaturated fatty acid regulation of gene expression. *Current Opinion in Lipidology* 11(1), 3-7.

Qin, Y.F., Dalen, K.T., Gustafsson, J.-Å. & Nebb, H.I. (2009). Regulation of hepatic fatty acid elongase 5 by LXRα-SREBP-1c. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1791(2), 140-147.

Reed, L.J. (1998). From lipoic acid to multi-enzyme complexes. *Protein science : a publication of the Protein Society* 7(1), 220-224.

Regost, C., Jakobsen, J.V. & Rørå, A.M.B. (2004). Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. *Food Research International* 37(3), 259-271.

Rhainds, D. & Brissette, L. (2004). The role of scavenger receptor class B type I (SR-BI) in lipid trafficking: Defining the rules for lipid traders. *The International Journal of Biochemistry & Cell Biology* 36(1), 39-77.

Ricketts, M.L., Moore, D.D., Banz, W.J., Mezei, O. & Shay, N.F. (2005). Molecular mechanisms of action of the soy isoflavones includes activation of promiscuous nuclear receptors. A review. *Journal of Nutritional Biochemistry* 16(6), 321-330.

Rosenlund, G. (2001). Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (Salmo salar L.). *Aquaculture Research* 32(SUPPL. 1), 323-328.

Ruxton, C.H.S., Calder, P.C., Reed, S.C. & Simpson, M.J.A. (2005). The impact of long-chain n-3 polyunsaturated fatty acids on human health. *Nutrition Research Reviews* 18(1), 113-129.

Ruyter, B., Andersen, Ø., Dehli, A., Östlund Farrants, A.-K., Gjøen, T. & Thomassen, M.S. (1997). Peroxisome proliferator activated receptors in Atlantic salmon (*Salmo salar*): Effects on PPAR transcription and acyl-CoA oxidase activity in hepatocytes by peroxisome proliferators and fatty acids. *Biochimica et Biophysica Acta - Lipids and Lipid Metabolism* 1348(3), 331-338.

Ruyter, B. & Thomassen, M.S. (1999). Metabolism of n-3 and n-6 fatty acids in Atlantic salmon liver: Stimulation by essential fatty acid deficiency. *Lipids* 34(11), 1167-1176.

Safari, M.-R. & Sheikh, N. (2003). Effects of flavonoids on the susceptibility of low-density lipoprotein to oxidative modification. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 69, 73–77.

Sales, J. (2010). Quantification of the Differences in Flesh Fatty Acid Components between Farmed and Wild Fish. *Journal of Aquatic Food Product Technology* 19(3), 298-309.

Sanden, M., Stubhaug, I., Berntssen, M.H.G., Lie, Ø. & Torstensen, B.E. (2011). Atlantic salmon (Salmo salar L.) as a net producer of long-chain marine ω-3 fatty acids. *Journal of Agricultural and Food Chemistry* 59(23), 12697-12706.

Sargent, J., Bell, G., McEvoy, L., Tocher, D. & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* 177(1-4), 191-199.

Sargent, J.R. & Tacon, A.G.J. (1999). Development of farmed fish: a nutritionally necessary alternative to meat. *Proceedings of the Nutrition Society* 58(2), 377-383.

Sargent, J.R.S. (2001). What will happen when the demand for fish oil will exceed supply within ten years? *European Journal of Lipid Science and Technology* 103(11), 711.

Sassi-Messai, S., Gibert, Y., Bernard, L., Nishio, S.I., Ferri Lagneau, K.F., Molina, J., Andersson-Lendahl, M., Benoit, G., Balaguer, P. & Laudet, V. (2009). The phytoestrogen genistein affects zebrafish development through two different pathways. *PLoS ONE* 4(3).

Schneider, R. & Grosschedl, R. (2007). Dynamics and interplay of nuclear architecture, genome organization, and gene expression. *Genes and Development* 21(23), 3027-3043.

Seglen, P.O. (1976). Preparation of isolated rat liver cells. *Methods in Cell Biology* Vol. 13, 29-83.

Shimizu, S., Akimoto, K., Shinmen, Y., Kawashima, H., Sugano, M. & Yamada, H. (1991). Sesamin Is a Potent and Specific Inhibitor of Delta-5 Desaturase in Polyunsaturated Fatty-Acid Biosynthesis. *Lipids* 26(7), 512-516.

Simopoulos, A.P. (1999a). Evolutionary aspects of omega-3 fatty acids in the food supply. *Prostaglandins Leukotrienes and Essential Fatty Acids* 60(5-6), 421-429.

Simopoulos, A.P. (1999b). New products from the agri-food industry: The return of n-3 fatty acids into the food supply. *Lipids* 34(6 SUPPL.), S297-S301.

Simopoulos, A.P. (2000). Human requirement for N-3 polyunsaturated fatty acids. *Poultry Science* 79(7), 961-970.

Simopoulos, A.P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy* 56(8), 365-379.

Singer, S.J. & Nicolson, G.L. (1972). The fluid mosaic model of the structure of cell membranes. *Science* 175(4023), 720-731.

Sprecher, H. (2000). Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1486(2-3), 219-231.

Stauffer, C.E. (1996). *Fats and Oils*. 2nd. ed. St. Paul, Minnesota, USA: American association of cereal chemists, Inc. Eagan press.

Tacon, A.G.J. (2005). State of information on salmon aquaculture feed and the environment *Report prepared for the WWF US Initiated Salmon Aquaculture Dialogue*.

Tacon, A.G.J. & Metian, M. (2009). Fishing for feed or fishing for food: Increasing global competition for small pelagic forage fish. *Ambio* 38(6), 294-302.

Taggart, J.B., Bron, J.E., Martin, S.A.M., Seear, P.J., Høyhein, B., Talbot, R., Carmichael, S.N., Villeneuve, L.A.N., Sweeney, G.E., Houlihan, D.F., Secombes, C.J., Tocher, D.R. & Teale, A.J. (2008). A description of the origins, design and performance of the TRAITS–SGPAtlantic salmon *Salmo salar* L. cDNA microarray. *Journal of Fish Biology* 72, 2071–2094.

Takahashi, Y., Odbayar, T.-O. & Ide, T. (2009). A comparative analysis of genistein and daidzein in affecting lipid metabolism in rat liver. *Journal of Clinical Biochemistry and Nutrition* 44(3), 223-230.

Thomassen, M.S., Rein, D., Berge, G.M., Østbye, T.-K. & Ruyter, B. (2012a). High dietary EPA does not inhibit Δ5 and Δ6 desaturases in Atlantic salmon (Salmo salar L.) fed rapeseed oil diets. *Aquaculture* 360–361(0), 78-85.

Thomassen, M.S., Rein, D., Berge, G.M., Østbye, T.K. & Ruyter, B. (2012b). High dietary EPA does not inhibit δ5 and δ6 desaturases in Atlantic salmon (Salmo salar L.) fed rapeseed oil diets. *Aquaculture* 360-361, 78-85.

Thomassen, M.S. & Røsjø, C. (1989). Different fats in feed for salmon: Influence on sensory parameters, growth rate and fatty acids in muscle and heart. *Aquaculture* 79(1-4), 129-135.

Tocher, D.R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* 11(2), 107-184.

Tocher, D.R., Bell, J.G., MacGlaughlin, P., McGhee, F. & Dick, J.R. (2001a). Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: Effects of dietary vegetable oil. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* 130(2), 257-270.

Tocher, D.R., Bell, J.G., MacGlaughlin, P., McGhee, F. & Dick, J.R. (2001b). Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: effects of dietary vegetable oil. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 130(2), 257-270.

Tocher, D.R., Bell, J.G., McGhee, F., Dick, J.R. & Fonseca-Madrigal, J. (2003). Effects of dietary lipid level and vegetable oil on fatty acid metabolism in Atlantic salmon (Salmo salar L.) over the whole production cycle. *Fish Physiology and Biochemistry* 29(3), 193-209.

Tocher, D.R. & Dick, J.R. (1990). Incorporation and metabolism of (n-3) and (n-6) polyunsaturated fatty acids in phospholipid classes in cultured atlantic salmon (Salmo salar) cells. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* 96(1), 73-79.

Todorčević, M., Vegusdal, A., Gjøen, T., Sundvold, H., Torstensen, B.E., Kjær, M.A. & Ruyter, B. (2008). Changes in fatty acids metabolism during differentiation of Atlantic salmon preadipocytes; Effects of n-3 and n-9 fatty acids. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1781(6-7), 326-335.

Torstensen, B.E., Bell, J.G., Rosenlund, G., Henderson, R.J., Graff, I.E., Tocher, D.R., Lie, O. & Sargent, J.R. (2005). Tailoring of Atlantic salmon (Salmo salar L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. *Journal of Agricultural and Food Chemistry* 53(26), 10166-10178.

Torstensen, B.E., Frøyland, L. & Lie, Ø. (2004). Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil - Effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. *Aquaculture Nutrition* 10(3), 175-192.

Torstensen, B.E., Øyvind, L. & Frøyland, L. (2000). Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) - Effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. *Lipids* 35(6), 653-664.

Trattner, S. (2009). Quality of lipids in fish fed vegetable oils. *Diss. (sammanfattning/summary) Uppsala : Sveriges lantbruksuniv., Acta Universitatis agriculturae Sueciae, 1652-6880 ; 2009:31*.

Trattner, S., Kamal-Eldin, A., Brannas, E., Moazzami, A., Zlabek, V., Larsson, P., Ruyter, B., Gjoen, T. & Pickova, J. (2008a). Sesamin Supplementation Increases White Muscle Docosahexaenoic Acid (DHA) Levels in Rainbow Trout (Oncorhynchus mykiss) Fed High Alpha-Linolenic Acid (ALA) Containing Vegetable Oil: Metabolic Actions. *Lipids* 43(11), 989-997.

Trattner, S., Pickova, J., Park, K.H., Rinchard, J. & Dabrowski, K. (2007). Effects of alpha-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. *Aquaculture* 273(1), 158-164.

Trattner, S., Ruyter, B., Ostbye, T.K., Gjoen, T., Zlabek, V., Kamal-Eldin, A. & Pickova, J. (2008b). Sesamin Increases Alpha-Linolenic Acid Conversion to Docosahexaenoic Acid in Atlantic Salmon (*Salmo salar* L.) Hepatocytes: Role of Altered Gene Expression. *Lipids* 43(11), 999-1008.

Trattner, S., Ruyter, B., Ostbye, T.K., Kamal-Eldin, A., Moazzami, A., Pan, J., Gjoen, T., Brannas, E., Zlabek, V. & Pickova, J. (2010). Influence of dietary sesamin, a bioactive compound on fatty acids and expression of some lipid regulating genes in Baltic Atlantic salmon (Salmo salar L.) juveniles. *Physiol Res*.

Trattner, S., Ruyter, B., Østbye, T.K., Kamal-Eldin, A., Moazzami, A., Pan, J., Gjøen, T., Brännäs, E., Zlabek, V. & Pickova, J. (2011). Influence of dietary sesamin, a bioactive compound on fatty acids and expression of some lipid regulating genes in Baltic Atlantic salmon (Salmo salar L.) juveniles. *Physiological Research* 60(1), 125-137.

Umeda-Sawada, R., Fujiwara, Y., Abe, H. & Seyama, Y. (2003). Effects of sesamin and capsaicin on the mRNA expressions of Delta 6 and Delta 5 desaturases in rat primary cultured hepatocytes. *Journal of Nutritional Science and Vitaminology* 49(6), 442-446.

Wada, H., Shintani, D. & Ohlrogge, J. (1997). Why do mitochondria synthesize fatty acids? Evidence for involvement in lipoic acid production. *Proceedings of the National Academy of Sciences of the United States of America* 94(4), 1591-1596.

Waples, R.S. (1988). Estimation of allele frequencies at isoloci. *Genetics* 118(2), 371-384.

Varanasi, U., Chu, R., Huang, Q., Castellon, R., Yeldandi, A.V. & Reddy, J.K. (1996). Identification of a peroxisome proliferator-responsive element upstream of the human peroxisomal fatty acyl coenzyme A oxidase gene. *Journal of Biological Chemistry* 271(4), 2147-2155.

Vegusdal, A., Sundvold, H., Gjøen, T. & Ruyter, B. (2003). An in vitro method for studying the proliferation and differentiation of Atlantic salmon preadipocytes. *Lipids* 38(3), 289-296.

Wollin, S.D. & Jones, P.J.H. (2003). α-Lipoic Acid and Cardiovascular Disease. *Journal of Nutrition* 133(11), 3327-3330.

Voss, A., Reinhart, M., Sankarappa, S. & Sprecher, H. (1991). The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *Journal of Biological Chemistry* 266(30), 19995-20000.

Yasuda, K., Ikushiro, S., Wakayama, S., Itoh, T., Yamamoto, K., Kamakura, M., Munetsuna, E., Ohta, M. & Sakaki, T. (2012). Comparison of metabolism of sesamin and episesamin by drug-metabolizing enzymes in human liver. *Drug Metabolism and Disposition* 40(10), 1917-1926.

Yi, X. & Maeda, N. (2006). α-Lipoic acid prevents the increase in atherosclerosis induced by diabetes in apolipoprotein E-deficient mice fed high-fat/low-cholesterol diet. *Diabetes* 55(8), 2238-2244.

Young, W.P., Wheeler, P.A., Coryell, V.H., Keim, P. & Thorgaard, G.H. (1998). A detailed linkage map of rainbow trout produced using doubled haploids. *Genetics* 148(2), 839-850.

Yuan, J.P., Wang, J.H. & Liu, X. (2007). Metabolism of dietary soy isoflavones to equol by human intestinal microflora - Implications for health. *Molecular Nutrition and Food Research* 51(7), 765-781.

Zeron, E.S. & Santillán, M. (2010). Distributions for negative-feedback-regulated stochastic gene expression: Dimension reduction and numerical solution of the chemical master equation. *Journal of Theoretical Biology* 264(2), 377-385.

Zheng, X., Seiliez, I., Hastings, N., Tocher, D.R., Panserat, S., Dickson, C.A., Bergot, P. & Teale, A. (2004). Characterization and comparison of fatty acyl Delta 6 desaturase cDNAs from freshwater and marine teleost fish species. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 139(2), 269-279.

Zheng, X., Tocher, D.R., Dickson, C.A., Bell, J.G. & Teale, A.J. (2005). Highly unsaturated fatty acid synthesis in vertebrates: New insights with the cloning and characterization of a Δ6 desaturase of atlantic salmon. *Lipids* 40(1), 13-24.

Zheng, X.Z., Leaver, M.J. & Tocher, D.R. (2009). Long-chain polyunsaturated fatty acid synthesis in fish: Comparative analysis of Atlantic salmon (Salmo salar L.) and Atlantic cod (Gadus morhua L.) Delta 6 fatty acyl desaturase gene promoters. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 154(3), 255-263.

Zhou, J., Febbraio, M., Wada, T., Zhai, Y., Kuruba, R., He, J., Lee, J.H., Khadem, S., Ren, S., Li, S., Silverstein, R.L. & Xie, W. (2008). Hepatic Fatty Acid Transporter Cd36 Is a Common Target of LXR, PXR, and PPAR[gamma] in Promoting Steatosis. *Gastroenterology* 134(2), 556-567.e1.