Quality of Lipids in Fish Fed Vegetable Oils

Effects of Bioactive Compounds on Fatty Acid Metabolism

Sofia Trattner
Faculty of Natural Resources and Agricultural Sciences
Department of Food Science
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2009

Acta Universitatis Agriculturae Sueciae
2009:31

Cover: The cover illustrate four main compartments in this thesis, oil, fish, sesame and linseed.
(picture: T. Bahnasy, photo: http://de.fotolia.com/ and
sesame drawing: Å. Lagerstedt)

ISSN 1652-6880
ISBN 978-91-86195-78-6

Print: SLU Service/Repro, Uppsala 2009

Quality of lipids in fish fed vegetable oils. Effects of bioactive compounds on lipid metabolism

Abstract

Healthy long chain polyunsaturated fatty acids are traditionally associated with fish. In farmed fish, the feed previously contained these 'marine' fatty acids. However, the dramatic increase in aquaculture production, accompanied by increased demand for fish-based raw materials for feed production and the static or decreasing supply of fish raw materials, poses a challenge for the aquaculture feed industry. When scarce fish oil is exchanged for vegetable alternates, this is reflected in the fatty acid profile of the fish. Fish fed a vegetable oil-based diet have a lower content of long chain omega 3 (n-3) fatty acids than those fed a fish oil-based diet.

This study examined the use of vegetable oils in combination with the bioactive compounds lipoic acid and sesamin in fish feeds. These compunds increased the proportion of long chain n-3 fatty acids in the muscle of pacu (*Piaractus mesopotamicus)* and rainbow trout (*Oncorhynchus mykiss*) and in hepatocytes of Atlantic salmon (*Salmo salar* L). Lipoic acid increased the proportion of eicosapentaenoic acid (EPA, 20:5n-3) in pacu muscle, while sesamin increased the proportion of docosahexaenoic acid (DHA, 22:6n-3) in rainbow trout muscle and Atlantic salmon hepatocytes. A study on lipid uptake and transport in cannulated rainbow trout showed that ingested and *de novo* synthesised fatty acids cannot be studied separately by blood sampling solely. Sesamin affected expression of lipid-related genes (PPARα and γ, cd36, SRB-I, CPT1, Δ5 and Δ6 desaturase) in Atlantic salmon hepatocytes and PPARα in rainbow trout liver. The desaturation and elongation of radiolabelled 18:3n-3 and radiolabelled β-oxidation products from 18:3n-3 increased in Atlantic salmon hepatocytes incubated with sesamin. The most increased β-oxidation product was acetate, indicating increased peroxisomal β-oxidation.

The results presented in this thesis provide information on how lipid metabolism in fish is affected by intake of bioactive compounds. This is useful in finding sustainable alternatives for production of aquaculture feeds and, in turn, of fish muscle with a healthy fatty acid profile.

Keywords: fatty acids, lipid classes, β-oxidation, elongation, desaturation, gene expression, sesamin, episesamin, lipoic acid.

Author's address: Sofia Trattner, Department of Food Science, SLU,
P.O. Box 7051, SE 750 07 Uppsala, Sweden
E-mail: sofia.trattner@lmv.slu.se

Kvalitet på lipider i fisk utfodrad med vegetabiliska oljor. Effekter av bioaktiva föreningar på lipidmetabolismen

Svensk sammanfattning

Under de senaste åren har produktionen av odlad fisk ökat dramatiskt, vilket även innebär ökad efterfrågan av fiskråvara för fodertillverkning. Tillgång på fiskråvara är begränsad och alternativa foderingredienser är nödvändiga för ett uthålligt vattenbruk. Foderingredienser baserade på fiskråvara innehåller höga halter omega-3 (n-3) fettsyror. Dessa n-3 fettsyror återfinns i fisken, som därmed traditionellt har betraktats som ett hälsosamt livsmedel rikt på n-3 fettsyror. När fiskråvaror (fiskolja) i fodret byts ut mot vegetabiliska oljor minskar tyvärr även mängden n-3 fettsyror i fisken.

I den här avhandlingen har vi använt vegetabiliska oljor i fiskfoder, vilket reflekterades i fiskens fettsyrasammansättning. Till det vegetabiliska fodret tillsattes två bioaktiva föreningar, liponsyra och sesamin. Detta ökade mängden n-3 fettsyror i pacu (*Piaractus mesopotamicus)* och regnbåge (*Oncorhynchus mykiss*) samt i lever celler (hepatocyter) från lax (*Salmo salar* L). Liponsyra ökade proportionen eikosapentaensyra (EPA, 20:5n-3) i pacumuskel och sesamin ökade mängden dokosahexaensyra (DHA, 22:6n-3) i muskel av regnbåge samt i hepatocyter från lax. I en studie på upptag och transport av lipider i regnbåge kunde vi genom blodprovtagning före och efter leverpassage visa att tillförda och *de novo* syntetiserade fettsyror inte kunde studeras separat enbart genom blodprover. Sesamin påverkade uttrycket av ett antal gener involverade i lipidmetabolismen (PPARα och γ, cd36, SRB-I, CPT1, Δ5 desaturas, Δ6 desaturas) i hepatocyter från lax samt även PPARα i lever hos regnbåge. Med hjälp av en radioaktivt inmärkt fettsyra (^{14}C 18:3n-3) kunde vi bevisa att sesamin ökar förmågan att bilda (elongera och desaturera) DHA, samt ökar förbränningen av lipider (ökad β-oxidation) i hepatocyter. Den β-oxidationsprodukt som ökade mest var acetat, vilket indikerar en peroximal β-oxidation.

Resultaten från detta arbete tillför information om hur lipidmetabolismen i fisk påverkas av bioaktiva föreningar, en information som kan komma att utnyttjas i sökandet efter uthålliga alternativ för fiskfoderproduktion och därigenom också odlad fisk med en hälsosam fettsyrasammansättning.

Fettqualität von Fisch gefuettert mit pflanzlichen Ölen. Effekte von bioaktiven Substanzen auf deren Fettmetabolismus.

Deutsche Zusammenfassung

Ein dramatischer Anstieg der Produktion von Fischen in Zuchtfarmen hat eine zunehmende Nachfrage für marine Rohstoffe für die Fischfutterproduktion zur Folge. Dies ist mit einem stabilen oder abnehmenden Angebot von Fischmehl und -öl die größte Herausforderung für die heutige und zukünftige Aquakultur. Die als gesund geltenden langkettigen, mehrfach ungesättigten omega-3 (n-3) Fettsäuren werden traditionell mit Fischprodukten assoziiert. Fischfutter, das in Fischzuchten verwendet wird, enthält normalerweise diese "marinen" Fettsäuren. Wird aber Fischöl im Futter gegen pflanzliche Alternativen substituiert, verändert sich entsprechend das Fettsäuremuster in Fisch. Aus diesem Grund enthält Fisch, der mit Futter mit pflanzlichen Ölen produziert wurde, einen geringeren Anteil an langkettigen n-3 Fettsäuren als Fisch, der mit Fischöl gefüttert wurde.

In dieser Studie wurde Fischfutter mit pflanzlichen Ölen verwendet. Die Fettsäurezusammensetzung im Muskelgewebe von Fische entspricht dem des Fischfutters. Darüber hinaus konnte festgestellt werden, dass die Kombination von pflanzlichen Fetten mit bioaktiven Substanzen, wie Liponsäure und Sesamin, die Produktion von langkettigen n-3 Fettsäuren in Muskelgewebe von Pacu (*Piaractus mesopotamicus*) und Regenbogenforelle (*Oncorhynchus mykiss*), und in den Leberephitelzellen von atlantischen Lachs (*Salmo salar L*) erhöht. Liponsäure führte zu erhöhten Gehalten von Eicosapentaensäure (EPA, 20:5n-3) in Pacu. Sesamin führte zu einer Erhöhung des Gehaltes von Docosahexaensäure (DHA, 22:6n-3) im Muskelgewebe von Regenbogenforellen und Leberephitelzellen von atlantischen Lachs. In einer Studie mit kanülierten Regenbogenforellen konnten wir zeigen, dass der Fetttransport von absorbierten und *de novo* synthetisierte Fettsäuren nicht mit Hilfe von Pfortader und arteriellen Blutproben studiert werden kann. Sesamin beeinflusste die Expression von Genen, die im Fettstoffwechsel in Leberephitelzellen (PPARa and γ, cd36, SRB-I, CPT1, Δ5 and Δ6 desaturase) von atlantischem Lachs, und in der Leber von Regenbogenforellen (PPARa) involviert sind. Sowohl die Desaturierung und Verlängerung von radioaktiv markierte 18:3n-3, als auch radioaktiv markierte ß-Oxidationsprodukte von 18:3n-3, waren in atlantischen Lachs erhöht. Das am meisten erhöhte ß-Oxidationsprodukt war Acetat, welches auf eine erhöhte peroxisomale ß-Oxidation hinweist.

Die Ergebnisse dieser Arbeit zeigen, wie der Fettmetabolismus in Fisch durch bioaktive Substanzen beeinflusst wird. Solche Ergebnisse können für die Fischindustrie im Bestreben einer nachhaltigen Fischfutterproduktion und Erzeugung von Fisch mit gesundem Fettsäureprofil, sehr wertvoll sein.

Dedication

To Ellen for being you

Contents

List of Publications

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

I Eliason, E.J., Djordjevic, B., Trattner, S., Pickova, J., Karlsson, A., Farrell, A. P. & Kiessling, A. The effect of hepatic passage on postprandial plasma lipid profile of rainbow trout (*Oncorhynchus mykiss*) after a single meal. *Aquaculture Nutrition* (accepted).

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

III Trattner, S., Kamal-Eldin, A., Brännäs, E., Moazzami, A., Zlabek, V., Larsson, P., Ruyter, B., Gjøen, T. & Pickova, J. (2008) 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, 989-997.

IV Trattner, S., Ruyter, B., Østbye, T.K., Gjøen, T., Zlabek, V., Kamal-Eldin, A. & Pickova, J. (2008) Sesamin increases alpha-linolenic conversion to docosahexaenoic acid in Atlantic salmon (*Salmo salar* L.) Hepatocytes: Role of altered gene expression. *Lipids* 43, 999-1008.

The contribution of Sofia Trattner to the papers included in this thesis was as follows:

I Participated in part of the analytical work, the evaluation of results and the preparation of the manuscript.

II Participated in the analytical work and data analysis. Prepared the manuscript.

III Participated in the planning of the study, was mainly responsible for the analytical part and data analysis and prepared the manuscript.

IV Participated in the planning of the study, was mainly responsible for the analytical part and data analysis and prepared the manuscript.

Abbreviations

AA	Arachidonic acid
ACO	Acyl-CoA oxidase
AMPK	Adenosine monophosphate kinase
ASP	Acid-soluble products
ATP	Adenosine-5´triphosphate
CoA	Coenzyme A
CPT	Carnitine palmitoyltransferase
cd36	Cluster of differentiation 36
cDNA	Complementary deoxyribonucleic acid
CYP	Cytochrome P450
DA	Dorsal aorta
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EROD	Ethoxyresorufin O-deethylase
FAT	Fatty acid translocase
FATP	Fatty acid transport protein
FABP	Fatty acid binding protein
GC	Gas chromatography
GLM	General Line Model
GMO	Gene modified organisms
HNF	Hepatic nuclear factor
HPLC	High performance liquid chromatography
HPV	Hepatic portal vein
LDL	Low-density lipoprotein
LO	Linseed oil
LXR	Liver X receptor
mRNA	Messenger ribonucleic acid
MO	Mixed oil

PCR	Polymerase chain reaction
PL	Phospholipid
PLA_2	Phospholipase A_2
PPAR	Peroxisome proliferator activated receptors
PPRE	Peroxisome proliferator response element
PUFA	Polyunsaturated fatty acid
REST	Relative expression software tool
RNA	Ribonucleic acid
RXR	Retinoid X receptor
SAS	Statistical analysis system for windows
SRB-I	Scavenger receptors type B
SREBP	Sterol regulatory element binding protein
TAG	Triacylglycerol
TLC	Thin-layer chromatography
VLDL	Very low density lipoprotein

1 Introduction

World aquaculture production is constantly increasing but is facing challenges due to the limited supply of fish raw material for fish feed production (Tacon & Metian, 2008). Therefore, efforts are being made to replace fish raw material in the diet of farmed fish with more vegetable-based ingredients. A natural drawback with this change is a decreased level of the healthy long chain n-3 fatty acids in the fish diet and in the fish as human food. Intake of long chain n-3 fatty acids, which are usually present in fish, is well correlated to health (Mozaffarian & Rimm, 2006). Consequently, any changes in ingredients in aquaculture feed production that affect the amount of long chain n-3 fatty acids in farmed fish may have long-term effects on human health. Fish is the human food with the highest content of long-chain n-3 fatty acids, in particular eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Season and diet both affect the lipid content of fish, while the fatty acid composition seems to be mostly affected by diet (Henderson & Tocher, 1987).

This thesis examines novel feed composition strategies to achieve sustainable aquaculture production by replacing fish oil in feeds with alternative oils, and investigates their effects on fish fatty acid quality. Plant and microbial oils (including genetically modified organisms, GMO) and krill oil are possible alternatives. The work presented here focuses on the use of vegetable oils and two bioactive compounds, lipoic acid and sesamin, and their effects on the fatty acid composition of fish tissues. It covers uptake, transport, elongation, desaturation, β-oxidation and composition of lipids. The sesamin compound used was an equi-mixture of sesamin and episesamin, hereafter referred to as sesamin. The use of these compounds can be a step towards sustainable aquaculture, preserving levels of long chain n-3 fatty acids in fish tissues.

Fish species are adapted to live in a wide range of environments, *e.g.* different temperatures and levels of salinity. The species differ in their ability to utilise carbohydrates, proteins and lipids and thus they also differ in lipid content and fatty acid composition. Fish are poikilotherm animals, *i.e.* dependent on the ambient temperature, and their metabolic activity is related to water temperature. Warm water freshwater fish, such as pacu (*Piaractus mesopotamicus)* and common carp (*Cyprinus carpio* L) can in general utilise carbohydrates better than salmonids such as rainbow trout (*Oncorhynchus mykiss)* and Atlantic salmon (*Salmo salar* L.), which are coldwater carnivorousfish and which use protein and lipids as their major energy source. Salmonids are fatty fish, storing lipids as triacylglycerols in their visceral fat and in adipocytes within the connective tissue between the muscle bundles (Zhou *et al.*, 1995). Pacu is a South American freshwater fish mostly feeding as an herbivore, but is actually an omnivore (Dal Pai *et al.*, 2000). Pacu has semi-lean meat and, like carp, stores most of its fat as adipose tissue in the abdominal wall (Mráz & Pickova, 2009). These fish have also evolved different strategies for metabolic response to xenobiotic compounds (van der Oost *et al.*, 2003)

It is well known that n-3 fatty acids, particularly EPA and DHA, have a number of beneficial effects on human health, *e.g.* reducing the risk of many diseases, such as cardiovascular disease, inflammatory diseases and possibly behavioural disorders (Connor, 2000). An overview of the some effects conferred by n-3 fatty acids at determined doses are presented in Table 1. Other effects include decreased total mortality and positive effects on early neurodevelopment (Mozaffarian & Rimm, 2006), inflammatory response (Massaro *et al.*, 2008) and some forms of cancer (Larsson *et al.*, 2004). The n-3 polyunsaturated fatty acids (PUFA) are important for cell membrane fluidity and regulation of cell signalling (Calder, 2008). Dietary intake of n-3 fatty acids is known to influence the expression of several genes, such as peroxisome proliferator activated receptors (PPARs) and sterol regulatory element binding protein (SREBP) (Price *et al.*, 2000). The n-6 and n-3 fatty acids are metabolically and functionally distinct and have many opposing physiological effects. The dietary balance of n-6 and n-3 fatty acids is important for homeostasis and normal development in humans (Simopoulos, 2000). Recently, EPA and DHA have been suggested to increase the production of the anti-inflammatory bioactive lipid modulators resolvins (Calder, 2006; Massaro *et al.*, 2008).

Table 1. *Examples of the effects of n-3 fatty acids at determined doses*

Beneficial effect on	**Amount**	**Reference**
Coronary heart disease	250-500 mg/day EPA+DHA	(Mozaffarian & Rimm, 2006)
Serum triglycerides	> 750 mg/day EPA+DHA	(Mozaffarian & Rimm, 2006)
Heart rate	> 300 mg/day EPA+DHA	(Mozaffarian *et al.*, 2006)
Depression	> 130 mg/day EPA+DHA	(Appleton *et al.*, 2008)
Inattention, hyperactivity	> 350 mg/day EPA+DHA	(Appleton *et al.*, 2008)

An adequate daily intake for adults is recommended to be 0.65 g DHA and EPA together, with at least 0.22 g of each fatty acid, while recommended daily intake is higher for pregnant and breast-feeding women (Simopoulos, 2002). The n-6 fatty acids are essential, but they are generally present in foods (Table 2). A recent study of common food products from fish, meat and chicken showed that the n-6/n-3 fatty acid ratio ranged from 6.5 to 43.2 (Sampels *et al.*, 2009). The intake of n-6 fatty acids is increasing and is suggested to be linked to the global increase in obesity (Ailhaud *et al.*, 2006; Strandvik *et al.*, 2008) and inflammatory action (Calder, 2006, 2008). In contrast, intake of n-3 fatty acids is decreasing and a number of scientific publications show this to be linked to several health problems (Simopoulos, 1999; Ailhaud *et al.*, 2006; Calder, 2008). Fish is traditionally a food rich in n-3 fatty acids. However, when feeds for farmed fish are altered by addition of vegetable oils, it is important to be aware of how the lipid quality of the fish muscle is affected. There is an obvious risk of the potential of fish as a source of long chain n-3 fatty acids being decreased. Therefore, to meet the recommended intake of n-3 fatty acids, measures to improve fish feeds while also creating sustainable and improved aquaculture practices are needed.

Table 2. *Fatty acid composition (% of total fatty acids) of animal food products from conventional feed without/with inclusion of n-3 fatty acid-rich sources in the diet. The main lipid source in the animal feed is defined in brackets*

Animal (feed)	18:2n-6	20:4n-6	18:3n-3	20:5n-3	22:5n-3	22:6n-3	n-6/n-3
Beef[a] (grain)	8.3	2.3	0.5	0.2	0.5	0.05	8.7
Beef[b] (pasture)	3.1	0.8	1.8	0.6	0.9	0.08	1.3
Deer[c] (game)	13.5	5.7	3.8	2.0	3.2	1.9	2.3
Pork[d] (grain)	9.7	1.5	0.5	-	0.3	0.3	12.1
Pork[e] (linseed)	22.6	3.5	3.3	1.8	2.2	0.8	3.5
Egg[f] (corn oil)	19.2	2.7	1.0	0.02	0.1	1.7	15.3
Egg[f] (algae)	14.2	2.1	1.9	0.2	0.2	2.5	4.3
Salmon[g] (rapeseed)	12.3	0.6	4.1	1.1	0.4	3.2	1.5
Salmon[g] (fish oil)	3.7	0.3	0.6	4.1	1.8	7.7	0.3

[a]Wood & Enser, 1997; [b]Eriksson & Pickova, 2007; [c]Cordain *et al.*, 2002; [d]Nilzen *et al.*, 2001; [e]Matthews *et al.*, 2000; [f]Fredriksson *et al.*, 2006; [g]Torstensen *et al.*, 2004.

1.1 General background to aquaculture

Fish consumption is increasing, as it is recommended as a healthy food by many national food authorities (EFSA, 2005; SLV, 2009). Aquaculture is the most rapidly growing animal food production sector, with an annual growth on a world basis of 8.8% since 1970, compared with 1.2% for fisheries and 2.8% for terrestrial animal production (SOFIA, 2006) (Figure 1). For example, in 2004 fisheries and aquaculture supplied the world with 106 million tonnes of fish, of which 43% came from aquaculture. Aquaculture production in Norway, the largest producer in Europe, has increased from almost nothing in the beginning of the 1970s to 700 000 tonnes in 2008. This rapid growth in production has made salmon production the second most important food crop in Norway. Globally, the fish species with the highest production volume is common carp, which is mostly produced in China (Holm, 2001).

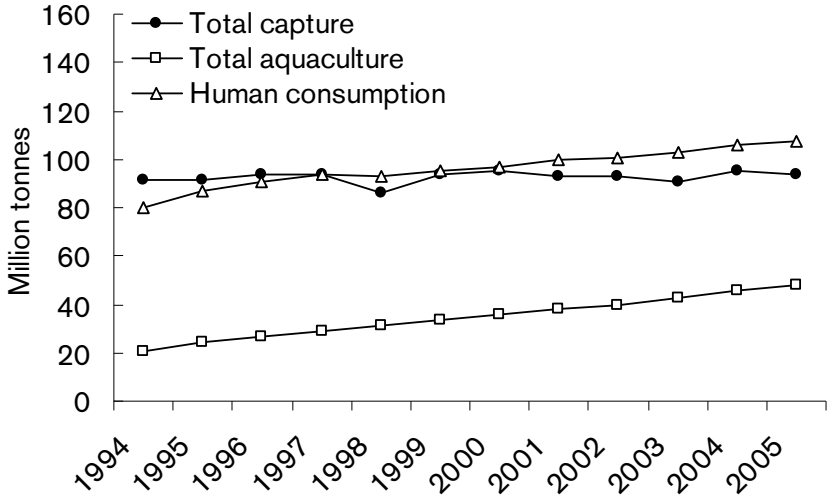

Figure 1. Total capture, total aquaculture production and human consumption of fish in the period 1994-2005. Data taken from SOFIA (2002, 2004, 2006).

Traditionally, fish raw materials are used in aquaculture feeds as a protein and oil source but the demand for fish oil for aquaculture feed production is expected to outstrip the supply within the next decade (Tacon, 2008). The production of fish oil and fish meal has been relatively stable since the 1970s, whereas consumption has dramatically increased. Even in 2003, the demand for fish oil was almost as high as its supply (FAO, 2006) (Figure 2). Replacement of fish oil with vegetable alternates is necessary and is being practised. The only drawback seems to be the decrease in long chain n-3 fatty acids (Thomassen & Røsjø, 1989; Torstensen *et al.*, 2005; Pickova & Mørkøre, 2007). Growth and fish health have been shown to not be negatively affected by replacement of up to 50% of fish oil in the diet with vegetable oils (Torstensen *et al.*, 2005). Vegetable oils also contain a number of minor compounds (Kamal-Eldin, 2005), which may affect the farmed fish, therefore, more knowledge of the metabolic effects of these compounds is needed. It is also of great interest to understand and maximise the innate ability of salmonids to synthesise long chain n-3 fatty acids (Sargent *et al.*, 2002).

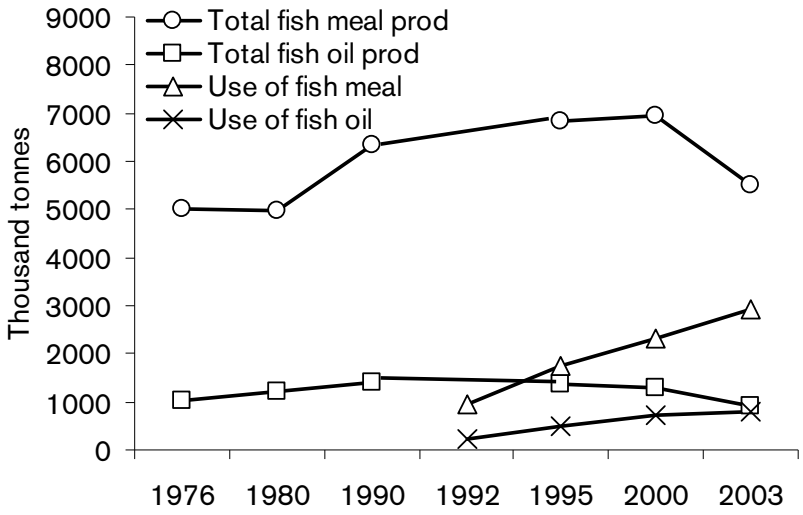

Figure 2. Production and use of fish oil and fish meal between 1976 and 2003. Data are taken from FAO (2006).

1.2 Lipids and lipid metabolism in fish

Lipids include a range of molecules that are soluble in organic solvents and insoluble in water (Dowhan & Bogdanov, 2002). Christie (1987) defined lipids as 'fatty acids and their derivates, and substances related biosynthetically or functionally to these compounds'. Lipids have structural functions in cell membranes, are precursors for eicosanoids, act as carriers for lipid-soluble substances, and are used for energy production. They can be divided into two main classes; neutral lipids and polar lipids. Neutral lipids serve mainly as an energy source and include triacyl-, diacyl- and monoacyl-glycerols, sterols, sterol esters, free fatty acids and wax esters. Polar lipids are mostly cell membrane constituents and include glycerophospholipids, glyceroglycolipids and sphingolipids (Henderson & Tocher, 1987; Tocher, 2003).

Fatty acids consist of a carbon chain with a methyl group at one end and a carboxyl group at the other end. The length and number of double bonds determine the properties of the fatty acids. Saturated fatty acids lack double bonds, monounsaturated fatty acids have one double bond and polyunsaturated fatty acids have two to six double bonds. The polyunsaturated fatty acids 18:2n-6 and 18:3n-3 (Figure 3) are considered

essential fatty acids in all vertebrate species, although it has been debated whether long chain n-3 fatty acids are essential for marine fish species (Sargent *et al.*, 1995). In salmonids, it has been shown that the length and degree of unsaturation of the fatty acids decide their metabolic fate (Kiessling & Kiessling, 1993; Bell *et al.*, 1997).

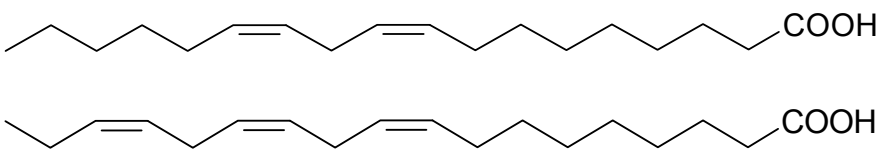

Figure 3. Chemical structure of the essential fatty acids 18:2n-6 (above) and 18:3n-3 (below).

1.2.1 Uptake and transport

When lipids are ingested, extracellular hydrolysis of lipids by lipase and colipase occurs in the lumen of the stomach, intestine and caecum, with the pyloric caecum and anterior intestine being the most important locations. Glycerol and fatty acids shorter than 10 carbons are absorbed through the brush border of the enterocytes, while fatty acids longer than 12 carbons are cleaved and emulsified by bile salts to form micelles (charged aggregates). These are transported from lumen to brush border, dissociated to fatty acid and diffused across the epithelial membrane. The scavenger receptor class B, type 1, has been identified in salmon intestine and is suggested to be involved in the uptake of dietary lipids (Kleveland *et al.*, 2006b). Inside the enterocytes, the fatty acids are reesterified and grouped with proteins to form chylomicrons. Lipids are transported from the enterocytes mainly as chylomicrons and partly as very low density lipoprotein (VLDL) of intestinal origin (Babin & Vernier, 1989; Roberts, 2002) through the hepatic portal vein to the liver, where they are further metabolised or directly transported as VLDL to other tissues through the dorsal aorta. A schematic overview of lipid uptake and transport is presented in Figure 4. It is unclear whether fish have a lymphatic system similar to that in mammals. Sheridan *et al.* (1985) and a review by Tocher (2003) suggest that the major lipoprotein is chylomicrons and that these are transported in the lymphatic system, but a proportion of lipoproteins may be transported directly to the liver via the portal vein. On the other hand, Torstensen *et al.* (2001) assumed that lipids are mainly transported in blood, as no lymphatic system has been confirmed. This is in agreement with Babin & Vernier (1989), who showed a high content of lipoproteins in fish plasma. Torstensen *et al.* (2001) also suggest that the direct effect of dietary fatty acids on muscle and adipose tissue could

indicate transport of lipids from the intestine to the tissues, possibly through a system similar to the lymphatic system in mammals.

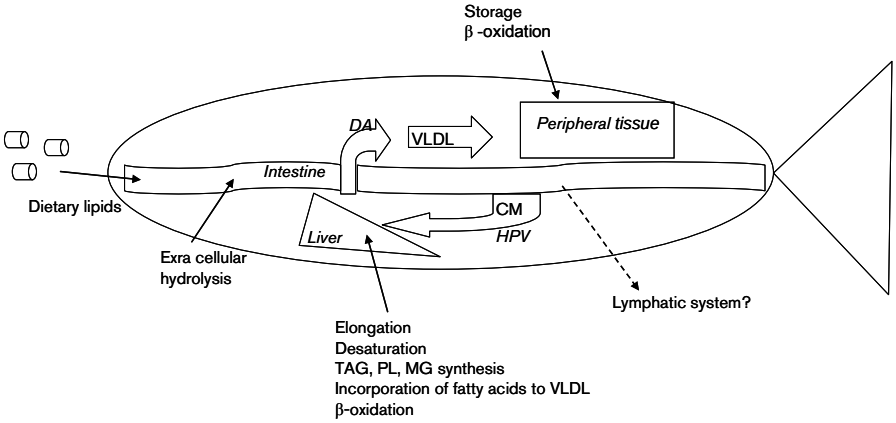

Figure 4. Schematic overview of the route of lipids. Once ingested, the lipids are taken up from the intestine and transported as chylomicrons (CM) in the hepatic portal vein (HPV) to the liver, where they can be modulated by elongation, desaturation or β-oxidation, or further transported as VLDL in the dorsal aorta (DA) to peripheral tissue for storage or for use in energy metabolism (β-oxidation).

1.2.2 Cellular uptake and storage of lipids

Two mechanisms for transport of lipids from the extracellular to the intracellular space have been proposed, diffusion or protein-mediated transport requiring a membrane-bound fatty acid translocase (FAT) or a fatty acid transport protein (FATP) (Van Nieuwenhoven *et al.*, 1996). Lipids can also be taken up into cells by the low density lipoprotein (LDL) receptor gene family (Hussain, 2001). Intracellular fatty acid binding proteins (FABP) transport long chain fatty acids and other hydrophobic compounds inside the cell (van der Vusse *et al.*, 2002). FABP have been reported in salmon muscle and in the intestine of fish (Andre *et al.*, 2000; Jordal *et al.*, 2006). When lipids are taken up into cells, they must be activated to acyl-CoA by acyl-CoA synthase for further modulation (described below) or storage as TAG. The pathways for phospholipid (PL) and TAG biosynthesis are generally the same in fish as in mammals, with synthesis occurring in the endoplasmic reticulum. Depending on cell type, TAG may be stored as lipid droplets in the cytosol. In salmonids lipids are stored as TAG in visceral adipose tissue and in adipocytes within muscle myosepta (Zhou *et al.*, 1995) or secreted in plasma as VLDL (*e.g.* hepatocytes) (Babin & Vernier, 1989), while PL may be used as cell membrane constituents.

1.2.3 Elongation and desaturation

The biosynthesis pathway from C_{18} to C_{22} is well described for many freshwater anadromous species, including rainbow trout and salmon (Bell *et al.*, 1997; Buzzi *et al.*, 1997; Tocher, 2003). Essential substrates for the elongation and desaturation of n-6 and n-3 fatty acids are the 18:2n-6 and 18:3n-3 fatty acids (Figure 3). The same enzymes are required for both n-6 and n-3 fatty acids, but the affinity of the enzymes is higher for fatty acids of the n-3 series (Figure 5).

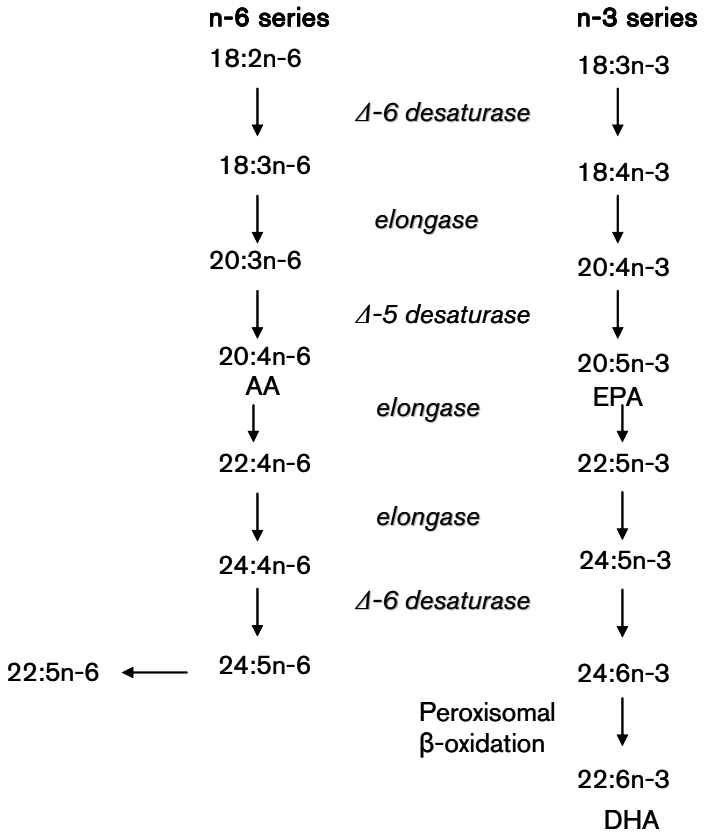

Figure 5. Elongation and desaturation pathway of n-6 and n-3 fatty acids. Adapted from Voss *et al.* (1991).

All this synthesis of long chain polyunsaturated fatty acids occurs in the microsomal fraction of the liver except for the chain shortening reaction to form 22:6n-3, which occurs in peroxisomes through β-oxidation. However,

the conversion is poorly understood, if present at all, in marine species. It has been suggested that the mechanism has been repressed in fish, which have an adequate supply of highly unsaturated n-3 fatty acids in the natural diet (Tocher, 2003).

1.2.4 β -Oxidation

When stored, lipids are catabolised through β-oxidation and either heat or ATP is produced. Heat is produced by peroxisomal β-oxidation, whereas ATP is produced by mitochondrial β-oxidation. In order to be β-oxidised, a fatty acid needs to be activated to its acyl-CoA derivative, which is converted to acylcarnitine by the enzyme carnitine palmitoyltransferase I (CPT1) in order to pass through the outer membrane of the mitochondria. The acylcarnitine is transferred over the inner membrane by carnitine/acylcarnitine translocase and converted to acyl-CoA by the enzyme CPT II on the inner surface of the inner mitochondrial membrane. The acyl-CoA then enters the β-oxidation process (Frøyland *et al.*, 1998). The β-oxidation in peroxisomes mostly oxidises long chain fatty acids, which can then be further oxidised in the mitochondria or as a step in the synthesis of DHA (Torstensen *et al.*, 2001). Fatty acids have been shown to be selectively β-oxidised with saturated fatty acids as the preferred substrate in rainbow trout mitochondria (Kiessling & Kiessling, 1993). The main product of acetyl-CoA generated from peroxisomal β-oxidation in rat hepatocytes is acetate, whereas oxaloacetate and malate originate from acetyl-CoA generated by mitochondrial β-oxidation (Leighton *et al.*, 1989). The total β-oxidation is highest in red muscle, followed by the liver, and the lowest activity is found in white muscle. Of these tissues, white muscle has the largest proportion of peroxisomal β-oxidation. A study on salmon has shown that white muscle, which contributes 60% of the total body mass, is important for total β-oxidation (Frøyland *et al.*, 2000).

1.2.5 Regulation of genes involved in lipid metabolism

The molecular mechanism by which lipid metabolism is regulated has been well investigated in mammals and some research has also been done on fish. The nuclear receptors PPARs represent one of the key regulators of lipid metabolism in mammals (Issemann & Green, 1990; Jump, 2002). After activation by a ligand, PPARs form heterodimers with the receptor retinoid X receptor (RXR) and bind to specific peroxisome proliferator response elements (PPREs) (Chinetti-Gbaguidi *et al.*, 2005). Four subtypes of PPARs (α, β/δ, γ1 and γ2) were first identified in mammals and have been well studied there. They have also been identified in fish such as Atlantic salmon, plaice (*Pleuronectes platessa*), sea bream (*Sparus aurata*), and sea bass (*Dicentrarchus labrax*), indicating similar functions in fish (Ruyter *et al.*, 1997; Boukouvala *et al.*, 2004; Leaver *et al.*, 2005). Each of the four subtypes is expressed in distinct tissues with different functions (Lee *et al.*, 2003). In mammals, PPARα is expressed in metabolically active tissues, *e.g.* the liver, and induces a range of genes involved in lipid transport, oxidation and thermogenesis (Clarke, 2001). Both isoforms of PPARγ are found in white adipose tissue, where they are involved in lipid synthesis (Ruyter *et al.*, 1997; Andersen *et al.*, 2000; Ferre, 2004). PPARβ is more broadly expressed and has less defined functions (Lee *et al.*, 2003).

SREBP is another key regulator in lipid metabolism, involved in cholesterol and lipid synthesis. Three SREBPs have been described; SREBP-1a, SREBP-1c and SREBP-2. SERBP-1a and SREBP-1c control genes involved in the lipogenesis and synthesis of TAG, whereas SRREBP-2 is involved in the control of genes coding for cholesterol synthesis. SREBP-1c is the subtype most expressed in the human and rodent liver (Jump, 2002).

The liver X receptors (LXRs), which regulate genes involved in hepatic bile acid synthesis and SREBP-1c gene transcription, and the hepatic nuclear factor-4α (HNF-4α), which regulates apo-lipoprotein synthesis, are other transcription factors that are potential targets for controlling the lipid metabolism and are controlled by *e.g.* dietary PUFA (Jump, 2002).

Nutritional factors such as content of fatty acids (Jump & Clarke, 1999; Jump, 2002; Zheng *et al.*, 2005) and bioactive compounds in the diet affect the expression of genes involved in lipid metabolism (Ide *et al.*, 2001; Kushiro *et al.*, 2002; Umeda-Sawada *et al.*, 2003; Huong & Ide, 2008). Furthermore, some drugs, *e.g.* lipid lowering fibrates, target PPARs (Jump & Clarke, 1999; Chinetti-Gbaguidi *et al.*, 2005). The expression of Δ5 desaturase and Δ6 desaturase is regulated by both PPARα and SREBP-1c

(Matsuzaka *et al.*, 2002). Environmental factors have also been shown to influence the expression of Δ6 desaturase in Atlantic salmon (Zheng *et al.*, 2005).

1.3 Effect of bioactive compounds on lipid metabolism

Bioactive compounds from plants occur naturally in small amounts in foods and appear to have beneficial effects on health (Kris-Etherton *et al.*, 2002). Tocopherols, carotenoids, sterols, certain fatty acids, sesamin, lipoic acid and other lipophilic compounds are present in foods and have been shown to affect lipid metabolism. The effects on lipid-related enzymes and genes in rats of sesamin and lipoic acid, the two bioactive compounds investigated in this work, are shown in Table 3. Low levels of dietary antioxidants have been suggested to increase the level of long chain n-3 fatty acids in catfish (*Ictalurus punctatus*) and in Atlantic salmon and salmon eggs (Baker & Davies, 1996; Pickova *et al.*, 1998; Bell *et al.*, 2000).

Table 3. *Comparison of the effects of sesamin and lipoic acid on enzymatic activities and genes involved in lipid metabolism in rats*

Effect on	Sesamin	Lipoic acid
Enzymatic activity		
Fatty acid synthase	↓[a]	↓[d]
Malic enzyme	↑[b]	↓[d]
Glucose 6-phosphate dehydrogenase	↓[a]	↓[d]
ATP-citrate lyase	↓[a]	↓[d]
Pyruvate kinase	↓[a]	↓[d]
Gene expression (mRNA level)		
Acetyl-CoA carboxylase	↓[a]	↓[d]
Fatty acid synthase	↓[a]	↓[d]
ATP-citrate lyase	↓[a]	↓[d]
Glucose 6-phosphate dehydrogenase	↓[a]	↓[d]
Malic enzyme	↑[b]	↓[d]
L-pyruvate kinase	↓[a]	↓[d]
Δ5 desaturase	-[c]	↓[d]
Δ6 desaturase	-[c]	↓[d]
LDL receptor	↓[b]	↓[d]
SREBP-1	↓[b]	-[d]

[a]Kushiro *et al.*, 2002; [b]Ide *et al.*, 2001; [c]Umeda-Sawada *et al.*, 2003; [d]Huong & Ide, 2008. a,b,d are *in vivo* studies, c is an *in vitro* study. ↓ = reduction, ↑ = increase and - = no effect.

1.3.1 Antioxidants

An antioxidant is a substance that prevents oxidation when present at low concentrations (Halliwell, 1990). Antioxidants can be divided into enzymatic and non-enzymatic (water-soluble and lipid-soluble) antioxidants. Their action may be as a radical scavenger, peroxide decomposer, enzyme inhibitor and/or catalytic metallic ion or oxygen or quenching singlet oxygen. Ascorbic acid, mostly present as ascorbate at physiological pH, and glutathione are cytosolic water-soluble antioxidants (Halliwell, 1996). These antioxidants regenerate the lipid-soluble antioxidant vitamin E (Packer *et al.*, 1979). Carotenoids, also lipid-soluble antioxidants, act mainly as singlet oxygen scavengers, but also as radical scavengers (Sargent *et al.*, 2002).

1.3.2 Lipoic acid

Lipoic acid (Figure 6) is a potent antioxidant in both hydrophilic and lipophilic environments (Navari-Izzo *et al.*, 2002). Synthesised *de novo*, lipoic acid is found as a cofactor in two different oxidative decarboxylation enzyme complexes, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (Reed, 1974; Bast & Haenen, 2003). It has been shown to protect against reactive oxygen species and to restore vitamin E, C and glutathione in mammals (Bast & Haenen, 2003). The vitamin C restoration effect has also been reported in fish (Park *et al.*, 2006). Physiological effects of lipoic acid include inhibition of phospholipase A_2 (PLA_2), hypoglycaemic and hypotriglycaemic effects (Hamano, 2002) and anti-inflammatory effects (Jameel *et al.*, 2006). Recently, dietary lipoic acid was shown to change both enzymatic activity and mRNA levels of lipogenic enzymes in rat hepatocytes, with the mRNA levels for stearoyl-CoA desaturase 1, Δ5 and Δ6 desaturase being dose-dependently decreased (Huong & Ide, 2008). That study also showed decreased levels of circulating leptin and insulin and increased serum adipokine after intake of lipoic acid. Increased adipokine increases the amount of the phosphorylated form of adenosine monophosphate kinase (AMPK). Activation of AMPK stimulates phosphorylation of acetyl-CoA carboxylase and decreases the enzyme activity, which enhances fatty acid oxidation through decreased hepatic concentration of malonyl-CoA, an inhibitor of CPT1.

Figure 6. Chemical structure of lipoic acid.

1.3.3 Sesamin

Sesamin (Figure 7) is an oil-soluble lignan found in sesame seed and oil. During the refining of sesame oil, episesamin (Figure 7) is formed from sesamin. Sesame lignans are well studied in mammals and have significant effects on lipid metabolism. For example they have been shown to increase β-oxidation (Ashakumary *et al.*, 1999; Jeng & Hou, 2005), to affect elongation and desaturation of fatty acids in rats (Fujiyama-Fujiwara *et al.*, 1995) and to lower serum levels of TAG and cholesterol in rats and humans (Kamal-Eldin *et al.*, 2000; Kushiro *et al.*, 2002; Jeng & Hou, 2005).

Enzymes involved in both the desaturation and β-oxidation of fatty acids are affected by sesamin, both at the level of enzymatic activity and at mRNA level, *e.g.* acyl-CoA oxidase (ACO) and CPT (Kushiro *et al.*, 2002; Jeng & Hou, 2005; Kiso *et al.*, 2005). Sesamin has been shown to reduce Δ5 desaturase index and to decrease enzymatic activity of Δ5 desaturase in the fungus *Mortierella alpina* and in primary rat hepatocytes (Shimizu *et al.*, 1991) but no effects could be detected on Δ5 desaturase mRNA levels in rats (Umeda-Sawada *et al.*, 2003). The lipid modulating effects are possibly through the activation of PPARs and inhibition of SREBP-1 (Ashakumary *et al.*, 1999; Ide *et al.*, 2004). In rats, sesamin and episesamin are absorbed by the lymph and metabolised by the liver, with sesamin being metabolised faster than episesamin. Both lignans are metabolised by cytochrome P450 2B and conjugated by uridine 5'-diphospho-glucuronosyltransferase 1A and 2B (Ikeda *et al.*, 2007) within 24 h and are not accumulated in rats (Umeda-Sawada *et al.*, 1999). Existing data indicate that sesamin does not have antioxidative effects, but that its metabolites (of catechol type) have strong antioxidative activity (Namiki, 2007).

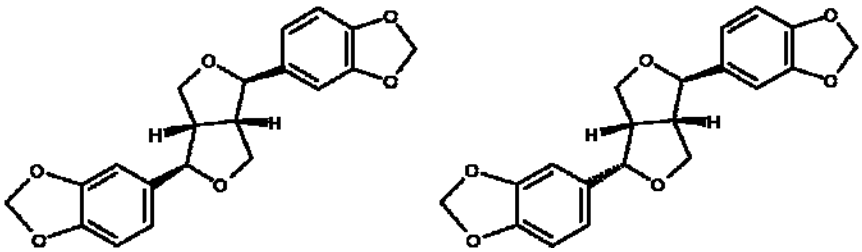

Figure 7. Chemical structure of sesamin (left) and episesamin (right).

1.4 Implications of n-3 fatty acids for humans

As summarised in Table 1, long chain n-3 fatty acids have several health implications. Fish is the main source of these fatty acids. In addition to the amounts of n-3 fatty acids in the diet, the n-6/n-3 fatty acid ratio has also been suggested to have impact on health, both because they are metabolically distinct and because the capacity to synthesise long chain derivates from the 18-carbon fatty acids is dependent on this ratio. The n-6/n-3 fatty acid ratio in the modern Western diet is currently between 10 and 20, whereas it used to be between 1 and 4 during evolution (Simopoulos, 2002). The long chain n-3 fatty acids DHA and EPA are also

found in other animals, with higher levels and lower n-6/n-3 fatty acid ratio when the animals are fed an n-3 fatty acid-rich diet (Fredriksson *et al.*, 2006; Eriksson & Pickova, 2007). Similarly, game meat has a higher content of n-3 fatty acids than meat from farmed animals (Cordain *et al.*, 2002). During the past 50 years, the level of n-3 fatty acids has been decreasing in all terrestrial animals due to intensive agriculture with increased amounts of cereals, and accordingly n-6 fatty acids, in the animal diet (Kyle & Arterburn, 1998; Wood *et al.*, 2004). Consumption of fish is low compared with consumption of meat from terrestrial animals. Approximately five times more meat than fish were consumed in Sweden in 2006, and in a study on 4-year-old children, 3% of the fat intake came from fish, whereas 22% came from meat and eggs (Sampels *et al.*, 2009). Table 2 shows how the content of n-3 fatty acids in animal products depends on their diet. It has been shown that consumption of animal products with a balanced n-6/n-3 fatty acid ratio (due to inclusion of linseed in the animal diet) significantly lowers the n-6/n-3 fatty acid ratio in human plasma (Weill *et al.*, 2002). Therefore, a balanced n-6/n-3 fatty acid ratio in the feed used for terrestrial animals and for fish will have long-term beneficial effects on human health.

2 Objectives

The overall aim of this work was to evaluate the increased use of alternative raw materials for sustainable aquaculture production. The goal is to maintain the lipid quality of the farmed fish while decreasing the use of fish oil in fish feeds. The use of vegetable oils leads to decreased levels of the healthy long chain n-3 fatty acids in the fish and countermeasures have to be taken to compensate for this effect. Improving our general knowledge of the lipid-modulating ability of bioactive dietary compounds was a major aim of these investigations.

Specific objectives were to:

Investigate the uptake and transport of lipids in cannulated rainbow trout after a single meal (Paper I).

Evaluate the use of the method used in Paper (I).

Study the effects of lipoic acid on fatty acid composition and antioxidant profile in pacu (Paper II).

Study the effects of sesamin on fatty acid composition in rainbow trout (Paper III)

Investigate the effect of sesamin on β-oxidation, elongation and desaturation of n-3 fatty acids in Atlantic salmon hepatocytes (Paper IV).

Study the effects of sesamin on gene expression *in vivo* in rainbow trout and *in vitro* in Atlantic salmon hepatocytes (Papers III, IV).

3 Materials and methods

There follows a short description of the materials and methods used in the studies included in this work. For a more detailed description of each method see Papers I-IV. A summary of the studies design is presented in Table 4 and the fatty acid composition of the diets is presented in Table 5.

3.1 Study design

In Paper I, rainbow trout (*Oncorhynchus mykiss*) (695-1483 g, n = 12) were held in a 1000-L indoor tank with fully aerated, recirculating water at 10 °C. Anaesthetised fish were placed on an operating table where their gills were continually irrigated with a chilled, aerated, buffered MS-222 solution (0.05 g l^{-1}) and the dorsal aorta (DA) (Soivio *et al.*, 1975 as modified by Kiessling et al., 1995, 2003) and hepatic portal vein (HPV) (Eliason et al., 2007) were cannulated. The fish were fed a single meal (1% of body mass) consisting of one of two experimental diets that differed in their fish meal and corn meal contents. The diets only differed slightly in fatty acid composition and with no significant value for the study, therefore the average fatty acid composition of the two diets are presented. Blood sampling (400-500 µl) from both cannulas occurred immediately prior to feeding (control = 0 h), and at 3, 6, 12, 24 and 48 h postprandium. Plasma samples were stored at -80 °C until analysed.

Table 4. *Summary of study design for Papers I - IV*

Study	**I**	**II**	**III**	**IV**
Species	Rainbow trout	Pacu	Rainbow trout	Atlantic salmon hepatocytes
Size (g)	694 - 1483	16.6	34.0	-
Sample	Plasma DA[a]	Muscle	White muscle	-
	Plasma HPV[b]	Brain		
Basic diet[c]	Fish meal[d]	Fish protein hydrolysate	Defatted fish meal	-
	Corn gluten[d]			
	Wheat starch	Cod liver oil	Linseed oil[e]	
	Fish oil	Soybean oil	Sunflower oil[e]	
Treatment	-	Lipoic acid	Sesamin	Sesamin
		Vitamin C		
Measurements	Fatty acids	Fatty acids	Fatty acids	Fatty acids
	Lipid classes	Vitamin E	Sesamin	Lipid classes
		Vitamin C	Episesamin	Elongation
			CYP 450	Desaturation
			EROD	β-oxidation
			Gene expression	ACO
				Gene expression

[a]DA = dorsal aorta,

[b]HPV = hepatic portal vein

[c] All diets contained recommended levels of vitamins and minerals

[d] The diet contained only fish meal or a mixture of fishmeal and corn gluten.

[e] The oil was only linseed oil or a mixture of linseed oil and sunflower oil (6:4 v/v)

In Paper II, juvenile pacu (*Piractus mesopotamicus*) (initial weight 16.6 g, n = 10) were selected randomly and fed one of four experimental diets in three replicate glass aquaria (40-L). The diets differed in their content of vitamin C (0 or 0.05 g 100 g^{-1} feed) and alpha lipoic acid (0 or 0.1 g 100 g^{-1} feed). Water temperature was maintained at 25.6 ± 1.2 °C throughout the experiment. Fish were fed at a readjusted-restricted rate (1.9-2.5% BW day^{-1}) for a total of 8 weeks to a final weight of 56.9 g. On the last sampling occasion, fish were fed a meal in the morning and sampling commenced 3 h postprandium. Immediately after sampling, the fish were dissected, frozen in liquid nitrogen and stored at -80 °C until analysed.

In Paper III, rainbow trout (initial weight 34 g, n = 8) were fed four different diets based on two vegetable oils (linseed oil, LO, or a mixture of

linseed oil and sunflower oil, MO) with or without sesamin addition (0.58 g 100 g^{-1} feed). The experimental diets were fed to the fish *ad libitum* for 35 days and the water temperature was 10 °C. At harvest the fish were dissected, frozen in liquid nitrogen and stored at -80 °C until analysed.

Table 5. *Fatty acid composition (%) in the experimental diets used in Papers I - III*

	Paper I	**Paper II**	**Paper III MO[a]**	**Paper III LO[b]**
14:0	6.1	2.8	0.2	0.2
16:0	12.2	16.9	6.0	6.0
16:1n-7	6.2	1.1	0.2	0.2
18:0	1.4	8.6	3.5	3.6
18:1n-11	1.1	-	-	-
18:1n-9	9.4	30.1	16.6	20.6
18:1n-7	2.4	1.0	0.6	0.6
18:2n-6	2.5	36.3	15.0	33.2
18:3n-3	0.9	-	53.4	31.7
20:0		-	0.1	0.2
20:1n-11	3.1	-	-	-
20:1n-9	11.3	0.9	0.1	0.1
20:1n-7	0.4	-	-	-
20:4n-6	0.3	0.1	-	-
20:4n-3	1.2	-	-	-
20:5n-3	7.5	-	-	-
22:0		-	0.2	0.3
22:1n-11	16.0	-	0.1	0.2
22:1n-9	1.4	-	-	-
22:1n-7	0.5	-	-	-
22:5n-3	0.6	0.3	0.2	0.2
22:6n-3	8.1	2.0	0.4	0.4
24:0	0.3	-	-	-
24:1n-9	0.7	-	-	-

[a]MO = mixture of linseed oil and sunflower oil (6:4 v/v)

[b]LO = linseed oil.

In Paper IV, hepatocytes were isolated from Atlantic salmon (*Salmo salar* L.) (800 g) kept in seawater at 10 °C. The isolated hepatocytes were incubated with 35 nmol, 2.5μCi of [1-^{14}C] 18:3n-3 as well as non-radiolabelled 18:3n-3, with or without sesamin (12 °C for 48 h). Incubations were performed to study the effect of sesamin concentration (0.005 mM, 0.05 mM, 0.075 mM

and 0.11 mM) and the effect of time (0 - 72 h at 0.05 mM sesamin concentration). The radiolabelled experiment flasks were used for analyses of β-oxidation, radiolabelled lipid classes and radiolabelled cellular fatty acids. The non-labelled flasks were used for gene expression, fatty acid and sesamin analyses. As a control in both the dose response study and the time course study, dimethyl sulphoxide was added to the cells without sesamin.

3.2 Lipid analysis

3.2.1 Extraction and lipid analysis

The plasma in Paper I, tissues and diets in Paper III and non-radiolabelled hepatocytes in Paper IV were extracted in hexane:isopropanol (3:2 v/v) following the method of Hara & Radin (1978), The radiolabelled hepatocytes and medium in Paper IV, tissues in Paper II and diet in Paper I were extracted by using chloroform:methanol (2:1 v/v) according to Folch *et al.* (1957).

Total lipids of plasma, tissues and medium in Papers I, III and IV were separated by thin layer chromatography (TLC) into separate lipid classes. The total lipids in Paper II were separated on Sep-Pak silica cartridges into neutral and polar lipids.

The lipids in Papers I, II, III and IV were converted to fatty acid methyl esters and analysed with gas chromatography (GC). The fatty acid composition of the radiolabelled cells in Paper IV was determined by reversed-phase high performance liquid chromatography (HPLC) as described by Narce *et al.* (1988).

3.2.2 Measurement of β-oxidation

The β-oxidation capacity was measured by determination of the ^{14}C-containing oxidation products, acid-soluble products (ASP) and CO_2 as described by Christiansen *et al.* (1976).

3.3 Protein determination and acyl-CoA oxidase (ACO) activity

The protein content of the cells was determined using the total protein kit (Micro Lowry/Peterson's modification) (Lowry *et al.*, 1951; Peterson, 1977) and measured at 540 nm in a 96-well plate reader. The ACO activity was determined according to Small *et al.* (1985).

3.4 Sesamin and episesamin analysis

For the analysis of sesamin and episesamin, the lipid extracts of tissues and feed were dissolved in hexane and analysed with HPLC (Moazzami & Kamal-Eldin, 2006). Separation was performed on a silica column using hexane/1,4-dioxane (94:4 v/v) as mobile phase and detection was achieved by fluorescence (excitation wavelength 296 nm and emission wavelength 324 nm).

3.5 Analysis of vitamins C and E

Vitamin C was analysed by a colorimetric method according to Dabrowski & Hinterleitner (1989). Tocopherols were analysed as described in Cort *et al.* (1983) and Zaspel & Csallaany (1983) on a HPLC system equipped with an FP-920 intelligent fluorescence detector and a C18 reversed-phase column.

3.6 Total content of cytochrome P450 and ethoxyresorufin O-deethylation

Cytochrome P450 (CYP) values were measured by the Co-difference method (Omura & Sato, 1964) and hepatic ethoxyresorufin O-deethylase (EROD) activity was determined according to a modified method of Jönsson *et al.* (2006). Protein contents of the microsomes were assayed by the method of Smith *et al.* (1985), adapted for microplate readers.

3.7 Gene expression analysis

Total RNA was purified using Trizol®, followed by DNase treatment. RNA quality and quantity were determined spectrophotometrically ($A_{260/280}$). The cDNA was synthesised following a modified protocol from the Taq Man Reverse Transcription Reagent kit. The oligo $d(T)_{16}$ primers were used. Real-time PCR was then performed in a Prism® 7000 system using gene-specific primers. Standard curves were made for each primer pair and efficiency (E) was calculated as $E=10^{(-1/slope)}$.

3.8 Statistical analysis

Relative expressions of the different genes, in relation to housekeeping genes, were determined using the Relative Expression Software Tool (REST-384©-version 1). For all other data in Papers I, III and IV, the

Statistical Analysis System for Windows (SAS), version 8.2, and the General Line Model (GLM) were used. The data in Paper II were analysed using the students' t-test in Microsoft Office Excel 2003.

4 Summary of results

4.1 Paper I

The lipid content and fatty acid profile in rainbow trout plasma did not differ between dorsal aorta (DA) and hepatic portal vein (HPV) at any point in time. The relative lipid class composition differed, with higher levels of cholesterol esters in DA after 3 h and also in HPV after 6 h, both compensated for by lower levels of TAG. After 24 h the proportion of TAG increased in both DA and HPV and the levels of cholesterol esters decreased.

4.2 Paper II

The inclusion of alpha lipoic acid in the pacu diet significantly increased the content of EPA in the muscle polar fraction (from 6.0 ± 0.4 to 6.7 ± 0.5 and from 5.8 ± 0.4 to 6.9 ± 0.4 in muscle polar fraction of –C+LA and +C+LA fed fish, respectively). A slight non-significant increase in EPA was also seen in the brain. The total lipid content and the proportion of neutral and polar lipids did not differ among groups of fish. No effects on the levels of the antioxidants vitamin E and C were detected. Therefore, these results suggest that alpha lipoic acid affects lipid metabolism rather than having antioxidative effects that could increase EPA. Furthermore, there is no effect on DHA, which is more prone to oxidation and would therefore be the first fatty acid to be affected positively by an antioxidant.

4.3 Paper III

The inclusion of an equi-mixture of sesamin and episesamin (0.58 g 100 g^{-1} diet) in rainbow trout diet increased the content of DHA in both the

phospholipid and triacylglycerol (TAG) fraction of white muscle, but not red muscle or liver. The effects were greater when the diet contained a mixture of linseed and sunflower oil (6:4 v/v) than when only linseed oil was used. The DHA level increased from 5.7% to 7.8% in TAG and from 40.9% to 43.9% in the PL fraction of fish fed the mixed oil and sesamin diet ($P<0.05$). The expression of PPARα was significantly ($P<0.05$) downregulated in the liver of fish fed the sesamin and mixed oil diet. Fish fed sesamin had elevated levels of cytochrome P450 and EROD activity in the liver. The metabolism of sesamin in fish might be different from that in mammals, as sesamin is found in tissues of fish sampled 48 h after feeding. However, we cannot be sure whether this is an effect of temperature or of actual differences in metabolic capacity.

4.4 Paper IV

Incubation of hepatocytes with an equi-mixture of sesamin and episesamin (0.05 M, final concentration) increased elongation and desaturation of ^{14}C 18:3n-3 to ^{14}C 22:6n-3, ($P<0.01$) and downregulated expression of Δ5 and Δ6 desaturases. In non-radiolabelled cells, the levels of n-PUFA, in particular DHA, were also elevated. The β-oxidation of ^{14}C 18:3n-3 was increased ($P<0.01$). The acid-soluble β-oxidation products (ASP) increased most and of the ASP, acetate was the compound with the highest increase. The expression of CPT1 was upregulated by a factor of 3.8 ($P<0.01$), in agreement with the increased β-oxidation. The uptake of lipids was significantly higher in the sesamin-treated cells than in control cells. In contrast, lower amounts of lipids were secreted by the sesamin-treated cells. It was mainly the triacylglycerol level in the medium that was lower after sesamin incubation. The expression of cd36, PPAR α and γ and SRB-I was downregulated. The results show that sesamin is a potent lipid modulator in salmon hepatocytes, a finding that may be of interest for the aquaculture industry.

5 General discussion

5.1 Lipid uptake and transport

The uptake and transport of lipids (Paper I) were clearly different from the uptake and transport of amino acids, which showed significant differences in plasma levels between dorsal aorta (DA) and hepatic portal vein (HPV) (Karlsson *et al.*, 2006). The lipid class composition differed over time, in agreement with a study in sea bass (Santulli *et al.*, 1988). The relative level of unsaturated and saturated cholesterol esters increased after a single meal and the level of TAG decreased correspondingly in DA and HPV (Figure 8). The level of TAG increased after 24 h and the level of unsaturated and saturated cholesterol esters decreased. The other lipid classes (PL, cholesterol and free fatty acids) were stable. No differences in lipid class composition were detected between DA and HPV (with one exception, PL at 6 h). The plasma lipid content was similar between diets and sampling sites. The fatty acid composition of plasma was slightly affected by diet, but did not differ between DA and HPV. There are three possible mechanisms behind this finding: *i*) that the circulating lipid level and composition are tightly controlled by regulated uptake or release from adipose or hepatic tissue; *ii*) that the absorption and metabolism of lipids are too slow and plasma reflects a steady state where changes only occur gradually; and *iii*) that there is a secondary system for lipid uptake and transport. Suggestion (*iii*) is in agreement with Sire *et al.* (1981), who found only small amounts of radiolabelled fatty acids in plasma of rainbow trout. However, others have suggested plasma as the primary lipid transporter, *e.g.* plasma in fed rainbow trout transported three times more lipids and cholesterol than plasma in fed rats (Babin & Vernier, 1989). We found no quantitative relationship between dietary fatty acids and plasma fatty acids, in agreement with results

as reviewed by Tocher (2003). To further investigate how lipids are taken up and transported in fish, it would be interesting to trace the route of radiolabelled dietary fatty acids after a single meal. Our cannulated fish method allowed small differences in fatty acid composition to be detected. In combination with ingestion of radiolabelled fatty acids, this would create an interesting design for future studies.

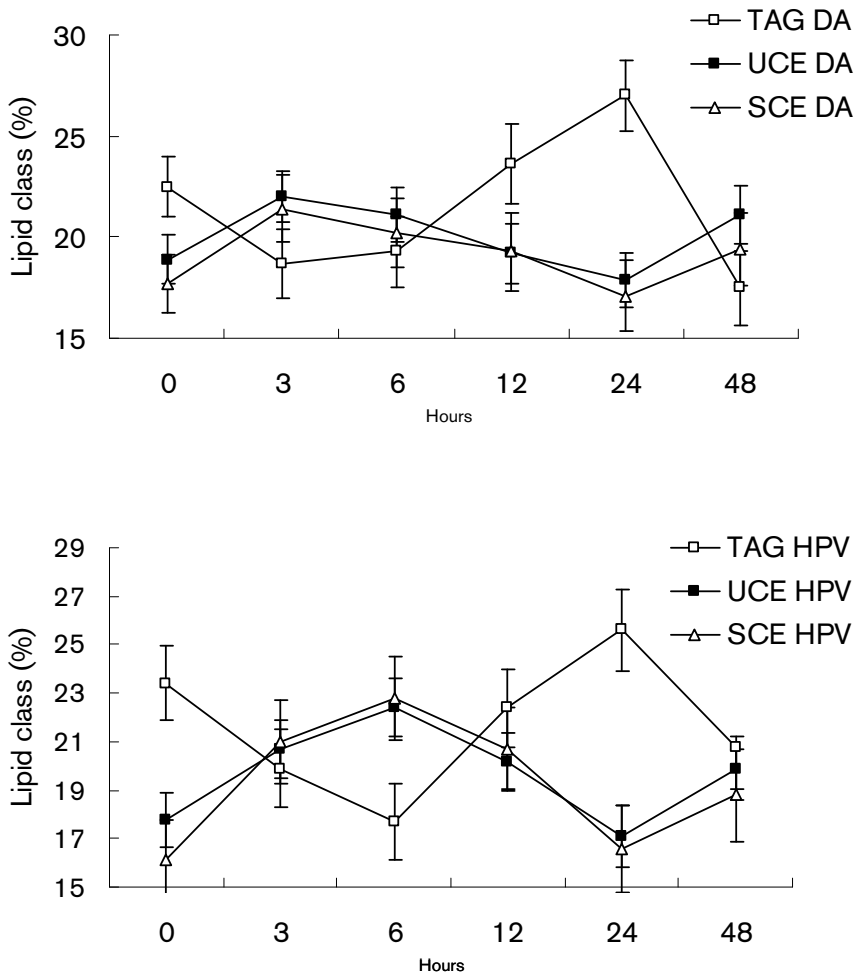

Figure 8. Relative percentage of triacylglycerols (TAG), saturated cholesterol esters (SCE) and unsaturated cholesterol esters (UCE) in rainbow trout plasma sampled from dorsal aorta (DA) and hepatic portal vein (HPV). Samples from 0-48 hours after feeding.

5.2 Fatty acid composition

The results of the present studies (Papers I, II, III) are in line with those of previous studies, which concluded that dietary fatty acid composition is reflected in fish tissues (Thomassen & Røsjø, 1989; Torstensen *et al.*, 2004). Furthermore, this work (Papers II, III) shows that bioactive compounds (lipoic acid and sesamin) in fish feed affect the fatty acid composition of the fish muscle. Interestingly, lipoic acid increased the proportion of EPA in the muscle polar lipid fraction, whereas sesamin increased the levels of DHA in the TAG and PL fractions. In both cases, there was a decrease in 18:3n-3 corresponding to the increase in EPA or DHA.

Lipoic acid increased the proportion of EPA in the muscle polar lipid fraction and slightly in the brain (Paper II). No changes were observed in the levels of n-6 fatty acids or in the total lipid content in fish fed lipoic acid. Lipoic acid has been shown to increase the level of the n-6 eicosanoid arachidonic acid (AA, 20:4n-6) in rat brains (Ozkan *et al.*, 2005) and to reduce peroxidation of PUFA in guinea pigs (Celik & Ozkaya, 2002). It is interesting to note that as in the case of sesamin, effects are found on the n-3 fatty acids in fish, whereas in mammals the effects are found on the n-6 fatty acids (discussed below). Other antioxidative effects of lipoic acid, such as the ability to restore vitamin E and C, have been reported (Arivazhagan *et al.*, 2002; Gonzalez-Perez & Gonzalez-Castaneda, 2006). The inhibitory effect of lipoic acid on PLA_2, an enzyme which cleaves AA from its sn-2 position on the glycerol structure for synthesis of eicosanoids (Jameel *et al.*, 2006), could explain the increase in EPA in fish. However other PUFA are also present in the sn-2 position of phospholipids, so an increase in other PUFA would also be expected if this were the sole mechanism. Lipoic acid possibly has an effect on desaturation and elongation of 18:3n-3 towards EPA. The increased EPA/18:3n-3 ratio in muscle polar lipids of fish fed lipoic acid indicates such an effect (Figure 9). Antioxidative effects or effects on β-oxidation cannot be excluded, although if lipoic acid mainly prevented fatty acid peroxidation, effects on other fatty acids (in particular the long chain and more unsaturated fatty acid DHA) and on the content of vitamins E and C and malondialdehyde could be expected. Neither of these effects has been observed in pacu muscle or brain (Park *et al.*, 2006). However, further research, *e.g.* with radiolabelled fatty acids and/or gene expression analysis of relevant genes, is necessary to confirm these speculations.

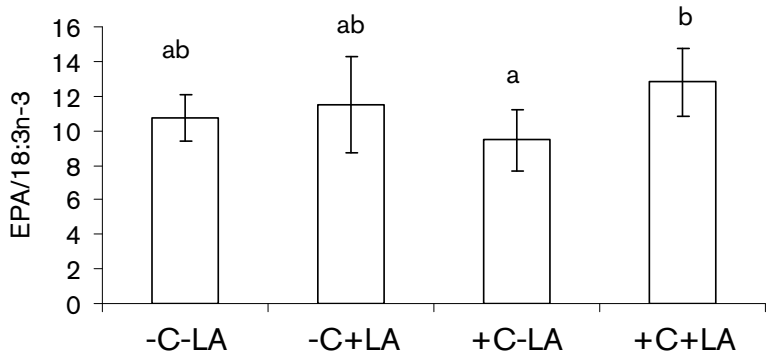

Figure 9. The EPA/18:3n-3 ratio in the muscle polar lipid fraction in pacu. Bars with different letters are significantly different ($P<0.05$).

Sesamin in the rainbow trout diet significantly increased the level of DHA ($P<0.05$) and decreased the level of 18:3n-3 in white muscle PL and TAG (Paper III), indicating desaturation and elongation of 18:3n-3 towards its longer chain derivates. The desaturation index (calculated as long chain n-3 fatty acids/18:3n-3) is shown in Figure 10. A decreased level of both 18:2n-6 and AA and total PUFA in sesamin-fed rainbow trout indicates that there was no elongation and desaturation of the n-6 fatty acid series, and the decrease was possibly due to increased β-oxidation.

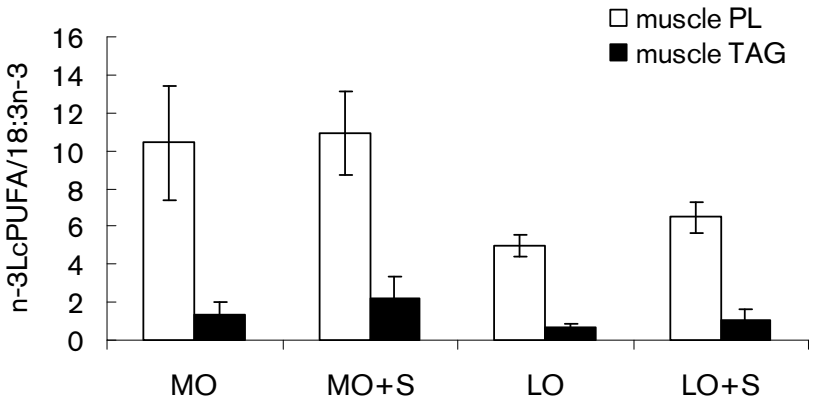

Figure 10. Desaturation index (n-3 long chain fatty acids, LcPUFA/18:3n-3) in rainbow trout muscle phospholipid (PL) and triacylglycerol (TAG) fraction after addition of sesamin (S) to the mixed oil diet (MO) and the linseed oil diet (LO).

The suggestion of increased elongation and desaturation of n-3 fatty acids in rainbow trout muscle was supported by our *in vitro* study, which showed that sesamin increased elongation and desaturation of ^{14}C18:3n-3 in Atlantic salmon hepatocytes (Paper IV). The fatty acid composition of the hepatocytes also showed increased levels of DHA ($P<0.01$), decreased levels of 18:3n-3 and increased desaturation index in both radiolabelled cells and non-radiolabelled cells with sesamin (Figure 11). The levels of ^{14}C18:4n-3 and of ^{14}C20:5n-3 were markedly lower in the sesamin-treated cells. This could be explained by either an increase in Δ6 desaturase activity and/or increased β-oxidation (see below). In sesamin-incubated cells, there were no differences in the n-6 fatty acids.

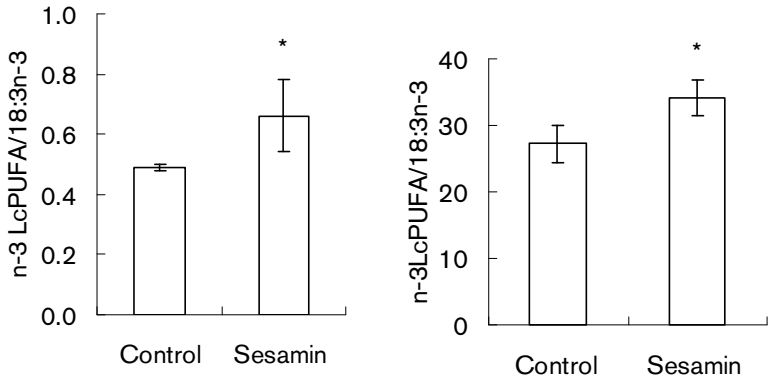

Figure 11. Desaturation index (n-3 long chain fatty acids, LcPUFA/18:3n-3) in radiolabelled hepatocytes (left) and in non-radiolabelled hepatocytes (right). Asterisks indicate significant differences in sesamin cells compared with control cells ($P<0.05$).

Lipid content in rainbow trout liver, red or white muscle and the fatty acid composition of liver and red muscle were not affected by dietary sesamin. In contrast, results from studies on rats show increased levels of n-6 long chain fatty acids after sesamin supplementation (Fujiyama-Fujiwara *et al.*, 1995). The higher lipid content and n-3 fatty acid content in the fish feed, the differing capacity of species to elongate and desaturate and the preferred affinity of the desaturases for the n-3 fatty acids (Tocher, 2003) may be factors causing the conflicting effects in fish.

The effects of sesamin were in general more pronounced when the fish were fed the MO diet than the LO diet. In the white muscle PL fraction (Paper III), we found a slightly higher content of DHA in the fish fed the MO diet than in the fish fed the LO diet. This could be due to the higher level of 18:3n-3 in the LO diet. Others have reported that a high level of

linseed oil decreases elongation and desaturation capacity (Tocher *et al.*, 2002), but the TAG data from Paper III are contradictory. Unpublished data from our group show that purified TAG from LO have the same effects as LO on the DHA content, but decrease the levels of 18:3n-3 and increase the level of EPA in white muscle. Therefore, one can speculate that it is not solely the amount of 18:3n-3 in LO that interferes with the elongation and desaturation capacity, but also other compounds in the linseed oil (Johnsson *et al.*, unpublished data).

The content of long chain n-3 fatty acids in the diet and the nutritional status of the individual affect the elongation and desaturation capacity of fish. Increased levels of long chain n-3 fatty acids in the diet decrease the elongation and desaturation capacity (Tocher *et al.*, 2002; Zheng *et al.*, 2005), while low levels of dietary long chain PUFA and fasting increase elongation and desaturation capacity in rats (Cook & McMaster, 2002). Temperature affects fatty acid composition, with the production of ^{14}C 22:6n-3 and ^{14}C 24:5n-3 from ^{14}C 20:5n-3 and the ratio of ^{14}C 24:5n-3 to ^{14}C 22:5n-3 being higher at 5 °C than at 12 °C, indicating higher elongation capacity at low temperature (Ruyter *et al.*, 2003). Environmental factors also have an effect on the fatty acid composition, with increased biosynthesis of long chain n-3 fatty acids prior to saltwater transfer in salmon (Zheng *et al.*, 2005) and higher Δ6 desaturase activity in common carp kept at 10 °C compared with 30 °C (Schuenke & Wodtke, 1983). The activity of desaturases declines with age in rat liver and brain (Cook & McMaster, 2002).

5.3 β-oxidation

In Paper IV, incubation of hepatocytes with sesamin increased β-oxidation products. Previously, sesamin has been reported to enhance mitochondrial and peroxisomal β-oxidation in rats (Ashakumary *et al.*, 1999; Jeng & Hou, 2005). In our study, it was mainly the level of acid-soluble β-oxidation products (ASP) that increased (Figure 12). In the sesamin-treated cells, acetate was 73% and oxaloacetate/malate 27%, while in the control cells acetate was 53% and oxaloacetate/malate 47%. The increase in acetate suggests increased peroxisomal β-oxidation. Acetate has been reported to be the main product of peroxisomal β-oxidation in rat hepatocytes (Leighton *et al.*, 1989). Peroxisomal β-oxidation is involved in the shortening of long chain fatty acids, including the synthesis of DHA (Voss *et al.*, 1991). The increased levels of DHA in the sesamin-treated cells and white muscle and

the decreased levels of PUFA in white muscle could be a result of increased β-oxidation (possibly peroxisomal). The β-oxidation rate is low in white muscle compared with red muscle and liver, but it is still important, as white muscle comprises a major proportion of total body mass. Peroxisomal β-oxidation is more active in white muscle than in the other tissues (Frøyland *et al.*, 2000). The lower level of 16:0 and the increased relative expression of CTP1 (by a factor of 3.8) in sesamin-treated hepatocytes indicates selective β-oxidation of saturated fatty acids in hepatocytes, as CPT1 is a rate-limiting step in mitochondrial β-oxidation. Kiessling & Kiessling (1993) reported saturated fatty acids to be the preferred substrate for β-oxidation in rainbow trout mitochondria. As for fatty acid synthesis, β-oxidation is dependent on temperature. The β-oxidation products from ^{14}C 18:1n-9 were 10-fold higher in hepatocytes kept at 5 °C than at 12 °C, with the main β-oxidation product being acetate (Moya-Falcon *et al.* 2006) Tocher *et al.* (2004) also found higher levels of β-oxidation products in rainbow trout kept at 7 °C than at 15 °C.

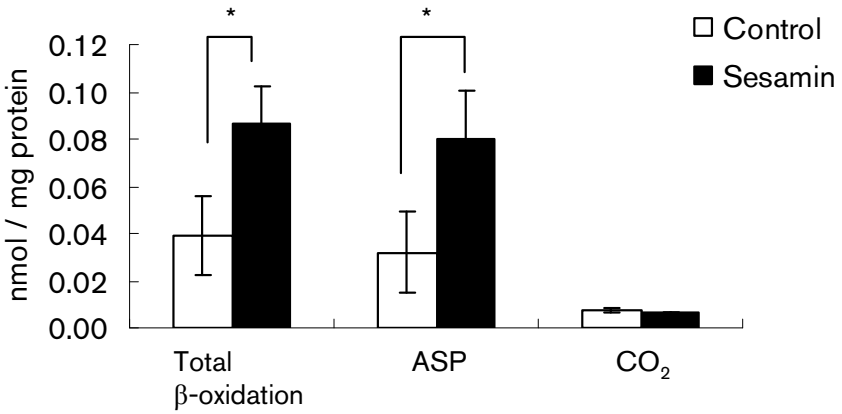

Figure 12. Total β-oxidation and amount of acid-soluble (ASP) and CO_2 β-oxidation products. Significant differences are indicated with an asterisk (P<0.01).

5.4 Lipid class composition

Sesamin treatment of cells tended to decrease the secretion of lipids, particularly TAG (Figure 13, Paper IV). TAG are mainly transported as VLDL (Sheridan, 1988; Tocher, 2003), and therefore the lower TAG level may indicate lower levels of VLDL. In rats, sesamin has been shown to reduce TAG and VLDL levels in serum (Umeda-Sawada *et al.*, 1998;

Kamal-Eldin *et al.*, 2000; Kushiro *et al.*, 2002; Jeng & Hou, 2005). In salmon hepatocytes and skeletal muscle cells, increased levels of n-3 PUFA reduce secretion of TAG (Vegusdal *et al.*, 2004; Kjaer *et al.*, 2008). Similarly, in rat hepatocytes 18:1n-9 increases and EPA and DHA decrease synthesis and secretion of TAG (Nossen *et al.*, 1986), while in humans the levels of circulating TAG-rich lipoproteins are decreased by EPA and DHA (Nestel, 1990). In the case of our sesamin-incubated cells, both the presence of sesamin and the increased levels of n-3 PUFA may explain the decreased TAG levels. It has been suggested that the serum lipid lowering effect is a response of decreased expression of lipogenic enzymes (Kushiro *et al.*, 2002). The decreased relative expression of PPARγ could play a role in the lower TAG level.

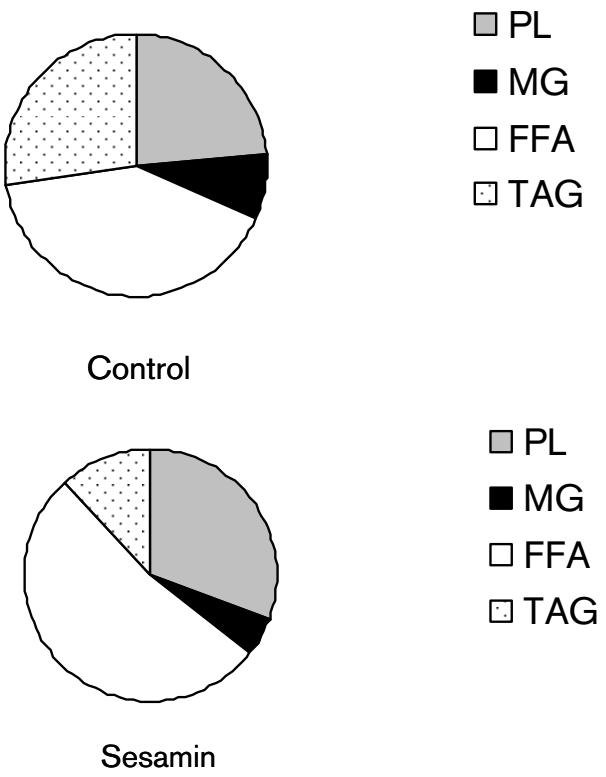

Figure 13. Lipid class composition (as relative %) in the medium of control cells and sesamin-treated cells after 48 h of incubation. Free fatty (FFA) acids are not taken up by the hepatocytes. Phospholipids (PL), monoglycerides (MG) and triacylglycerols (TAG) are secreted lipids.

5.5 Sesamin content in tissues

Sesamin was found in liver and red and white muscle (Figure 14) after 24 hours of fasting (Paper III). Even after 5 days of fasting, sesamin could still be detected in these tissues, but the content was lower (unpublished data). Sesamin is most likely removed from the muscle and catabolised in the liver when fish use the stored lipids as energy reserves during starvation. These results contradict those found in rats and humans, where sesamin is catabolised rapidly and the catecol metabolites are found within 24 hours (Nakai *et al.*, 2003; Moazzami & Kamal-Eldin, 2006). Whether this difference between fish and mammals is an effect of different temperatures in fish and mammals or whether fish handle sesamin catabolism in a different way remains to be investigated.

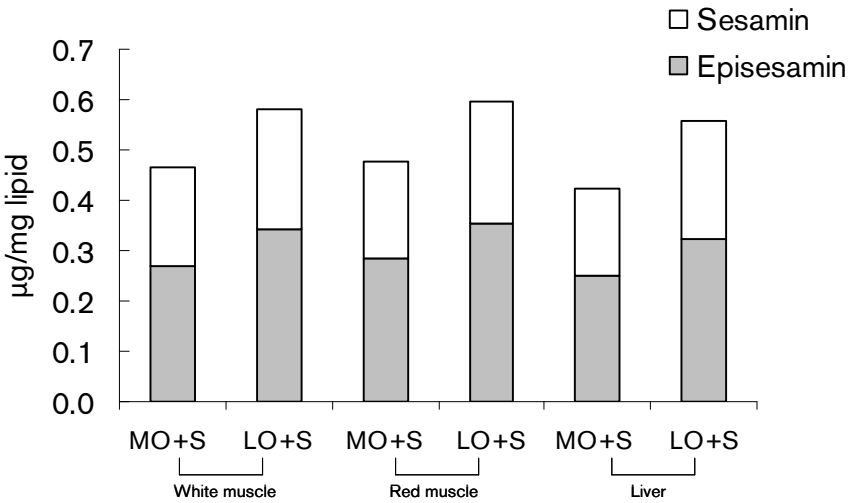

Figure 14. Content of sesamin and episesamin (µg mg^{-1} lipid) in muscle and liver of rainbow trout after 24 h of fasting.

Our results are in agreement with those from a study in rats (Umeda-Sawada *et al.*, 1999), which showed lower sesamin/episesamin ratio in tissues than in the diet, indicating higher metabolism of sesamin than episesamin or different rates of absorption. Assuming that the tissues were exposed to sesamin and episesamin in the same ratios, the lower sesamin/episesamin ratio in red muscle compared with white muscle and liver could indicate that sesamin is metabolised and deposited at different rates in different tissues. Kushiro *et al.* (2002) reported more potent effects of episesamin than sesamin on β-oxidation rate, enzymatic activity and expression of β-

oxidation related genes and enzymes in rat liver. This aspect would be interesting to investigate in fish.

We do not know how fish metabolise sesamin. In humans, sesamin is known to inhibit cytochrome P450 (von Moltke *et al.*, 2004). The increased level of CYP and EROD activity suggests induction of certain cytochrome systems in fish, possibly because the fish recognise sesamin as a xenobiotic compound. However, there was a lack of statistical power in the pooled material used here, and further studies of longer duration are necessary to draw any final conclusions. In addition, it would be worthwhile evaluating the response in omnivorous and herbivorous fish.

5.6 Gene expression

Sesamin affected several genes involved in fish lipid metabolism, both *in vivo* and *in vitro* (Papers III, IV) (Figure 15). The expression of CTP1, which is a rate-limiting step in mitochondrial β-oxidation, was upregulated ($P<0.01$) in hepatocytes. The relative expression of PPARα was downregulated in the *in vivo* study and in the *in vitro* dose response study of hepatocytes ($P<0.05$). The relative expression of PPARγ was downregulated in the hepatocytes, dose response study and incubation with 0.05mM sesamin ($P<0.05$), and non-significant downregulation was also noted *in vivo*. PPARγ is expressed in adipose tissue in rodents, humans and Atlantic salmon, where it is involved in *e.g.* the synthesis of lipids (Ruyter *et al.*, 1997; Andersen *et al.*, 2000; Ferre, 2004; Todorcevic *et al.*, 2008). Moreover, PPARγ regulates the expression of cd36, a gene involved in lipid storage and uptake (Lee *et al.*, 2003), which was also downregulated in our dose response study of hepatocytes incubated with sesamin ($P<0.05$). The general conclusion of the dose response study was that no gene was more specifically affected by higher concentrations of sesamin.

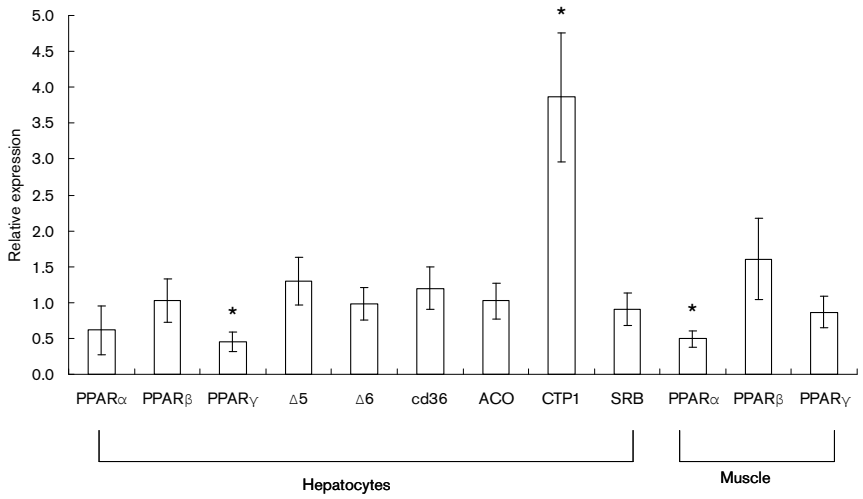

Figure 15. Relative expression of some lipid-related genes in Atlantic salmon hepatocytes after 48 h incubation with sesamin and in rainbow trout white muscle after sesamin supplementation in the diet. Asterisks indicate significant differences between control group and sesamin group ($P<0.05$). Abbreviations: PPARα, β, γ = peroxisome proliferator-activated receptor alpha, beta, gamma, Δ5 = Δ 5 desaturase, Δ6 = Δ 6 desaturase, cd36 = cluster of differentiation 36, ACO = acyl-CoA oxidase, CTP1 = carnitine palmitoyltransferase I, SRB-I = scavenger receptor type B, (n=3).

In rats, sesamin has been associated with upregulation of PPARs (Ashakumary *et al.*, 1999), but we found the opposite in fish. Similarly, 3-thia fatty acids are also associated with activation of PPARs in mammals, but have been found to have the opposite effect in fish (Raspe *et al.*, 1999; Berge *et al.*, 2001; Kleveland *et al.*, 2006a). The relative expression of Δ5 and Δ6 desaturase was significantly downregulated compared with the control cells after 42, 48, 66, 72 h, and for Δ6 desaturase also at timepoint 24 h (Figure 16).

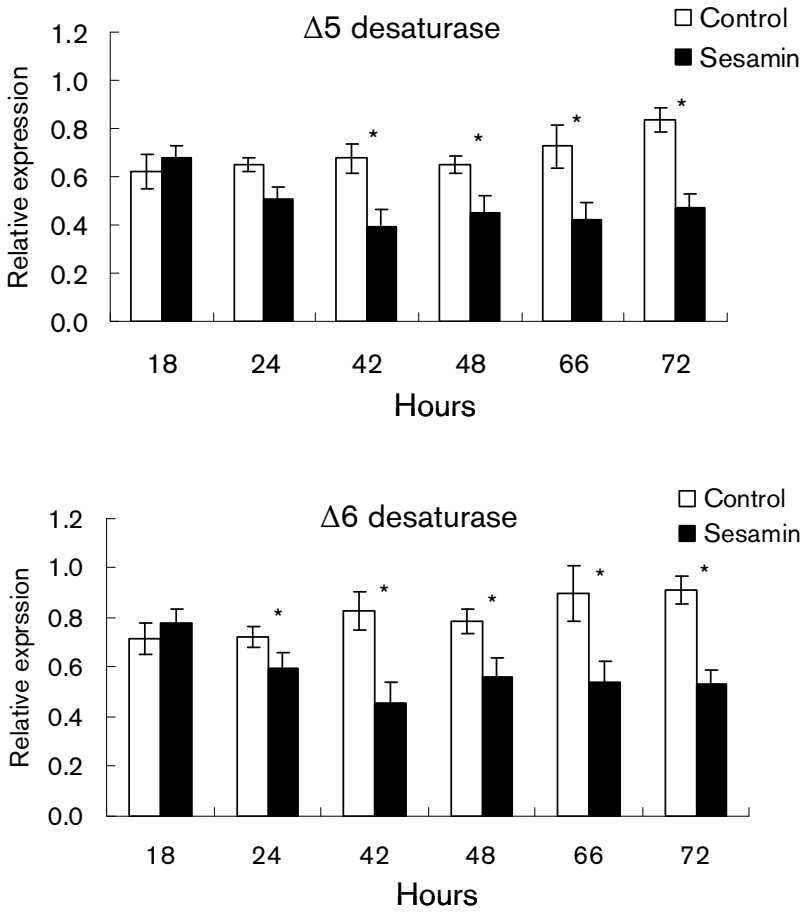

Figure 16. Relative expression of Δ5 and Δ6 desaturase over 72 h. Significant differences between control and sesamin-treated cells are denoted with an asterisk ($P<0.05$).

The first measurements were made after 18 h, so we do not have information about the expression of these genes during the first 18 h of incubation. The ^{14}C18:3n-3 analysis strongly indicated increased elongation and desaturation, and therefore one could speculate that the response on mRNA level was faster than 18 h and that at the time of measurement the expression was already downregulated. Another hypothesis is that there is increased translation of mRNA to protein. At this point we cannot demonstrate whether there is increased transcription or translation of Δ5 and Δ6 desaturase or whether there is another mechanism involved. We found

effects of sesamin on several of the lipid-related metabolic pathways studied in hepatocytes, as summarised in Figure 17.

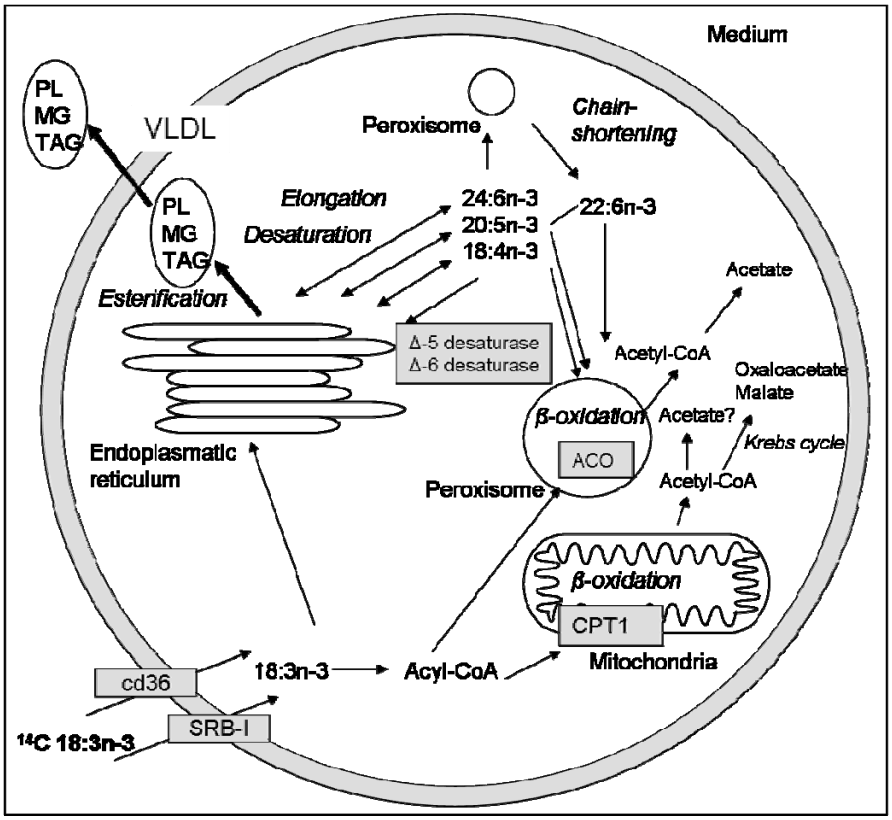

Figure 17. Schematic drawing of the metabolic pathways analysed in salmon hepatocytes. The genes analysed are shown in grey boxes and biochemical reactions are written in italics. The β-oxidation products, lipid classes and fatty acids shown are products from ^{14}C 18:3n-3. Abbreviations: SRB-I = scavenger receptor type B, cd36 = cluster of differentiation 36, Δ5 = Δ5 desaturase, Δ6 = Δ6 desaturase, ACO = acyl-CoA oxidase, CPT1 = carnitine palmitoyl transferase I, PL = phospholipids, MG = monoglycerides, TAG = triacylglycerols, VLDL = very low density lipoprotein

5.7 Implications for aquaculture

Lack of sustainable aquaculture production and heavy use of fish raw materials are well-recognised problems. Several efforts have been made to overcome these problems and to find alternative ways to secure the supply of fish for human consumption. Fish is generally accepted as being a long chain n-3 fatty acid-rich food. However, as mentioned before, this is due to a high content of these fatty acids in the diet, either in the natural prey of wild fish or as fish meal or fish oil in the diet of farmed fish. At present, when the fish oil supply is decreasing and the price of fish oil is increasing, the aquaculture industry is being forced to find alternatives for producing fish with a high content of long chain n-3 fatty acids. Unicellular algae, pelagic organisms and benthic invertebrates have high contents of long chain n-3 fatty acids, but due to high production costs they are not a viable alternative for commercial use (Turchini *et al.*, 2009). Genetically modified vegetable oilseeds containing long chain n-3 fatty acids have been produced but are not a commercially acceptable solution at present. Therefore the focus at the moment is on vegetable oils with a beneficial fatty acid composition (high content of 18:3n-3) (Turchini *et al.,* 2009). This thesis showed that the use of bioactive compounds can increase the levels of long chain n-3 fatty acids in farmed fish. Sesamin affects the lipid metabolism on both gene expression and enzymatic level, which indicates that it is possible to further improve the lipid quality of fish fed vegetable oils by adding potent lipid modulators to the diet.

6 Main findings and conclusions

- Plasma lipid composition in cannulated rainbow trout does not differ dramatically pre and post liver passage. The plasma lipid class composition differs over time after a single meal.

- Dietary lipoic acid increases the proportion of EPA (20:5n-3) in the muscle polar fraction of pacu, but has no effect on dietary vitamin C.

- Sesamin in fish feed increases the proportion of DHA (22:6n-3) in the white muscle of rainbow trout, while incubation of Atlantic salmon hepatocytes with sesamin has a similar effect.

- Incubation of Atlantic salmon hepatocytes with sesamin and ^{14}C 18:3n-3 increases elongation and desaturation of ^{14}C 18:3n-3 to ^{14}C 22:6n-3 and increases the amount of β-oxidation products, in particular acetate, which indicates peroxisomal β-oxidation.

- Sesamin has effects on expression of several lipid-related genes (PPARα and γ, cd36, SRB-I, CPT1, Δ5 and Δ6 desaturase) in Atlantic salmon hepatocytes and on expression of PPARα in liver of rainbow trout.

- Dietary additives can be used to improve lipid metabolism in fish, and thereby to increase the proportions of long chain n-3 fatty acids in fish products. Sesamin is a strong lipid modulator in fish, with effects on both mRNA and enzymatic level of the lipid metabolism. The inclusion of bioactive compounds in fish feed may be one way to meet the demand for novel aquaculture feeds.

The n-3 fatty acids, especially EPA and DHA, have a broad spectrum of positive effects on human health. Exploiting the ability of fish to synthesise EPA and DHA from 18:3n-3 is an effective way of maintaining the healthy fatty acid composition of farmed fish for human food.

7 Future perspectives

This thesis evaluated the possibility of maintaining the levels of healthy lipids in farmed fish without the extensive use of fish oils in fish feeds. Some specific areas of future interest are:

The mechanisms, e.g. the metabolic effects on mitochondria and peroxisomes behind the observed effects, adding the two selected bioactive compounds to fish feeds based on vegetable oils.

The effects of lipoic acid on fatty acid composition in salmonids.

The effects of lipoic acid on lipid metabolism and gene expression in fish.

The effects and catabolism of sesamin in salmonid fish at different life stages and different water temperatures.

Compare the effects on lipid metabolism of episesamin /sesamin and sesamin solely.

The effects of sesamin with different n-3/n-6 ratios in feeds.

Identification of other bioactive substances that are potent candidates as lipid modulators.

References

Ailhaud, G., Massiera, F., Weill, P., Legrand, P., Alessandri, J.M. & Guesnet, P. (2006). Temporal changes in dietary fats: Role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Progress in Lipid Research* 45(3), 203-236.

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.

Andre, M., Ando, S., Ballagny, C., Durliat, M., Poupard, G., Briancon, C. & Babin, P.J. (2000). Intestinal fatty acid binding protein gene expression reveals the cephalocaudal patterning during zebrafish gut morphogenesis. *International Journal of Developmental Biology* 44(2), 249-252.

Appleton, K.M., Rogers, P.J. & Ness, A.R. (2008). Is there a role for n-3 long-chain polyunsaturated fatty acids in the regulation of mood and behaviour? A review of the evidence to date from epidemiological studies, clinical studies and intervention trials. *Nutrition Research Reviews* 21(1), 13-41.

Arivazhagan, P., Shila, S., Narchonai, E. & Panneerselvam, C. (2002). alpha-lipoic acid enhances reduced glutathione, ascorbic acid, and alpha-tocopherol in aged rats. *Journal of Anti-Aging Medicine* 5(3), 265-269.

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.

Babin, P.J. & Vernier, J.M. (1989). Plasma-lipoproteins in fish. *Journal of Lipid Research* 30(4), 467-489.

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 alpha-tocopheryl acetate. *Journal of Fish Biology* 49(4), 748-752.

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. & Sargent, J.R. (2000). Depletion of alpha-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., Tocher, D.R., Farndale, B.M., Cox, D.I., McKinney, R.W. & Sargent, J.R. (1997). The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (Salmo salar) undergoing Parr-Smolt transformation. *Lipids* 32(5), 515-525.

Berge, K., Tronstad, K.J., Flindt, E.N., Rasmussen, T.H., Madsen, L., Kristiansen, K. & Berge, R.K. (2001). Tetradecylthioacetic acid inhibits growth of rat glioma cells ex vivo and in vivo via PPAR-dependent and PPAR-independent pathways. *Carcinogenesis* 22(11), 1747-1755.

Boukouvala, E., Antonopoulou, E., Favre-Krey, L., Diez, A., Bautista, J.M., Leaver, M.J., Tocher, D.R. & Krey, G. (2004). Molecular characterization of three peroxisome proliferator-activated receptors from the sea bass (Dicentrarchus labrax). *Lipids* 39(11), 1085-1092.

Buzzi, M., Henderson, R.J. & Sargent, J.R. (1997). Biosynthesis of docosahexaenoic acid in trout hepatocytes proceeds via 24-carbon intermediates. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 116(2), 263-267.

Calder, P.C. (2006). Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukotrienes and Essential Fatty Acids* 75(3), 197-202.

Calder, P.C. (2008). Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Molecular Nutrition & Food Research* 52(8), 885-897.

Celik, S. & Ozkaya, A. (2002). Effects of intraperitoneally administered lipoic acid, vitamin E, and linalool on the level of total lipid and fatty acids in guinea pig brain with oxidative stress induced by H2O2. *Journal of Biochemistry and Molecular Biology* 35(6), 547-552.

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), 177-183.

Christiansen, R., Borrebaek, B. & Bremer, J. (1976). The effect of (-)carnitine on the metabolism of palmitate in liver cells isolated from fasted and refed rats. *FEBS Letters* 62, 313-317.

Christie, W.W. (1987). *HPLC and lipids*. Oxford: Pergamon press.

Clarke, S.D. (2001). Polyunsaturated fatty acid regulation of gene transcription: A molecular mechanism to improve the metabolic syndrome. *Journal of Nutrition* 131(4), 1129-1132.

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: Vance, D.E., *et al.* (Eds.) *Biochemistry of lipids, lipoproteins and membranes, 4th edition*. Amsterdam: Elsevier. pp. 181-204.

Cordain, L., Watkins, B.A., Florant, G.L., Kelher, M., Rogers, L. & Li, Y. (2002). Fatty acid analysis of wild ruminant tissues: evolutionary implications for reducing diet-related chronic disease. *European Journal of Clinical Nutrition* 56(3), 181-191.

Cort, W.M., Vicente, T.S., Waysek, E.H. & Williams, B.D. (1983). Vitamin E content of feedstuffs determined by high performance liquid chromatographic fluorescence. *Journal of Agricultural and Food Chemistry*. 31(6), 1330-1333.

Dabrowski, K. & Hinterleitner, S. (1989). Simultaneous analysis of ascorbic acid, dehydroascorbic acid and ascorbic sulfate in biological materials. *Analyst* 114, 83-87.

Dal Pai, V., Dal Pai-Silva, M., Carvalho, E.D., Fujihara, C.Y., Gregorio, E.A. & Curi, P.R. (2000). Morphological, histochemical and morphometric study of the myotomal muscle tissue of the pacu (Piaractus mesopotamicus Holmberg 1887: Serrasalminae, Characidae, Teleostei). *Anatomia Histologia Embryologia-Journal of Veterinary Medicine Series C* 29(5), 283-289.

Dowhan, W. & Bogdanov, M. (2002). Functional roles of lipids in membranes. In: Vance, D.E., *et al.* (Eds.) *Biochemistry of Lipids, Lipoproteins and Membranes, 4th edition*. Amsterdam: Elsevier.

EFSA (2005). *Press release EFSA provides advice on the safety and nutritional contribution of wild and farmed fish* [online]. Available from: http://www.moh.gov.cy/Moh/SGL/SGL.nsf/All/74EBB1CEB3BA55E8C225710F0033332C/$file/Safety%20and%20Nutritional%20contribution%20of%20wild%20and%20farmed%20fish.pdf?OpenElement [Accessed 2009-04-08].

Eriksson, S.F. & Pickova, J. (2007). Fatty acids and tocopherol levels in M-Longissimus dorsi of beef cattle in Sweden - A comparison between seasonal diets. *Meat Science* 76(4), 746-754.

FAO (2006). *FAO Fisheries Circular No 1018.* [online] Available from: ftp://ftp.fao.org/docrep/fao/009/a0604e/a0604e00.pdf [Accessed 2009-04-09]

Ferre, P. (2004). The biology of peroxisome proliferator - Activated receptors - Relationship with lipid metabolism and insulin sensitivity. *Diabetes* 53(1 Suppl), S43-S50.

Folch, J., Lees, M. & Stanley, S.G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226(1), 497-509.

Fredriksson, S., Elwinger, K. & Pickova, J. (2006). Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. *Food Chemistry* 99(3), 530-537.

Frøyland, L., Madsen, L., Eckhoff, K.M., Lie, O. & Berge, R.K. (1998). Carnitine palmitoyltransferase I, carnitine palmitoyltransferase II, and Acyl-CoA oxidase activities in Atlantic salmon (Salmo salar). *Lipids* 33(9), 923-930.

Frøyland, L., Lie, O. & Berge, R.K. (2000). Mitochondrial and peroxisomal beta-oxidation capacities in various tissues from Atlantic salmon Salmo salar. *Aquaculture Nutrition* 6(2), 85-89.

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. *Journal of Nutritional Sciences and Vitaminology*. 41(2), 217-225.

Gonzalez-Perez, O. & Gonzalez-Castaneda, R.E. (2006). Therapeutic perspectives on the combination of α-lipoic acid and vitamin E. *Nutrition Research* 26(1), 1-5.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research Communications* 9(1), 1-32.

Halliwell, B. (1996). Antioxidants in human health and disease. *Annual Review of Nutrition* 16, 33-50.

Hamano, Y. (2002). Influence of lipoic acid on lipid metabolism and beta-adrenergic response to intravenous or oral administration of clenbuterol in broiler chickens. *Reprod Nutr Dev*. 42(4), 307-316.

Hara, A. & Radin, N.S. (1978). Lipid extraction of tissue with low toxicity solvent. *Analytic Biochemistry* 90(1), 420-426.

Henderson, R.J. & Tocher, D.R. (1987). The lipid-composition and biochemistry of fresh-water fish. *Progress in Lipid Research* 26(4), 281-347.

Holm, J.C. (2001). Utviklingen av oppdrettsarter globalt. In: Waagbo, R.*, et al*. (Eds.) *Fiskeernaering*. Bergen: Kystnaeringen forlag och bokklubb AS. pp. 323-340.

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.

Hussain, M.M., Kedees, M.H., Singh, K., Athar, H. & Jamali, N.Z. (2001). Signposts in the assembly of chylomicrons. *Frontiers in Bioscience* 6, D320-D331.

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.

Ikeda, S., Abe, C., Uchida, T., Ichikawa, T., Horio, F. & Yamashita, K. (2007). Dietary sesame seed and its lignan increase both ascorbic acid concentration in some tissues and urinary excretion by stimulating biosynthesis in rats. *Journal of Nutritional Science and Vitaminology* 53(5), 383-392.

Issemann, I. & Green, S. (1990). Activation of a member of the steroid-hormone receptor superfamily by peroxisome proliferators. *Nature* 347(6294), 645-650.

Jameel, N.M., Shekhar, M.A. & Vishwanath, B.S. (2006). alpha-lipoic acid: An inhibitor of secretory phospholipase A(2) with anti-inflammatory activity. *Life Sciences* 80(2), 146-153.

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

Jordal, A.E.O., Hordvik, I., Pelsers, M., Bemlohr, D.A. & Torstensen, B.E. (2006). FABP3 and FABP10 in Atlantic salmon (Salmo salar L.) - General effects of dietary fatty acid composition and life cycle variations. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 145(2), 147-158.

Jump, D.B. (2002). Dietary polyunsaturated fatty acids and regulation of gene transcription. *Current Opinion in Lipidology* 13(2), 155-164.

Jump, D.B. & Clarke, S.D. (1999). Regulation of gene expression by dietary fat. *Annual Review of Nutrition* 19, 63-90.

Jönsson, M., Abrahamson, A., Brunström, B. & Brandt, I. (2006). Cytochrome P4501A induction in rainbow trout gills and liver following exposure to waterborne indigo, benzo(a)pyrene and 3,3´,4´,5-pentachlorobiphenyl. *Aquatic Toxicology* 79(3), 226-232.

Kamal-Eldin, A. (2005). Minor components of vegetable oils. In: Shahidi, F. (Ed.) *Bailey´s Industrial Oil and Fat Products*. Sussex, UK,: John Wiley & Sons Inc.III). pp. 483-523.

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.

Karlsson, A., Eliason, E.J., Mydland, L.T., Farrell, A.P. & Kiessling, A. (2006). Postprandial changes in plasma free amino acid levels obtained simultaneously from the hepatic portal vein and the dorsal aorta in rainbow trout (Oncorhynchus mykiss). *Journal of Experimental Biology* 209(24), 4885-4894.

Kiessling, K.H. & Kiessling, A. (1993). Selective utilization of fatty-acids in rainbow-trout (Oncorhynchus-Mykiss Walbaum) red muscle

mitochondria. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 71(2), 248-251.

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), 116S-120S.

Kjaer, M.A., Vegusdal, A., Gjøen, T., Rustan, A.C., Todorcevic, 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 (BBA) - 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 B-Biochemistry & Molecular Biology* 145(2), 239-248.

Kleveland, E.J., Syvertsen, B.L., Ruyter, B., Vegusdal, A., Jorgensen, S.M. & Gjøen, T. (2006b). Characterization of scavenger receptor class B, type I in Atlantic salmon (Salmo salar L.). *Lipids* 41(11), 1017-1027.

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*. Basel: Karger.83. pp. 117-131.

Larsson, S.C., Kumlin, M., Ingelman-Sundberg, M. & Wolk, A. (2004). Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *American Journal of Clinical Nutrition* 79(6), 935-945.

Leaver, M.J., Boukouvala, E., Antonopoulou, E., Diez, A., Favre-Krey, L., Ezaz, M.T., Bautista, J.M., Tocher, D.R. & Krey, G. (2005). Three

peroxisome proliferator-activated receptor isotypes from each of two species of marine fish. *Endocrinology* 146(7), 3150-3162.

Lee, C.H., Olson, P. & Evans, R.M. (2003). Minireview: Lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. *Endocrinology* 144(6), 2201-2207.

Leighton, F., Bergseth, S., Rortveit, T., Christiansen, E.N. & Bremerll, J. (1989). Free Acetate production by rat hepatocytes during peroxisomal fatty acid and dicarboxylic acid oxidation⋆. *The Journal of Biological Chemistry* 264(18), 10347-10350.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193(1), 265-275.

Massaro, M., Scoditti, E., Carluccio, M.A. & De Caterina, R. (2008). Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. *Prostaglandins Leukotrienes and Essential Fatty Acids* 79(3-5), 109-115.

Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Yoshikawa, T., Hasty, A.H., Tamura, Y., Osuga, J., Okazaki, H., Iizuka, Y., Takahashi, A., Sone, H., Gotoda, T., Ishibashi, S. & Yamada, N. (2002). Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPAR alpha. *Journal of Lipid Research* 43(1), 107-114.

Matthews, K.R., Homer, D.B., Thies, F. & Calder, P.C. (2000). Effect of whole linseed (Linum usitatissimum) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues. *British Journal of Nutrition* 83(6), 637-643.

Moazzami, A. & Kamal-Eldin, A. (2006). Sesame seed is a rich source of dietary lignans. *Journal of the American Oil Chemists Society* 83(8), 719-723.

Moya-Falcon, C., Hvattum, E., Tran, T.N., Thomassen, M.S., Skorve, J. & Ruyter, B. (2006). Phospholipid molecular species, beta-oxidation, desaturation and elongation of fatty acids in Atlantic salmon hepatocytes: Effects of temperature and 3-thia fatty acids. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 145(1), 68-80.

Mozaffarian, D., Prineas, R.J., Stein, P.K. & Siscovick, D.S. (2006). Dietary fish and n-3 fatty acid intake and cardiac electrocardiographic parameters in humans. *Journal of the American College of Cardiology* 48(3), 478-484.

Mozaffarian, D. & Rimm, E.B. (2006). Fish intake, contaminants, and human health - Evaluating the risks and the benefits. *JAMA-Journal of the American Medical Association* 296(15), 1885-1899.

Mráz, J. & Pickova, J. (2009). Differences between lipid content and composition of different parts of fillets from crossbred farmed carp (*Cyprinus carpio*). *Fish Physiology and Biochemistry* in press, 1-9.

Nakai, M., Harada, M., Nakahara, K., Akimoto, K., Shibata, H., Miki, W. & Kiso, Y. (2003). Novel antioxidative metabolites in rat liver with ingested sesamin. *Journal of Agricultural and Food Chemistry* 51(6), 1666-1670.

Namiki, M. (2007). Nutraceutical functions of sesame: A review. *Critical Reviews in Food Science and Nutrition* 47(7), 651-673.

Narce, M., Gresti, J. & Bezard, J. (1988). Method for evaluating the bioconversion of radioactive polyunsaturated fatty acids by use of reversed-phase liquid chromatography. *Journal of Chromatography A* 448(2), 249-264.

Navari-Izzo, F., Quartacci, M.F. & Sgherri, C. (2002). Lipoic acid: a unique antioxidant in the detoxification of activated oxygen species. *Plant Physiology and Biochemistry* 40(6-8), 463-470.

Nestel, P.J. (1990). Effects of n-3 fatty-acids on lipid-metabolism. *Annual Review of Nutrition* 10, 149-167.

Nilzen, V., Babol, J., Dutta, P.C., Lundeheim, N., Enfalt, A.C. & Lundstrom, K. (2001). Free range rearing of pigs with access to pasture grazing - effect on fatty acid composition and lipid oxidation products. *Meat Science* 58(3), 267-275.

Nossen, J.O., Rustan, A.C., Gloppestad, S.H., Malbakken, S. & Drevon, C.A. (1986). Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. *Biochimica Et Biophysica Acta* 879(1), 56-65.

Omura, T. & Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *Journal of Biological Chemistry* 239(7), 2379-2385.

Ozkan, Y., Yllmaz, O., Ozturk, A.I. & Ersan, Y. (2005). Effects of triple antioxidant combination (vitamin E, vitamin C and alpha-lipoic acid) with insulin on lipid and cholesterol levels and fatty acid composition of brain tissue in experimental diabetic and non-diabetic rats. *Cell Biology International* 29(9), 754-760.

Packer, J.E., Slater, T.F. & Willson, R.L. (1979). Direct observation of a free-radical interaction between Vitamin-E and Vitamin-C. *Nature* 278(5706), 737-738.

Park, K.H., Terjesen, B.F., Tesser, M.B., Portella, M.C. & Dabrowski, K. (2006). alpha-Lipoic acid-enrichment partially reverses tissue ascorbic acid depletion in pacu (Piaractus mesopotamicus) fed vitamin C-devoid diets. *Fish Physiology and Biochemistry* 32(4), 329-338.

Parker, R.S., Sontag, T.J. & Swanson, J.E. (2000). Cytochrome P4503A-dependent metabolism of tocopherols and inhibition by sesamin. *Biochemical and Biophysical Research Communications* 277(3), 531-534.

Peterson, G.L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analytical Biochemistry* 83(2), 346-356.

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.

Pickova, J. & Mørkøre, T. (2007). Alternate oils in fish feeds. *European Journal of Lipid Science and Technology* 109(3), 256-263.

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.

Raspe, E., Madsen, L., Lefebvre, A.M., Leitersdorf, I., Gelman, L., Peinado-Onsurbe, J., Dallongeville, J., Fruchart, J.C., Berge, R. & Staels, B. (1999). Modulation of rat liver apolipoprotein gene expression and serum lipid levels by tetradecylthioacetic acid (TTA) via PPAR alpha activation. *Journal of Lipid Research* 40(11), 2099-2110.

Reed, L.J. (1974). Multienzyme complex. *Accounts of Chemical Research*. 7(2), 40-46.

Roberts, R.J. (2002). Nutritional physiology. In: Halver, J.*, et al.* (Eds.) *Fish Nutrition*. San Diego: Academic Press. pp. 368-453.

Ruyter, B., Andersen, O., Dehli, A., Farrants, A.K.O., 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., Røsjø, C., Grisdale-Helland, B., Rosenlund, G., Obach, A. & Thomassen, M.S. (2003). Influence of temperature and high dietary linoleic acid content on esterification, elongation, and desaturation of PUFA in Atlantic salmon hepatocytes. *Lipids* 38(8), 833-840.

Sampels, S., Strandvik, B. & Pickova, J. (2009). Processed animal products with emphasis on polyunsaturated fatty acid content. *European Journal of Lipid Sciences and Technology* in press, 1-20.

Santulli, A., Curatolo, A., Modica, A. & Damelio, V. (1988). Time-course changes of plasma-lipid levels and lipoprotein pattern after feeding in cultured sea bass, Dicentrarchus-Labrax L. *Journal of Fish Biology* 32(6), 859-867.

Sargent, J.R., Bell, J.G., Bell, M.V., Hendersson, R.J. & Tocher, D.R. (1995). Requirement criteria for essential fatty acids. *Journal of applied Ichthyology* 11(3-4), 183-198.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002). The lipids. In: Halver, J.*, et al.* (Eds.) *Fish Nutrition*. San Diego: Academic Press. pp. 182-257.

Schuenke, M. & Wodtke, E. (1983). Cold-induced increase of D9- and D6-desaturase activities in endoplasmic membranes of carp liver. *Biochima et Biophysica Acta* 734, 70-75.

Sheridan, M.A. (1988). Lipid Dynamics in fish - aspects of absorption, transportation, deposition and mobilization. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 90(4), 679-690.

Sheridan, M.A., Friedlander, J.K.L. & Allen, W.V. (1985). Chylomicra in the serum of postprandial steelhead trout (Salmo-Gairdnerii). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 81(2), 281-284.

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. (1999). New products from the agri-food industry: the return of n-3 fatty acids into the food supply. *Lipids* 34, 297-301.

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 & Pharmacotherapy* 56(8), 353-379.

Sire, M.F., Lutton, C. & Vernier, J.M. (1981). New views on intestinal-absorption of lipids in teleostean fishes - an ultrastructural and biochemical-study in the rainbow-trout. *Journal of Lipid Research* 22(1), 81-94.

SLV (2009). *Kostråd* [online]. Available from: http://www.slv.se/templates/SLV_Page.aspx?id=2559&epslanguage=SV [Accessed 2009-04-08].

Small, G.M., Burdett, K. & Connock, M.J. (1985). A sensitive spectrophotometric assay for peroxisomal acyl-coA oxidase. *Biochemical Journal* 227(1), 205-210.

Smith, P.K., Krohn, R.I., Hermansson, G.T., Malilia, A.K., Gartner, F.H., Provenzano, M.D., Fujimito, E.K., Goeke, N.M., Olson, B.J. & Klenk, D.C. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry* 150(1), 76-85.

SOFIA (2002). *The state of world fisheries and aquaculture* [online] Available from: http://www.fao.org/docrep/005/y7300e/y7300e00.htm [Accessed 2009-02-10].

SOFIA (2004). *The state of world fisheries and aquaculture* [online] Available from: http://www.fao.org/DOCREP/007/y5600e/y5600e00.htm [Accessed 2009-02-10].

SOFIA (2006). *The state of world fisheries and aquaculture* [online] Available from: http://www.fao.org/docrep/009/A0699e/A0699e00.htm [Accessed 2009-02-10].

Strandvik, B., Eriksson, S., Garemo, M., Palsdottir, V., Sampels, S. & Pickova, J. (2008). Is the relatively low intake of omega-3 fatty acids in Western diet contributing to the obesity epidemics? *Lipid Technology* 20(3), 57-59.

Tacon, A.G.J. (2008). *State of information on salmon aquaculture feed and the environment*. Report prepared for the WWF US Initiated Salmon Aquaculture Dialogue 80p. [online] Available from: http://www.westcoastaquatic.ca/Aquaculture_feed_environment.pdf [Accessed 2008-03-01]

Tacon, A.G.J. & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285(1-4), 146-158.

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., Fonseca-Madrigal, J., Bell, J.G., Dick, J.R., Henderson, R.J. & Sargent, J.R. (2002). Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (Salmo salar). *Fish Physiology and Biochemistry* 26(2), 157-170.

Tocher, D.R., Fonseca-Madrigal, J., Dick, J.R., Ng, W.K., Bell, J.G. & Campbell, P.J. (2004). Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (Oncorhynchus mykiss). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 137(1), 49-63.

Todorcevic, M., Vegusdal, A., Gjøen, T., Sundvold, H., Torstensen, B.E., Kjaer, 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, O. (2001). Lipider. In: Waagbo, R.*, et al.* (Eds.) *Fiskeernaering*. Bergen: Kystnaeringen Forlag & Bokklubb. pp. 57-75.

Torstensen, B.E., Frøyland, L., Ornsrud, R. & Lie, O. (2004). Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (Salmo salar) fed vegetable oils. *Food Chemistry* 87(4), 567-580.

Turchini, G.M., Torstensen, B.E. & Ng, W.K. (2009). Fish oil replacement in finfish nutrition. *Reviews in Aquaculture* 1, 10-57.

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.

Umeda-Sawada, R., Ogawa, M. & Igarashi, O. (1998). The metabolism and n-6/n-3 ratio of essential fatty acids in rats: effect of dietary arachidonic acid and mixture of sesame lignans (sesamin and episesamin). *Lipids* 33(6), 567-527.

Umeda-Sawada, R., Ogawa, M. & Igarashi, O. (1999). The metabolism and distribution of sesame lignans (sesamin and episesamin) in rats. *Lipids* 34(6), 633-637.

van der Oost, R., Beyer, J. & Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13(2), PII S1382-6689(02)00126-6.

van der Vusse, G.J., van Bilsen, M., Glatz, J.F.C., Hasselbaink, D.M. & Luiken, J. (2002). Critical steps in cellular fatty acid uptake and utilization. *Molecular and Cellular Biochemistry* 239(1-2), 9-15.

Van Nieuwenhoven, F.A., VanderVusse, G.J. & Glatz, J.F.C. (1996). Membrane-associated and cytoplasmic fatty acid-binding proteins. *Lipids* 31(S Suppl), S223-S227.

Vegusdal, A., Østbye, T.K., Tran, T.N., Gjøen, T. & Ruyter, B. (2004). beta-oxidation, esterification, and secretion of radiolabeled fatty acids in cultivated Atlantic salmon skeletal muscle cells. *Lipids* 39(7), 649-658.

Weill, P., Schmitt, B., Chesneau, G., Daniel, N., Safraou, F. & Legrand, P. (2002). Effects of introducing linseed in livestock diet on blood fatty acid composition of consumers of animal products. *Annals of Nutrition and Metabolism* 46(5), 182-191.

von Moltke, L.L., Weemhoff, J.L., Bedir, E., Khan, I.A., Harmatz, J.S., Goldman, P. & Greenblatt, D.J. (2004). Inhibition of human cytochromes P450 by components of Ginkgo biloba. *Journal of Pharmacy and Pharmacology* 56(8), 1039-1044.

Wood, J.D. & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition* 78(1), S49-S60.

wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R. & Enser, M. (2004). Effects of fatty acids in meat quality: a review. *Meat Science* 66(1), 21-32.

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.

Zaspel, B.J. & Csallaany, A.S. (1983). Determination of α-tocopherol in tissues and plasma by high-performance liquid chromatography. *Analytical Biochemistry* (130), 145-150.

Zheng, X.Z., Torstensen, B.E., Tocher, D.R., Dick, J.R., Henderson, R.J. & Bell, J.G. (2005). Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids* 1734(1), 13-24.

Zhou, S.Y., Ackman, R.G. & Morrison, C. (1995). Storage of lipids in the myosepta of Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* 14(2), 171-178.

Acknowledgements

Först av allt vill jag rikta ett stort tack till mina handledare. Min huvudhandledare, professor **Jana Pickova** för att du delat med dig av din kunskap om lipider och inspiration för fisk. För ditt sätt att handleda så lagom, som ger mig utrymme för egna idéer, men ändå stöd, det är både roligt och lärorikt att jobba tillsammans med dig! Tack också för din vänskap och för att du är så omtänksam. My second supervisor, professor **Afaf Kamal Eldin,** thanks for sharing your knowledge, inspiration and for your never ending support and always open door. I also appreciate your warm and generous personality. Min tredje handledare docent **Torbjörn Lundh**, tack för att du alltid har ställt upp när jag bett om hjälp.

Sedan vill jag tacka alla på avdelningen för kött- och fiskkvalitet. Vår avdelningschef, professor **Kerstin Lundström,** tack för att du har gett mig möjligheten att jobba på institutionen samt för värdefull statistisk support. **Christina** och **Sabine** tack för att ni lärt mig allt jag kan på labbet, and to all of you in the lipid group, **Andreas, Honza, Pernilla, Tyler and Vlada,** thank you for all good time in the lab. It is nice to have some friends that also can be crazy happy for a small peak in a chromatogram☺ Tack till min rumskompis **Galina,** för alla skratt och mikropauser and to **Gang,** my first room mate for good time and nice dinners.

Tack till **alla** mina nuvarande och tidigare **kollegor på LMV** för att ni är så hjälpsamma och trevliga. Ett särskilt tack till **Veronica,** för alla trevliga resor och små utflykter vi har hittat på, vänner som dig får man leta efter! **Rikard**, tack för att du följde med mig och jagade mat mitt i natten i Granada. **Arwa**, thanks for all nice dinners, and for your warm friendship.

Till professor **Eva Brännäs** för alla fantastiska fiskförsök.

Till professor **Bente Ruyters** norska lipidgrupp**, Tone-Kari, Inger, Marte, Marijana** and **Brankica** det är otroligt inspirerande och lärorikt att jobba tillsammans med er och det alltid lika trevligt att komma tillbaks till Ås, tack för ert generösa bemötande och alla goda skratt!

To professor **Konrad Dabrowski**, thanks for the opportunity to work in your lab.

To **Tom Bahnasy**, thanks for your skilled help with the cover page.

Till alla vänner utanför jobbet, **Caroline, Cecilia, Ingela, Jeanette, Jenny, Karin, Karolina, Maria, Martina, Sara, Susanna, Tilda** ni är alla fantastiska och jag är glad att ha er, jag hoppas vi hinner ses mer nu när den här boken är klar...

Marie och **Leia,** tack för delad vårdnad av tjejerna och alla fina dagar i stallet och timmar på golvet. Det gångna året är det bästa i mitt liv, det planerade vi bra ☺

Tone, tack för att du alltid ställer upp lika entusiastiskt när det behövs hjälp med såväl hästar som Ellen, en Tone underlättar livet!

Åsa och **Jonna** min tappra hästskötare, inget oväder i världen skulle stoppa er, tack, ni är guld värda!

Min kära **Mamma** och **Lasse**, tack för all värdefull hjälp på sluttampen, Ellen har haft mysiga dagar med mormor, men mamma om jag vore så effektiv som du skulle jag inte behövt ha någon barnvakt ☺ Tack också för att ni alltid finns där för oss och rycker in när det behövs, vad vi än hittar på!

Min syster **Evelina**, för att du är så snäll, nu lovar jag att nästa ”tjejkväll” inte ska sluta med att du leker med Ellen och att jag sitter framför datorn. Min bror **Joakim** för att du finns.

Farmor och **Mormor** just för att ni är farmor och mormor.

Rosi, meine liebe Schwiegermutter, danke fuer deine grosse und abenteuerliche Reise in den Norden! Ich bin dankbar, dass Ellen so viel Spass mit ihrer Oma gehabt hat! Und auch danke an meinen lieben Schwiegervater, **Othmar**. Ihr zwei seit weit weg, aber doch immer mit uns! Das ist viel wert, danke!

An Familie Zapfel, **Silke, Harry, Selina** und **Elena**, danke fuer eure Freundschaft und wunderschöne Einladungen, wenn wir auf Besuch in Österreich sind.

Min familj, **Ellen** och **Norbert** som gör mitt liv så fullt av glädje och kärlek, det bästa som finns är att vara tillsammans med er, jag älskar er över allt annat på jorden! Nu hoppas jag på en lång skön sommar tillsammans med er, **Raffe** och **Disan,** våra fyrbenta familjemedlemmar som löser frågan om vad vi ska göra med tiden som blir över…