Effects of Non-Fish Based Raw Materials on the Fish Muscle Quality of Salmonids

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Abstract

Salmonids are considered as fatty fish and a healthy food. They are characterized by a high proportion of n-3 long chain polyunsaturated fatty acids (n-3 LCPUFA). There is great interest in producing high-quality salmonids with a reduced use of fish-based materials and a challenge to adjust feeds towards more sustainable.

This thesis investigated the effects of sesamin, linseed oil (LO), rapeseed oil (RO), krill oil (KO), krill meal (KM), mussel meal (MM), and zygomycete meal (ZM) (Rhizopus oryzae) on fish performance, fatty acid profiles, carotenoids, cytochrome P450 (CYP450) and, colour properties and oxidation in the white muscle of Atlantic salmon (*Salmo salar L*), rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*).

No negative effects on fish performance were found when KM, KO, MM, ZM and sesamin were included in the fish feeds. The FA profile was modified significantly by these feed compounds. LO and RO increased the α-linolenic acid (ALA) level and decreased the n-3 LCPUFA portion. Sesamin significantly decreased the ALA level and slightly increased the DHA level in some groups of Atlantic salmon and rainbow trout. Stripped-LO showed different effects on the portion of EPA and ALA compared to the LO group. The sesamin content in fish liver was consistent with its level in feeds, while the content in white muscle was similar across all groups. KO, KM and MM contributed a high portion of n-3 LCPUFA and under 3.5 mg/kg astaxanthin to the white muscle of Arctic charr, enhancing a* value. Some groups with a high level of astaxanthin showed high level of oxidation products (thiobarbituric reactive substances). Sesamin and ZM significantly affected the level or activity of CYP 450.

The results of this thesis reveal the different effects of the non-fish-based materials on the fatty acid profile and colour properties in salmonids, suggesting that these feed compounds can be used to improve fish quality with an optimised formula. Results also indicate that new feed raw materials need further evaluation before the full application in commercial fish feeds.

Keywords: salmonid, sesamin, krill, mussel, zygomycete, fatty acids, carotenoids, TBARS, CYP450.

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Dedication

To my parents

Is it not delightful to acquire knowledge and put it into practice from time to time?

Confucius

學而時習之，不亦說乎?

孔子

Contents

List of Publications

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

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

II Vestergren, A., Trattner, S., Pan, J., Johnsson, P., Kamal-Eldin, A., Brännäs, E., Moazzami, A. & Pickova, J. (2013). The effect of combining linseed oil and sesamin on the fatty acid composition in white muscle and on expression of lipid-related genes in white muscle and liver of rainbow trout (*Oncorhynchus mykiss*). Aquaculture International, 21(4), 843-859.

III Pan, J., Trattner, S., Zamaratskaia, G., Brännäs, E., Lundhc, T., Kiessling, A., Pickova, J. Effect of a diet including zygomycetes meal, mussel meal on performance, fatty acid profile and CYP450 activity of Arctic charr (*Salvelinus alpinus*) (manuscript).

IV Pan, J., Wagner, L., Trattner, S., Brännäs, E., Brunheim, I., Pickova, J. Effect of krill, mussel and fish meals on growth, fatty acid profile, carotenoid content, colour and oxidation property in white muscle of Arctic charr (*Salvelinus alpinus*) (manuscript).

Jinfeng Pan's contribution to the papers included in this thesis was as follows:

I Participated in part of the analytical work (analysis of fatty acid, sesamin and tocopherol), and part of the preparation of the manuscript.

II Participated in the part of the analytical work (analysis of fatty acid, sesamin and tocopherol), part of the data analysis and the preparation of the manuscript.

III Performed the analytical work, data analysis and prepared the manuscript.

IV Participated in the planning of the study, performed the analytical work, analysed data and prepared the manuscript.

Abbreviations

AA	Arachidonic acid
AC	Astaxanthin content
ACO	Acyl-CoA oxidase
AHA	American Heart Association
ALA	α-linolenic acid
ANHMRC	Australian National Health and Medical Research Council
AST	Astaxanthin
CD	Commercial diet
CF	Condition factor
CPT	Carnitine palmitoyltransferase
CYP	Cytochrome
DGC	Daily growth coefficient
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
ED	Experimental diet
Elovl	Elongase of very long chain fatty acid
EPA	Eicosapentaenoic acid
EROD	Ethoxyresorufin O-deethylase
FA	Fatty acid
FAO	Food and Agriculture Organization
FM	Fish meal
FO	Fish oil
GLM	General linear model
HPLC	High performance liquid chromatography
HSL	Hormone sensitive lipase
ISSFAL	International Society for the Study of Fatty Acids and Lipids
KM	Krill meal
KO	Krill oil

LA	Linoleic acid
LCPUFA	Long chain polyunsaturated fatty acid
LO	Linseed oil
MM	Mussel meal
MO	Mixture of linseed:sunflower oil
mRNA	Messenger ribonucleic acid
MUFA	Monounsaturated fatty acid
PCR	Polymerase chain reaction
PLS-DA	Partial least squares-discriminant analysis
PNPH	P-nitrophenol
PPAR	Peroxisome proliferator activated receptor
PUFA	Polyunsaturated fatty acid
RO	Rapeseed oil
RTC	Retention rate of total carotenoids
S	Sesamin/episesamin
SAS	Statistical Analysis System
SD	Standard deviation
SesO	Sesame oil:linseed oil (V:V=1:1)
SFA	Saturated fatty acid
SRB	Scavenger receptor type B
SREBP	Sterol regulatory element binding protein
TAG	Purified linseed oil triacylglycerol fraction
TBARS	Thiobarbituric reactive substances
TC	Total carotenoids content
VO	Vegetable oil
WHO	World Health Organization
ZM	Zygomycete meal

1 Introduction

1.1 Fish as food

Fish consumption is steadily increasing world-wide and fish is known to be healthy and beneficial for human health. Fish contains protein with a balanced amino acid profile, lipids, especially n-3 long chain polyunsaturated fatty acids (LCPUFA), antioxidants such as carotenoids, tocopherol, vitamins D and B12, essential minerals and trace elements, *e.g.* selenium, iodine (Bell & Waagbø, 2008). Over the past five decades, fish consumption has increased by an average rate of 3.2 % per year. In 2011, fish consumption per capita was 18.8 kg, accounting for 16.7 % of the world population's intake of animal protein (SOFIA, 2012). Therefore, fish is an important animal food source in human diets.

Muscle is the main edible part of fish, accounting for about 60 % of the fish body mass. Fish have mainly two different types of muscle fibers: white muscle and red muscle. Most fish have more of the glycogenic white muscle, which is usually leaner than the oxidative, fattier red muscle. Fish muscle is composed of parallel muscle fiber bunches called myotomes, which are separated by layers of connective tissue called myosepta (Figure 1). Myosepta is the main reservoir for lipid deposition in fatty species (Zhol *et al.*, 1995). Carotenoids in muscle bind to actomyosin by hydrophobic bonds rather than associate with lipids (Sigurgisladottir *et al.*, 1994).

Lipid content and lipid class as well as fatty acid (FA) composition differ in various tissues. The abdominal wall has the highest portion of lipids, mainly triacylglycerols, with a large portion of monounsaturated fatty acids (MUFA). White muscle contains lipids characterized by a high level of phospholipids rich in n-3 LCPUFA. Henderson and Tocher (1987) concluded that MUFA are positively correlated to fattiness, while n-3 LCPUFA are negatively correlated to fattiness in fish. Lipid content and FA composition also depends on the fish

species. Some species have a lipid content below 2 % in muscle (wet weight), *e.g.* pikeperch (*Stizostedion lucioperca*), burbot (*Lota Iota*) and, cod (*Gadus morhua*), while species such as Atlantic salmon (*Salmo salar*) and, buffalo catfish (*Ictiobus sp*.) contain 10 % lipids or more (Henderson & Tocher, 1987). The FA profile of muscle from marine fish such as Atlantic salmon and herring (*Clupea harengus*) is characterized by a high portion of n-3 LCPUFA, but in freshwater fish *e.g*. carp (*Cyprinus carpio*), n-3 LCPUFA levels are often lower. It should be pointed out that fish nutrition greatly affects the quality of the fish. n-3 LCPUFA and carotenoids in fish muscle greatly depend on the corresponding nutrients in the feed.

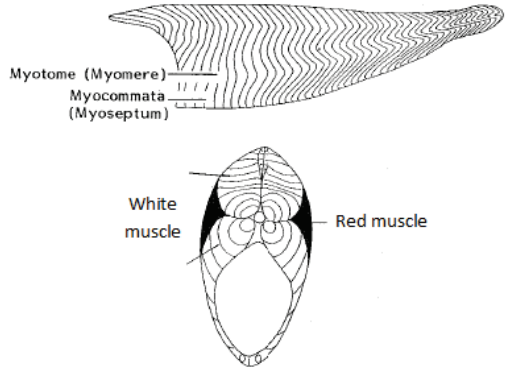

Figure 1. Schematic picture of general salmonid muscle. Adapted from (FAO, 1995)

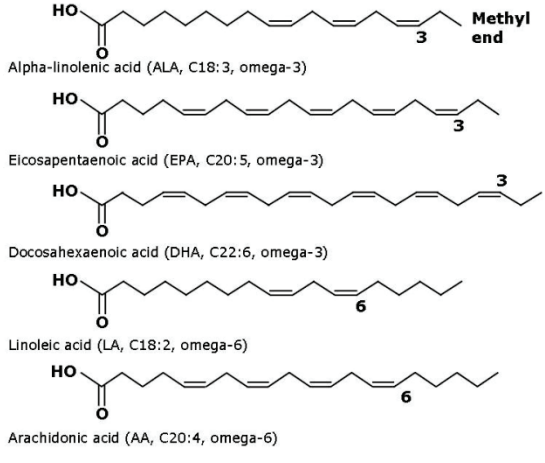

Figure 2. Some important n-6 and n-3 polyunsaturated fatty acids

1.2 Fish FAs and human health

1.2.1 n-3 PUFA and n-6 PUFA

Polyunsaturated fatty acids (PUFA) are FAs that contain more than one double bond. PUFA can be divided into n-3 series and n-6 series fatty acids (Figure 2), which have their first double bond at the third or sixth carbon, counting from the methyl end of the molecule, respectively.

n-3 LCPUFA, mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are necessary constituents of the cell membrane and play an important role in the regulation of cell signaling (Calder, 2009). They show beneficial effects on the prevention or treatment of diabetes (Nettleton & Katz, 2005), cardiovascular disease (Von Schacky & Harris, 2007), inflammatory diseases (Connor, 2000), some cancers (Larsson *et al.*, 2004), obesity (Garaulet *et al.*, 2001) as well as abnormal cognitive functions (Kröger *et al.*, 2009). Studies have shown that the n-6/n-3 ratio of FAs is important in human diets. n-6 and n-3 FAs are metabolically and functionally distinct and often have opposite roles. Metabolites from n-6 PUFA are important in immune response and inflammation, while metabolites of n-3 PUFA are involved in anti-inflammatory functions (Nagao & Yanagita, 2008).

1.2.2 Recommendations for the intake of FAs

Recommendations on optimal lipid intake are often re-evaluated to minimize metabolic syndrome in humans as an effect of lifestyle and according to new research findings. General guidelines recommend total fat intake to be around 30 % of the daily energy intake (Linseisen *et al.*, 2009). Different agencies, such as the European Food Safety Authority (EFSA), American Heart Association (AHA), and World Health Organization (WHO) recommend the consumption of at least two portions (or 400 grams) of fatty fish per week to maintain good health. The recommendation for the daily intake of individual fatty acids is as follows: 2 g α-linolenic acid (ALA), 10 g linoleic acid (LA) (n-3/n-6 ratio 1:5) and 200-500 mg EPA + DHA for normal population (EFSA, 2009; EFSA Panel on Dietetic Products, 2010; WHO/FAO, 2003a). Pregnant women, infants, children and person in risk of cardiovascular disease should consume higher doses (Kris-Etherton *et al.*, 2002) (Table 1).

During the last century, cereal grains rich in n-6 PUFA were and still are widely fed to animals to meet the increasing demand of animal-origin products such as meat, milk and eggs world-wide. This change in food production led to a remarkable increase in n-6 FAs in animal foods and caused a high n-6/n-3 ratio in human diet. The current western diet includes < 150 mg n-3 FAs per

day and the n-6/n-3 ratio is 10:1-20:1, far above the recommended level (Simopoulos, 2000).

Table 1. *International recommendations for n-3 long chain polyunsaturated fatty acids*

Organization (year)	Amount of EPA and DHA	Object
WHO/FAO (2003b)	450 – 1000 mg/day	Adult
ANHMRC (2006)	> 610 mg/day; > 410 mg/day	Men; women
ISSFAL (2004)	> 500 mg /day	Pregnant adult
AHA (2002)	1000 mg /day	CHD patient

Abbreviation: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; WHO, World Health Organization; FAO, Food and Agriculture Organization; AHA, American Heart Association; ISSFAL, International Society for the Study of Fatty Acids and Lipids; ANHMRC, Australian National Health and Medical Research Council; CHD, Coronary heart diseases.

Table 2. *Fatty acids composition (% of total fatty acids) of animal food products from conventional feed with different main lipid source (defined in brackets)*

Animal (feed)	ALA	EPA	DPA	DHA	Total	n-6/n-3
Beef[a] (grain)	0.5	0.2	0.5	0.05	1.25	8.7
Beef[b] (pasture)	1.8	0.6	0.9	0.08	3.38	1.3
Pork[c] (grain)	0.5	-	0.3	0.3	1.1	12.1
Pork[d] (linseed)	3.3	1.8	2.2	0.8	8.1	3.5
Chicken[e] (rapeseed)	4.52	0.5	0.18	0.48	5.68	3.68
Chicken[e] (fish oil)	1.60	7.63	2.29	5.59	17.1	0.79
Egg[f] (corn oil)	1.0	0.02	0.1	1.7	2.82	15.3
Egg[f] (algae)	1.9	0.2	0.2	2.5	4.8	4.3
Salmon[g] (rapeseed)	4.1	1.1	0.4	3.2	8.8	1.5
Salmon[g] (fish oil)	0.6	4.1	1.8	7.7	14.2	0.3

[a]Wood and Enser (1997); [b]Fredriksson and Pickova (2007); [c]Nilzén et al. (2001); [d]Matthews et al. (2000); [e]Lopez-Ferrer et al. (1999); [f]Fredriksson et al. (2006); [g]Torstensen et al. (2004).
Abbreviation: ALA, α-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

1.2.3 n-3 LCPUFA in animal origin food products

As a matter of priority, fish deposit n-3LCPUFA in tissues. At present, marine fish are the major contributors of n-3 LCPUFA in the human diet. Compared with other animal origin food, marine fish provide a much higher portion of n-3 LCPUFA (Table 2). The majorities of n-3 LCPUFA is synthesized by microalgae and transferred into fish or other aquatic animals *via* the food chain in aquatic systems. In farmed fish, these FAs are traditionally provided via fish feed in a diet containing fish oil (FO). If FO is replaced by other lipid sources with smaller amounts of n-3 LCPUFA, their levels in edible tissues will decrease (Table 2). In studies by Pettersson (2009a; 2009b), Arctic charr (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) were fed feeds

with different amounts of DHA and EPA. A clear decrease of n-3 LCPUFA was recorded with decreased levels in the feed. Mráz et al. (2010) concluded the same trend in dietary studies in common carp (*Cyprius caprio*).

1.2.4 Carotenoids and human health

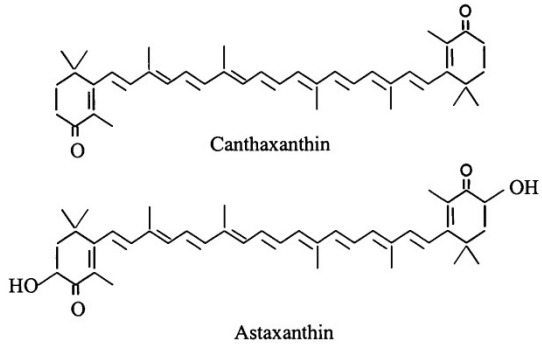

Figure 3. Chemical structure of canthaxanthin and astaxanthin.

Carotenoids are a group of fat-soluble pigments. They are divided into two classes, containing either no oxygen as highly unsaturated carotene hydrocarbons or containing oxygenated group substitutes at particular sites on the terminal rings as xanthophylls (Shahidi & Brown, 1998). Carotenoids are functional compounds for human health due to their excellent antioxidative activity. Astaxanthin (3,3´-dihydroxy-β,β-carotene-4,4´-dione) is the most common carotenoids in salmonids and canthaxanthin (β,β-carotene-4,4´-dione) is also often found in salmonids (Choubert *et al.*, 2009). Astaxanthin (AST) has terminal carbonyl groups conjugated to a polyene backbone that enhances the antioxidant features (Jackson *et al.*, 2008) (Figure 3). Its antioxidant activity is considered to be higher than other carotenoids (lutein, β-carotene and canthaxanthin) (Beutner *et al.*, 2001), 100 times more so than α-tocopherol (Miki, 1991). A large number of studies prove its potential effects on the prevention or treatment of cardiovascular disease (Hussein *et al.*, 2005) , some cancers (Jyonouchi *et al.*, 2000), diabetes (Naito *et al.*, 2004), ocular and skin health (Chitchumroonchokchai *et al.*, 2004; Lyons & O'Brien, 2002) and other inflammatory response and immunological system diseases (Lee *et al.*, 2003).

1.3 Aquaculture, fish oil and fish meal

Aquaculture is the systematic farming of aquatic organisms such as fish, crustaceans and aquatic plants. Two key sources of feeds for aquaculture are fish meal (FM) and FO. FM represents the most suitable protein source with

good digestibility and contains all the essential amino acids in appropriate proportions for fish nutrition (Cho & Kim, 2011). FO is considered an excellent lipid source, especially in the context of n-3 LCPUFA (Rice, 2009). Its composition mirrors the food chain Fas. n-3 LCPUFA produced mostly by microalgae are magnified in the food chain and consumed by humans mainly in the form of fish. These compounds are highly valued with regard to human nutrition. In addition, the content of fat-soluble carotenoid pigments and vitamin E (tocopherol), A and D (Rice, 2009) are of importance in fish oil. These raw materials have their origin in traditional ocean capture fisheries (De Silva *et al.*, 2011). In the 1980s, most of the feed resources needed for the cultivation of carnivorous and omnivorous fish and crustaceans originated from pelagic reduction fisheries (anchovy, capelin, horse mackerel, menhaden, sand eel, pilchard and herring and sprat) (Olsen, 2011). Some of them are actually threatened with extinction (Naylor *et al.*, 1998; Naylor *et al.*, 2000). Thanks to major investment in research, there has been a change in this threat over the past decade, with a tendency towards greater use of agricultural feed resources for fish (Gatlin *et al.*, 2007; Naylor *et al.*, 2009). Plant resources are more widely available and cost less than marine feed resources. There has been a strategy of increasing the fraction of plant products in formulated pelleted feeds in aquaculture (Olsen, 2011; Tacon *et al.*, 2011).

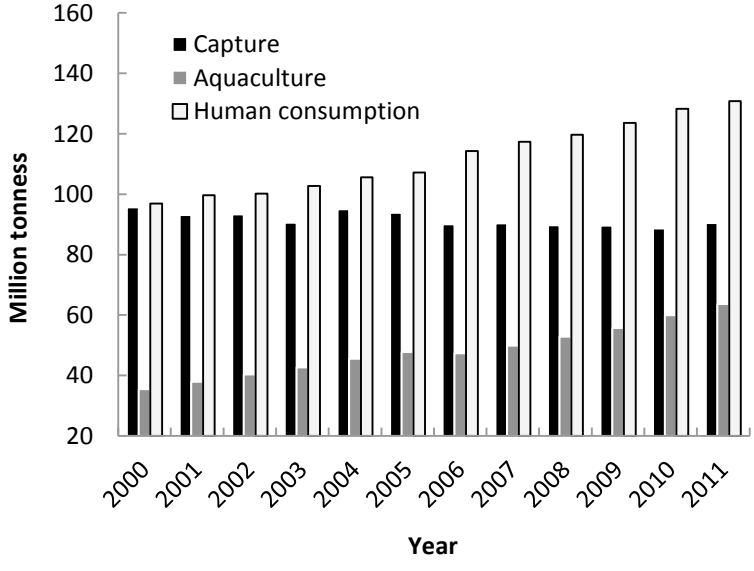

Figure 4. Capture, aquaculture production and human consumption of fish in the periods 2000-2011; Data taken from SOFIA (2012).

Aquaculture is the fastest-growing sector of animal production worldwide. In the last 10 years, aquaculture production has risen to 8.5 % annually while capture production has seen little change (Figure 4). Currently, fish and fish products for human consumption have risen up to 130.8 million tonnes and aquaculture covers roughly half of the supply (FAO, 2012). Thus, the requirement for FO and FM as traditional components of feedstuffs is steadily growing. Since aquaculture production continues to grow while the production of FM and FO is stagnating, the demand for them in aquaculture may outstrip supply within the next few years, and so has already done for fish oil (Tacon & Metian, 2008) (Figure 5).

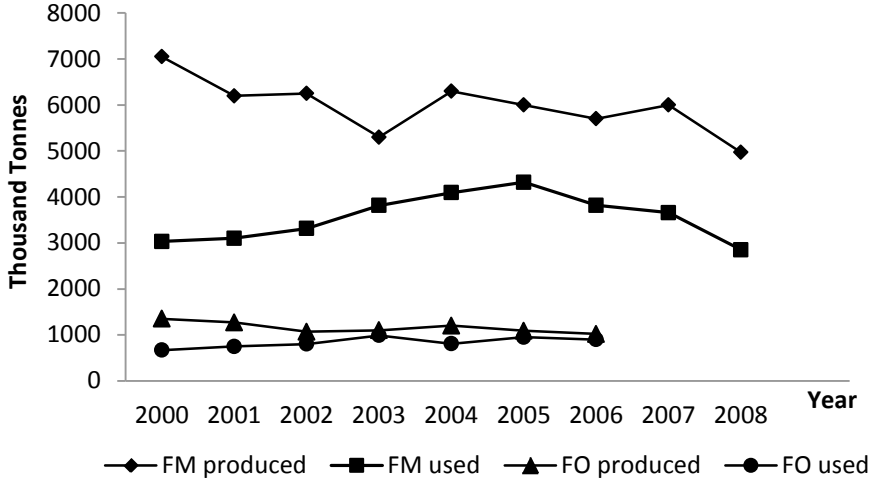

Figure 5. Total production and consumption of fish meal (FM) and fish oil (FO). Data taken from IFFO (2009).

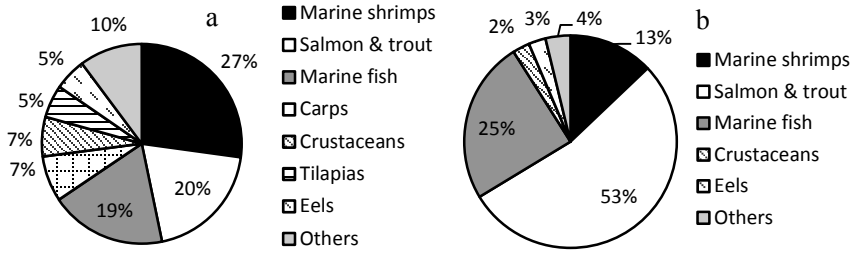

Figure 6. Usage of fish meal (a) and fish oil (b) in different species in aquaculture. Data taken from SOFIA (2012).

Most fish species farmed in European aquaculture are predatory fish species. Feeds for them traditionally contain FM and FO. Over half the entire production of FO is utilized in feeds for salmonids, mostly Atlantic salmon and rainbow trout, which represent only 4 % or so of entire fish production in global aquaculture (Figure 6).

By developing new feeding strategies, more than 50 % of the lipid source in feed can be replaced by vegetable oils (VO) such as rapeseed oil (RO) for FO during the main on-growth period (Bell *et al.*, 2003a) and today the commercial feeds contain up to 90 % VO. To ensure that n-3 LCPUFA content stays to the similar level compared to levels in wild fish, FO can be used in the finishing period (10-12 weeks) (Mráz *et al.*, 2012; Robin *et al.*, 2003).

FO is obtained as a by-product in the production of FM. Generally, 100 kg of fish (or by-products from fish processing, wet weight), produce 20-23 kg of FM and 5 kg of FO (Pike & Jackson, 2010). In recent years, around 25 million tonnes of fish per year are directly aimed at and used in the production of FM and FO, which represents about 25 % of captured fish worldwide (FAO, 2012). This leads to discussions about whether this fish should be used for direct human consumption instead, especially in developing countries (Tacon & Metian, 2008).

1.4 Non-fish feed ingredients

Plants, such as soy and other leguminosae are alternative feed sources, but their use in fish feed is hampered by some disadvantages. The low protein content, high level of carbohydrate and some anti-nutritional factors may cause problems in the digestion and uptake in fish, such as inflammation, protease inhibition and reduced fish growth (Francis *et al.*, 2001). Extrusion, fermentation and other processing techniques can be used to improve the nutritional value and quality of plant feed stuff. Plant protein from soybean and peas for example has been successfully tested in feeds for salmonid species (Brinker & Reiter, 2011; Carter & Hauler, 2000).

Today VO is the most abundant lipid source for aquaculture. However PUFA in VO, such as linseed, rapeseed, soy and hemp oil, is mainly carbon 18 PUFA such as ALA and LA. Although fish performances are not affected by replacing FO with a high level of VO, the n-3 LCPUFA portion in fish muscle decreases significantly, causing a decline to in the nutritional value of fish as human foods (Bell *et al.*, 2003b; Pettersson *et al.*, 2009a; Turchini *et al.*, 2009). A suggested way of improving the use of VO is to increase the conversion efficiency of ALA into n-3 LCPUFA by the synthesis pathway of n-3 FAs (see section 1.5.2).

Transgenic plants are also proposed as a possible source of n-3 LCPUFA (Robert, 2006). However their application in aquaculture and food is still a subject of intense debate given ethical issues concerning the environment and human health in most parts of the world, and Europe in particular (Myhr & Dalmo, 2005).

Another potential source for aquafeed is aquatic organisms further down the food chain, including aquatic invertebrates such as copepods, mussels, krill (*Euphausia superba*) and the byproducts of seafood. These sources contain a high portion of n-3 LCPUFA and protein and have an amino acid profile similar to FM. Some of them are also rich in carotenoids. Oil or meal produced from these sources can be used as FM or FO substitutes. Extracts from krill, salmonid viscera and calanoid copepod (*Calanus finmarchicus*) have been tested in different fish species (Olsen *et al.*, 2004; Suontama *et al.*, 2007; Turchini *et al.*, 2003). It should be noted that there is a significant difference between the catch quota for krill (8 million tonnes) and the present catch (0.2 million tonnes) (Nicol *et al.*, 2012). There is great potential in exploring this further, but the consequences of this on the catch and ecology should be closely monitored to avoid damage to the ecological system in the Southern Ocean (Kawaguchi & Nicol, 2007).

Micro-organisms might be the next source for aquafeed. Single-cell micro-organisms such as thraustochytrids, diatoms and microalgae are able to produce a high portion of n-3 LCPUFA with optimized strains and growth conditions. Oil extracted from thraustochytridea (*Schizochytrium sp.*) and microalgae (*Crypthecodinium cohnii*; *Phaeodactylum tricornutum*) have been tested in Atlantic salmon parr and juvenile seabream (*Sparus aurata*) which have demonstrated a strong capability for retaining n-3 LCPUFA in muscle (Atalah *et al.*, 2007; Ganuza *et al.*, 2008; Miller *et al.*, 2007) with this single cell origin. Micro-organisms are also a good protein source for fish feed. Protein accounts for a high proportion in the biomass of bacteria, yeast and microalgae. They could be used as substitutes for FM or as valuable additives in aquafeeds. Single-cell protein from some bacteria (*Bacterium glutamaticum*), zygomycete (*Rhizopus oryzae*) and wasted microalgae from nutraceutical production have been tested in salmonids (Ju *et al.*, 2012; Mydland *et al.*, 2007; Storebakken *et al.*, 2004). Additionally, micro-organisms are also a potential carotenoid source. Some species like green freshwater microalgae (*Haematococcus pluvialis*) and red yeast (*Phaffia rhodozyma*) can synthesize AST efficiently (Schmidt *et al.*, 2011; Wang *et al.*, 2013). Single-cell biomass is an interesting candidate for aquafeed as it provides n-3 LCPUFA-accompanying protein and carotenoids due to lowered processing costs as suggested by some studies (Miller *et al.*, 2008).

1.5 Fish FA metabolism

1.5.1 FAs in fish

FA is a carbon chain with a carboxyl group at one end and a methyl group at the other. Depending on the length of the carbon chain and the site and number of double bonds, it can be divided into saturated fatty acids (SFA) and unsaturated fatty acids. FAs with one double bond are called MUFA and those with two or more double bonds are called PUFA. Depending on whether the first double bond is at the third or sixth carbon counting from the methyl end, they are either n-3 PUFA or n-6 PUFA. PUFA with more than twenty carbons are considered LCPUFA.

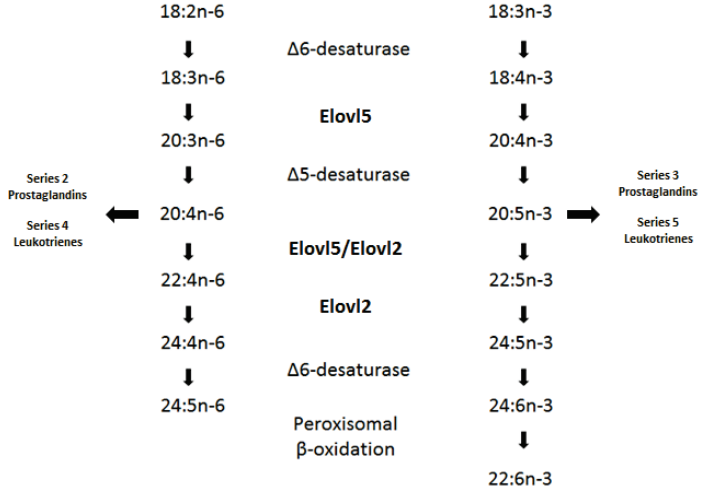

Figure 7. Elongation and desaturation pathway of n-6 and n-3 fatty acids and precursors of eicosanoids. Elovl, elongase of very long chain fatty acids. Adapted from Voss *et al.* (1991) and (Tocher, 2003).

FAs play important roles in the physiological activity of fish. They are an important energy source. FAs produce more than double the amount of energy as carbohydrate and protein with the same mass. Short-chain SFA and medium-chain MUFA such as 16:0, 18:1n-9, 20:1n-9 and 22:1n-9 are usually burned for energy production in salmonids through β-oxidation (Henderson & Sargent, 1984; Kiessling & Kiessling, 1993; Wanders *et al.*, 2001). FAs are also structural components of cell membrane lipids, such as phospholipids and glycolipids. Phospholipids in cell membranes generally contain high levels of

16:0, 18:1n-9, EPA and especially DHA (Henderson & Tocher, 1987). In addition, LCPUFA, mainly arachidonic acid (AA) and EPA are precursors of eicosanoid products such as prostaglandin, thromboxane, prostacyclin, leukotriene (Figure 7). These are mediators in physiological processes related to inflammatory and immunological responses and are involved in the reproductive function, hormone release and stress coping in fish (Stanley-Samuelson, 1994).

1.5.2 Elongation and desaturation

Fish are able to synthesize SFA (16:0 and 18:0) endogenously and go on to form MUFA (16:1n-9 and 18:1n-9) through Δ-9 desaturase (Sargent, 2002). However, like to other vertebrates, fish cannot add a second double bond and therefore cannot synthesize ALA and LA *de novo*. Thus, the two are essential fatty acids. In almost all fish, carbon 18 polyunsaturated fatty acids, ALA and LA can be elongated and desaturated into LCPUFA as EPA, DPA, DHA or AA, but to a limited degree (Voss *et al.*, 1991) (Figure 7). This provides a background and an approach for increasing n-3 LCPUFA in fish body. The biosynthesis process is regulated by desaturases and elongases, and occurs in the microsomal fraction of the liver except for the last step shortening 24:6n-3 to DHA through β-oxidation, which occurs in peroxisomes. Freshwater fish are considered to have a greater ability to convert shorter-chain PUFAs to LCPUFA than marine fish do (Sargent, 2002; Tocher, 2003). The effects in salmonids of VO rich in ALA have been tested and an enhanced conversion ability found in some cases (Bell *et al.*, 2001; Mourente *et al.*, 2005; Stubhaug *et al.*, 2005). However, the reduction in dietary n-3 LCPUFA in VO-based diets cannot generally be compensated by *in vivo* synthesis from the ALA.

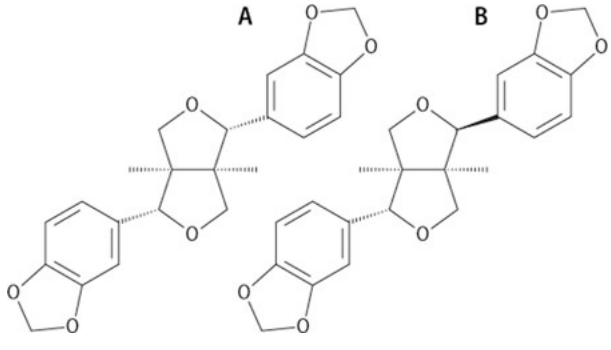

Figure 8. Structure of sesamin (A) and episesamin (B)

1.5.3 Effect sesamin on FA metabolism

Some bioactive compounds have been found to modulate fish FA metabolism to increase the conversion of ALA to n-3 LCPUFA. Sesamin is a lignan found in sesame seed and oil. It has significant effects on fatty acid metabolism, regulating FA oxidation and synthesis in humans and rats (Ide *et al.*, 2001; Jeng & Hou, 2005; Kushiro *et al.*, 2002). Trattner *et al.* (2008a) found that sesamin significantly enhanced the conversion of ALA to n-3 LCPUFA and the DHA level in rainbow trout was increased by 37 %. In another *in vitro* study with Atlantic salmon hepatocytes, sesamin/episesamin exposure led to increased elongation and desaturation of ALA to DHA (Trattner *et al.*, 2008b). Some genes related to lipid metabolism including peroxisome proliferator-activated receptor α (PPARα), Acyl-CoA oxidase (ACO) and carnitine palmitoyl transferase I (CPT1), scavenger receptor (SRB) type B, and delta-6 fatty acid desaturase (Δ6 fad) were also regulated significantly. Alhazzaa *et al.* (2012) also found an increased level of n-3 LCPUFA in total lipids in the whole body by up to 25 % in juvenile teleost (*Lates calcarifer*) fed with sesamin addition to feed containing ALA. The effect of sesamin on lipid modulation is enabled through the activation of the PPAR system and the sterol regulatory element binding protein (SREBP) (Ashakumary *et al.*, 1999; Ide *et al.*, 2004). Sesamin is able to regulate the activity and mRNA level of enzymes involved in β-oxidation, lipogenesis and desaturation (Ide *et al.*, 2003).

1.6 Carotenoids in fish

1.6.1 Carotenoids and colour properties

Colour is a factor that influences the acceptability of food to consumers. For salmonids, the redness of white muscle is considered to be an important criterion of their quality (Baker & Günther, 2004; Choubert, 2010). This pink colour is given by the carotenoids deposited in white muscle (Bjerkeng, 2000). AST is the most important carotenoid in salmonids (Choubert *et al.*, 2009). The system of the Commission Internationale de Éclairage, which includes the L*, a*, and b* values is often used to evaluate the colour properties. In this system, a* value represents redness, b* value reflects yellowness and L* value suggests lightness. A transformed system considered to be more accurate for colour properties measuring L*, C* and H values is also applied. Carotenoid content shows strong correlations with these parameters. Linear or logarithmic relationships between a*, b* and C* values and AST content as well as a negatively linear or logarithmic correlation between L* and H values and AST content have been found (Bjerkeng, 2000; Christiansen *et al.*, 1995; Teimouri *et al.*, 2013).

1.6.2 Carotenoid sources for fish

AST plays an important role in sexual mutuality, egg survival, embryo development, alleviation of oxidative stress and the immune system in fish (Higuera-Ciapara *et al.*, 2006). Fish cannot synthesize carotenoids *de novo* and have to obtain these via feed (Caballo *et al.*, 2012). Synthesized AST (Carophyll Pink, F.Hoffmann-La Roche Ltd., Switzerland) is the most widely used carotenoid for farmed salmonids. The growing interest in natural food and the high cost of this compound have driven the aquaculture industry to seek alternatives. AST extracted from microorganism such as red yeast (Schmidt *et al.*, 2011), green algae (Wang *et al.*, 2013) and aquatic organism rich in these pigments such as crustaceans (López *et al.*, 2004), shrimp shell (Franco-Zavaleta *et al.*, 2010) and krill (Arai *et al.*, 1987) have been tested.

1.6.3 Carotenoid metabolism and utilization in fish

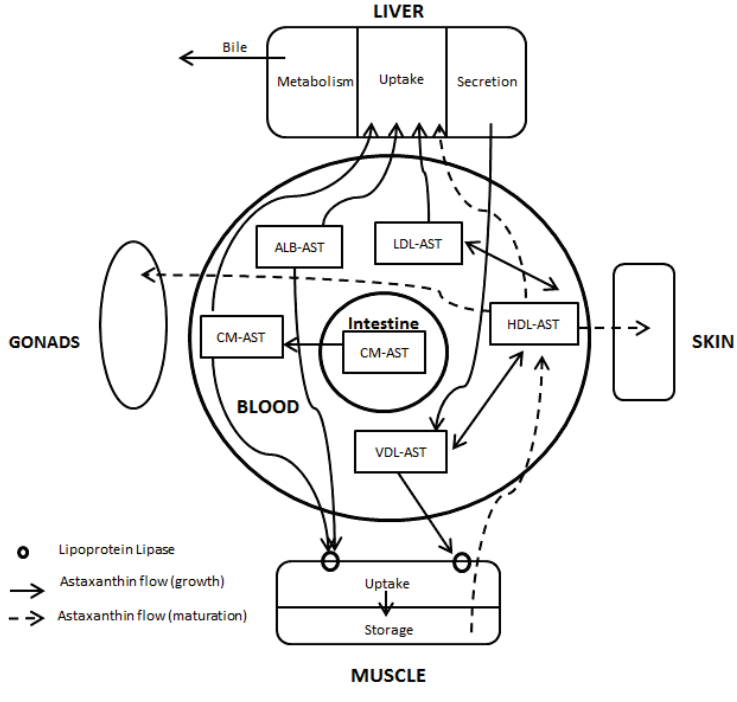

Figure 9. Astaxanthin uptake, transport and deposition. Astaxanthin (AST) is taken from the intestine, transported in the blood and deposited in muscle by the various lipoproteins (ALB, albumin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein); CM, chylomicron; CMR, chylomicron remnant. During sexual maturation/spawning migration (dotted lines), AST is redistributed from the muscle to the skin and gonads by HDL. Adapted from Rajasingh *et al.* (2006).

The processes of regulating carotenoid absorption and metabolism in fish are still not completely clear. For AST, it is generally taken from the intestine as micellar, incorporated into chylomicrons with triacylglycerol, transported in blood, and deposited in muscle by the various lipoproteins (Rajasingh *et al.*, 2006) (Figure 9). Liver also metabolizes AST, resulting in degradation and finally loss of AST (Page & Davies, 2003).

The efficiency of AST utilization in salmonids is low (the retention rate is usually below 15 %) (Rørvik *et al.*, 2010). Its utilization depends on fish species, physiological status, dietary composition, pigment source and environmental conditions (Torrissen *et al.*, 1989). There are differences in the efficiency of pigment retention among Atlantic salmon, rainbow trout and Arctic charr (Chimsung *et al.*, 2012). Small fish are reported to have a lower ability for AST utilization, probably due to the plateau level in the pigment capacity (Skrede & Storebakken, 1986). Fish in low-temperature water may have higher AST absorption than fish living in high-temperature aquatic areas (Olsen & Mortensen, 1997). During sexual maturation, AST is redistributed to the skin and gonads and the AST level in muscles then decreases (Bjerkeng *et al.*, 1992).

AST source and dietary composition, especially lipid content and FA profile, affect AST absorption. A high lipid level and PUFA in feed contribute to better utilization of AST (Bjerkeng *et al.*, 1999). For matrix like krill oil and algae oil, AST occurs in ester form. It needs to be hydrolyzed into free AST and incorporated into micelles and then absorbed into the intestine, before being transported to the liver and deposited in muscle (Rørvik *et al.*, 2010). Intestinal hydrolysis or cleavage is a limiting step for the absorption of AST (White *et al.*, 2003). A lower retention rate of AST is found when salmonids are fed AST ester compared with free AST (Albrektsen *et al.*, 2006; Hynes *et al.*, 2009). AST has several isomers and this also influences utilization. *Trans* one is considered to have higher apparent digestibility than *cis* isomer (Bjerkeng *et al.*, 1997). For yeast and algae, their cell wall may inhibit the digestibility of AST, and cell disruption enhances absorption (Barbosa *et al.*, 1999; Bjerkeng, 2000). Work on increasing the bioavailability of AST from these substitutes in fish feeds need to be continued to increase AST retention.

1.7 Oxidation of foods containing polyunsaturated fatty acids

Due to the positive health effects of n-3 LCPUFA in humans, there is increasing interest in producing n-3 LCPUFA-enriched food. However, n-3 LCPUFA is susceptible to oxidation, forming secondary oxidation products such as aldehydes, ketones, epoxidesand volatile organic acids. These

oxidation products reduce flavour (Kamal-Eldin & Pickova, 2008) and some are harmful to human health (Ames *et al.*, 1993). They may also start reactions with some amino acids to form carbonyls and protein aggregates, resulting in a deterioration in texture (Uchida & Stadtman, 1993). The colour of muscle is also compromised during the oxidation period (Ruff *et al.*, 2002; Scaife *et al.*, 2000). In summary, lipid oxidation in fish muscle may result in a reduction of sensory properties, a restriction of storage possibilities and nutritional losses. It is therefore necessary to maintain a balance between n-3 LCPUFA fortification in food for human nutrition and food product stability.

Antioxidants such as tocopherol, ascorbic acid, glutathione, and plant extracts containing substances such as phenols and anthocyanins have been applied to improve lipid stability in animal food. They can be included in the animal órigin food products matrix post mortem during processing. Many of these antioxidant compounds are suitable to add to animal feeds. The latter approach is common in aquaculture. Tocopherol is the most used antioxidant. It demonstrates an excellent ability to inhibit lipid oxidation, protecting the sensory properties such as taste and colour of white muscle of Atlantic salmon (Harare *et al.*, 1998; Sigurgisladottir *et al.*, 1994), and rainbow trout (Frigg *et al.*, 1990; Jittinandana *et al.*, 2006). Thiobarbituric reactive substances (TBARS) value determines the secondary oxidation product, which is malondialdehyd formed from PUFA. It is widely used to reflect the oxidation status in the food matrix (Botsoglou *et al.*, 1994).

1.8 CYP 450 in fish

Dietary components affect various physiological processes including detoxification ability of fish. Cytochrome P450 (CYP450) which plays an important role in the biotransformation of endogenous and exogenous compounds can be influenced by diet. Activities of some CYP450 isoforms can be changed by dietary manipulations (Trattner *et al.*, 2008a; Wagner *et al.*, 2012). Thus it is helpful to include the analysis of CYP450 activities in tests of new dietary ingredients to obtain better understanding of their effects.

CYP1A is inducible by aryl hydrocarbon receptor agonists and the activity measured as ethoxyresorufin O-deethylase (EROD) is used as a biomarker for exposure to xenobiotic compounds, such as dioxins, furanes, polychlorinated biphenyls and polyaromatic hydrocarbons in fish (Sarasquete & Segner, 2000). It has been reported that EROD was induced by sesamin in rainbow trout liver (Trattner *et al.*, 2008a). CYP2E1 is involved in the metabolism of ethanol, acetaminophen, carbon tetrachloride, N-nitroso dimethylamine and other low-molecular weight toxicants (Gonzalez, 1988). Activity of this isoform is often

measured as the rate of hydroxylation of p-nitrophenol (PNPH) to 4-nitrocatechol. Its activity in fish liver has been reported in several species, implying the possibility of its involvement in the metabolism of these compounds (Wagner *et al.*, 2012; Zamaratskaia & Zlabek, 2011).

2 Objectives

The overall aim of this thesis was to evaluate the use of non-fish-based raw materials on the fish muscle quality of salmonids. The goal is to maintain the lipid quality and colour property of farmed fish while decreasing the use of FO and FM in feeds. The effect of sesamin on the FA profile is of great interest; the effects of some non-fish-based materials on FA profile, AST content and colour property are also of interest, while the influences of these materials on fish performance are also attracting attention.

The specific objectives were:

to study the effects of sesamin together with different vegetable oils (LO, purified LO, MO) on the FA profile in Baltic Atlantic salmon and rainbow trout (paper I, II)

to study the effects of KM, MM, ZM and KO on the FA profile (paper III, IV) in Arctic charr

to study the effects of KM, MM and KO on carotenoids and colour properties in Arctic charr (paper IV)

to study the effects of sesamin, MM and ZM on CYP content, EROD and PNPH in Baltic Atlantic salmon and Arctic charr (paper I, III)

to study the effects of the FA profile and AST on the oxidation property of fish muscle (paper IV)

3 Materials and methods

This section contains a brief description of the materials and methods used in the studies. For a more detailed description of each method, see Papers I-IV. A summary of the materials and methods is presented in Table 3.

3.1 Study design

3.1.1 Study I

Atlantic salmon juveniles (n=15) were fed six different diets. Four groups were fed diets based on a mixture of linseed:sunflower oil (MO, 6:4, v/v) supplemented with a sesamin/episesamin mixture (S, 1:1, w/w) at different levels (MO, MO + S 0.29 g 100 g^{-1}, MO + S 0.58 g 100 g^{-1}, MO + S 1.16 g 100 g^{-1}), while one group was fed a diet based on FO and another group was fed a diet based on a mixture of sesame oil:linseed oil (SesO, 1:1, v/v). Fish were tagged and fed at a water temperature of 10 ºC for 77 days. Initial weight and length and final weight and length were recorded and the daily growth coefficient (DGC) was calculated. White muscle and liver were sampled. An analysis was performed of the FA profile in white muscle, tocopherol and sesamin in white muscle and liver, and the total content of CYP, EROD activity and gene expression in the liver.

3.1.2 Study II

Rainbow trout (initial weight 36.5 g, n=18) were fed six diets in duplicate based on three vegetable oils (linseed oil, LO; purified linseed oil triacylglycerol fraction, TAG; a linseed oil and sunflower oil mixture 6:4 v/v, MO) with or without a sesamin supplement (0.58 g 100 $^{-1}$ g). One group fed the fish oil diet was used as a control group. Fish were tagged and fed at a water temperature of 14.5 ºC for 58 days. Initial weight and length and final weight and length were recorded, and DGC was calculated. White muscle and liver were harvested. The former was used for analysis of FA profile, tocopherol, sesamin

content and gene expression. Liver was only used for the gene expression analysis.

Table 3. *Summary of the study designs in Papers I-IV*

Study	I	II	III-Trial 1	III-Trial 2	IV
Species	Atlantic salmon	Rainbow trout	A. charr	A. charr	A. charr
size	Juvenile	36.5 g	30.5 g	105 g	100 g
Trail length	11 weeks	58 days	24 weeks	4 weeks	15 weeks
Treatment	FO	FO	CD	FM	RO + meals (6 groups)
	SesO	LO	ED	MM	FKO + meals (4 groups)
	MO	LO + S		ZM	MO + meals (2 groups)
	MO + S 0.29	TAG			Standard (1 group)
	MO + S 0.58	TAG + S			
	MO + S 1.16	MO			
		MO + S			
Sample	White muscle	White muscle	White muscle		White muscle
	Liver	Liver	Liver	Liver	
Analysis	Fish performance	Fish performance	Fish performance	Fish performance	Fish performance
	Lipid content	Lipid content	Lipid content		Lipid content
	Fatty acids	Fatty acids	Fatty acids		Fatty acids
	Sesamin, tocopherol	Sesamin, tocopherol	Sesamin		Carotenoids
	CYP, EROD		EROD, PNPH	EROD, PNPH	L*, a*, b*, C*, H*
	Gene expression	Gene expression			TBARS

Abbreviations: FO, fish oil; FM, fish meal; CD, commercial diet; ED, experimental diet; SesO, sesame oil+linseed oil; LO, linseed oil; MM, mussel meal; MO; linseed oil+sunflower oil; ZM, zygomycete meal; S, sesamin; TAG, purified linseed oil triacylglycerol fraction; CYP, cytochrome P450; EROD, ethoxyresorufin O-deethylase; PNPH, hydroxylation of p-nitrophenol to 4-nitrocatechol; TBARS, thiobarbituric reactive substances.

3.1.3 Study III

In Trial 1, Arctic. charr (*Salvelinus alpinus*) (initial average weight 30.5 g, n=20) were fed an experimental diet (ED) with the protein source composed of fish meal (FM), mussel meal (MM) and zygomycete meal (ZM) and lipid sources composed of FO, rapeseed oil (RO) and sesame oil (SO) in triplicate.

A commercial diet (CD) with similar lipid and protein content (Skretting Nutra Parr 2 mm, Stavanger, Norway) was fed as a control. Fish were fed at a water temperature of 4 ± 1 °C for 24 weeks. Initial weight and length and final weight and length were recorded and DGC was calculated. White muscle and liver were harvested. White muscle was used for the FA profile and sesamin analysis, and liver was used for the EROD and PNPH activity analysis.

In Trial 2, three diets with FM, ZM, MM respectively as the main protein source were fed to A. charr (initial average weight 105 g, n=20) at a water temperature of 6 ± 1 °C for 4 weeks in triplicate. Liver was taken for EROD and PNPH activity analysis.

3.1.4 Study IV

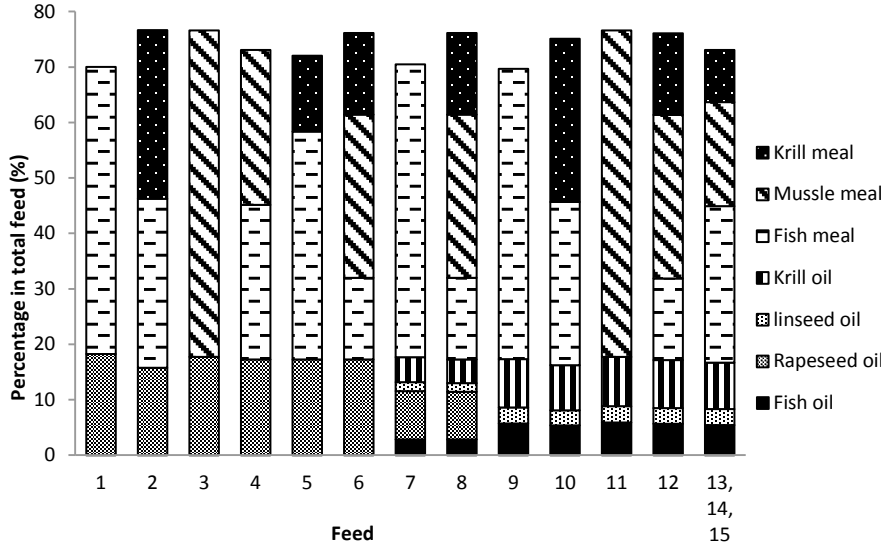

Figure 10. Lipid source and protein source of feeds in Paper IV

Arctic. charr (initial weight 100 g, n=12) were fed 13 experimental diets based on different oil sources (RO, krill oil (KO), FO, LO) and protein sources (krill meal (KM), MM, FM) for 15 weeks. In order to obtain an optimal response, a 13-type star model was used, while the centre-point was the standard reference in the experiment. Six feeds included RO as the lipid source (RO groups), four feeds used FO, KO and LO as the lipid source (FKO groups), two feeds mixed FO, KO with RO and LO as the lipid source (MO groups) and one feed included all these materials tagged as standard (Figure 10). Twelve feeds were fed to 12 tanks of fish, while the standard feed was fed to three tanks of fish.

The water temperature was 5-17°C during the rearing period. Initial length and weight and final length and weight were recorded, and average DGC was calculated. Nine fish were sampled from each tank, and white muscle was deselected and used for analysis of FA, carotenoid, colour and TBARS.

3.2 Lipid extraction and fatty acid composition analysis

In Papers I, II, III and IV, lipids of muscle and feed were extracted in hexane:isopropanol following the method of Hara and Radin (1978). Lipids in Papers I, II and III were separated into PL and TAG fractions by thin layer chromatography (TLC). Total lipids of feeds and white muscle in Paper IV and PL and TAG in Papers I, II, III were methylated with boron trifluoride according to Appelqvist (1968), and then analyzed using a gas chromatograph system (GC).

3.3 Sesamin and tocopherol analysis

For sesamin and tocopherol analysis, the lipid extracts of tissues and diets were dissolved in hexane and analyzed by HPLC (Moazzami & Kamal-Eldin, 2006). Separation was performed on a silica column using hexane/1, 4-dioxane (94:6 v/v) as mobile phase and detection was achieved by fluorescence (excitation wavelength 296 nm and emission wavelength 324 nm).

3.4 CYP450 content, EROD activity and PNPH activity analysis

In Paper I liver microsomes was prepared for analysis while in Paper III liver S9 was prepared. CYP values were measured by the co-difference method (Omura & Sato, 1964). Hepatic EROD activity and hydroxylation of PNPH activity was determined according to a modified method from Zamaratskaia and Zlabek (2009) and Zamaratskaia and Zlabek (2011). The protein contents of the microsomes or S9 were assayed by the method of Smith *et al.* (1985).

3.5 Gene expression analysis

Total RNA was purified using an RNA isolation kit (Trizol® used in Paper I, Z3105 Promega used in Paper II), followed by DNase treatment. RNA quality and quantity were determined spectrophotometrically ($A_{260/280}$). The cDNA was synthesised using the TaqMan Reverse Transcription Reagent kit (Paper I) or High-Capacity cDNA Archive kit (Paper II). Real-time PCR was performed with SYBR® Green PCR Mastermix (Paper I) or Power SYBR Green PCR

Master Mix (Paper II) using gene-specific primers. Standard curves were made for each primer pair and efficiency (E) was calculated as $E=10^{(-1/slope)}$.

3.6 Colour properties analysis

The L*, a*, b* C* and H values of white muscle were measured using a Minolta Chroma Meter CR-300. The Chroma Meter was equipped with an 8 mm diameter aperture and calibrated on a white reference plate. Measurements were processed at four points of the fillet.

3.7 Carotenoid analysis

3.7.1 Total carotenoid analysis

The lipid extracts were used for carotenoids analysis. The total carotenoid content (TC) of muscle and diet was analyzed by the spectrophotometric method as Tolasa *et al.* (2005). TC was expressed as mg/kg muscle (or feed). The average retention rate of total carotenoids (RTC) was calculated.

3.7.2 Specific carotenoid analysis

Specific carotenoid was analyzed suing a HPLC with UV detector (480 nm). Separation was performed with a normal phase column using isocratic mobile phase, hexane:isopropanol (94:6 v/v). AST, canthaxanthin and lutein were used as standards. Carotenoid content was expressed as mg/kg.

3.8 TBARS analysis

TBARS was determined by a forced oxidation method with modification as ikn Sigurgisladottir *et al.* (1994). Fish muscle was heated at 90 °C for 5 min to accelerate oxidation. Trichloroacetic acid was used to extract malonaldehyde. Thiobarbituric acid was used to generate pink, and absorbance at 532 nm was measured. TBARS value was expressed as nmol/kg muscle.

3.9 Statistical analysis

Data from the biochemical analyses in all papers are presented as mean values ± standard deviation. In Paper I and II, the General Linear Model (GLM) was used to compare the physiological responses to the different diets in Statistical Analysis System (SAS) 9.3 for Windows. In Paper III, data in Trial 1 was analyzed by student t-test while data in Trail 2 was analyzed by GLM in SAS. In Paper IV, data was firstly analyzed in the first instance by partial least

squares-discriminant analysis (PLS-DA) in SIMCA-P+13.0. To evaluate the effects of different components, three comparisons including different groups were made by using different statistical methods: the *Bonferroni test* (comparison 1), *Scheffe test* (comparison 2) and *Duncan test* (comparison 3). Differences were considered as significant if $P < 0.05$.

4 Summary of results

4.1 Study I

No significant differences in fish growth were found between the groups. Compared with the FO group, all MO groups showed lower levels of EPA and DHA both in phospholipids and triacylglycerols. Sesamin decreased the levels of ALA in white muscle phospholipids of all the groups fed sesamin ($P < 0.05$) and slightly increased the levels of DHA in some of the sesamin-fed groups. Compared with the MO group, sesamin significantly upregulated the expression of PPARα in the MO 1.16 group, SRB and HSL in MO 0.29 and MO 0.58 group. Total cytochrome P450 enzymes were higher in MO 0.29 ($P = 0.02$) and slightly higher in MO 1.16 ($P = 0.07$) compared to MO. Sesamin content in liver differed with changes in the dose in feed, while its content in white muscle is similar in all the sesamin-fed groups. The amounts of α- and γ-tocopherols in liver and the amounts of γ-tocopherol in white muscle were significantly lower in fish fed the FO diet compared to the MO diet ($P < 0.05$).

4.2 Study II

No significant differences in fish growth parameters were found in the six groups. Compared to the FO group, the FA profile changed from a high content of n-3 LCPUFA (EPA and DHA) towards a high content of ALA, with a decreased ratio of n-3/n-6 in all the vegetable oil-fed groups ($P < 0.05$). Although no significant increase in EPA or DHA was observed, sesamin in the LO diet significantly decreased the ALA level in triacylglycerols ($P < 0.05$), and the EPA level in sesamin-fed groups was slightly higher than their counterpart without sesamin. Compared to the LO group, the stripped-LO group (TAG group) showed higher levels of EPA, lower levels of ALA in triacylglycerol and an increased DPA level in phospholipid. In liver, PPARβ1A and ACO were upregulated ($P < 0.05$) in all the groups compared to the FO

group. The addition of sesamin upregulated PPARβ1A, CPT1 and ACO in the LO+S group; downregulated PPARβ1A and upregulated Δ6 FAD in TAG+S group; and downregulated PPARα, γ and ACO, and upregulated Δ6 FAD in the MO+S group. In white muscle, sesamin downregulated most genes in the LO+S and TAG+S groups, but upregulated CPT1, and the decreases of gene expression in the TAG+S group were more pronounced than in the LO+S group. Only PPARβ1A was upregulated in the MO+S group. Similar levels of sesamin and tocopherol was found in all the sesamin-fed groups. The MO group showed a lower level of γ-tocopherol than the LO and TAG groups ($P < 0.05$).

4.3 Study III

In Trial 1, no difference in fish growth was observed in the two groups. ED decreased the level of EPA and DHA in TAG fraction ($P < 0.05$). In PL, ED also decreased the EPA level ($P < 0.05$), but increased the DHA portion ($P < 0.05$). Sesamin was detected in ED (≈ 6400 mg/kg) and white muscle of the ED group (21 mg/kg). Increased activity of CYP1A and CYP 2E1 in liver were observed in the ED group. In Trial 2, the ZM group exhibited higher EROD activity than the FM and MM groups, and the PNPH activity in the MM and ZM groups was higher than that in the FM group ($P < 0.05$ or $P < 0.01$).

4.4 Study IV

The FA profile of the RO groups (groups 1-6) was characterised by MUFA, ALA and LA, while FKO groups (groups 9-12) mainly had SFA, 16:1n-7 and EPA with a high n-3/n-6 ratio. The ALA level was lower in the FKO groups than in the RO groups. In the RO groups, the FA profile of group 2 and group 6 were mainly composed of SFA, EPA, DPA and 18:1n-7. However, group 4 and group 1 contained a high level of ALA, LA, 18:3n-6, 20:3n-6, 18:1n-9 and 20:1n-9. Group 1 showed comparable levels of AA, EPA and DHA compared with the other RO groups, although feed 1 contained lower levels of these FAs than the other feeds. In the FKO groups, the FA profile of group 9 was characterised by MUFA, n-6 PUFA and ALA. In contrast, group 10 showed an FA profile rich in EPA, DPA and DHA with a high n-3/n-6 ratio. Groups 7 and 9 had higher a*, b*, C* and TC values and a lower H value than group 1. Group 1 exhibited lower a*, b*, C* and TC values than groups 2 and 4. A lower a* and TC value and higher H value were observed in group 4 than in group 2. Group 10 had the highest AST content and a*, b* and C* values in the FKO groups. The average retention rate of AST in all the groups is 1.32 %-

5.08 % and the average AST content is < 3.5 mg/kg. The TBARS value of groups 2 and 10 was higher than that of other groups in comparisons 2 and 3.

5 General discussion

5.1 Fish growth

The results from Papers I and II coincided with the findings from the study of Mráz *et al.* (2010) on carp and the study of Trattner *et al.* (2008a) on rainbow trout, in which no effect of sesamin on fish growth was found. However, a recent study on *Lates calcarifer* reported that sesamin inhibited fish growth in early juveniles (Alhazzaa *et al.*, 2012). Thus, fish size could be one reason for the different effects. Alhazzaa *et al.* (2012) hypothesised that sesamin may increase the activity of enzymes involved in FA oxidation and decrease the enzymes involved in lipogenesis, leading to problems in energy balance and thus slowing growth. It is possible that the fish in the early lifecycle are more sensitive to dietary intervention and their metabolism is more easily affected than larger fish. Another study on Atlantic salmon found reduced growth with a high dose of sesamin in feed (5.8 g/kg), but no distinct effect with low dose sesamin (1.16 g/kg) (Schiller Vestergren *et al.*, 2012).

In Paper III, mycelium meal of a filamentous fungus, *Rhizopus oryzae,* was included as a protein source in experimental feed for fish and had no effect on fish growth. Previous studies on the utilisation of micro-organisms such as yeast meal in aquafeed show limited utilisation due to the deficiency of amino acids, high carbohydrate content and low digestibility caused by cell structure (Olvera-Novoa *et al.*, 2002; Rumsey *et al.*, 1992). Growth reduction has been reported in the fish fed feed containing yeast meal as the sole or main protein source (Perera *et al.*, 1995; Storebakken *et al.*, 2004). However, it is suggested that by altering microbe production or process conditions, a suitable composition of micro-organisms can be achieved for different fish species (Kiessling, 2009). The fungal biomass used in the present study has balanced nutrient composition, particularly the amino acid profile is close to that of FM, which makes it suitable as a fish feed ingredient (Thorarinsdottir *et al.*, 2011).

In the Baltic Sea area, blue mussels (*Mytilus edulis*) are filtrating the excessive algal growth caused by the eutrophication from surrounding agricultural production (Jönsson & Elwinger, 2009). These mussels are of small size and of no or low interest for human consumption. They can be used as organic feedstuff replacing FM in feed for poultry or fish or as a fertiliser. MM contains a high portion of protein with a similar amino acid pattern to FM and a certain amount of lipids (≈10%) with a beneficial FA profile. It is considered to be a high-quality protein and lipid source for animal feed. However, Berge and Austreng (1989) have reported that there was a tendency towards poorer growth with an increased level of MM in the diet for rainbow trout due to the low energy density of the diet caused by its high ash content contributed by the shell. MM used in Papers III and IV was de-shelled with no negative ash content and thereby of higher nutritional quality.

In Paper IV, KM and KO were used in the feed for Arctic charr. No significant difference on fish growth was found, which is in agreement with previous studies (Julshamn *et al.*, 2004; Tibbetts *et al.*, 2011; Yoshitomi *et al.*, 2007). However, some studies found a reduced growth rate and weight when KM was included in fish feed (Hansen *et al.*, 2010; Rungruangsak-Torrissen, 2007). The negative effect on growth was mainly caused by fluoride accumulated in the exoskeleton of krill (Yoshitomi & Nagano, 2012). In the KM and KO used in this study, most of the fluoride was removed by purification. The removal of fluoride in krill-based raw material may be one possible way of avoiding negative effects on fish growth.

5.2 Fatty acids

When fish are fed vegetable oils, the decrease in n-3 LCPUFA reduces the nutritional value of fish with regard to consumers, which is a common disadvantage of VO usage in fish feed (Bell *et al.*, 2003b; Caballero *et al.*, 2002). The results in Papers I, II and IV confirm this conclusion. The response to the dietary FA is usually more pronounced in triacylglycerols than in phospholipids, which is in agreement with previous studies (Bell *et al.*, 2001; Pettersson, 2010; Pettersson *et al.*, 2009b; Torstensen *et al.*, 2004). It is known that fish can maintain a homeostatic FA profile in their bodies to maintain certain physiological functions. In phospholipids, EPA and DHA are essential components, playing an important role in physiological functions, thus their homeostasis becomes a priority (Turchini *et al.*, 2009). However, triacylglycerols mainly function as storage lipids and their profile is more flexible and easier to modify through feed (Olsen & Henderson, 1997).

The fatty acid profile of fish muscle can be altered by adding bioactive compounds such as the sesame seed lignan sesamin. It is suggested that by activating PPARs and inhibiting SREP, sesamin could affect a wide range of enzymes involved in desaturation and β-oxidation both at activity and mRNA levels (Ashakumary *et al.*, 1999; Ide *et al.*, 2004). PPARs are expressed in tissues, *e.g.* liver and adipose, and could induce genes involved in lipid transport, oxidation and thermogenesis such as CPT1 and ACO (Clarke, 2001). Gene expression analysis in the present studies showed that sesamin regulated the gene expression of PPARα, PPARβ1A, PPARγ, Δ5 and Δ6 desaturase, CPT1 and ACO and resulted in an increase of DPA and DHA in liver (Table 4) (Paper I). In Paper II, the LO+S group had a decreased ALA level and slightly increased EPA level in triacylglycerols, showing upregulation of PPARβ1A, CPT1 and ACO in liver (Table 4). It is suggested that sesamin activated PPARα and PPARβ1A, and they regulated the other genes involved in β-oxidation or elongation and desaturation, leading to the increase in EPA, DPA or DHA. Trattner *et al.* (2008b) also found an increased β-oxidation product and upregulated CPT1 with an increased DHA level.

Table 4. *Changes in some fatty acids and gene expressions regulated by sesamin compared to their sesamin-free groups in some studies.*

Study	**Paper I**	**Paper II**		**Trattner *et al.* (2008b)**
Group	**MO1.16**	**LO+S**	**MO+S**	**Sesamin**
PPARα	↑		↓	
PPARβ1A		↑		
PPARγ			↓	↓
CPT1		↑		↑
ACO		↑	↓	
Δ5 desaturase				↓
Δ6 desaturase			↑	↓
ALA	↓	↓	↓	↓
EPA		↑	↑	
DPA	↑			
DHA	↑			↑

↑, upregulated; ↓, downregulated. Abbreviations: LO, linseed oil; MO, linseed oil+sunflower oil; S, sesamin; PPAR, Peroxisome proliferator activated receptor; CPT1, Carnitine palmitoyltransferase; ACO, Acyl-CoA oxidase; ALA, α-linolenic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid.

However, the gene expression level is not always directly related to a corresponding increase in n-3 LCPUFA. In Paper II, downregulation of PPARα, PPAEγ and ACO in liver were observed with a slight decrease in ALA

and increase in EPA in MO+S group (Table 4). In the studies by Trattner *et al.* (2008b), downregulated PPARα and Δ5 and Δ6 desaturase were also found with an increased level of DHA (Table 4). Thus the gene expression cannot explain all the effects on the FA profile. Further studies focusing on the mechanism of the sesamin effect on FA metabolism at a molecular level, including gene expression and proteome expression, need to be continued.

In Paper II the effect was studied of LO and stripped-LO (minor polar compounds removed) on the role of sesamin. It was concluded that the stripped-LO group did not have a major influence on the sesamin's effect on FAs. However, differences in the expression of some genes were found between the TAG+S and LO+S groups. These results indicate that the removed polar compounds in LO could have some effect on the metabolism of PUFA and may affect the role of sesamin. However, no further conclusions can be drawn from the results.

Krill is as a known source of n-3 LCPUFA. Its positive effect on n-3 LCPUFA has been reported in previous studies (Roncarati *et al.*, 2011; Suontama *et al.*, 2007). Results in Paper IV confirm that KO and KM improved the FA profile towards more desirable for human dietary purpose. The results also reveal that KM had a more pronounced effect on preserving n-3 LCPUFA than MM.

In Paper IV, the AA level was higher in the fish muscle of group 1 than in groups 7 and 9, but its level in feed 1 was lower and the LA level was higher than those in feeds 7 and 9. This result contradict the finding of Pettersson *et al.* (2009b) where no concomitant increase of AA was observed with an increasing LA portion in feed. Meanwhile, the ALA level was higher in group 1 than in groups 7 and 9, and the level of EPA and DHA was similar. EPA and DHA proportions were lower in feed 1 than in feeds 7 and 9. Since EPA, DHA and AA are important FAs for fish (Bell & Sargent, 2003; Pettersson *et al.*, 2009b), it is hypothesised that Arctic charr synthesised them from the substrates (LA and ALA). Since there was a much higher level of LA than ALA in feed 1, the elongation and desaturation of LA led to the increase of AA and concomitantly to an accumulation of ALA. This hypothesis questions the conclusion given by Tocher *et al.* (2001) that Arctic charr has a weak ability in the desaturation and elongation of carbon 18 FAs to AA, EPA and DHA. In addition, ALA in groups 7 and 9 may mainly be catabolised by β-oxidation, but not converted into n-3 LCPUFA due to the sufficient amount of n-3 LCPUFA in feeds 7 and 9.

The high conversion rate of carbon 18 FAs to long-chain AA and DHA in group 1 could partly be explained by the deficiency of AST in the feed. Bell *et al.* (2000) found that dietary deficiency of vitamin E and AST increased the

recovery of desaturated and elongated products of ALA and EPA in isolated hepatocytes from Atlantic salmon, and the absence of AST had a greater stimulatory effect on the conversion of EPA to DHA than did the absence of vitamin E. An increase in AA and DHA was also observed in the liver total lipids of African catfish (*Clarias gariepinus*) fed feeds containing oxidised FO with vitamin E compared with fish fed oxidised FO without vitamin E (Baker & Davies, 1996). In this study, feeds 6, 7, 8, 9 included KO or KM and these contributed a certain amount of AST to the feeds (> 50 mg/kg). However, feed 1 had no source of carotenoids (< 10 mg/kg). Thus it is possible that the deficiency of AST stimulated the conversion of carbon 18 FA to long-chain FA. A further study focusing on the effect of AST on elongation and desaturation is of interest.

5.3 Carotenoid and colour properties

In Paper IV, a clear distinction between the RO, MO and FKO groups was found. The results indicate that RO replacement decreased redness and TC, while KO contributed positive effects with regard to AST content and colour property in white muscle. Differences between groups 1 and 2 and groups 9 and 10 indicate that KM had a significant effect on colour properties. It is not surprising to see the positive effect of KO and KM on pigmentation since they contain a huge amount of AST (KO, 884 mg/kg; KM, 482 mg/kg) which is the dominant carotenoid in salmonids. The MM effect on colour property was also confirmed by a slightly higher value of a* and TC in groups 3 and 4 than in group 1. Furthermore, the comparison between group 2 and group 4 suggests that the pigmentation effect of KM was more pronounced than the effect of MM.

For salmonid quality, redness of muscle is important. Carotenoid content in muscle determines the depth of redness. Most studies report a linear relationship between a*, b* and C* values and carotenoid content (Bjerkeng, 2000). In Paper IV, a highly logarithmic correlation was found between a*, H and TC values, in agreement with results from the study of Christiansen *et al.* (1995). FA and lipid content was reported to affect colour property (Einen & Skrede, 1998), but this was not observed in the present study.

The efficiency of AST utilisation is of interest in Paper IV. Feeds used in the study contained sufficiently high AST content for fish to obtain the expected level of AST (6 mg/kg). However, RTC and AST content in the study were lower than the results from previous studies on salmonids (Nickell & Bromage, 1998; Synowiecki *et al.*, 1994). Species differences may exist in pigmentation retention efficiencies between Atlantic salmon, rainbow trout and Arctic charr

(Bjerkeng *et al.*, 2000; Bjerkeng *et al.*, 1999). Fish size is one possible reason for the poor deposition of AST in this study. Storebakken *et al.* (1986) and Olsen and Mortensen (1997) found that small fish had a lower AST content than large fish in the muscle of Atlantic salmon. The authors concluded that small size salmonids may reach a plateau level in their pigmenting capacity. The present results confirmed that fish size affects pigmentation.

Pigment source could be another important factor affecting the retention and utilisation of AST. This analysis of carotenoid in feeds showed that AST mainly exists in di-ester form in KO and KM (not showed). Free AST is usually absorbed and deposited more effectively than AST ester (Foss *et al.*, 1987). AST ester needs to be hydrolysed into free form, incorporated into mixed micelles or lipoproteins, and then absorbed in the intestine before finally being transported to the liver and deposited in muscle (Rørvik *et al.*, 2010). During this process, intestinal hydrolysis or cleavage is a limiting step for absorption (Storebakken *et al.*, 1987). White *et al.* (2003) found that AST content in serum was higher in salmon fed free or mono-esterified AST than in salmon fed di-esterified AST. Since AST in KO and KM is mainly di-esterified, it may have a low hydrolysis rate in Arctic charr. Further steps in the transport of AST may also play an important role in AST deposition. A future study focusing on the bioavailability of AST and the mechanisms of its absorption, transportation and metabolism is of interest.

5.4 Sesamin, tocopherol in tissues

Both sesamin and tocopherol are able to influence the metabolism of FAs. They or their metabolites have anti-oxidative properties, thus it is of interest to study their portion in fish tissue from the perspective of food nutrition and quality. In Paper I, the sesamin content in fish liver increased with the increase in sesamin addition, but its content in white muscle was similar. This indicates that most sesamin is catabolised by the liver, but not deposited in muscle. In rats, it has been proved that sesamin catabolises rapidly (Moazzami & Kamal-Eldin, 2006). CYP has also been reported to be induced by sesamin (Trattner *et al.*, 2008a). Thus, sesamin could be recognised as a xenobiotic compound and fish may try to metabolise it to avoid its accumulation in the body. If this is true, the expectation of keeping sesamin in fish muscle to enhance the nutrition value of fish through dietary sesamin may not be achieved.

Tocopherol is also found in fish liver and white muscle in Papers I and II. Studies have shown that tocopherol is deposited in the fish tissue of fish fed VO-based feed (Ng *et al.*, 2004; Pettersson *et al.*, 2009a). This is because VO, like palm oil and rapeseed oil, contains a certain amount of tocopherol. The

accumulation of tocopherol in fish could prolong the shelf life and improve fish quality. Sesamin has been reported to influence the level of tocopherol in the tissue and plasma of rats (Kamal-Eldin *et al.*, 1995), however this effect was not observed between the fish fed feeds with or without added sesamin in Paper II. Only a slightly lower level of γ-tocopherol appeared in the group's white muscle with the lowest sesamin addition in the feed compared to the other two sesamin-added groups in Paper I.

5.5 Oxidation

In Paper IV, the TBARS value was measured to investigate the effect of LC, the FA profile and carotenoids on oxidation in white muscle. Results showed that the LC and FA profile did not strongly affect the TBARS value, nor did n-6 PUFA. However n-3 PUFA showed a slight correlation with the TBARS value. This could be explained by the more sensitive vulnerability of n-3 PUFA than n-6 PUFA (Cosgrove *et al.*, 1987). Some groups (groups 2, 10 and 11) with a high TBARS value were found to have a high TC. This suggests that AST did not exhibit the expected antioxidant action. Contradictory results of the effect of AST on oxidation have been reported in previous studies (Fabio Brambilla *et al.*, 2009; Ingemansson *et al.*, 1993; Larsen, 2011; Sigurgisladottir *et al.*, 1994). AST mainly acts as a singlet oxygen quencher (Shahidi & Zhong, 2010). For oxidation in meat products, free radical is a more important factor than singlet oxygen. Thus the protective effect of AST may be of minor importance. On the other hand, high levels of antioxidants may also exhibit pro-oxidant effects, such as β-carotene (Young & Lowe, 2001). This would suggest a pro-oxidant-like effect, but not an antioxidant effect of AST in this study. Further study on the *in vitro* anti-oxidative effect and mechanism of AST needs to be undertaken.

5.6 CYP 450

In Paper I, sesamin affected the content of total CYP 450 and EROD activity. In previous studies, both total CYP content and EROD activity were also found to be affected by sesamin (Schiller Vestergren *et al.*, 2012; Trattner *et al.*, 2008a). In the study of Wagner *et al.* (2014), a high dose of sesamin caused reduced growth and several metabolites associated with energy metabolism (*e.g.* glucose, glycogen, leucine, valine, creatine, carnitine, lactate and nucleosides) in liver and muscle were affected by sesamin. Another study by (Kokushi *et al.*, 2012) found similar metabolites in carp fed the feed containing environmental pollutant-heavy oil. Since EROD is often used as a biomarker

for exposure to xenobiotic compounds in fish, the induced CYP indicates that sesamin may be recognised as a xenobiotic compound by fish, negatively affecting the metabolism. Although no distinct reduction in fish growth was found in the studies, the results from the other studies showed effects which might be important for fish welfare.

In Trial 1 in Paper III, the activity of EROD and PNPH in liver was determined to expose the ED effect on fish. Higher EROD and PNPH activity in the ED group indicates that the ingredients in ED affect physiological activity in fish. EROD and PNPH activity was measured in Trial 2 to study further the effect of MM and ZM on fish response. Increased activities of the two enzymes in the ZM group suggest that ZM caused a response similar to toxicological exposures. The fungus used in the study was grown on the spent sulphite liquid from the paper pulp industry. This medium may contain some pollutants that might lead to the toxicological-like response. Thus, it is possible that some toxicants deposited or produced from ZM should not be excluded. The application of ZM as protein sources for fish feed requires further study. The MM used in the study is food grade. This may explain its less pronounced effect on this enzyme activity. Additionally, although EROD is widely studied and used as a biomarker for exposure to xenobiotic compounds (Sarasquete & Segner, 2000), there are very few studies on PNPH in fish. The present study confirmed in the first instance the existence of PNPH activity in Arctic charr. A further study focusing on the enzyme property with dose-response of a known xenobiotic would provide more information about this enzyme and its metabolic action in fish.

6 Main findings and conclusions

The findings and conclusions can be summarised as follows. Different compounds of feeds are changing from fish meal and fish oil towards plant materials and microfungi, and towards lower food chain organisms from the aquatic environment. Such compounds include proteins, oils and binders.

In this thesis the possible effects of these non-evolutionary compounds of salmonid diets on their performance were investigated.

- Salmonid growth was not affected by KM, KO, MM, ZM or sesamin in the studies in this thesis.

- Sesamin decreased the ALA level in the white muscle of Atlantic salmon and rainbow trout. It slightly increased the level of DHA in some groups. Sesamin affected lipid metabolism.

- Krill and mussel meal and oil can be used to increase n-3 LCPUFA and enhance pink in the white muscle of Arctic charr. The effect of KM was more pronounced than that of MM.

- The utilisation rate of AST in KM and KO was low in Arctic charr. The low bioavailability of AST could be due to the AST di-ester in KO and KM.

- Sesamin affected the level and activity of CYP 450 in Atlantic salmon. ZM and MM increased the activity of EORD and PNPH in liver microsomes of Arctic charr.

7 Future perspectives

This thesis investigated the effects of some non-fish-based raw materials on the fish muscle quality of salmonids. Information has been gained about FA, carotenoid and CYP 450. Based on the findings from this thesis, it may be useful to focus more attention on the following areas of research:

- Dose-response study on the influences of ZM, MM, KM and KO on fish growth and CYP 450
- The effect on FA metabolism of sesamin/episesamin and sesamin with fish of different sizes and life stages
- The mechanism on a molecular level, including gene expression and proteome expression, for the role of sesamin in FA metabolism
- The identification of potential bioactive compounds in VO affecting FA metabolism
- The effect of AST on the conversion of carbon 18 FAs into LCPUFA
- The bioavailability of AST in KO and KM and the mechanism of its absorption, transportation and metabolism
- The effect of KM, KO, MM on sensory properties and the stability of fish muscle during storage
- Consequences of the new feeding strategies in aquaculture on human nutrition and health.

References

AHA (2002). *Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease*.

Albrektsen, S., Amlund, H., Langmyhr, E. & Hemre, G.I. (2006). *Astaxanthin digestibility, tissue concentration and body retention in Atlantic salmon (Salmo salar) fed graded diet inclusion levels of Antarctic krill (Euphausia superba) meal or Carophyll Pink as pigment sources*. (Report) Bergen, Norway: Protevs AS.

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

Ames, B.N., Shigenaga, M.K. & Hagen, T.M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences* 90(17), 7915-7922.

ANHMRC (2006). *Nutrient reference values for australia and new zealand - including recommended dietary intakes*.

Appelqvist, L.-Å. (Ed.) (1968). *Rapid methods of lipid extractions and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipids contaminants.* Stockholm Almqvist & Wiksell (Lipids; Extraction (Chemistry); Fatty acids; Methyl esters; Analysis

Arai, S., Mori, T., Miki, W., Yamaguchi, K., Konosu, S., Satake, M. & Fujita, T. (1987). Pigmentation of juvenile coho salmon with carotenoid oil extracted from Antarctic krill. *Aquaculture* 66(3–4), 255-264.

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* 48(10), 1303-1313.

Atalah, E., Cruz, C.M.H., Izquierdo, M.S., Rosenlund, G., Caballero, M.J., Valencia, A. & Robaina, L. (2007). Two microalgae *Crypthecodinium cohnii* and *Phaeodactylum tricornutum* as alternative source of essential

fatty acids in starter feeds for seabream (*Sparus aurata*). *Aquaculture* 270(1-4), 178-185.

Baker, R. & Günther, C. (2004). The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption. *Trends in Food Science & Technology* 15(10), 484-488.

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

Barbosa, M.J., Morais, R. & Choubert, G. (1999). Effect of carotenoid source and dietary lipid content on blood astaxanthin concentration in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 176(3–4), 331-341.

Bell, J., Tocher, D., Henderson, R., Dick, J. & Crampton, V. (2003a). Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *Journal of Nutrition* 133, 2793 - 2801.

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

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

Bell, J.G., McGhee, F., Campbell, P.J. & Sargent, J.R. (2003b). Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". *Aquaculture* 218(1-4), 515-528.

Bell, J.G. & Sargent, J.R. (2003). Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture* 218(1–4), 491-499.

Bell, J.G. & Waagbø, R. (2008). Safe and nutritious aquaculture produce: benefits and risks of alternative sustainable aquafeeds. In: Holmer, M*., et al.* (Eds.) *Aquaculture in the Ecosystem*. pp. 185-225 Springer Netherlands. ISBN 978-1-4020-6810-2.

Berge, G.M. & Austreng, E. (1989). Blue mussel in feed for rainbow trout. *Aquaculture* 81(1), 79-90.

Beutner, S., Bloedorn, B., Frixel, S., Hernández Blanco, I., Hoffmann, T., Martin, H.-D., Mayer, B., Noack, P., Ruck, C., Schmidt, M., Schülke, I., Sell, S., Ernst, H., Haremza, S., Seybold, G., Sies, H., Stahl, W. & Walsh, R. (2001). Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β-carotene in antioxidant functions. *Journal of the Science of Food and Agriculture* 81(6), 559-568.

Bjerkeng, B. (2000). *Carotenoid pigmentation of salmonids - recent progress*. Avances en Nutricion Acuicola:Nuevo Leon. (Avances en Nutricion Acuicola.

Bjerkeng, B., Folling, M., Lagocki, S., Storebakken, T., Olli, J.J. & Alsted, N. (1997). Bioavailability of all-E-astaxanthin and Z-isomers of astaxanthin in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 157(1), 63-82.

Bjerkeng, B., Hatlen, B. & Jobling, M. (2000). Astaxanthin and its metabolites idoxanthin and crustaxanthin in flesh, skin, and gonads of sexually immature and maturing Arctic charr (*Salvelinus alpinus L.*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 125(3), 395-404.

Bjerkeng, B., Hatlen, B. & Wathne, E. (1999). Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. *Aquaculture* 180(3–4), 307-319.

Bjerkeng, B., Storebakken, T. & Liaaen-Jensen, S. (1992). Pigmentation of rainbow trout from start feeding to sexual maturation. *Aquaculture* 108(3–4), 333-346.

Botsoglou, N.A., Fletouris, D.J., Papageorgiou, G.E., Vassilopoulos, V.N., Mantis, A.J. & Trakatellis, A.G. (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *Journal of Agricultural and Food Chemistry* 42(9), 1931-1937.

Brinker, A. & Reiter, R. (2011). Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: Effects on feed utilization and fish quality. *Aquaculture* 310(3–4), 350-360.

Caballero, M., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. & Izquierdo, M. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 214, 253 - 271.

Caballo, C., Costi, E.M., Sicilia, M.D. & Rubio, S. (2012). Determination of supplemental feeding needs for astaxanthin and canthaxanthin in salmonids by supramolecular solvent-based microextraction and liquid chromatography–UV/VIS spectroscopy. *Food Chemistry* 134(2), 1244-1249.

Calder, P.C. (2009). Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie* 91(6), 791-795.

Carter, C.G. & Hauler, R.C. (2000). Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar L*. *Aquaculture* 185(3–4), 299-311.

Chimsung, N., Tantikitti, C., Milley, J.E., Verlhac-Trichet, V. & Lall, S.P. (2012). Effects of various dietary factors on astaxanthin absorption in Atlantic salmon (*Salmo salar*). *Aquaculture Research*, n/a-n/a.

Chitchumroonchokchai, C., Bomser, J.A., Glamm, J.E. & Failla, M.L. (2004). Xanthophylls and α-tocopherol decrease UVB-induced lipid peroxidation

and stress signaling in human lens epithelial cells. *The Journal of Nutrition* 134(12), 3225-3232.
Cho, J.H. & Kim, I.H. (2011). Fish meal – nutritive value. *Journal of Animal Physiology and Animal Nutrition* 95(6), 685-692.
Choubert, G. (2010). Response of rainbow trout (*Oncorhynchus mykiss*) to varying dietary astaxanthin/canthaxanthin ratio: colour and carotenoid retention of the muscle. *Aquaculture Nutrition* 16(5), 528-535.
Choubert, G., Cravedi, J.-P. & Laurentie, M. (2009). Effect of alternate distribution of astaxanthin on rainbow trout (*Oncorhynchus mykiss*) muscle pigmentation. *Aquaculture* 286(1–2), 100-104.
Christiansen, R., Struksnæs, G., Estermann, R. & Torrissen, O.J. (1995). Assessment of flesh colour in Atlantic salmon, *Salmo salar L*. *Aquaculture Research* 26(5), 311-321.
Clarke, S.D. (2001). Polyunsaturated Fatty Acid Regulation of Gene Transcription: A Molecular Mechanism to Improve the Metabolic Syndrome. *The Journal of Nutrition* 131(4), 1129-1132.
Connor, W.E. (2000). Importance of n-3 fatty acids in health and disease. *The American Journal of Clinical Nutrition* 71(1), 171S-175S.
Cosgrove, J., Church, D. & Pryor, W. (1987). The kinetics of the autoxidation of polyunsaturated fatty acids. *Lipids* 22(5), 299-304.
De Silva, S.S., Francis, D.S. & Tacon, A.G.J. (2011). Fish Oil in Aquaculture in Retrospect. In: Turchini, G.M*., et al.* (Eds.) *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*. pp. 1-20. Boca Raton, FL: Taylor and Francis Group, LLC.
EFSA (2009). Scientific opinion - Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *EFSA Journal* 1176, 1-11.
EFSA Panel on Dietetic Products, N.a.A.N. (2010). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *The EFSA Journal* 8(3), 1461-1568.
Einen & Skrede (1998). Quality characteristics in raw and smoked fillets of Atlantic salmon, *Salmo salar*, fed high-energy diets. *Aquaculture Nutrition* 4(2), 99-108.
Fabio Brambilla, Andrea Forchino, Micaela Antonini, Simona Rimoldi, Genciana Terova & Saroglia, M. (2009). Effect of dietary Astaxanthin sources supplementation on muscle pigmentation and lipid peroxidation in rainbow trout (*Oncorhynchus mykiss*). *Italian Journal of Animal Science* 8(2), 845-847.
FAO (1995). Quality and quality changes in fresh fish. In: Huss, H.H. (Ed.) Food and Agriculture Organization (FAO)
FAO (2012). *The State of World Fisheries and Aquaculture*. Rome, Italy.
Foss, P., Storebakken, T., Austreng, E. & Liaaenjensen, S. (1987). Carotenoids in diets for salmonids: V. Pigmentation of rainbow trout and sea trout with astaxanthin and astaxanthin dipalmitate in comparison with canthaxanthin. *Aquaculture* 65(3–4), 293-305.

Francis, G., Makkar, H.P.S. & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199(3–4), 197-227.

Franco-Zavaleta, M.E., Jiménez-Pichardo, R., Tomasini-Campocosio, A. & Guerrero-Legarreta, I. (2010). Astaxanthin extraction from shrimp wastes and its stability in 2 model systems. *Journal of Food Science* 75(5), C394-C399.

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.

Fredriksson, S. & 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.

Frigg, M., Prabucki, A.L. & Ruhdel, E.U. (1990). Effect of dietary vitamin E levels on oxidative stability of trout fillets. *Aquaculture* 84(2), 145-158.

Ganuza, E., Benítez-Santana, T., Atalah, E., Vega-Orellana, O., Ganga, R. & Izquierdo, M.S. (2008). *Crypthecodinium cohnii* and *Schizochytrium sp*. as potential substitutes to fisheries-derived oils from seabream (*Sparus aurata*) microdiets. *Aquaculture* 277(1-2), 109-116.

Garaulet, M., Pérez-Llamas, F., Pérez-Ayala, M., Martínez, P., de Medina, F.S., Tebar, F.J. & Zamora, S. (2001). Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. *The American Journal of Clinical Nutrition* 74(5), 585-591.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., J Souza, E., Stone, D., Wilson, R. & Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research* 38(6), 551-579.

Gonzalez, F.J. (1988). The molecular biology of cytochrome P450s. *Pharmacological Reviews* 40(4), 243-288.

Hansen, J.Ø., Penn, M., Øverland, M., Shearer, K.D., Krogdahl, Å., Mydland, L.T. & Storebakken, T. (2010). High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 310(1–2), 164-172.

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

Harare, K., Berge, R.K. & Lie, Ø. (1998). Oxidative stability of Atlantic salmon (*Salmo salar, L.*) fillet enriched in α-, γ-, and δ-tocopherol through dietary supplementation. *Food Chemistry* 62(2), 173-178.

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

Henderson, R.J. & Tocher, D.R. (1987). The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* 26(4), 281-347.
Higuera-Ciapara, I., Félix-Valenzuela, L. & Goycoolea, F.M. (2006). Astaxanthin: a review of its chemistry and applications. *Critical Reviews in Food Science and Nutrition* 46(2), 185-196.
Hussein, G., Sankawa, U., Goto, H., Matsumoto, K. & Watanabe, H. (2005). Astaxanthin, a carotenoid with potential in human health and nutrition. *Journal of Natural Products* 69(3), 443-449.
Hynes, N., Egeland, E.S., Koppe, W., Baardsen, G. & Kiron, V. (2009). Calanus oil as a natural source for flesh pigmentation in Atlantic salmon (*Salmo salar L.*). *Aquaculture Nutrition* 15(2), 202-208.
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 (BBA) - 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 (BBA) - Molecular and Cell Biology of Lipids* 1682(1–3), 80-91.
Ide, T., Kushiro, M., Takahashi, Y., Shinohara, K., Fukuda, N. & Sirato-yasumoto, S. (2003). Sesamin, a sesame lignan, as a potent serum lipid-lowering food component. *Japan agricultural research quarterly* 37(3), 151-158.
IFFO (2009). *International fish meal and fish oil organization statistical yearbook*.
Ingemansson, T., PelTersson, A. & Kaufmann, P. (1993). Lipid hydrolysis and oxidation related to astaxanthin content in light and dark muscle of frozen stored rainbow trout (*Oncorbynchus mykiss*). *Journal of Food Science* 58(3), 513-518.
ISSFAL (2004). *Recommendations for intake of polyunsaturated fatty acids in healthy adults*.
Jackson, H., Braun, C.L. & Ernst, H. (2008). The chemistry of novel xanthophyll carotenoids. *The American Journal of Cardiology* 101(10, Supplement), S50-S57.
Jeng, K.C.G. & Hou, R.C.W. (2005). Sesamin and sesamolin: Natures therapeutic lignans. *Current Enzyme Inhibition* 1(1), 11-20.
Jittinandana, S., Kenney, P.B., Slider, S.D. & Hankins, J.A. (2006). Effect of high dietary vitamin E on lipid stability of oven-cooked and hot-smoked trout fillets. *Journal of Food Science* 71(3), C130-C136.
Ju, Z.Y., Deng, D.-F. & Dominy, W. (2012). A defatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (*Litopenaeus vannamei,* Boone, 1931). *Aquaculture* 354–355(0), 50-55.
Julshamn, K., Malde, M.K., Bjorvatn, K. & Krogedal, P. (2004). Fluoride retention of Atlantic salmon (*Salmo salar*) fed krill meal. *Aquaculture Nutrition* 10(1), 9-13.

Jyonouchi, H., Sun, S., Iijima, K. & Gross, M.D. (2000). Antitumor activity of astaxanthin and its mode of action. *Nutrition and Cancer* 36(1), 59-65.
Jönsson, L. & Elwinger, K. (2009). Mussel meal as a replacement for fish meal in feeds for organic poultry – a pilot short-term study. *Acta Agriculturae Scandinavica, Section A - Animal Science* 59(1), 22-27.
Kamal-Eldin, A., Pettersson, D. & Appelqvist, L.-Å. (1995). Sesamin (a compound from sesame oil) increases tocopherol levels in rats fedad libitum. *Lipids* 30(6), 499-505.
Kamal-Eldin, A. & Pickova, J. (2008). Balance between polyunsaturated fatty acids and antioxidants in nutrition. *Lipid Technology* 20(4), 80-83.
Kawaguchi, S. & Nicol, S. (2007). Learning about Antarctic krill from the fishery. *Antarctic Science* 19(02), 219-230.
Kiessling, A. (2009). *Feed-The key to sustainable fish farming*. Stockholm, Sweden: Royal Swedish Academy of Agriculture and Forestry (KSLA). (Fisheries sustainability development.
Kiessling, K.H. & Kiessling, A. (1993). Selective utilization of fatty acids in rainbow trout (*Oncorhynchus mykiss Walbaum*) red muscle mitochondria. *Canadian Journal of Zoology* 71(2), 248-251.
Kokushi, E., Uno, S., Harada, T. & Koyama, J. (2012). 1H NMR-based metabolomics approach to assess toxicity of bunker a heavy oil to freshwater carp, *Cyprinus carpio*. *Environmental Toxicology* 27(7), 404-414.
Kris-Etherton, P.M., Harris, W.S., Appel, L.J. & Committee, f.t.N. (2002). Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Circulation* 106(21), 2747-2757.
Kröger, E., Verreault, R., Carmichael, P.-H., Lindsay, J., Julien, P., Dewailly, É., Ayotte, P. & Laurin, D. (2009). Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. *The American Journal of Clinical Nutrition* 90(1), 184-192.
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.
Larsen, D.S. (2011). *Thermal treatment of New Zealand king salmon (Oncorhynchus tshawytscha): physio-chemical and sensory properties and the role of astaxanthin in lipid oxidation.* Diss.:The University of Auckland.
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. *The American Journal of Clinical Nutrition* 79(6), 935-945.
Lee, S.J., Bai, S.K., Lee, K.S., Namkoong, S., Na, H.J., Ha, K.S., Han, J.A., Yim, S.V., Chang, K., Kwon, Y.G., Lee, S.K. & Kim, Y.M. (2003). Astaxanthin inhibits nitric oxide production and inflammatory gene

expression by suppressing I(kappa)B kinase-dependent NF-kappaB activation. *Molecules and cells* 16(1), 97-105.

Linseisen, J., Welch, A.A., Ocke, M., Amiano, P., Agnoli, C., Ferrari, P., Sonestedt, E., Chajes, V., Bueno-de-Mesquita, H.B., Kaaks, R., Weikert, C., Dorronsoro, M., Rodriguez, L., Ermini, I., Mattiello, A., van der Schouw, Y.T., Manjer, J., Nilsson, S., Jenab, M., Lund, E., Brustad, M., Halkjar, J., Jakobsen, M.U., Khaw, K.T., Crowe, F., Georgila, C., Misirli, G., Niravong, M., Touvier, M., Bingham, S., Riboli, E. & Slimani, N. (2009). Dietary fat intake in the European Prospective Investigation into Cancer and Nutrition: results from the 24-h dietary recalls. *Eur J Clin Nutr* 63(S4), S61-S80.

Lopez-Ferrer, S., Baucells, M., Barroeta, A. & Grashorn, M. (1999). n-3 enrichment of chicken meat using fish oil: alternative substitution with rapeseed and linseed oils. *Poultry Science* 78(3), 356-365.

López, M., Arce, L., Garrido, J., Ríos, A. & Valcárcel, M. (2004). Selective extraction of astaxanthin from crustaceans by use of supercritical carbon dioxide. *Talanta* 64(3), 726-731.

Lyons, N.M. & O'Brien, N.M. (2002). Modulatory effects of an algal extract containing astaxanthin on UVA-irradiated cells in culture. *Journal of Dermatological Science* 30(1), 73-84.

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 qualityand fatty acid composition of various tissues. *British Journal of Nutrition* 83(06), 637-643.

Miki, W. (1991). Biological functions and activities of animal carotenoids. *Pure and Applied Chemistry* 63, 141-146.

Miller, M.R., Nichols, P.D. & Carter, C.G. (2007). Replacement of fish oil with thraustochytrid Schizochytrium sp. L oil in Atlantic salmon parr (*Salmo salar L*) diets. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 148(2), 382-392.

Miller, M.R., Nichols, P.D. & Carter, C.G. (2008). n-3 Oil sources for use in aquaculture – alternatives to the unsustainable harvest of wild fish. *Nutrition Research Reviews* 21(02), 85-96.

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.

Mourente, G., Dick, J.R., Bell, J.G. & Tocher, D.R. (2005). Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and beta-oxidation of [1-C-14]18 : 3n-3 (LNA) and [1-C-14]20 : 5n-3 (EPA) in hepatocytes and enterocytes of European sea bass (Dicentrarchus labrax L.). *Aquaculture* 248(1-4), 173-186.

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

Mráz, J., Zajíc, T. & Pickova, J. (2012). Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy. *Neuroendocrinology Letters* 33(Supply.2), 60-67.

Mydland, L.T., Landsverk, T., Zimonja, T., Storebakken, T., Edsbo, L. & Kiessling, A. (2007). Mycelium biomass from fungi (*Rhizopus oryzae*) grown on spent sulphite liquor from paper pulp as a protein source in diets for rainbow trout (*Oncorhynchus mykiss*). In: *Aquaculture Europe 2007.* Istanbul,Turkey. pp. Book of Abstracts, pp 375-376.

Myhr, A.I. & Dalmo, R.A. (2005). Introduction of genetic engineering in aquaculture: Ecological and ethical implications for science and governance. *Aquaculture* 250(3–4), 542-554.

Nagao, K. & Yanagita, T. (2008). Bioactive lipids in metabolic syndrome. *Progress in Lipid Research* 47(2), 127-146.

Naito, Y., Uchiyama, K., Aoi, W., Hasegawa, G., Nakamura, N., Yoshida, N., Maoka, T., Takahashi, J. & Yoshikawa, T. (2004). Prevention of diabetic nephropathy by treatment with astaxanthin in diabetic db/db mice. *BioFactors* 20(1), 49-59.

Naylor, R.L., Goldburg, R.J., Mooney, H., Beveridge, M., Clay, J., Folke, C., Kautsky, N., Lubchenco, J., Primavera, J. & Williams, M. (1998). Nature's Subsidies to Shrimp and Salmon Farming. *Science* 282(5390), 883-884.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature* 405(6790), 1017-1024.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K. & Nichols, P.D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences* 106(36), 15103-15110.

Nettleton, J.A. & Katz, R. (2005). n-3 long-chain polyunsaturated fatty acids in type 2 diabetes: A review. *Journal of the American Dietetic Association* 105(3), 428-440.

Ng, W.-K., Wang, Y., Ketchimenin, P. & Yuen, K.-H. (2004). Replacement of dietary fish oil with palm fatty acid distillate elevates tocopherol and tocotrienol concentrations and increases oxidative stability in the muscle of African catfish, Clarias gariepinus. *Aquaculture* 233(1–4), 423-437.

Nickell, D.C. & Bromage, N.R. (1998). The effect of timing and duration of feeding astaxanthin on the development and variation of fillet colour and efficiency of pigmentation in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 169(3–4), 233-246.

Nicol, S., Foster, J. & Kawaguchi, S. (2012). The fishery for Antarctic krill – recent developments. *Fish and Fisheries* 13(1), 30-40.

Nilzén, V., Babol, J., Dutta, P.C., Lundeheim, N., Enfält, A.C. & Lundström, 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.
Olsen, R.E. & Henderson, R.J. (1997). Muscle fatty acid composition and oxidative stress indices of Arctic charr, *Salvelinus alpinus (L.)*, in relation to dietary polyunsaturated fatty acid levels and temperature. *Aquaculture Nutrition* 3(4), 227-238.
Olsen, R.E., Henderson, R.J., Sountama, J., Hemre, G.I., Ringø, E., Melle, W. & Tocher, D.R. (2004). Atlantic salmon, *Salmo salar*, utilizes wax ester-rich oil from Calanus finmarchicus effectively. *Aquaculture* 240(1–4), 433-449.
Olsen, R.E. & Mortensen, A. (1997). The influence of dietary astaxanthin and temperature on flesh colour in Arctic charr *Salvelinus alpinus L*. *Aquaculture Research* 28(1), 51-58.
Olsen, Y. (2011). Resources for fish feed in future mariculture. *Aquaculture Environment Interactions* 1(3), 187-200.
Olvera-Novoa, M.A., MartÍNez-Palacios, C.A. & Olivera-Castillo, L. (2002). Utilization of torula yeast (*Candida utilis*) as a protein source in diets for tilapia (*Oreochromis mossambicus Peters*) fry. *Aquaculture Nutrition* 8(4), 257-264.
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.
Page, G.I. & Davies, S.J. (2003). Hepatic carotenoid uptake in rainbow trout (*Oncorhynchus mykiss*) using an isolated organ perfusion model. *Aquaculture* 225(1–4), 405-419.
Perera, W.M.K., Carter, C.G. & Houlihan, D.F. (1995). Feed consumption, growth and growth efficiency of rainbow trout (*Oncorhynchus mykiss (Walbaum)*) fed on diets containing a bacterial single-cell protein. *British Journal of Nutrition* 73(04), 591-603.
Pettersson, A. (2010). *Effects of replacing fish oil with vegetable oils in feed for rainbow trout (Oncorhynchus mykiss) and Arctic charr (Salvelinus alpinus)* Diss. Uppsala, Sweden:Swedish University of Agricultural Sciences
Pettersson, A., Johnsson, L., Brännäs, E. & Pickova, J. (2009a). Effects of rapeseed oil replacement in fish feed on lipid composition and self-selection by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* 15(6), 577-586.
Pettersson, A., Pickova, J. & Brännäs, E. (2009b). Effects of crude rapeseed oil on lipid composition in Arctic charr *Salvelinus alpinus*. *Journal of Fish Biology* 75(6), 1446-1458.
Pike, I.H. & Jackson, A. (2010). Fish oil: production and use now and in the future. *Lipid Technology* 22(3), 59-61.
Rajasingh, H., Øyehaug, L., Våge, D. & Omholt, S. (2006). Carotenoid dynamics in Atlantic salmon. *BMC Biology* 4(1), 1-15.

Rice, R. (2009). Nutritional value of fish oils. In: Rossell, B. (Ed.) *Fish oils*. pp. 131-154. Chichester, UK: Blackwell Publishing.

Robert, S. (2006). Production of eicosapentaenoic and docosahexaenoic acid-containing oils in transgenic land plants for human and aquaculture nutrition. *Marine Biotechnology* 8(2), 103-109.

Robin, J.H., Regost, C., Arzel, J. & Kaushik, S.J. (2003). Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. *Aquaculture* 225(1–4), 283-293.

Roncarati, A., Sirri, F., Felici, A.A., Stocchi, L., Melotti, P. & Meluzzi, A. (2011). Effects of dietary supplementation with krill meal on pigmentation and quality of flesh of rainbow trout (*Oncorhynchus mykiss*). *Italian Journal of Animal Science* 10(2).

Ruff, N., FitzGerald, R.D., Cross, T.F. & Kerry, J.P. (2002). Fillet shelf-life of Atlantic halibut *Hippoglossus hippoglossus L*. fed elevated levels of α-tocopheryl acetate. *Aquaculture Research* 33(13), 1059-1071.

Rumsey, G.L., Winfree, R.A. & Hughes, S.G. (1992). Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 108(1–2), 97-110.

Rungruangsak-Torrissen, K. (2007). Digestive efficiency, growth and qualities of muscle and oocyte in Atlantic salmon (*Salmo salar l.*) fed on diets with krill meal as an alternative protein source. *Journal of Food Biochemistry* 31(4), 509-540.

Rørvik, K.A., Ytrestøyl, T., Lundberg, E., Jakobsen, F.A., Jakobsen, A.A. & Bjerkeng, B. (2010). How apparent digestibility of carotenoids, macronutrients, and minerals are differently affected by ration level in Atlantic salmon, *Salmo salar*. *Journal of Applied Aquaculture* 22(2), 123-139.

Sarasquete, C. & Segner, H. (2000). Cytochrome P4501A (CYP1A) in teleostean fishes. A review of immunohistochemical studies. *Science of The Total Environment* 247(2–3), 313-332.

Sargent, J.R., Tocher, D.R., Bell, J.G. (2002). The lipids. In: Halver, J.E*., et al.* (Eds.) *Fish Nutrition*. pp. 181-257. San Diego: Academic Press, Elsevier.

Scaife, Onibi, Murray, Fletcher & Houlihan (2000). Influence of α-tocopherol acetate on the short- and long-term storage properties of fillets from Atlantic salmon *Salmo salar* fed a high lipid diet. *Aquaculture Nutrition* 6(1), 65-71.

Schiller Vestergren, A., Wagner, L., Pickova, J., Rosenlund, G., Kamal-Eldin, A. & Trattner, S. (2012). Sesamin Modulates Gene Expression Without Corresponding Effects on Fatty acids in Atlantic Salmon (*Salmo salar L.*). *Lipids* 47(9), 897-911.

Schmidt, I., Schewe, H., Gassel, S., Jin, C., Buckingham, J., Hümbelin, M., Sandmann, G. & Schrader, J. (2011). Biotechnological production of astaxanthin with Phaffia rhodozyma/Xanthophyllomyces dendrorhous. *Applied Microbiology and Biotechnology* 89(3), 555-571.

Shahidi, F. & Brown, J.A. (1998). Carotenoid pigments in seafoods and aquaculture. *Critical Reviews in Food Science and Nutrition* 38(1), 1-67.
Shahidi, F. & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews* 39(11), 4067-4079.
Sigurgisladottir, S., Parrish, C.C., Lall, S.P. & Ackman, R.G. (1994). Effects of feeding natural tocopherols and astaxanthin on Atlantic salmon (*Salmo salar*) fillet quality. *Food Research International* 27(1), 23-32.
Simopoulos, A.P. (2000). Human requirement for N-3 polyunsaturated fatty acids. *Poultry Science* 79(7), 961-970.
Skrede, G. & Storebakken, T. (1986). Instrumental colour analysis of farmed and wild Atlantic salmon when raw, baked and smoked. *Aquaculture* 53(3–4), 279-286.
Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, 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 *The state of world fisheries and aquaculture 2012*. [online] Available from: http://www.fao.org/docrep/009/a0699e/a0699e00.htm. [Accessed 2013-07-30].
Stanley-Samuelson, D.W. (1994). The biological significance of prostaglandins and related eicosanoids in invertebrates. *American Zoologist* 34(6), 589-598.
Storebakken, T., Baeverfjord, G., Skrede, A., Olli, J.J. & Berge, G.M. (2004). Bacterial protein grown on natural gas in diets for Atlantic salmon, *Salmo salar*, in freshwater. *Aquaculture* 241(1–4), 413-425.
Storebakken, T., Foss, P., Huse, I., Wandsvik, A. & Berg Lea, T. (1986). Carotenoids in diets for salmonids: III. Utilization of canthaxanthin from dry and wet diets by Atlantic salmon, rainbow trout and sea trout. *Aquaculture* 51(3–4), 245-255.
Storebakken, T., Foss, P., Schiedt, K., Austreng, E., Liaaen-Jensen, S. & Manz, U. (1987). Carotenoids in diets for salmonids: IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. *Aquaculture* 65(3–4), 279-292.
Stubhaug, I., Tocher, D.R., Bell, J.G., Dick, J.R. & Torstensen, B.E. (2005). Fatty acid metabolism in Atlantic salmon (*Salmo salar L.*) hepatocytes and influence of dietary vegetable oil. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids* 1734(3), 277-288.
Suontama, J., Karlsen, Ø., Moren, M., Hemre, G.I., Melle, W., Langmyhr, E., Mundheim, H., RingØ, E. & Olsen, R.E. (2007). Growth, feed conversion and chemical composition of Atlantic salmon (*Salmo salar L.*) and Atlantic halibut (*Hippoglossus hippoglossus L.*) fed diets supplemented with krill or amphipods. *Aquaculture Nutrition* 13(4), 241-255.
Synowiecki, J., Shahidi, F. & Penney, R.W. (1994). Nutrient composition of meat and uptake of carotenoids by Artic charr (*Savelinus alpinus*). *Journal of Aquatic Food Product Technology* 2(3), 37-58.

Tacon, A.G.J., Hasan, M.R. & Metian, M. (2011). *Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects*. Rome: FAO; Technical paper No. 564).

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.

Teimouri, M., Amirkolaie, A.K. & Yeganeh, S. (2013). The effects of Spirulina platensis meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 396–399(0), 14-19.

Thorarinsdottir, R.I., Jokumsen, A., Bjornsson, B.T. & Torrissen, O. (2011). *Local raw materials for production of fish feed for aquaculture*. Oslo, Norway: Nordic Innovation Centre.

Tibbetts, S.M., Olsen, R.E. & Lall, S.P. (2011). Effects of partial or total replacement of fish meal with freeze-dried krill (*Euphausia superba*) on growth and nutrient utilization of juvenile Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*) fed the same practical diets. *Aquaculture Nutrition* 17(3), 287-303.

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

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

Tolasa, S., Cakli, S. & Ostermeyer, U. (2005). Determination of astaxanthin and canthaxanthin in salmonid. *European Food Research and Technology* 221(6), 787-791.

Torrissen, O.J., Hardy, R.W. & Shearer, K.D. (1989). Pigmentation of salmonids-carotenoid deposition and metabolism. *Reviews in Aquatic Science* 1, 209-225.

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

Trattner, S., Kamal-Eldin, A., Brännäs, E., Moazzami, A., Zlabek, V., Larsson, P., Ruyter, B., Gjøen, T. & Pickova, J. (2008a). Sesaminsupplementation increases white muscle docosahexaenoic acid (DHA) levels in rainbow trout (*Oncorhynchus mykiss*) fed high alpha-linolenic acid (ALA) containing vegetable oil: metabolic actions. *Lipids* 43(11), 989-997.

Trattner, S., Ruyter, B., Østbye, T., Gjøen, T., Zlabek, V., Kamal-Eldin, A. & Pickova, J. (2008b). Sesamin increases alpha-Linolenic acid conversion to docosahexaenoic acid in Atlantic salmon (*Salmo salar L.*) hepatocytes: role of altered gene expression. *Lipids* 43(11), 999-1008.

Turchini, G.M., Gunasekera, R.M. & De Silva, S.S. (2003). Effect of crude oil extracts from trout offal as a replacement for fish oil in the diets of the Australian native fish Murray cod *Maccullochella peelii peelii*. *Aquaculture Research* 34(9), 697-708.

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

Uchida, K. & Stadtman, E.R. (1993). Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction. *Journal of Biological Chemistry* 268(9), 6388-6393.

Wagner, L., Trattner, S., Pickova, J., Gómez-Requeni, P. & Moazzami, A.A. (2014). 1H NMR-based metabolomics studies on the effect of sesamin in Atlantic salmon (*Salmo salar*). *Food Chemistry* 147(0), 98-105.

Wagner, L., Zlabek, V., Trattner, S. & Zamaratskaia, G. (2012). In vitro inhibition of 7-ethoxyresorufin-O-deethylase (EROD) and p-nitrophenol hydroxylase (PNPH) activities by sesamin in hepatic microsomes from two fish species. *Molecular Biology Reports*, 1-6.

Wanders, R.J., Vreken, P., Ferdinandusse, S., Jansen, G.A., Waterham, H.R., van Roermund, C.W. & Van Grunsven, E.G. (2001). Peroxisomal fatty acid alpha- and beta-oxidation in humans: enzymology, peroxisomal metabolite transporters and peroxisomal diseases. *Biochemical Society transactions* 29(Pt 2), 250-267.

Wang, J., Han, D., Sommerfeld, M., Lu, C. & Hu, Q. (2013). Effect of initial biomass density on growth and astaxanthin production of Haematococcus pluvialis in an outdoor photobioreactor. *Journal of Applied Phycology* 25(1), 253-260.

White, D.A., Moody, A.J., Serwata, R.D., Bowen, J., Soutar, C., Young, A.J. & Davies, S.J. (2003). The degree of carotenoid esterification influences the absorption of astaxanthin in rainbow trout, *Oncorhynchus mykiss* (*Walbaum*). *Aquaculture Nutrition* 9(4), 247-251.

WHO/FAO (2003a). Diet, nutrition and the prevention of chronic diseases. *WHO technical report series* 916, 1-150.

WHO/FAO (2003b). Diet, nutrition, and the prevention of chronic diseases. *WHO technical report series* 916, 89-90.

Von Schacky, C. & Harris, W.S. (2007). Cardiovascular benefits of omega-3 fatty acids. *Cardiovascular Research* 73(2), 310-315.

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(01), S49-S60.

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.

Yoshitomi, B., Aoki, M. & Oshima, S.-i. (2007). Effect of total replacement of dietary fish meal by low fluoride krill (*Euphausia superba*) meal on

growth performance of rainbow trout (*Oncorhynchus mykiss*) in fresh water. *Aquaculture* 266(1–4), 219-225.
Yoshitomi, B. & Nagano, I. (2012). Effect of dietary fluoride derived from Antarctic krill (*Euphausia superba*) meal on growth of yellowtail (*Seriola quinqueradiata*). *Chemosphere* 86(9), 891-897.
Young, A.J. & Lowe, G.M. (2001). Antioxidant and Prooxidant Properties of Carotenoids. *Archives of Biochemistry and Biophysics* 385(1), 20-27.
Zamaratskaia, G. & Zlabek, V. (2009). EROD and MROD as markers of cytochrome P450 1A activities in hepatic microsomes from entire and castrated male pigs. *Sensors* 9(3), 2134-2147.
Zamaratskaia, G. & Zlabek, V. (2011). Para-nitrophenol hydroxylation by fish liver microsomes: kinetics and effect of selective cytochrome P450 inhibitors. *Fish Physiology and Biochemistry* 37(4), 969-976.
Zhol, S., Ackman, R. & Morrison, C. (1995). Storage of lipids in the myosepta of Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* 14(2), 171-178.

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