Effects of Replacing Fish Oil with Vegetable Oils in Feed for Rainbow Trout (*Oncorhynchus mykiss*) and Arctic Charr (*Salvelinus alpinus*)

Andreas Pettersson
Faculty of Natural Resources and Agricultural Sciences
Department of Food Science
Uppsala

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Cover: Illustration of rainbow trout and Arctic charr and the vegetable oils used in the studies presented in this thesis.
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Abstract

As global capture of fish has stagnated and fish consumption is increasing due to a growing human population, the demand can only be met by increased aquaculture production. Fish oil (FO), derived exclusively from wild pelagic fish, has traditionally been used as the primary lipid source in fish feeds. For a number of reasons, more sustainable development of aquaculture is necessary where FO needs to be replaced with a more sustainable lipid source.

This thesis investigated the effects of FO replacement with two vegetable oils on growth, lipid content, feed preference and swimming performance of two salmonid species; rainbow trout and Arctic charr. In addition, a comparison of lipid content and composition with wild fish was performed to highlight the importance of natural food webs for successful production of specific fish species.

The results obtained showed no negative effects on growth of fish fed vegetable oils. However, significant changes in fatty acid profiles were observed in fish tissues, with reduced levels of long-chain polyunsaturated fatty acids (LCPUFA), mainly EPA and DHA, and increased levels of 18:1n-9 and 18:2n-6. Comparisons of fatty acid profiles of wild and farmed Arctic charr showed significant differences in individual n-3 and n-6 fatty acids. The largest difference was found in arachidonic acid (20:4n-6) content with 7-fold higher levels in the phospholipid fraction in white muscle of wild Arctic charr compared with Arctic charr fed marine FO. Cholesterol-lowering effects were observed in fish fed rapeseed oil, possibly explained by the presence of phytosterols in the diet. Swimming performance at 4 °C was significantly reduced in Arctic charr fed a blend of rapeseed oil and palm oil. This outcome is suggested to be an effect of the different levels of n-3 LCPUFA and saturated fatty acids due to their temperature influenced properties.

The results in this thesis imply that an appropriate mix of vegetable oils and FO can replace the sole use of FO in fish feeds. However, researchers and feed manufacturers should be encouraged to continue to increase feed diversification in order to optimize the nutritional requirements of farmed fish.

Keywords: salmonids, fish oil, vegetable oil, n-3 LCPUFA, phospholipid, DHA, arachidonic acid, swimming performance, phytosterols.

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

Effekter av att ersätta fiskolja med vegetabiliska oljor i foder för regnbåge (*Oncorhynchus mykiss*) och röding (*Salvelinus alpinus*)

Svensk sammanfattning

Med en växande befolkning och en begränsad tillgång på vild fisk, kan efterfrågan på sikt bara tillgodoses genom att odla fisk. Fiskolja, som uteslutande utvinns från marina pelagiska arter, har traditionellt använts som huvudsaklig lipidkälla i fiskfoder. För att vattenbruket ska kunna expandera måste utvecklingen ske på ett uthålligt sätt där bl.a. fiskoljan delvis eller helt ersätts med alternativa lipidkällor.

I denna doktorsavhandling beskrivs effekterna av att ersätta fiskolja med två vegetabiliska oljor (raps- och palmolja) på tillväxt, lipidinnehåll, foderpreferens och simkapacitet hos regnbåge och röding. En jämförelse i lipidsammansättning mellan vildfångad och odlad fisk har dessutom genomförts för att få underlag för en förfinad foderkomposition i syfte att efterlikna den vilda fiskens sammansättning.

Tillväxten påverkades inte hos fisk som blivit utfodrade med foder innehållande vegetabiliska oljor. Däremot observerades signifikanta skillnader i fettsyraprofiler i fiskvävnader, med lägre halter av långkedjiga fleromättade fettsyror, främst eikosapentaensyra (EPA, 20:5n-3) och dokosahexaensyra (DHA, 22:6n-3) och högre halter av oljesyra (18:1n-9) och linolsyra (18:2n-6). Stora skillnader i enskilda n-3 och n-6 fettsyror förekom mellan vild och odlad röding. Den största och mest anmärkningsvärda skillnaden var halten arakidonsyra (20:4n-6) i fosfolipidfraktionen av vit muskel som var 7 gånger högre i vild röding än i odlad. Kolesterolhalten var lägre i röding som utfodrats med rapsoljebaserat foder än i de som utfodrats med traditionellt fiskfoder. Detta kan möjligen förklaras av förekomsten av växtsteroler i rapsoljan och/eller ett lägre innehåll av kolesterol i fodret. Acceptansen hos regnbåge för rapsoljebaserat foder var förhållandevis hög men den föredrog fiskoljebaserat foder när den hade valmöjlighet. Simkapaciteten vid 4 °C reducerades signifikant hos röding som utfodrats med ett foder där 75% av fiskoljan ersatts med en blandning av raps- och palmolja. Responsen antas vara en effekt av de mättade och fleromättade fettsyrornas olika egenskaper vid olika temperaturer vilket påverkar muskelfysiologin och därmed simförmågan.

Resultaten i denna avhandling visar att en balanserad blandning av vegetabiliska oljor och fiskolja kan användas vid produktion av fiskfoder, men visar även att nya foder bör artanpassas för att nå bästa resultat.

Trots lägre halter av n-3 fettsyror i muskel hos fisk utfodrad med växtoljebaserat foder, kan fisken ändå anses vara en nyttig matprodukt med märkbart högre halter av n-3 fettsyror och andra hälsofrämjande födoämnen än i andra livsmedelsprodukter som konsumeras i dagens samhälle.

Dedication

To Maja for being the most beautiful thing in the world.

Det är bättre att ställa frågor och verka okunnig än att inte göra det och förbli det.
Kinesiskt ordspråk

Contents

List of Publications

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

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

II Pettersson, A., Pickova, J., Brännäs, E. (2009). Effects of crude rapeseed oil on lipid composition in Arctic charr (*Salvelinus alpinus*). *Journal of Fish Biology* 75, 1446-1458.

III Pettersson, A., Pickova, J., Brännäs, E. (2010). Swimming performance at different temperatures and fatty acid composition of Arctic charr (*Salvelinus alpinus*) fed rapeseed and palm oils. *Aquaculture* 300, 176-181.

IV Pettersson, A., Pickova, J., Ask, P., Byström, P., Brännäs, E. (2010). Fatty acid profiles of wild and farmed Arctic charr (*Salvelinus alpinus*). *Manuscript*

Papers I-III are reproduced with the permission of the publishers.

The contribution of Andreas Pettersson to the papers included in this thesis was as follows:

I Produced the experimental diets, sampled the tissue of interest and performed all lipid analyses. Evaluated analytical data, performed statistical analysis and prepared the major part of the manuscript.

II Produced the experimental diets together with the co-supervisor, prepared samples for analysis and performed all analytical work. Performed statistical evaluation of the results and prepared the main part of the manuscript.

III Sampled fish for analytical tissues and performed the swimming performance experiment. Carried out all lipid analyses and statistical evaluations and prepared the main part of the manuscript.

IV Sampled fish and performed the lipid analyses. Performed and interpreted the statistical analysis and took a major part in writing the manuscript.

Abbreviations

ANOVA	Analysis of Variance
ATP	Adenosine-5´triphosphate
CoA	Coenzyme A
DGC	Daily Growth Coefficient
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
$FADH_2$	Flavin Adenine Dinucleotide
FAME	Fatty Acid Methyl Ester
FID	Flame Ionization Detector
FO	Fish Oil
GC	Gas Chromatography
GC-MS	Gas Chromatograph Mass Spectrometry
GLM	General Linear Model
HPLC	High Performance Liquid Chromatography
HUFA	Highly Unsaturated Fatty Acid(s)
LCPUFA	Long Chain Polyunsaturated Fatty Acid(s)
LDL	Low Density Lipoprotein
MUFA	Monounsaturated Fatty Acid(s)
NADH	Nicotinamide Adenine Dinucleotide
PIT	Passive Integrated Transponder
PL	Phospholipids
PO	Palm Oil
PUFA	Polyunsaturated Fatty Acid(s)
RO	Rapeseed Oil
SAS	Statistical Analysis System
SFA	Saturated Fatty Acid(s)
SPSS	Statistical Package for the Social Sciences
TAG	Triacylglycerols

TLC	Thin Layer Chromatography
VLDL	Very Low Density Lipoprotein

1 Introduction

With an annual increase of ~10% since the 1950s, the aquaculture industry is the fastest growing food producing sector in the world and accounts for nearly 50% of the world's fish consumption today (SOFIA, 2008). Estimates show that the growth will continue over forthcoming decades as the demand increases with the growth in the human population (SOFIA, 2006). One of the main concerns encountered by the aquaculture industry is the great dependence on fish oil (FO) produced from wild fish as sole lipid source in the feeds. Given the estimated growth together with a production of FO that is estimated to be static (SOFIA, 2006), the dependence upon this finite resource could be risky to the aquaculture sector (Tacon, 2004). Consequently, there is a need for sustainable alternatives to FO. In fact, research efforts have been made to identify potential raw lipid materials that could act as substitutes to FO. The most successful alternatives have been oils of plant origin due to their global availability and favorable price and the fact that their nutritional properties can satisfy the nutritional requirements of the fish. However, the use comes with certain disadvantages, mainly in terms of altered fatty acid composition in the muscle of fish (Bell *et al.*, 2001; Torstensen *et al.*, 2005; Pettersson *et al.*, 2009).

This thesis investigates the effects of replacing FO with vegetable oils on lipid composition in salmonid tissues. Salmonids are considered fatty fish, containing high amounts of the long-chain polyunsaturated fatty acids (LCPUFA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) mainly derived from prey of wild fish or from FO included in the diet of farmed fish. These fatty acids are of great importance not only to the fish but also to human nutrition. It is well documented that these n-3 fatty acids have numerous beneficial effects on human health and fish is the greatest provider of these fatty acids (Connor, 2000). They have been suggested to decrease the risk of developing coronary heart disease

(Mozaffarian *et al.*, 2006), cancer (Leitzmann *et al.*, 2004), anxiety and depression (Raeder *et al.*, 2007), as well as having positive effects on early neurodevelopment, etc. (Mozaffarian & Rimm, 2006). When altering the lipid source in the feed of farmed fish by replacing FO with vegetable oil it is very important to be acquainted with how the lipid quality is affected in fish muscle in terms of human nutrition as well as fish welfare.

1.1 Aquaculture

As fish consumption is increasing due to a growing human population and health recommendations, the demand is mainly being met by the aquaculture industry as capture fishery production remains relatively stable (SOFIA, 2006). Aquaculture is the fastest growing food industry in the world, with an annual growth of approximately 10% since the 1950s (SOFIA, 2008). Although the majority of the growth increase is explained by the very large increase in Chinas aquaculture production, significant growth of 7% per annum still occurred in the rest of the world during 1970-2006 (SOFIA, 2008). In 2006, 110 million tonnes of food fish were supplied to the world by capture fisheries and the aquaculture industry. Of these, 51.7 million tonnes came from aquaculture, which thus accounted for 47%

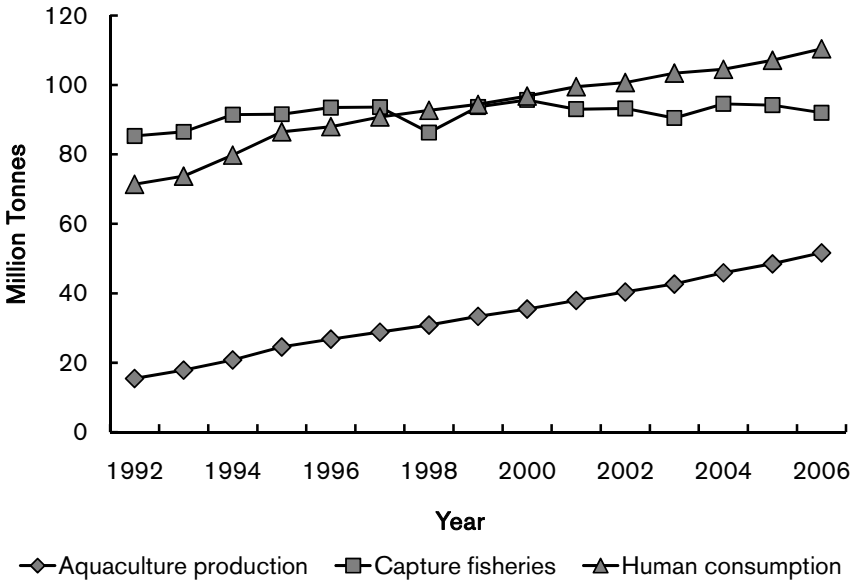

Figure 1. Total aquaculture production, global capture and human consumption of fish in 1992-2006 (aquatic plants excluded). Data obtained from SOFIA (1998, 2002, 2006, 2008).

of the total fish supply for human consumption (SOFIA, 2008) (Figure 1). As a comparison, in 1970 aquaculture only accounted for 4% of fish available for human consumption (SOFIA, 2006). Expressed per capita, the supply from aquaculture has increased from 0.7 kg in 1970 to 7.8 kg in 2006 (SOFIA, 2008). The production within different regions in the world is diverse (Table 1). China is by far the top producing country accounting for 67% of the total aquaculture production in 2006 with carp as the main fish species produced (SOFIA, 2008). Norway and Chile are the world's leading producers of salmonids accounting for 33% and 31% of world production, respectively (SOFIA, 2008). More than half the world's aquaculture production comprises freshwater fish, while marine fish only account for 3.5% of total aquaculture production (SOFIA, 2008). Given the high probability that the catch of capture fisheries will remain stagnant in the

Table 1. *Aquaculture production (aquatic plants excluded) in million tonnes (MT) of the world's top ten producing countries in 2004 and 2006. Data taken from SOFIA (2008)*

Country	Aquaculture Production (MT)	
	2004	2006
China	30.6	34.4
India	2.8	3.1
Vietnam	1.2	1.7
Thailand	1.3	1.4
Indonesia	1.1	1.3
Bangladesh	0.9	0.9
Chile	0.7	0.8
Japan	0.8	0.7
Norway	0.6	0.7
Philippines	0.5	0.6
Total	45.9	51.7

coming decades, aquaculture remains the most obvious sector for meeting the high demand for fish products for human consumption. FAO projections show that in order to maintain the present level of per capita consumption, world aquaculture production will need to produce 80 million tonnes by the year 2050 (FAO, 2006). Aquaculture has great potential for meeting this demand, but many difficult challenges remain to be addressed before this potential can be realised. One of the most important issues in this context is that the intensification and modification of the

industry must be implemented with consideration given to the responsible use of resources and to the environment.

1.2 Aquafeed

Feed is one of the major constraints for the expansion of aquaculture in terms of key raw materials such as fish meal and FO (SOFIA, 2006). Fish meal and FO are produced from a variety of wild small pelagic fish species. Commonly used species include anchovies, blue-whiting, herring, mackerel, capelin, menhaden, sardines, sprat and others. Some of these species are also used for direct human consumption, while others are solely used for production of fish meal and FO. A seven-fold increase in the global capture of these pelagic species has occurred since the 1950s with the sole purpose of supplying the fish meal and FO producers (Karalazos, 2007). In the past two decades there has been a stabilisation in captures at 20-25 million tonnes, possibly as a result of stock over-fishing, weather changes (e.g. El Nino) and the implementation of fish quotas (FAO Code of Conduct for Responsible Fisheries) (Silva & Turchini, 2008). In 2006, the global total production of fish meal was 5150 tonnes and that of FO 944 thousand tonnes (IFFO, 2008) (Figure 2). The aquaculture industry absorbed 56% of the total amount of FO produced and 87% of the fishmeal (SOFIA, 2008). FO provides the main source of metabolic energy in feed for a number of

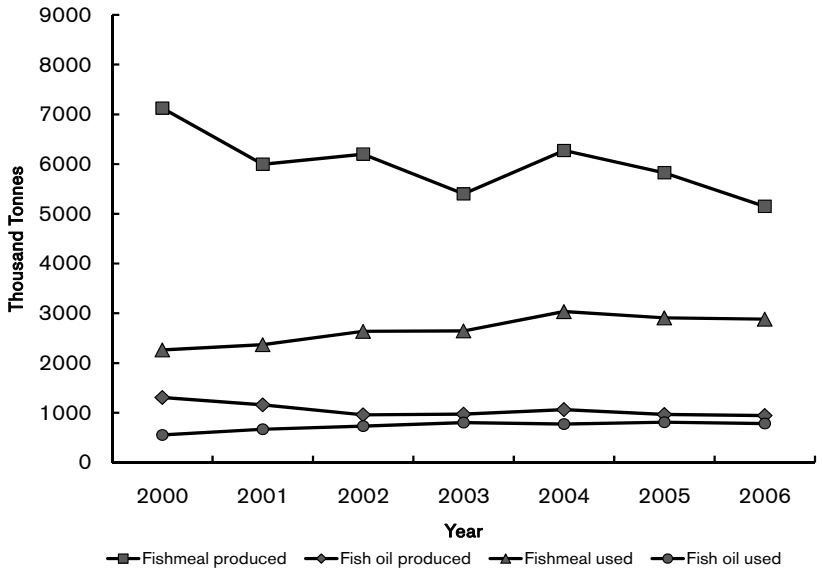

Figure 2. Total production and consumption of fishmeal and fish oil in 2000-2006. Data obtained from IFFO (2008).

cultured predatory fish, especially salmonids. Estimates show that salmonid production alone uses around 50% of the total FO production in the world (SOFIA, 2008). In order to produce a high quality product for the consumer in a short time, current extrusion techniques produce feed containing up to 40% lipid for salmonid production, hence the high usage of FO. The use of such a high energy diet in salmonid production is mainly designed to increase growth of the fish by maximizing the utilization of lipids and thereby using protein for growth instead of energy (Sargent *et al.*, 2002).

With a stagnant landing by global capture fisheries together with an increasing demand from the aquaculture feed industry as a consequence of increasing human consumption, the future availability of fish meal and fish oil is uncertain.

1.3 Replacement of fish oil

It is clear that the expansion of aquaculture is heavily dependent on the availability of fish meal and FO. As described above, this especially applies to the salmonid industry as it is the main user of total FO production due to its high FO content in the feed. Consequently, this generates a general concern since the annual production of FO is fairly constant and comes from a finite source. Therefore, there is currently a great need within the aquafeed industry to find and implement sustainable alternatives to FO. In recent years, substantial research efforts have been devoted to finding suitable and sustainable alternatives to FO. The major challenge in the search for substitutes is to maintain the recognised positive health effects of EPA and DHA from consuming fish, while simultaneously considering the importance of sustainability, economic benefits and fish welfare. One potential solution could be the use of fish offal or even use of by-catch, as these are resources that would otherwise be lost. Unfortunately, this has not been taken into practice yet. Other suggestions are the use of unicellular algae (Hertrampf & Piedad-Pascual, 2000), pelagic organisms (Carter *et al.*, 2003) or benthic invertebrates (Olsen *et al.*, 2004) containing similar fatty acid profiles as FO. However, the most promising alternatives so far in the search for suitable and sustainable substitutes are vegetable oils.

1.3.1 Vegetable oils

In contrast to FO production, which has basically remained stable in recent decades, the production of vegetable oils has increased considerably (IFFO, 2008; Malaysian Palm Oil Board, 2008) (Table 2). Consequently, this makes

vegetable oils a good alternative to FO in terms of availability, cost effectiveness and sustainability. In addition, research has shown that many vegetable oils have nutritional properties that have the potential of satisfying the nutritional and energy requirements of fish (NRC, 1993; Powell, 2003).

Table 2. *World production in million tonnes of selected vegetable oils in 2000-2006. (Malaysian Palm Oil Board, 2008)*

Year	Palm oil	Soyabean oil	Sunflower oil	Rapeseed oil	Olive oil	Fish oil
2000	21.9	25.6	9.8	14.5	2.5	1.3
2001	24.0	27.8	8.2	13.7	2.8	1.2
2002	25.4	29.9	7.6	13.3	2.8	1.0
2003	28.3	31.2	8.9	12.7	2.9	1.0
2004	31.0	30.7	9.4	15.1	3.1	1.1
2005	33.8	33.6	9.8	16.3	3.0	1.0
2006	37.1	35.3	11.2	18.5	2.8	0.9

Fatty acid composition

When considering the chemical composition of vegetable oils, some properties might cause some problems if vegetable oil is the only lipid source in feed. Vegetable oils lack the long-chain highly unsaturated fatty acids, HUFA (fatty acids $\geq C_{20}$ and with ≥ 3 double bonds, (Sargent *et al.*, 1989)), EPA and DHA, which are present in high amounts in FO (Sargent *et al.*, 2002). These fatty acids are essential for growth and a number of other physiological processes in the fish (Sargent *et al.*, 1995, 2002). Instead, vegetable oils are in general rich in n-6 and n-9 fatty acids, mainly linoleic acid (18:2n-6) and oleic acid (18:1n-9), and with moderate or low levels of n-3 (except linseed oil), mainly α-linolenic acid (18:3n-3) (Regost *et al.*, 2004). Depending on species, fish have different abilities to endogenously convert 18:3n-3 to 20:5n-3 and 22:6n-3 in the form of elongation and desaturation (described in section 1.5.2). Since marine prey already contains high amounts of HUFA, marine predatory fish generally have an insufficient ability to produce EPA and DHA *in vivo* for optimal growth while maintaining health (Sargent *et al.*, 1995). Freshwater fish, however, have been suggested to have a higher ability. Consequently, when choosing a potential vegetable oil for replacing FO in fish feeds, certain criteria have to be met. The substitute oil should provide sufficient energy in the form of saturated and monounsaturated fatty acids to maintain high growth rates. In addition, it should contain moderate levels of 18:3n-3, the fatty acid precursor for the endogenous conversion into EPA and DHA, and low

amounts of 18:2n-6 since it is poorly oxidized and therefore should be avoided (Bell *et al.*, 2002). Two vegetable oils fulfilling these criteria are rapeseed oil (RO) and palm oil (PO). The high amount of monounsaturated fatty acids (i.e. 18:1n-9 in RO) and saturated fatty acids (i.e. 16:0 in PO) makes them suitable since these fatty acids have been reported to be preferred for energy production in fish (Henderson & Sargent, 1985; Kiessling & Kiessling, 1993). In addition, moderate levels of 18:3n-3 are present in RO, suggesting a great potential for endogenous conversion to EPA and DHA. Several studies on these vegetable oils have been performed and the results show that they are successful in terms of growth but with an altered fatty acid composition compared with fish fed FO (Bell *et al.*, 2001, 2002; Caballero *et al.*, 2002; Tocher *et al.*, 2003; Torstensen *et al.*, 2005; Pettersson *et al.*, 2009). These changes mainly involve reduced levels of the HUFA, EPA and DHA and increased levels of 18:2n-6 and 18:1n-9 in the muscle of salmonid species.

Other lipid compounds

Vegetable oils also contain other minor lipid components such as phytosterols and tocopherols (Kamal-Eldin, 2005), which are not present in the natural diet of fish. These compounds are poorly investigated and could further affect physiological processes in fish. Phytosterols are naturally occurring compounds found in vegetable oils and are structurally related to cholesterol (Figure 3). The most abundant phytosterol is β-sitosterol, followed by campesterol and stigmasterol. Phytosterols are known to affect

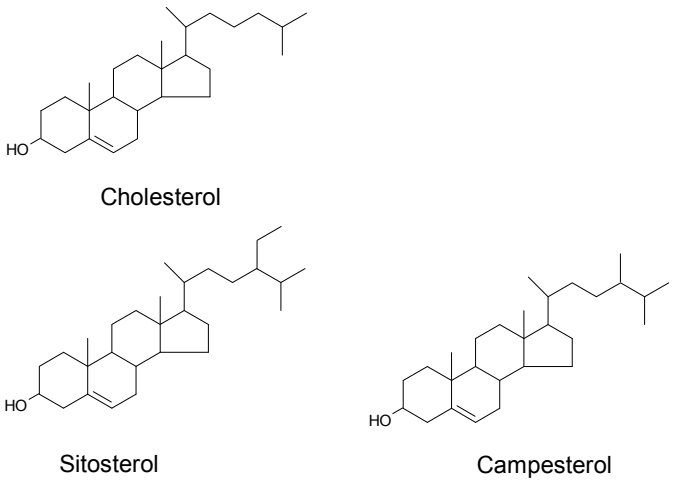

Figure 3. Chemeical structures of cholesterol, sitosterol and campesterol.

cholesterol metabolism and compete with cholesterol for binding sites, thereby reducing the low density lipoprotein (LDL) cholesterol levels in humans and resulting in a reduced risk of coronary heart disease (Ostlund, 2004; Earnest *et al.*, 2007). They have been implemented in the food industry by including them in margarines, butters, cereals, etc., referred to as 'functional food' to reduce heart diseases (Kuhlmann *et al.*, 2005). Phytosterols are also found in pulp and paper mill effluents and have been shown to affect the behavior, reproduction, endocrine function and development of fish (Mattsson *et al.*, 2001; Honkanen *et al.*, 2005).

Vegetable oils also contain tocopherols, of which α-tocopherol and γ-tocopherol are the major vitamin E compounds (Kamal-Eldin, 2005). Tocopherols are not generally the major components of vegetable oils, but their presence is crucial for preventing unsaturated fatty acids in vegetable oils from oxidation. Subsequently, the same ability can be used to enhance shelf-life of fish products. Studies have shown that increased amounts of vitamin E in the muscle of fish, due to dietary inclusion of vegetable oils, prolong the shelf life of frozen and fresh fish fillets (Jensen *et al.*, 1998). That in combination with the health benefits when consuming vitamin E has resulted in an oil industry keen on preserving the natural content of vitamin E during the refining stages of vegetable oils (Mag & Reichert, 2002).

1.4 Lipids

Lipids are a diverse group of compounds that are insoluble in water due to their chemical structure. They can be classified into several groups or lipid classes, but two main classes are commonly used; polar and neutral lipids. Polar lipids mainly include phospholipids (PL) and are generally considered to be structural or functional lipids, which are incorporated to a large extent in the membrane structure of cells. Neutral lipids mainly include triacylglycerols (TAG), diacylglycerols, monoacylglycerols and sterols and usually serve as energy sources. The triacylglycerols are more often storage lipids and reflect the fatty acid composition of the diet to a greater extent than PL (Olsen & Henderson, 1997). A TAG molecule consists of a glycerol molecule with each hydroxyl group esterified to a fatty acid. PL have a similar structure, with the exception that one of the free fatty acids has been exchanged for an alcohol such as choline, inositol, ethanolamine, serine or glycerol. Fatty acids contain a carbon chain with a methyl group at one end and a carboxyl group at the other. The length of the carbon chain and the site and number of double bonds determines the properties of the fatty acid. Saturated fatty acids (SFA) have no double bonds and have a straight

structure whereas unsaturated fatty acids can have up to six double bonds and various conformational structures. Fatty acids with one double bond are commonly called monounsaturated fatty acids (MUFA), while fatty acids with two or more double bonds are called polyunsaturated fatty acids (PUFA). The position of the first double bond in relation to the methyl end of the carbon chain in PUFA is important for the nomenclature. If the first double bond is in the third carbon atom from the methyl end, the fatty acid is termed an omega-3 (n-3) fatty acid, while on the sixth carbon it is termed an omega-6 fatty (n-6) acid (Figure 4). As described earlier, the conformational structures of the fatty acid molecule determine the properties of the fatty acid. For example, the straight molecular structure of saturated fatty acids enables them to be 'stacked', which results in close intermolecular interactions and these in turn result in a high melting point. However, the introduction of one or more double bonds in the hydrocarbon chain in unsaturated fatty acids results in one or more 'bends' in the molecule. These molecules do not 'stack' very well and the intermolecular interactions are much weaker than saturated molecules. As a

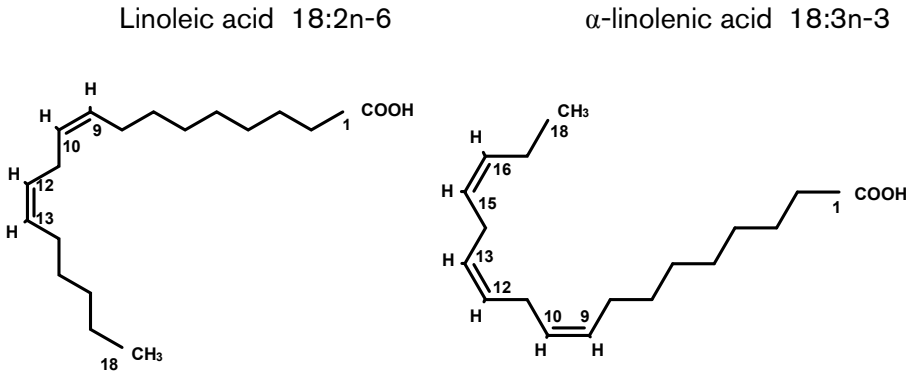

Figure 4. Chemical structures of the two essential fatty acids; Linoleic acid, 18:2n-6 (left) and α-linolenic acid, 18:3n-3 (right).

result, the melting points are much lower for unsaturated fatty acids. The different conformational structures of fatty acids and the properties that come with it are used for a number of different and vital functions in the body. Some fatty acids are termed essential fatty acids since they are critically important for cellular structure and function. They cannot be synthesized *de novo* in the body, but need to be included in the diet. In vertebrates, 18:3n-3 (α-linolenic acid) and 18:2n-6 (linoleic acid) (Figure 4) are considered essential fatty acids.

1.5 Lipid metabolism in fish

Lipids and their constituent fatty acids along with their metabolic derivatives are important compounds in the fish body for a number of functions such as growth, reproduction, health, etc. Fish also have a unique capability of storing and utilizing lipids in different ways depending on species. Different species are exposed to a variety of environmental conditions such as differences in temperature and changes in salinity levels and have subsequently developed special lipid mechanisms for dealing with the effects of these environmental factors.

The following sections mainly focus on lipid metabolism in salmonid species since they are used as experimental fish in this thesis.

1.5.1 Dietary uptake of lipids

Once lipids have been ingested by the fish, different digestive processes occur depending on species. The general digestive process for lipids is extracellular hydrolysis of lipids in the stomach, intestinal and caecal lumen by a variety of lipases and colipases. The primary site of lipid hydrolysis for salmonid species appears to be in the pyloric caecum and anterior intestine (Denstadli *et al.*, 2004). It is generally understood that short-chain fatty acids and glycerol are absorbed directly through the brush border of the enterocytes. Long-chain fatty acids are cleaved by lipases and emulsified by bile salts to form negatively charged aggregates called micelles, which are transported from the lumen to the brush border where they dissociate and fatty acids diffuse across the epithelial membrane. Once inside the enterocyte, the fatty acids are re-esterified into PL and TAG and grouped with proteins to form complexes called chylomicrons, which are transported to the liver via the hepatic portal vein and/or the lymphatic system as lipoproteins or very low density lipoproteins (VLDL) (Babin & Vernier, 1989; Tocher, 2003). Other possible transportation routes have been suggested. Torstensen *et al.* (2001) proposed direct transport of lipids from the intestine straight to muscle and adipose tissue, possibly through a transport system similar to the lymphatic system in mammals. In the liver lipids are further metabolized or transported to other tissues via the dorsal aorta as VLDL. The final metabolic fates of the dietary fatty acids differ depending on the nutritional status of the fish, etc. The following sections describe these fates in more detail and an overview is shown in Figure 5.

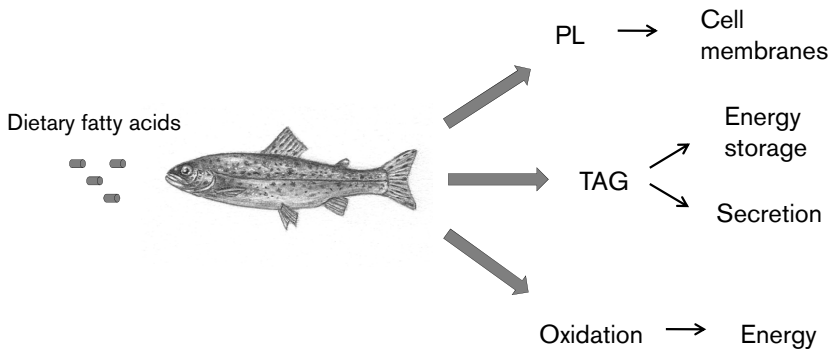

Figure 5. Metabolic fates of dietary fatty acids. (PL; phospholipids, TAG; triacylglycerols)

1.5.2 Biosynthesis, elongation and desaturation

Fish, like all known organisms, are able to endogenously synthesize the SFA 16:0 and 18:0 (Sargent *et al.*, 2002). This synthesis takes place in the cytoplasm, where acetyl-CoA is utilized in a pathway catalyzed by fatty acid synthase. From the *de novo* synthesis of 16:0 and 18:0, fish are able to produce 16:1n-9 and 18:1n-9, respectively, through desaturation by microsomal Δ^9 desaturase. Since fish lack desaturases beyond Δ^9, 18:2n-6 and 18:3n-3 cannot be synthesized endogenously and are therefore termed essential fatty acids. However, 18:2n-6 and 18:3n-3, once obtained from the feed, can be further elongated and desaturated into LCPUFA such as 20:4n-6 (arachidonic acid), EPA and DHA. The pathways of n-3 and n-6 elongation and desaturation have been well described in fish due to the interest in the health properties of these fatty acids (Tocher *et al.*, 2001, 2002, 2003; Zheng *et al.*, 2005; Tocher *et al.*, 2006a; Zheng *et al.*, 2009). They involve several elongation and desaturation steps mediated by the activity of Δ^5 and Δ^6 desaturases (Figure 6). The synthesis of these LCPUFA occurs in the microsomal fraction of the liver except for the chain shortening from 24:6n-3 to 22:6n-3, which occurs in the peroxisomes by β-oxidation. This ability for elongation and desaturation is considered to be more effective in freshwater fish than in marine fish. In marine fish, the mechanism is poorly understood and it has been suggested that marine fish have lost this ability or that it is severely repressed due to the high content of LCPUFA already present in their natural diet (Mourente *et al.*, 2005; Tocher *et al.*, 2006b). Therefore, it is also debateable whether 20:4n-6, 20:5n-3 and 22:6n-3 should be considered essential fatty acids in marine species (Sargent *et al.*, 1995).

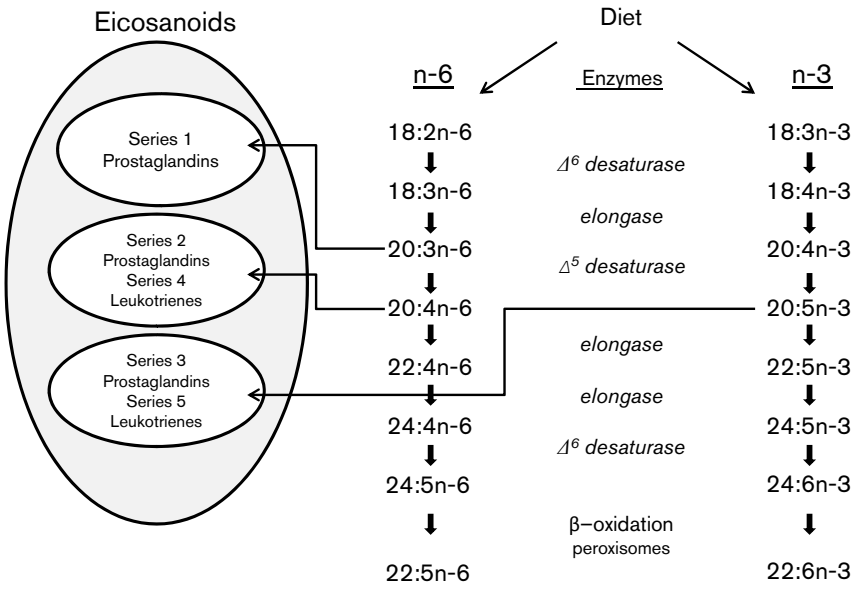

Figure 6. Elongation and desaturation pathway of n-6 and n-3 fatty acids and the precursors of eicosanoids.

1.5.3 Role of PL and TAG

Due to the high energy feed produced for farmed salmonids today, the ability of the fish to produce their own lipids from carbohydrates (lipogenesis) is thought to be minor and consequently has no significant influence on tissue lipid composition (Henderson, 1996). The high lipid intake is more than enough to satisfy the lipid requirements of the fish. The excess is generally stored as TAG in lipid droplets in the cytosol of fish tissue. The primary storage sites in salmonids are the visceral adipose tissue, the adipose tissue within the white muscle and, to a smaller extent the liver, although the liver can be the major lipid storage tissue for many marine species (Zhou *et al.*, 1995) (e.g. sharks, Atlantic cod, etc.). In addition, many studies have reported that TAG are much more influenced by dietary fatty acid composition in comparison with PL. As a result, tissues with high TAG content, such as lipid stores, may be more affected by dietary fatty acid composition than tissues low in TAG. PL, on the other hand, is less influenced by dietary fatty acids, confirming its role as a membrane lipid. PL in cell membranes generally contain high levels of 16:0, 18:1n-9, 20:5n-3 and especially 22:6n-3, which are of great importance for cell functioning (Henderson & Tocher, 1987).

1.5.4 Physiological role of n-3 and n-6

Long-chain n-3 and n-6 fatty acids generally have three main functions in fish: to act as an energy source (β-oxidation, described in next section), as structural components in cell membranes and as precursors of eicosanoids (Trautwein, 2001). As already described, the LCPUFA (mainly 20:5n-3 and 22:6n-3) are important structural constituents of the PL in cell membranes of fish. It is believed that the high content of DHA in cell membranes is due to the adaptation to cold environments. Since DHA is highly unsaturated, the conformational structure enables low melting point and fast response to certain processes. Arachidonic acid (20:4n-6) and to a smaller extent EPA are the precursors of prostaglandins, thromboxanes, prostacyclins and leukotrienes, commonly classified as eicosanoids (Figure 6). These are highly bioactive compounds with a number of diverse activities (Sargent *et al.*, 1999). They are reported to be important mediators in many physiological processes such as inflammatory and immunological responses etc. (Tocher *et al.*, 1996). In fish, there is also evidence supporting their involvement in reproductive function, hormone release, stress coping, etc. (Stanley-Samuelson, 1994).

1.5.5 β-oxidation

As mentioned earlier, one of the major roles of lipids and especially their fatty acids is to provide energy. In brief, this is accomplished by the catabolism of ingested and stored fatty acids through β-oxidation which occurs primarily in the inner space of the mitochondria (matrix) or in the peroxisomes. The name β-oxidation refers to the sequential removal of 2 carbon units by oxidation at the β-carbon position of the fatty acyl-CoA molecule. Every cycle of the β-oxidation generates 1 NADH, 1$FADH_2$ and one acetyl-CoA. After continuous oxidation of the acetyl-CoA to CO_2 in the tricarboxylic acid cycle, 3 NADH, 1 $FADH_2$ and 1 ATP are produced. The red muscle, liver and heart are generally known as the tissues with the highest β-oxidation capacity (Henderson & Tocher, 1987). However, considering the high amount of white muscle in fish, the total β-oxidation activity is very high in white muscle and should be considered an important (if not the most) tissue in energy production (Froyland *et al.*, 2000). However, other factors such as fish size, maturation and seasonal variation affect the catabolizing capability. It is well documented that certain fatty acids are preferentially utilized for β-oxidation in fish. Studies have shown that mitochondrial β-oxidation has a preference for short- and medium-chain SFA and MUFA over LCPUFA (more than C_{20}), which are generally oxidized in peroxisomes (Wanders *et al.*, 2001). 16:0, 18:1n-9, 20:1n-9 and

22:1n-9 have been reported to be readily metabolized for energy production in salmonids (Henderson & Sargent, 1984; Kiessling & Kiessling, 1993).

1.5.6 Other lipid compounds in fish

Other important lipid compounds found in fish tissues are cholesterol and α-tocopherol (Vitamin E). Cholesterol is found in all animal tissues and is an important component of biological cell membranes with functions such as precursor to bile acids, hormones and vitamins, providing mechanical strength as well as controlling different phase behaviors of membranes (Rog *et al.*, 2009). FO is rich in cholesterol with values ranging from 5 g kg^{-1} in menhaden oil to 7.7 g kg^{-1} in herring and sprat oil (Pettersson *et al.*, 2009; Turchini *et al.*, 2009). As described in section 1.3.1, tocopherols have important properties for lipid conservation. α-tocopherol is the main vitamin E compound found in fish and is required in the diet since it cannot be synthesized *de novo*. It has also been suggested that the vitamin E requirements of fish may be higher at low temperatures due to the increased amount of membrane PUFA which is associated with environmental adaptation (Henderson & Tocher, 1987).

1.5.7 Effects of temperature adaptation

Temperature has a significant influence on membrane and storage lipids in exothermic animals, such as fish, which are forced to adapt to seasonal variations and sudden changes in environmental temperature (Henderson & Tocher, 1987). It is well documented that fish can alter the composition of their biomembrane lipids in response to alterations in environmental temperatures. This is generally known as homeoviscous adaptation, a phenomenon by which fish maintain their cell membranes in a constant fluid state independent of the surrounding temperature (Hazel, 1984). This is accomplished by alteration of the fatty acid composition of structural lipids (PL) in the cell membranes. Previous studies have reported that the incorporation of unsaturated fatty acids is increasing in proportion to decreasing temperatures (Henderson & Tocher, 1987). The extent to which this process occurs is highly dependent on species and tissue. Thus, the major adaptation to decreasing temperature mainly takes place in the PL within the cell membranes. TAG are also affected by decreasing temperature but to a lesser extent than PL. Lipid digestibility in salmonids has also been proposed to be affected by shifts in temperature. The digestibility of SFA and MUFA in salmonids has been reported to be lower compared with PUFA (Ng *et al.*, 2003). Decreasing the temperature may reduce fatty acid digestibility further and subsequently reduce energy availability. Olsen *et al.*

(1998) reported that the digestibility of SFA was significantly reduced in Arctic charr (*Salvelinus alpinus*) maintained at 0.6 °C compared with Arctic charr held at 10 °C. A tendency towards a small reduction was observed for the MUFA, while the digestibility of PUFA was not affected at all by the temperature change. However, opposing results have also been reported. Austreng *et al.* (1979) reported that lipid digestibility in rainbow trout was almost the same at 3 and 11 °C. It has been observed that the digestibility of fatty acids increases with the degree of unsaturation but decreases with increasing chain length (Olsen & Ringo, 1997). Olsen and Ringo (1998) also reported that lowering the environmental temperature may cause some dietary oils to solidify in the fish gastrointestinal tract. Thus, the impact of dietary lipid sources appears to be determined by water temperature for salmonids.

1.5.8 Dietary effects on swimming performance

Studies on replacing FO with vegetable oils in salmonid diets have shown that high replacements rates are possible without any adverse effects on growth and feed efficiency, provided that the essential fatty acid requirements are met. However, the degree to which such replacements might influence the physiology of salmonids has not been extensively studied. Main concerns have been cardiac myopathy and impeded ability to handle stress in Atlantic salmon (*Salmo salar*) that had been fed excessive amounts of n-6 fatty acids in combination with low intake of n-3 fatty acids (Bell *et al.*, 1991, 1993; Seierstad *et al.*, 2005). Subsequently, suggestions have also been made that swimming performance is affected by modification of the diet. Wagner *et al.* (2004) found effects on swimming performance of Atlantic salmon fed diets containing different supplemental oils (anchovy oil and poultry fat) depending on the different n-3 HUFA/SFA ratios. The fish fed anchovy oil performed significantly better than the fish fed poultry fat, which had a low muscle ratio of n-3 HUFA to SFA. However, contradicting results have been reported by McKenzie *et al.* (1998), who found that increasing the amount of rapeseed oil in the diet resulted in incremental increases in swimming performance of Atlantic salmon, possibly explained by the energy from 18-carbon unsaturated fatty acids.

The relationship between temperature and swimming performance has been described as bell shaped with an optimal swimming speed at 14-17 °C for rainbow trout (Randall & Brauner, 1991). However, to date no study has been performed where both diet and temperature are considered. Thus, additional research is needed to elucidate the effects of dietary modifications

as well as the combination of diet and temperature on the physiological status of fish.

1.6 Natural freshwater food chain

As previously mentioned, the lipid source in intensive culture of salmonids has to date been supplied by FO originating from marine pelagic species containing high amounts of n-3 LCPUFA, particularly, EPA and DHA. The reason for using FO of marine origin is to provide a diet that is similar to the marine organisms which would be the natural prey of wild fish. However, many farmed salmonid species are freshwater species, spending their whole lifecycle or parts of it in freshwater. Using marine FO in the diet formulation of these species must be considered less desirable due to the different fatty acid composition between marine FO and the fatty acid composition found in freshwater invertebrates that wild freshwater fish feed upon (Bell *et al.*, 1994). It has been reported that the natural diet of freshwater fish, which mainly constitutes crustaceans and insects, generally has higher levels of 18:3n-3 and 18:2n-6 and lower levels of DHA, i.e. resulting in decreased n-3/n-6 dietary ratios, in comparison with marine organisms and FO (Sargent *et al.*, 1999). In this context, it has been proposed that some vegetable oil diets resemble the fatty acid profile of freshwater prey more than marine FO, which could benefit freshwater fish (Bell *et al.*, 1994). Therefore, when choosing a potential vegetable oil for substitution of FO in feeds for farmed fish, especially coldwater fish, special consideration should be given to the natural food chain for each individual species in an attempt to mimic the fatty acid composition of the species' natural prey.

1.7 Fish for human consumption

In the past two decades there has been great interest in the nutritional value of fish as the evidence of the unique health benefits of n-3 LCPUFA is continually increasing. This was first recognized in nutritional studies on Inuit subjects, who displayed low plasma levels of cholesterol and low incidence of heart disease, which was highly correlated with the high dietary intake of FOs from marine fish and mammals (Bang *et al.*, 1971). This finding stimulated scientists worldwide and has resulted in a number of studies investigating the effects of the intake of fish and FO and their high content of the n-3 fatty acids, EPA and DHA. n-3 fatty acids are intimately involved in the control of inflammation, cardiovascular health, cerebral

development, immune response, hormone modulation etc. (Bourre *et al.*, 1993; Stanley-Samuelson, 1994; Simopoulos, 1999; Calder, 2006; Mozaffarian & Rimm, 2006). n-6 fatty acids are generally known to promote inflammatory responses in the body and are therefore considered less desirable in the diet. In fact, both n-3 and n-6 fatty acids are essential for body functions (described in section 1.5.4) and it is the balance of the two in relation to each other that is of importance. However, an excess of n-6 fatty acids can create an imbalance of the ratio and result in pro-inflammatory response, propagation of cancer, heart disease, stroke, etc. (Connor, 2000; Leitzmann *et al.*, 2004; Calder, 2008). The recommended n-3/n-6 ratio of humans is in the range 1:1-1:4, which is also the ratio occurring in most wild animals (Simopoulos, 2002). The Western diet today has resulted in a n-3/n-6 ratio ranging from 1:10 to 1:25. The increased consumption of n-6 fatty acids in past century is mainly due to the large-scale production of hydrogenated vegetable oils and the introduction of grain feeds for domestic livestock (Simopoulos, 2002).

2 Objectives

The overall aims of this project were to investigate the effects of two vegetable oils as substitutes for FO in fish feeds on growth and lipid composition in different tissues, and to examine the feed preferences of two salmonid species important for Swedish aquaculture. Attention was focused on fish welfare and on the nutritional quality of fish for human consumption.

Specific objectives were to:

- Study the effects of rapeseed oil on fatty acid composition, uptake of minor lipid compounds and feed preference by rainbow trout (Paper I)

- Study the effects of rapeseed oil on fatty acid composition and sterol content of Arctic charr (Paper II)

- Investigate the effects of palm oil and rapeseed oil on lipid content and swimming ability of Arctic charr (Paper III)

- Compare the fatty acid composition of wild freshwater Arctic charr with that of farmed fish fed a diet supplemented with marine fish oil (Papers II and IV)

3 Materials and methods

This section describes the material and methods used in the studies included in this thesis. For a more detailed description of the procedures presented below, see Papers I-IV. An overview of the material and methods used in all studies is shown in Table 3.

3.1 Fish rearing, treatments and sampling

3.1.1 Experimental diets

The diets used in Papers I and II were prepared in our laboratory facilities using the formula described by Sanchez-Vazquez *et al.* (1999). Each kilogram of the diets produced was formulated to contain 435 g of proteins, 97.5 g of carbohydrates and 217.5 g of lipid. Four experimental diets were produced. The control diet contained 100% FO while the remaining diets contained increasing levels of RO at 25%, 50% and 75% of total lipid added. The RO used was organically produced at Julita Farm (Julita, Sweden) where the seeds were cold-pressed without any additives. The FO was produced from sprat (*Sprattus sprattus*) at Triplenine, Esbjerg, Denmark. The average total lipid, fatty acid composition, tocopherol and sterol content of the diets used in Papers I and II are shown in Table 4. The diets used in Paper III were produced in a similar way to the diets in Papers I and II. However, the dietary ingredients were different in an attempt to mimic the diets commonly used for farmed Arctic charr. Here we also implemented PO as a potential substitute for FO. The lipid content was in the range 15-16% instead of 20-22%. The control diet contained 100% FO (FO) and the two experimental diets contained 25%FO:75%RO (RO) and 25%FO:37.5%RO:37.5%PO (ROPO). The RO and FO were obtained from the same producers as in Papers I and II and the PO was bought from a grocery shop (Crude Red Palm oil, RACINES.SA®, Montpellier, France).

Table 3. *Schematic overview of the experimental material and parameters studied in Papers I-IV*

Study	I	II	III	IV
Species	Rainbow trout	Arctic charr	Arctic charr	Arctic charr (wild+farmed)
Sample size	216	216	300	–
Initial size (g)	75.6 ± 16.4	47.0 ± 13.0	85.7 ± 16.5	–
Treatment	Rapeseed oil (four diets)	Rapeseed oil (four diets)	Rapeseed and palm oil (three diets)	–
Sample size (lipid analyses)	6	6	6	6
Tissues	White muscle	White muscle	White muscle	White muscle
	Red muscle	Liver		
	Liver			
Measurements	Total lipid	Total lipid	Total lipid	Total lipid
	Fatty acids	Fatty acids	Fatty acids	Fatty acids
	Lipid classes	Sterols	Swimming performance	
	Sterols			
	Tocopherols			
	Preference			

Table 4. *Average total lipid (g 100g^{-1} wet weight), fatty acid composition (% of total fatty acids), vitamin content (mg kg^{-1} lipid) and sterol content (mg g^{-1} lipid) in the four experimental diets containing different levels of replacement with rapeseed oil (RO) used in Papers I and II*

Parameter	0% RO	25% RO	50% RO	75% RO
Total lipid	2.0	1.9	2.1	1.9
Fatty acids				
14:0	8.2	6.0	4.3	2.2
16:0	18.7	14.9	12.0	8.2
18:0	2.8	2.5	2.2	1.9
16:1n-7	7.1	5.2	3.8	1.9
18:1n-9	10.9	23.0	33.4	45.2
20:1n-9	4.2	3.5	2.7	1.8
22:1[1]	7.1	5.5	3.7	1.8
18:2n-6	1.7	6.8	11.4	16.7
20:4n-6	0.6	0.5	0.3	0.2
18:3n-3	1.1	3.9	6.3	9.1
18:4n-3	3.4	2.5	1.6	0.8
20:5n-3	11.1	8.4	5.7	2.8
22:6n-3	9.6	7.3	5.1	2.6
n-3/n-6	10.4	3.2	1.7	1.0
Vitamins[2]				
α-tocopherol	205.0	202.8	222.8	248.2
γ-tocopherol	n.d.	39.0	79.6	120.4
Sterols				
Cholesterol	5.5	4.4	3.4	2.0
Campesterol	n.d.	0.4	0.8	1.1
Sitosterol	n.d.	0.5	0.9	1.3

Abbreviation: n.d.= not detected.

[1] Includes 22:1n-9 and 22:1n-11.

[2] Only analyzed in Paper I.

3.1.2 Reared fish

In Paper I, 216 juvenile rainbow trout (*Oncorhynchus mykiss*) (75.6±16.4 g) and in Paper II, 216 juvenile Arctic charr (*Salvelinus alpinus*) (47.0±13.0g) were evenly distributed into 12 groups and put into six divided 1m^3 tanks. Each section was supplied with brackish water (3 g L^{-1}) maintaining 10 ºC at a flow rate of 5 L min^{-1}. The water level was set to 0.7 m, which gave a water volume of 350 L in each half. Triplicate groups were fed one of the four diets containing either 0%, 25%, 50% or 75% RO of the total lipids added for 51 days (Paper I) or 79 days (Paper II) until a twofold weight increase was obtained (Paper I, 142.5±24.5g; Paper II, 92.3±28.2g). On the day of final sampling, two fish from each triplicate group (six from each diet) were selected based on whether they had increased at least two-fold in weight, which was possible to detect as the fish were PIT-tagged (Passive Integrated Transponder). The fish were anesthetized and killed by a blow to the head and the fillet and liver were dissected from each fish. The fillets were stored on ice and the liver was washed in sodium chloride before being frozen in liquid nitrogen. All samples were then stored at -80 ºC until further analysis.

In Paper III, 300 juvenile PIT-tagged Arctic charr with an initial weight of 85.7±16.5g were equally distributed into 12 groups (25 fish in each group) and put into circular tanks supplied with slightly brackish water (3‰) maintaining 10 ºC at a flow rate of 5 L min^{-1}. The water level was set to 0.5 m, which gave a water volume of 500L. Each of the formulated diets described in section 3.1.1 was fed to four replicate groups until at least a twofold weight increase had occurred (173.1±33.8g), which was obtained after 14 weeks. Six fish from each diet were anesthetized and killed by a blow to the head and the fillet was dissected from each fish. The fillets were put on ice before being stored at -80 ºC until further lipid analysis.

3.1.3 Wild fish

As a complement to the dietary experiment in Paper II, four wild Arctic charr (176.8±48.6g) from two coldwater lakes in northern Sweden were obtained from a local fisherman in order to compare fatty acid profiles between wild and farmed individuals. However, the sample size was small and the weights were not matched against the experimental fish. As a result, a larger study (Paper IV) was planned and performed one year later. In that study, wild Artic charr were caught in nets in three clearwater lakes in the sub-Arctic region of northern Sweden. Lake Ruozutjaure and Lake Vuorejaure are located in the low alpine belt while Lake Almberga is located in the birch forest belt. Arctic charr was the only fish species

inhabiting these lakes. Six fish from each lake were sampled resulting in 18 individuals with an average weight of 70.6±12.1g. In both Paper II and IV, fillets were dissected and stored at -80 °C until further lipid analysis.

3.2 Lipid analysis

3.2.1 Lipid extraction

White muscle samples in all papers, red muscle in Paper I, liver in Papers I and II and feed in Papers I-III, were homogenized and extracted in hexane:isopropanol (3:2 v/v) according to Hara & Radin (1978). The total lipid of all tissues was fractioned on TLC (Thin Layer Chromatography) silica-coated plates 20 x 20 cm 60 F 254 (MERCK, Darmstadt, Germany) into phospholipids and triacylglycerols by placing the plates in a hexane:diethylether:acetic acid (85:15:1 v/v/v) solution (Dutta & Appelqvist, 1989).

3.2.2 Determination of lipid classes

In Paper I, white and red muscle and liver lipids were analyzed by TLC to determine the composition of different lipid classes. TLC glass plates (20 x 10 cm; silica gel 60; 0.20 mm layer, Merck, Darmstadt, Germany) were used the stationary phase. The analysis was performed according to Olsen & Henderson (1989) with minor modifications. Lipid classes were identified by comparing the samples to an external standard (TLC 18-4A, Nu-Chek Prep, Elysian, USA).

3.2.3 Fatty acid analysis

Phospholipid and triacylglycerol fractions from all tissues in all studies were converted to fatty acid methyl esters (FAME) in order to be analyzed on gas chromatography (GC). The FAME were prepared according to Appelqvist (1968) and then analyzed with GC according to Fredriksson-Eriksson & Pickova (2007). In Papers I and II, peak areas were integrated using Star chromatography workstation software version 5.5 (Varian AB, Stockholm, Sweden) while in Papers III and IV, peak areas were integrated using Galaxie chromatography software version 1.9 (Varian AB, Stockholm, Sweden).

3.2.4 Sterol analysis

In Papers I and II, sterols were analyzed according to Savage *et al.* (1997). Samples of 10 mg lipids from white muscle and liver were hydrolyzed in aqueous ethanolic alkali solution by heating and the non-saponifiables were

dissolved in the organic phase, evaporated to dryness and silylated. The silylated sterols were separated and quantified by GC-flame ionization detector (FID) in Papers I and II and GC-mass spectrometry (MS) in Paper I (Johnsson & Dutta, 2003).

3.2.5 Analysis of tocopherols

The vitamin E content in white and red muscle and liver in Paper I was analyzed on HPLC according to Hogberg *et al.* (2002). In brief, 5 mg lipid were saponified and extracted with hexane, evaporated and diluted with the mobile phase consisting of 95% methanol:acetonitrile (1:1 v/v) and 5% chloroform. Analyses were performed with a Merck Hitachi L7100 pump, a F1 L-7485 detector and an L-7200 autosampler (Merck Hitachi, Eurolab, Darmstadt, Germany). The HPLC column was a 4.0 x 250 mm RP-18 LiChroCART (Merck KGaA, Darmstadt, Germany). Quantification and identification of the vitamins were carried out by comparison with external standards.

3.3 Preference test

In order to investigate the fish's own choice of feed, a self-selection preference test was performed on individual fish as a complement to the feeding experiment in Paper I. Four 170 L glass aquaria were divided into three sections by opaque plastic walls with an opening in the middle that enabled the fish to swim between the three sections. Six individuals from each dietary group were selected after the dietary experiment giving in total 24 individuals. The self-selection was based on offering one of three diets in each of the three sections. Each diet was given as three daily meals from 6.00 to 13.00 h by battery-driven aquarium feeders (Fish mate F 14 aquarium fish feeder, Pet mate Ltd, England). Each fish had a choice of three of the four available diets. Two of the diets were always the extremes 0% and 75% RO, and for those fish not fed these diets prior to the self-selection test, the third option was the diet composition from the previous dietary study. For the fish that were fed 0 and 75% RO, the third diet option was the diet with least resemblance to the growth diet. Thus, a fish that was previously fed 0% RO was a given a choice of 0%, 75% and 50% RO. During an experimental period of 10 days, rejected feed was collected in each section between 14.00 and 15.00 h, dried and separated from faeces and measured by mass.

3.4 Swimming performance

In Paper III, prolonged swimming performance was measured to determine whether the vegetable oil diets had any significant effect on the physiological status of the experimental fish. Thirty-six individuals (12 from each diet) were selected, with an average weight and length of 221.0±43.3 g; 25.8±1.4 cm, and put in nine 170 L glass aquaria maintaining a temperature of 10 °C (same temperature as in the feeding experiment). Fish were kept separated according to previous diet and were gradually acclimatised to two additional temperatures: 4 °C and 17 °C during approximately 12 h. The 4 °C temperature was maintained by keeping fish in a flowthrough system in a climate room maintained at 3 °C and the 17 °C temperature was achieved by heat exchangers. Swimming performance of fish from the different experimental groups was measured in a 150 L swim tunnel (Swim Tunnel 150, Loligo Systems, Tjele Denmark) at all experimental temperatures. A 45 minute practice swim was performed before the actual swimming test to provide an estimate of the critical swimming speed (U_{crit}) for each temperature (Jain *et al.*, 1997). The day after the practice swim, individual Arctic charr were tested with a ramp–U_{crit} protocol according to Jain *et al.* (1997). In brief, fish were ramped to 75% of the estimated U_{crit} (determined from the practice swim) by speed increments every 2 min. Thereafter, speed increments of ~0.1m s^{-1} were applied every 30 min until the fish fatigued. In all tests, fatigue swimming velocities were defined as the moment when the fish was unable to remove itself from the rear grid during 20 sec.

3.5 Calculations and statistics

Individual daily growth coefficient (DGC) was determined in Papers I and II and calculated according to:

$$DGC = 100 \times (w2^{1/3} - w1^{1/3})\ D^{-1}$$

where w2 was the final weight, w1 the initial weight and D the number of days (Cowey, 1992)

Critical swimming speed (U_{crit}) was used as an indicator of the physiological status of fish in Paper III and was calculated according to Brett (1964):

$$U_{crit} = u_i + (t_i/t_{ii} \times u_{ii})$$

where U_{crit} = critical swimming speed (cm s^{-1})
u_i = highest velocity at which the fish swam the entire time period (cm s^{-1})
u_{ii} = incremental speed increase (cm s^{-1})
t_i = time the fish swam at fatigue velocity (min)
t_{ii} = prescribed time interval for swimming at a given velocity (min)

One-Way ANOVA in SPSS (Vers. 11.5 in Papers I and II; Vers. 15.0 in Paper III) was used to test the differences in weight (Papers I-III), growth rate (Papers I and II), proportion of rejected pellets depending on diet (Paper I) and swimming performance (Paper III). For all the biochemical analyses, General Linear Model (GLM) of SAS (SAS Institute Inc., Cary, N.C., USA, Vers. 9.1) was used to determine significant differences between dietary treatments (Papers I-III) and differences between wild and farmed fish (Paper IV).

4 Results and discussion

4.1 Growth

Replacing FO with different amounts of RO did not affect growth in rainbow trout (Paper I) or Arctic charr (Paper II). Furthermore, 75% replacement of FO by RO or a 75 % blend of RO and PO did not have any significant effect on growth of Arctic charr in Paper III. This is in agreement with many other studies on salmonid species that have concluded that vegetable oils are good substitutes for FO in terms of growth (Bell *et al.*, 2001; Rosenlund *et al.*, 2001; Bell *et al.*, 2002; Caballero *et al.*, 2002; Bell *et al.*, 2003a; Tocher *et al.*, 2003; Ng *et al.*, 2004; Torstensen *et al.*, 2004; Fonseca-Madrigal *et al.*, 2005; Torstensen *et al.*, 2005; Bell *et al.*, 2006; Richard *et al.*, 2006).

4.2 Total lipid

Fat content of all experimental fish is shown in Table 5. No significant differences were found in total lipid content of white, red muscle and liver between rainbow trout fed the dietary treatments containing different levels of RO in Paper I. Similarly, in Paper II, no effect of the RO diets was found on total lipid content in white muscle of Arctic charr. However, a significant effect was found in total lipid content in the liver between fish fed 0% RO (100% FO) and 75% RO, with the highest content in the latter. In Paper III, the results of total lipid content of white muscle in Arctic charr showed a small but significant difference between fish fed FO and those fed ROPO (2.0% compared with 2.7%).

The results in Paper I indicate that no excessive lipid deposition occurred in any of the analyzed tissues from rainbow trout fed RO diets containing as much as 75% RO.

Table 5. *Fat content (g 100g^{-1}) of white muscle (WM), red muscle (RM) and liver in rainbow trout and Arctic charr in Papers I-IV.*

Paper	Species	Tissue	0% RO	25% RO	50% RO	75% RO
I	Rainbow trout	WM	2.0 ± 0.2	2.0 ± 0.3	1.9 ± 0.2	2.0 ± 0.3
		RM	2.1 ± 0.3	2.3 ± 0.5	2.3 ± 0.4	2.3 ± 0.3
		Liver	3.1 ± 0.4	3.3 ± 0.3	3.4 ± 0.4	3.0 ± 0.4
II	Arctic charr	WM	2.2 ± 0.7	1.9 ± 0.2	2.1 ± 0.3	1.8 ± 0.3
		Liver	4.6 ± 0.8^{a}	6.4 ± 2.0^{ab}	6.1 ± 1.1^{ab}	6.5 ± 0.8^{b}
			FO	RO	ROPO	
III	Arctic charr	WM	2.0 ± 0.4^{a}	2.1 ± 0.4^{ab}	2.7 ± 0.6^{b}	
			Almberga	Ruozotjaure	Vuorejaure	
IV	Arctic charr	WM	0.8 ± 0.1^{a}	1.1 ± 0.2^{a}	1.6 ± 0.3^{b}	

Abbreviations: RO=rapeseed oil, FO=fish oil, ROPO = rapeseed oil/palm oil.
Values are means ± S.D. (n=6). Different superscripts indicate significant differences ($p<0.05$)

In Paper II, even though the white muscle was not affected by the dietary inclusions of RO, a significant effect was found in total lipid content in the liver between fish fed 0% RO and 75% RO. Previous studies have reported that salmonids fed diets low in essential fatty acids tend to develop signs of swollen, pale and fatty livers (Henderson & Tocher, 1987). This has also been reported in rats and is assumed to be the result of dysfunctional lipoprotein metabolism (Fukuzawa *et al.*, 1971). In addition, Takeuchi & Watanabe (1982) found higher liver lipid content in chum salmon (*Oncorhynchus keta*) and coho salmon (*Oncorhynchus kisutch*) fed a diet rich in 18:2n-6 compared those fed a PUFA-deficient diet. Although no sign of pale or swollen liver that would suggest a dysfunctional liver was observed in this study, lipid content was still affected.

The findings in Paper III may indicate excessive lipid deposition in white muscle of Arctic charr fed a blend of RO and PO. The inclusion of 37.5% PO seemed to be responsible for the higher lipid content, since no such increase in lipid content was seen in fish fed only RO. In contrast to this study, an earlier study performed by Bell *et al.* (2002) showed a lower lipid content in white muscle of Atlantic salmon fed 50% PO at the expense of FO. One explanation could be species dependence, with Arctic charr perhaps having a lipid metabolism different from that of other salmonid

species in metabolizing PO. It is worth mentioning in this context that none of the fish in these studies were grown to slaughter weight so no major conclusions should be drawn on lifetime metabolism.

The analysis of wild Arctic charr (Paper IV) showed some variation in total lipid content in fish between lakes of origin. Leanest fish were found in Lake Almberga and Lake Ruozutjaure compared with fish from Lake Vuorejaure which had a significantly higher lipid content. When wild fish were compared with control fish (0% RO) from Paper II, representing farmed fish, higher lipid content was found in the farmed fish irrespective of lake origin. This is in accordance with many studies demonstrating higher lipid content in farmed fish compared with wild. However, the differences in lipid content in this study were fairly small. Larger differences would most likely be expected if the fish had reached slaughter weight since the fat content is much higher in the commercial diet compared with the prey on which wild fish feed naturally.

4.3 Lipid class composition

The lipid content in white and red muscle and liver of rainbow trout in Paper I was analyzed for lipid class composition. No significant differences in lipid classes in white muscle were found between the RO treatments. In red muscle, a significantly higher proportion of PL and lower proportion of TAG were found in the control fish (0% RO) compared with all fish fed the RO diets. Furthermore, in the liver, a significantly higher amount of sterols was found in 0% RO and 75% RO fish compared with fish fed 25% RO. Although significant, the differences were small and can only be explained by the total lipid in the individual fish within each dietary treatment, which correlates with the proportion of PL, TAG and sterol in the same fish.

4.4 Fatty acid composition

A number of studies on salmonids have shown that the fatty acid composition of fish tissue clearly reflects the composition found in the diet. In fact, a linear relationship has been observed between individual fatty acids in the diet and the concentrations found in total lipid in fish tissue (Bell *et al.*, 2001, 2002, 2003b; Torstensen *et al.*, 2004). The results of the dietary experiments in this thesis showed no exception to those findings. Feeding rainbow trout and Arctic charr different diets where FO had been replaced by different levels of vegetable oils clearly influenced the fatty acid profile of fish tissue.

In the PL fraction of white muscle of rainbow trout, SFA generally decreased and MUFA increased, mainly due to 18:1n-9, with higher RO content in the feed (Table 6). Total n-3 PUFA was not affected, but individual n-3 fatty acids differed; 18:3n-3 increased while 20:5n-3 decreased with higher RO levels. 22:6n-3, the most predominant fatty acid, was not affected by the dietary treatments. Due to the increase in 18:2n-6, the n-3/n-6 ratio was significantly reduced with every inclusion of RO. The results for red muscle and liver showed a similar pattern, but with different percentages.

White and red muscle of rainbow trout TAG responded very similarly to the RO treatments (Figure 7). In general, total SFA and total n-3 PUFA decreased, while total MUFA and total n-6 PUFA increased, with more RO in the diet. The main contributor to the decrease in total n-3 PUFA was 22:6n-3 which decreased with every RO inclusion in both muscle types. Here too, 18:2n-6 was most responsible for the increase in total n-6 PUFA resulting in a decreased ratio of n-3/n-6 with higher RO content. The fatty acid composition in the TAG fraction of the liver differed slightly from the muscle types. Significant effects on total MUFA, 18:2n-6, 20:5n-3, 22:6n-3 and n-3/n-6 were only found in fish fed 0% RO compared with the other diets. These results are in agreement with a study by Torstensen *et al.* (2000), who reported that white and red muscle are more affected by dietary fatty acids than liver. In addition, the results of the present study are further supported by Olsen and Henderson (1997) and Torstensen *et al.* (2004) who reported that PL are less affected by dietary treatments than TAG due to their functional roles as membrane and storage lipids, respectively. This statement is especially supported in the present study by the relatively stable amounts of 22:6n-3 in the PL of all tissues analyzed irrespective of diet. The less pronounced effects of dietary fatty acids on the fatty acid composition of the PL fraction of tissue lipid can most likely be explained by the homeostatic relationship that the fish attempts to maintain within the fatty acid profile. This is in order to sustain optimal membrane function by actively modifying dietary fatty acids by selective metabolism to maintain specific levels in their muscle. The selective deposition of 22:6n-3 in the TAG fraction of muscle is also noteworthy, since a two-fold decrease was observed in the muscle between the extreme diets (0% RO and 75% RO) compared with a four-fold decrease in the feed.

In Paper II, the fatty acid composition of the TAG fraction of white muscle (Figure 7) and liver of Arctic charr responded in a very similar way to the RO treatments as the corresponding tissues in rainbow trout in

Table 6. *Fatty acid profile (% of total fatty acids) in phospholipid fraction of white muscle from rainbow trout (Paper I) and Arctic charr (Paper II) fed four experimental rapeseed oil (RO) diets*

Fatty acids	0% RO	25% RO	50% RO	75% RO
Rainbow trout (I)				
16:0	22.6±1.4^{a}	21.5±1.6^{a}	21.3±1.4^{ab}	19.8±1.0^{b}
18:1n-9	3.6±0.3^{a}	5.5±0.4^{b}	6.6±1.0^{c}	8.4±1.2^{d}
18:2n-6	0.6±0.1^{a}	1.6±0.2^{b}	2.3±0.4^{c}	3.4±0.6^{d}
20:4n-6	0.9±0.1^{ab}	0.8±0.1^{a}	0.8±0.1^{a}	0.9±0.1^{b}
18:3n-3	0.5±0.1^{a}	1.1±0.1^{b}	1.5±0.2^{c}	2.2±0.4^{d}
20:5n-3	7.2±0.4^{a}	7.1±0.6^{a}	6.1±0.4^{b}	5.0±0.3^{c}
22:6n-3	49.8±1.6	49.4±2.1	48.8±4.0	48.0±3.2
Total SFA[1]	28.3±1.3^{a}	26.7±1.6^{ab}	26.3±2.0^{bc}	24.6±1.1^{c}
Total MUFA[2]	8.0±0.8^{a}	9.3±0.8^{ab}	10.2±1.6^{bc}	11.8±1.3^{c}
Total PUFA	61.8±1.4	62.8±2.2	62.4±3.4	62.5±1.9
Total n-3[3]	60.0±1.6	60.0±2.2	58.8±3.8	57.5±2.6
Total n-6[4]	1.8±0.3^{a}	2.7±0.2^{b}	3.6±0.4^{c}	5.0±0.7^{d}
n-3/n-6	33.2±5.4^{a}	22.0±1.8^{b}	16.6±3.1^{c}	11.7±2.3^{d}
Arctic charr (II)				
16:0	21.8±0.8^{a}	21.6±1.0^{a}	21.1±1.1^{ab}	20.3±1.1^{b}
18:1n-9	5.8±0.3^{a}	8.0±0.7^{b}	10.3±0.6^{c}	12.1±0.8^{d}
18:2n-6	0.9±0.1^{a}	1.9±0.2^{b}	3.1±0.2^{c}	4.6±0.2^{d}
20:4n-6	1.4±0.1^{ab}	1.4±0.1^{ab}	1.3±0.1^{a}	1.4±0.1^{b}
18:3n-3	0.4±0.1^{a}	0.9±0.1^{b}	1.7±0.1^{c}	2.4±0.2^{d}
20:5n-3	11.3±0.7^{a}	10.1±0.3^{b}	9.6±0.3^{b}	8.6±0.6^{c}
22:6n-3	41.0±1.9^{a}	39.7±2.4^{ab}	37.5±1.2^{b}	35.8±1.6^{b}
Total SFA[1]	27.3±0.9^{a}	27.2±1.0^{a}	26.4±1.0^{ab}	25.2±1.2^{b}
Total MUFA[2]	11.6±0.6^{a}	13.2±1.0^{b}	15.3±0.8^{c}	16.6±0.9^{d}
Total PUFA	57.8±1.1^{a}	56.5±2.2^{ab}	55.7±1.1^{b}	55.7±1.2^{b}
Total n-3[3]	55.3±1.3^{a}	53.0±2.3^{b}	50.9±1.1^{c}	49.0±1.3^{d}
Total n-6[4]	2.4±0.2^{a}	3.5±0.2^{b}	4.8±0.2^{c}	6.7±0.3^{d}
n-3/n-6	22.8±2.0^{a}	15.1±1.5^{b}	10.6±0.6^{c}	7.3±0.4^{d}

Values are means ± S.D. (n = 6). Values in the same row but with different superscripts are significantly different ($p<0.05$). Abbreviations: SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids.

[1] Includes 12:0, 14:0, 15:0, 17:0, 20:0, 22:0, 24:0.

[2] Includes 16:1n-7, 18:1n-9trans, 18:1n-7, 18:1n-5, 20:1n-9, 22:1n-11, 24:1.

[3] Includes 18:4n-3, 20:3n-3, 20:4n-3, 22:5n-3.

[4] Includes 18:3n-6, 20:2n-6, 20:3n-6, 22:4n-6, 22:5n-6.

Paper I, confirming the role of TAG as a storage lipid that clearly reflects the fatty acid composition of the diet.

Considering the fatty acid composition in the PL fraction of white muscle (Table 6) and liver, less evident differences were found between the dietary groups, again verifying the importance of preserving the membrane structure and function. In general, significantly higher levels of n-6 and lower levels of n-3 fatty acids were observed with each increase in RO content and as a result, a decrease in n-3/n-6 ratio occurred. In general, the fatty acid composition of the PL fraction of white muscle and liver in Arctic charr was affected in a similar way as in rainbow trout. However, some interesting exceptions were observed. The percentage of 22:6n-3 decreased significantly already at 50% inclusion of RO in both white muscle and liver of Arctic charr. As a consequence, the total n-3 fatty acids decreased with

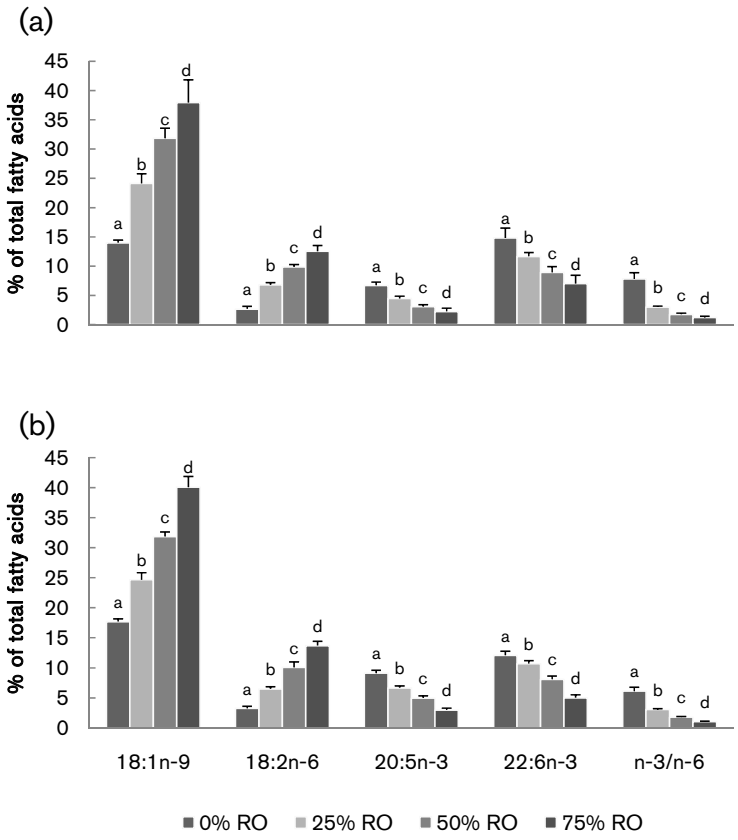

Figure 7. Fatty acid profiles in the triacylglycerol fraction of white muscle of rainbow trout (a) and Arctic charr (b) fed four rapeseed oil (RO) diets. Values are means ± S.D. (n=6). Different letters indicate significant differences between diets.

every increase in RO content in the diet. The corresponding trend was not seen in rainbow trout, where the contents of 22:6n-3 and total n-3 fatty acids were relatively stable irrespective of diet. In addition, the level of 22:6n-3 was higher in rainbow trout. These results are further supported by Yang & Dick (1994) who also reported lower 22:6n-3 content in tissue PL of Arctic charr compared with rainbow trout, which could suggest less effective desaturation and elongation in Arctic charr. Similar results were found by Tocher *et al.* (2001), who reported hepatic desaturation ability in three salmonid species in the ranking: brown trout > Atlantic salmon > Arctic charr, which further indicates a lower ability to desaturate in Arctic charr compared with other salmonid species.

The dietary experiment in Paper III resulted in considerable changes in fatty acid composition in both PL and TAG fraction of white muscle in Arctic charr after being fed the RO and ROPO diets (Table 7). The PL fraction of white muscle in fish fed ROPO had the highest level of total SFA, while the control fish (FO) were intermediate and had a higher level than fish fed RO. The proportion of total n-6 fatty acids was twice as high in fish fed the vegetable oil as in the fish fed FO due to the high levels of 18:2n-6 found in the dietary vegetable oils. 22:6n-3 was the predominant fatty acid in the PL fraction, but the levels were significantly decreased with vegetable oil inclusion. The same trend was seen in the levels of 20:5n-3, resulting in a significant difference in total n-3 fatty acids between fish fed vegetable oil and those fed FO. The increase in n-6 fatty acids and the decrease in n-3 fatty acids consequently led to a dramatic decrease in the n-3/n-6 ratio of the fish fed vegetable oil. A similar pattern was seen in the TAG faction, but the effects were more pronounced and clearly reflected the fatty acid composition of the diet. As expected, a higher content of total SFA and n-3 PUFA was found in the control fish compared with the fish fed vegetable oil, mainly due to the high levels of 16:0 and 20:5n-3, 22:6n-3, respectively. The fish fed vegetable oil contained significantly higher amounts of total MUFA due to the high content of 18:1n-9 in the vegetable oil diets. Significantly higher levels of 18:2n-6 were found in fish on vegetable oil diets, with the highest in fish fed RO followed by those fed ROPO which resulted in the same trend in total n-6 fatty acids. This great shift in total n-3 and n-6 fatty acids resulted in a four-fold lower n-3/n-6 ratio in the fish fed vegetable oil. The main differences between the fish fed RO and ROPO were seen in the content of 16:0 which was highest in fish fed ROPO, resulting in a significantly higher content of total SFA. The high content of 18:3n-3 present in the RO diet led to a concomitantly higher content in fish fed RO than in the fish fed ROPO. Even though the

Table 7. *Fatty acid profiles in phospholipid (PL) and triacylglycerol (TAG) fraction of white muscle from Arctic charr fed 100% fish oil (FO), 75% rapeseed oil (RO) and 75% rapeseed oil/palm oil (ROPO) for 14 weeks*

Fatty acids	PL			TAG		
	FO	RO	ROPO	FO	RO	ROPO
16:0	20.2±1.2[a]	18.5±0.8[b]	21.6±0.5[c]	13.8±0.5[a]	10.4±0.4[b]	14.2±0.5[a]
18:1n-9	6.1±0.3[a]	11.6±0.8[b]	9.9±0.3[c]	17.8±0.8[a]	33.8±1.9[b]	32.0±1.8[b]
18:2n-6	0.9±0.0[a]	3.6±0.3[b]	3.1±0.1[c]	3.5±0.2[a]	10.5±1.0[b]	8.7±0.8[c]
20:4n-6	1.4±0.1	1.4±0.1	1.4±0.1	0.6±0.0[a]	0.3±0.0[b]	0.3±0.0[b]
18:3n-3	0.3±0.0[a]	1.8±0.2[b]	1.1±0.1[c]	1.1±0.1[a]	4.0±0.6[b]	2.5±0.3[c]
20:5n-3	10.3±0.9[a]	7.6±0.5[b]	8.2±0.8[b]	7.6±0.6[a]	3.6±0.2[b]	3.9±0.4[b]
22:6n-3	44.2±0.9[a]	41.4±1.8[b]	41.2±1.3[b]	10.9±0.5[a]	6.2±0.5[b]	6.5±0.3[b]
SFA[1]	25.9±1.3[a]	23.5±1.0[b]	26.3±0.5[a]	22.0±0.6[a]	16.2±0.5[b]	20.3±0.7[c]
MUFA[2]	11.7±0.3[a]	15.9±0.7[b]	14.0±0.3[c]	43.5±1.3[a]	52.2±0.4[b]	50.8±0.7[c]
PUFA[3]	60.2±1.7[a]	58.9±1.6[ab]	58.0±0.7[b]	29.7±1.1[a]	29.3±0.8[a]	26.3±0.8[b]
n-3HUFA[4]	57.1±1.7[a]	51.1±2.0[b]	51.6±0.7[b]	21.3±1.1[a]	11.4±0.9[b]	12.0±0.8[b]
n-3	57.7±1.7[a]	53.3±1.8[b]	52.9±0.7[b]	25.0±1.1[a]	17.5±0.4[b]	16.4±0.8[c]
n-6	2.5±0.1[a]	5.6±0.3[b]	5.1±0.1[c]	4.7±0.2[a]	11.8±1.0[b]	9.9±0.8[c]
n-3/n-6	23.2±0.9[a]	9.6±0.8[b]	10.4±0.4[b]	5.4±0.4[a]	1.5±0.2[b]	1.7±0.2[b]
n-3HUFA/SFA	2.2±0.2[a]	2.2±0.2[a]	2.0±0.1[b]	1.0±0.0[a]	0.7±0.0[b]	0.6±0.0[c]
n-3HUFA/n-6	22.9±0.9[a]	9.2±0.8[b]	10.2±0.4[b]	4.6±0.4[a]	1.0±0.2[b]	1.2±0.2[b]

Values are means ± S.D. (n = 6). Values in the same row but with different superscripts are significantly different ($p<0.05$).

Abbreviations: SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, HUFA=highly unsaturated fatty acids.

[1] Includes 12:0, 14:0, 15:0, 17:0, 20:0, 22:0; [2] Includes 14:1, 16:1n-7, 17:1, 18:1n-9, 18:1n-7, 18:1n-5, 20:1n-9, 22:1n-11, 22:1n-9, 24:1

[3] Includes , 18:4n-3, 20:3n-3, 20:4n-3, 22:5n-3, 18:3n-6, 20:2n-6, 20:3n-6, 22:5n-6; [4] Includes 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.

main interest in Paper III was the swimming ability of Arctic charr and not the possible substitution of FO for industrial use, it can still be concluded that RO and a blend of RO and PO affected muscle fatty acid composition in an expected way. Earlier studies on RO and PO have reported similar results with an altered fatty acid composition when replacing FO (Torstensen *et al.*, 2000; Bell *et al.*, 2002; Caballero *et al.*, 2002; Ng *et al.*, 2004; Tocher *et al.*, 2004; Fonseca-Madrigal *et al.*, 2005).

Despite the small sample size and weight differences of the wild individuals compared with the experimental fish in Paper II, the results of the lipid analysis turned out to be very similar to those reported in Paper IV and are not presented here. The results of PL fatty acid analysis in Paper IV (Table 8) showed that total SFA and MUFA levels were fairly similar between lakes and compared with farmed fish, with no individual fatty acid being exceptionally different. Similarly, the total PUFA content was comparable between all experimental groups. Only fish from Lake Almberga had a slightly higher PUFA content than the other experimental groups. However, when the PUFA where separated into total n-3 and n-6 fatty acids, remarkable differences were found in the PL between wild and farmed Arctic charr. Farmed fish contained significantly more n-3 fatty acids than wild fish. The most contributing n-3 fatty acid was 22:6n-3 and the highest content was found in farmed fish. Differences were also found in 20:5n-3 content between the experimental groups, but not as pronounced as for the 22:6n-3 content. The most significant differences between wild and farmed fish were found in the n-6 series. All n-6 fatty acids identified were present in considerably lower amounts in farmed fish compared with wild individuals, which resulted in 7-8-fold lower total n-6 content in farmed fish compared with wild. The most predominant n-6 fatty acid and one of the fatty acids that differed the most was 20:4n-6 (arachidonic acid), with levels around 7-fold lower in farmed fish. A noteworthy finding was the stable level of 20:4n-6 for all three lakes. Small differences were also found in the total n-6 fatty acids among the lakes, with a lower content in fish from Lake Almberga than in those from the other lakes. This is mainly explained by the higher 18:2n-6 content in the latter. The large differences in n-3 and n-6 levels were expressed in the n-3/n-6 ratio which was 8-10-fold higher in farmed fish compared with. The latter displayed similar values to each other.

In the TAG fraction of white muscle, fairly uniform variation was found among the lakes (Figure 8). Only small differences could be found, which were most probably related to the different feeding habitats among the lakes. More interesting results were observed when comparing wild fish with their

Table 8. *Total lipid (% of wet weight) and fatty acid profile (% of total fatty acids) of phospholipid fraction of white muscle of wild Arctic charr from three coldwater lakes and farmed Arctic charr fed marine fish oil in Pettersson et al.(2009)*

	Almberga	Ruozutjaure	Vuorejaure	Farmed
Total lipid	0.8±0.1[a]	1.1±0.2[a]	1.6±0.3[b]	2.0±0.4[c]
Fatty acids				
14:0	0.3±0.0[a]	0.5±0.1[a]	0.4±0.1[a]	1.5±0.3[b]
16:0	20.4±0.8	22.2±1.6	21.6±1.9	21.8±0.8
18:0	5.1±0.3[a]	6.2±0.8[b]	5.4±0.4[a]	3.5±0.3[c]
Total SFA[1]	26.4±0.8[a]	29.6±1.8[c]	28.3±2.3[bc]	27.3±0.9[ab]
16:1n-7	1.3±0.4[a]	1.3±0.2[a]	2.2±0.7[b]	1.2±0.2[a]
18:1n-9	4.4±0.3[a]	5.5±0.7[b]	5.3±1.4[ab]	5.8±0.3[ab]
18:1n-7	2.8±0.5[a]	2.7±0.4[a]	3.9±0.5[b]	2.2±0.1[c]
Total MUFA[2]	8.9±1.0[a]	10.2±1.2[ab]	12.1±2.5[c]	11.6±0.6[bc]
18:3n-3	1.2±0.1[a]	1.3±0.1[ab]	1.5±0.4[b]	0.4±0.1[c]
20:5n-3	11.0±1.4[a]	7.6±0.9[b]	9.7±0.5[c]	11.3±0.7[a]
22:5n-3	3.0±0.2[a]	2.9±0.5[a]	2.4±0.2[b]	2.2±0.1[b]
22:6n-3	31.0±1.0[a]	28.6±2.7[b]	25.6±1.8[c]	41.0±1.9[d]
Total n-3[3]	47.0±2.2[a]	41.3±3.2[b]	39.8±2.1[b]	55.3±1.3[c]
18:2n-6	2.4±0.4[a]	3.6±0.8[b]	4.1±0.9[b]	0.9±0.1[c]
20:4n-6	9.8±0.5[a]	9.5±0.4[a]	9.9±0.3[a]	1.4±0.1[b]
22:5n-6	2.3±0.3[a]	2.6±0.3[b]	2.3±0.3[ab]	0.2±0.0[c]
Total n-6[4]	15.8±1.0[a]	17.5±0.7[b]	18.0±1.3[b]	2.4±0.2[c]
Total PUFA	62.8±1.4[a]	58.7±3.0[b]	57.8±1.5[b]	57.8±1.1[b]
n-3/n-6	3.0±0.3[a]	2.4±0.2[a]	2.2±0.2[a]	22.8±2.0[b]

Values are means ± S.D. (n = 6). Values in the same row but with different superscript letters are significantly different ($p<0.05$). Abbreviations: SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids.

[1] Includes 15:0, 17:0, 20:0.

[2] Includes 14:1, 17:1, 18:1n-5, 20:1n-9, 24:1.

[3] Includes 18:4n-3, 20:3n-3, 20:4n-3.

[4] Includes 20:2n-6, 20:3n-6, 22:4n-6.

farmed counterparts. Although many individual fatty acids fell within the range of the wild values, some diverged considerably from the pattern. The levels of the monounsaturated fatty acid 20:1n-9 were significantly lower in wild fish compared with farmed (results not shown). Furthermore, 22:1n-11 was not detected in the wild fish, while it constituted around 4% in the farmed. This is explained by the high proportion of these MUFA found in commercial diets containing marine fish oils produced in the northern

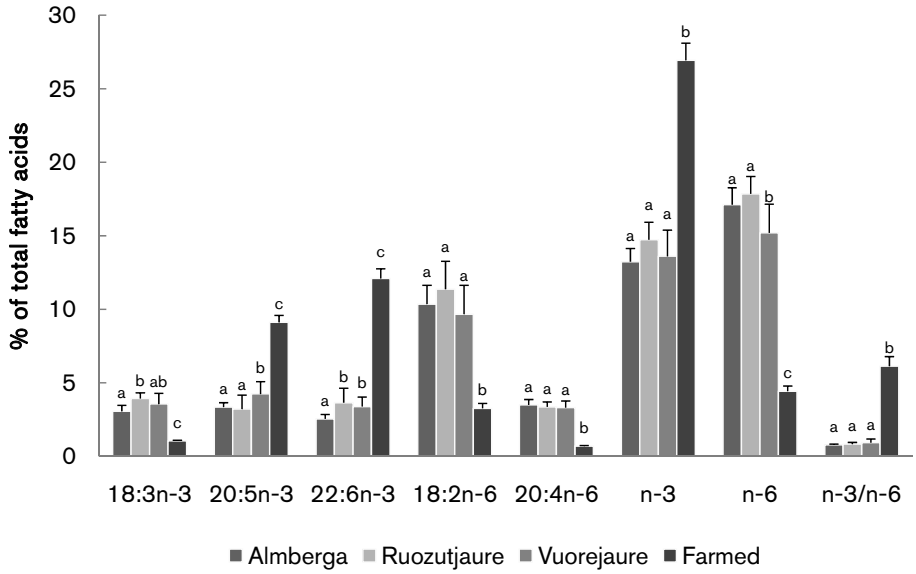

Figure 8. Fatty acid profiles in the triacylglycerol fraction of white muscle of wild Arctic charr from three coldwater lakes and farmed Arctic charr from Pettersson *et al*. (2009). Values are means ± S.D. (n=6). Different letters indicate significant differences between fish origin.

hemisphere. Twice as much total n-3 fatty acid was found in farmed fish compared with wild, with 20:5n-3 and 22:6n-3 being the n-3 fatty acids contributing the most in the farmed fish. In terms of the total levels of n-6 fatty acids, a similar pattern as seen for the PL fraction emerged, with significantly higher values found for every n-6 fatty acid identified in muscle of wild fish. 18:2n-6 and 20:4n-6 were the two major n-6 fatty acids, with 3- and 5-fold higher values in wild fish compared with farmed. This skewed distribution of individual n-3 and n-6 fatty acids resulted in a n-3/n-6 ratio that was 7-fold higher in farmed individuals compared with wild.

The differences in TAG fraction could be expected, since the fatty acid profiles of storage lipids are known to mimic the composition of the diet, which in this case was different. However, the remarkable differences observed in PL between wild and farmed Arctic charr were less expected, since fish usually maintain a uniform PUFA profile by selectively incorporating fatty acids into membranes. This regulation was observed in Paper I, where rainbow trout maintained even levels of 22:6n-3 in their PL when they were fed diets with a low 22:6n-3 content.

Large differences in 20:4n-6 content between wild and farmed salmonids have been observed in earlier studies. Yang & Dick (1994) found that wild

Arctic charr had 10 times more 20:4n-6 in their muscle PL than farmed. The same study also reported only small differences between wild and farmed rainbow trout. These results highlight the importance of species characteristics in the sense that the response to dietary modification may be significantly different for species within the salmonid family. Furthermore, differences in 20:4n-6 content have been reported in PL of eggs between wild and farmed Arctic charr (Pickova *et al.*, 2007), with approximately 5-fold less 20:4n-6 in the latter. This deficiency of 20:4n-6 in farmed Arctic charr has in fact been suggested to be responsible for the unpredictable variation in hatching of eggs and survival success of fry. In addition, it has been proposed that there is a preference for n-3 PUFA over n-6 PUFA at the Δ^6 desaturase binding site thereby inhibiting the conversion of 18:2n-6 to 20:4n-6 in salmonids (Bell *et al.*, 1994). Thus feeding farmed freshwater fish marine oils with high levels of 20:5n-3 and 22:6n-3 will further prevent conversion of 18:2n-6 to 20:4n-6 by feedback inhibition of Δ^6 desaturase. Since 20:4n-6 is the precursor for the biologically active eicosanoids which are involved in many processes in the body, it should perhaps be considered important when formulating diets for farmed fish. Sargent *et al.* (1999) reported that the ratio of 20:4n-6/20:5n-3 was significantly increased in liver and gills PL of salmon parr fed vegetable oils and that the amount of eicosanoids produced from 20:4n-6 was substantially elevated in gills in the same species. As a result, fish fed vegetable oil improved their osmoregulation when challenged with sea water compared with fish fed FO. These results clearly support the claim that 20:4n-6 and its derived eicosanoids play a crucial role in fish metabolism and should be considered very important, despite the relatively low levels found in fish cell membranes compared with 20:5n-3 and 22:6n-3.

It has been proposed that some vegetable oils resemble the profile of freshwater prey and are thereby more suitable for farmed freshwater fish. Although not statistically investigated, a comparison between fish fed 75% RO and 100% FO in Paper II and wild individuals in Paper IV showed that the fatty acid profile of white muscle TAG in fish fed RO actually resembled the profile of wild to a higher degree than that of fish fed the commercial diet (Figure 9). The exception was 20:4n-6, which was not in the range of wild fish. This is an interesting paradox, since many arguments against feeding fish vegetable oils cite the fact that vegetable oils are not a natural part of the fish diet.

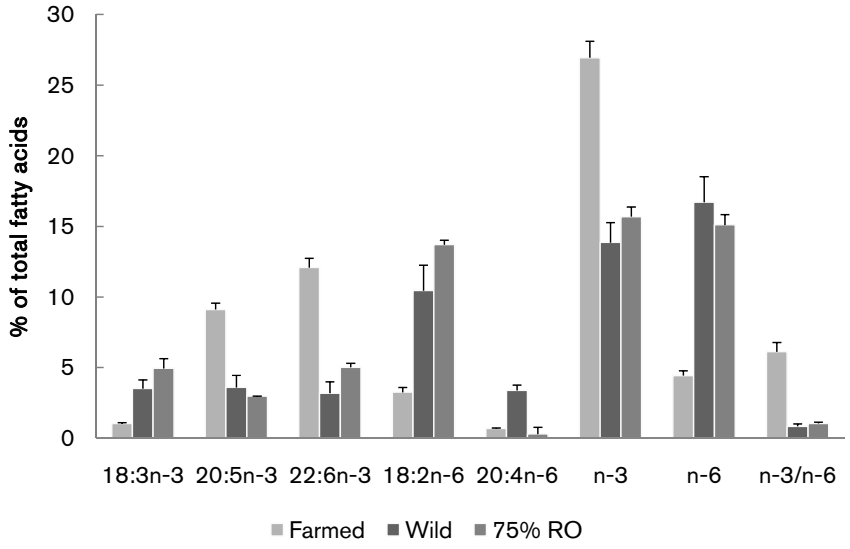

Figure 9. Fatty acid profiles in triacylglycerol fraction of muscle in farmed, wild and 75% rapeseed oil (RO) fed Arctic charr. Values are means ± S.D. (n=6).

4.5 Minor lipid compounds

Feeding rainbow trout diets containing varying amounts of RO in Paper I gave no significant effect on the cholesterol levels in white muscle and liver (Figure 10). A tendency towards a small reduction from 69.5 mg g^{-1} in 0% RO fish to 58.1 mg g^{-1} in 75% RO fish was seen in the liver, although it was not statistically significant ($p=0.11$). No phytosterols were determined in the white muscle, despite significant amounts in the RO diets. The results of the GC-MS analysis on rainbow trout liver showed that there were more sterol compounds in fish fed 75% RO compared with 0% RO. Campesterol was identified as one of these but the rest could only be identified as sterol metabolites (molecular ion, $m/z=129$) and need further investigation for proper identification. In Paper II, significant differences in cholesterol content were found in both white muscle and liver of Arctic charr fed the RO diets (Figure 10). In the white muscle, there was a lower amount of cholesterol in the 25% RO and 50% RO fish compared with the 0% RO and 75% RO fish. Clearer results were found in the liver, with a significant drop already at 25% RO inclusion. No traces of phytosterols were found in any of the tissues analyzed.

Earlier studies have reported that cholesterol uptake in the rat intestine isconsiderably higher than phytosterol uptake (Child & Kuksis, 1982). The preferential absorption of cholesterol over phytosterols in humans has also been reported by Ling & Jones (1995). They stated that the absorption rate of phytosterols is usually less than 5% of dietary levels compared with 40% of cholesterol. Although absorbed in tiny amounts, phytosterols may still compete with cholesterol for binding sites on cell level thereby lowering the cholesterol levels in tissues. Such an effect was observed in the liver of Atlantic cod (*Gadus morhua*) fed soy oil by Pickova & Mörköre (2007). A similar effect was not seen in white muscle of rainbow trout, although a tendency towards a reduction was observed in the liver of fish fed 75% RO. More pronounced effects of vegetable oils were seen in white muscle and liver of Arctic charr. It is difficult to conclude whether the reduction in cholesterol was an effect of phytosterols, since the vegetable oils themselves contain less cholesterol than FO. In the white muscle of Arctic charr the cholesterol level decreased already at 25% inclusion of RO but back to control levels again in fish fed 75% RO. This can probably be explained by

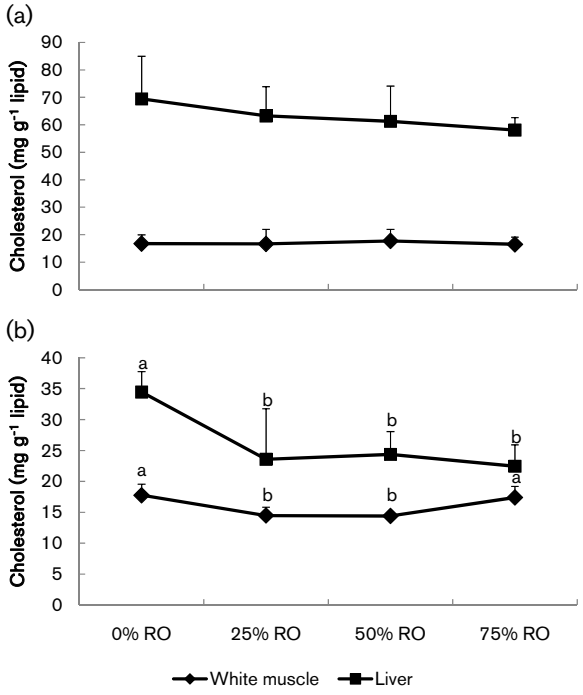

Figure 10. Cholesterol content in white muscle and liver of rainbow trout (a) and Arctic charr (b) fed four different rapeseed oil (RO) diets. Values are means ± S.D. (n=6). Different letters indicate significant differences between diets.

compensation synthesis in the latter which is supported in a study where an up-regulation in genes associated with cholesterol synthesis occurred in Atlantic salmon fed vegetable oil (Leaver *et al.*, 2008). In the liver, however, the compensation synthesis was probably not enough, which resulted in a significant drop in cholesterol between control fish and the fish fed RO. Additional effects of phytosterols and their metabolites, as seen in Paper I, can be assumed since they are not part of the natural fish diet.

The tocopherol content generally increased in the white and red muscle and liver of rainbow trout with higher amount of RO in the diet (Paper I). α-tocopherol was the major vitamin E compound in all tissues, while γ-tocopherol was found in smaller amounts only in fish fed RO. This confirms the natural occurrence of α-tocopherol in fish and the dietary effect of RO in the case of γ-tocopherol. The addition of γ-tocopherol to the diet by substituting FO with RO may have beneficial effects for shelf-life and human health, since γ-tocopherol is a good antioxidant. However, its effects on fish physiology and metabolism are poorly understood.

4.6 Fish behavior and welfare

Giving rainbow trout the freedom to choose from whatever diet they prefer resulted in a significantly lower proportion of rejected 0% RO compared with the other RO diets (Figure 11). This comparison was made without considering the previous diet during the feeding period. When taking the previous diet into consideration, the proportion of rejected pellets was compared between individuals fed 0% RO and RO (Figure 12). Rainbow trout rejected less 0% RO than RO pellets, irrespective of the previous diet, but the differences were only significant in fish previously fed 0% and 50% RO. These results confirm findings by Geurden *et al.* (2005) where rainbow trout were given a choice by self-feeders and were able to discriminate between diets containing FO and vegetable oils. In the same study, RO was preferred over linseed and sunflower oil when the FO diet was excluded. However, the rejection of RO diets by rainbow trout was not high (10-15%), which is also in line with results reported in Geurden *et al.* (2005). This suggests that substituting FO with RO only has minor effect on the feed preferences of rainbow trout.

In Paper III, the main interest was to investigate the dietary effects of vegetable oils on the physiological status of Arctic charr by measuring the critical swimming speed at three temperatures. Fish fed the blend of RO and PO (ROPO) showed significantly poorer swimming performance at 4 °C compared with fish fed the FO diet and the RO diet (Figure 13). No

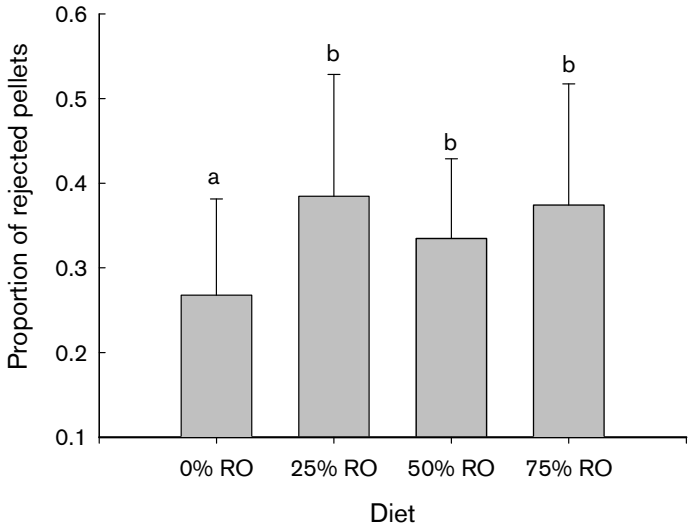

Figure 11. Proportion of feed rejected by rainbow trout allowed to choose from three separate rapeseed oil (RO) diets. Values are means ± S.D. (n=24). Different letters indicate significant differences between diets.

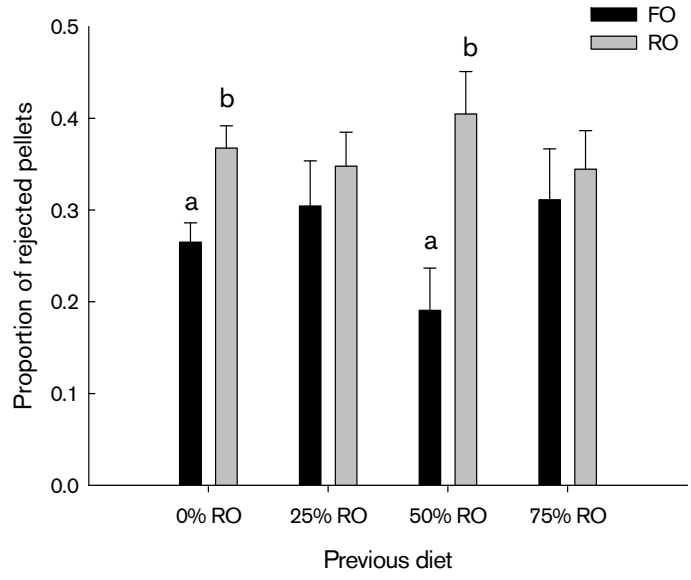

Figure 12. Mean proportion of fish oil (FO) and rapeseed oil (RO) diet rejected considering previous fed diet of individually rainbow trout. Values are means ± S.D. (n=24). Different letters indicate significant differences between FO and RO fed fish.

significant differences were found between the dietary treatments at 10 °C and 17 °C. However, a similar pattern as was seen at 4 °C was observed, with poorer performance of the fish fed vegetable oil compared with those fed FO. The effects of dietary manipulation on swimming performance of fish have been studied previously, but the studies are few and the effect of temperature was never considered. In addition, contradicting results have been observed. Increased swimming performance has been reported in Atlantic salmon fed diets with increasing amounts of rapeseed oil compared with those fed FO (McKenzie *et al.*, 1998). Another study on the same species reported improved swimming ability when fed a diet rich in anchovy oil (Wagner *et al.*, 2004). In both studies, the fatty acid composition was suggested to be responsible for the outcome of the swimming performance. In the latter study, the worst swimming performance was observed in fish fed a diet where FO had been supplemented with poultry fat. The low ratio of n-3 HUFA/SFA in the muscle of the fish fed poultry fat was suggested to account for the poorer

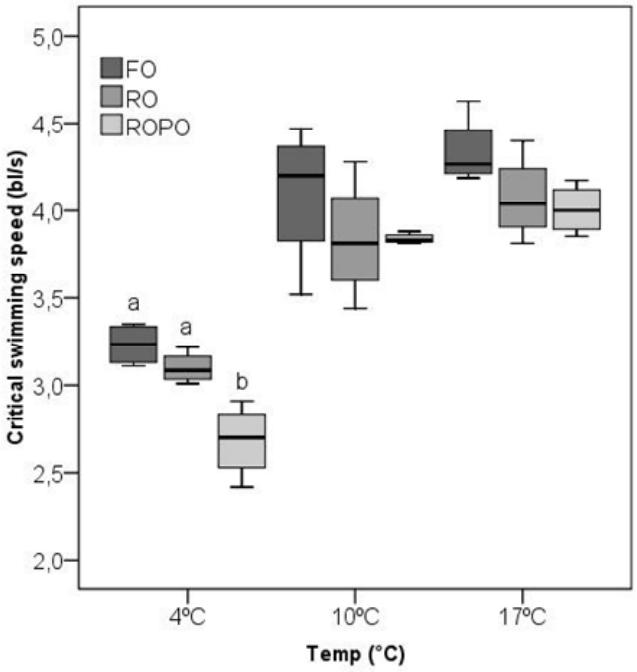

Figure 13. Box plot of critical swimming speed (bl/s) at three different temperatures of Arctic charr fed three diets containing 100% fish oil (FO), 75% rapeseed oil (RO) and 75% rapeseed oil/palm oil (ROPO) for 14 weeks (n=4). Different letters indicate a significant difference ($p<0.05$).

swimming ability. This would be in accordance with our study, since a significantly higher n-3 HUFA/SFA ratio was found in the fish fed FO and RO than in those fed ROPO. However, it is important to understand that it is only possible to speculate about the relationship between fatty acid compositions and swimming performance, since the mechanisms behind it have not been well investigated. A fact supporting the positive effects of a high n-3 HUFA/SFA on swimming performance is the well-studied properties of n-3 HUFA. They are incorporated and used in the cell membranes to maintain membrane structure and fluidity, which is crucial for fish survival, especially in coldwater species. It has also been reported that lowering the water temperature may cause some saturated and monounsaturated fatty acids to solidify in the gastrointestinal tract of Arctic charr, but this can perhaps be avoided by the counteracting effects of high levels of PUFA (Olsen & Ringo, 1998). This would further support the results of this study, since the effect of ROPO on the swimming ability of fish was particularly evident at 4 °C and since fish fed FO and RO contained significantly higher amounts of PUFA than those fed ROPO. Negative effects of n-6 fatty acids have also been observed in Atlantic salmon, which developed cardiac lesions when fed diets low in n-3 HUFA/n-6 fatty acid ratios (Bell *et al.*, 1991, 1993). The effects on cardiac condition were not specifically examined in our study, but fish fed vegetable oil had significantly lower n-3 HUFA/n-6 ratios in both lipid fractions compared with fish fed FO.

Although the main reason why fatty acids influence the swimming performance of Arctic charr remains unknown, PO inclusion in the diet had a remarkable effect on the swimming exercise, especially at 4 °C. Taking previous studies (Bell *et al.*, 1991, 1993; Olsen & Ringo, 1998) into consideration, the effect of n-3 HUFA is most likely involved in influencing the outcome of the swimming performance.

5 Conclusions

This thesis investigated the effects of vegetable oils as a more economically and sustainable lipid source in the diet of farmed fish than FO on growth, lipid content, feed preference and swimming performance of two salmonid species. The main focus was on fatty acid composition in the phospholipid and triacylglycerol fractions of the tissue analyzed, in order to provide further knowledge about fish physiology and fish as a food product. Significant efforts were also devoted to comparing the fatty acid profiles of farmed and wild individuals, in order to recommend a more suitable dietary composition for the fish feed industry that takes the evolutionary perspective of salmonid species into account.

In general, the results obtained showed no negative effects on growth of rainbow trout and Arctic charr when FO was replaced by RO or a blend of RO and PO, suggesting that the essentials for good growth are present in the vegetable oils used. However, it was clear that alterations in dietary formulation affected the fatty acid composition of fish tissue. This was demonstrated by a linear correlation between fatty acids in the diet and those in fish tissue analyzed in the studies presented here in this thesis. In general, lower amounts of n-3 HUFA, mainly EPA and DHA, and higher amounts of 18:1n-9 and 18:2n-6 were found in tissues of fish fed different levels of vegetable oils. Phospholipids in fish tissue were also affected by vegetable oil inclusion, although not to the same extent as TAG, despite the consequences of the homeostatic relationship that fish try to maintain in membrane lipids.

The results also showed that other minor lipid components, such as phytosterols and tocopherols found in vegetable oils, may have further effects on lipid metabolism in fish. Although phytosterols were not found in appreciable amounts within fish tissues, they may still compete with cholesterol for binding sites and thereby lower cholesterol levels, as seen

here in liver and white muscle of Arctic charr. However, further studies on sterol metabolism in fish fed vegetable oil are necessary to determine the actual mechanisms behind this effect.

The effects of vegetable oils on swimming performance of Arctic charr were found to be pronounced. Fish fed a blend of RO and PO for 14 weeks were particularly affected and displayed significantly poorer swimming performance at 4 °C compared with fish fed the other diets. The consequences of this result in fish farming conditions may be of minor importance for growth and survival, but it reveals a side-effect of using alternative feed ingredients.

Comparing the fatty acid profiles between wild and farmed fish showed some dramatic differences in n-3 and n-6 fatty acid content. Fish farming today, aims to produce a healthy product for human consumption within a short time. This is accomplished by feeding fish high-fat diets (sometimes up to 40% lipid) containing marine fish oil with high levels of HUFA, mainly EPA and DHA. Feeding such diets to freshwater fish can alter the fatty acid composition to such an extent that it is no longer within the natural variation found in wild freshwater fish. This shift in fatty acid composition may be beneficial for humans, but a disadvantage for fish physiology. Many body functions and metabolic processes are dependent on dietary fatty acids, especially PUFA, and a disturbance of the 'natural' fatty acid profile may have consequences that affect biochemical processes, resulting in less optimal physiological functions.

The similarities in fatty acid profiles of wild fish and fish fed RO and the low rejection of RO diets compared with FO diets by rainbow trout suggest that RO might be a good alternative to FO in the search for a more sustainable lipid source. The feed industry may need to balance fish feed ingredients more carefully in order to produce a sustainable feed that is species-specific, while simultaneously maintaining a high nutritional value, in order to optimize the conditions for fish farmers to produce high quality products for human consumption.

The altered fatty acid composition of fish muscle caused by vegetable oil inclusion in the diet can influence the nutritional and quality parameters of fish as a food product. Fish muscle, representing the edible part of the fish, has traditionally been the unique source of n-3 HUFA for human consumers. This thesis showed considerable reductions (4-5-fold) in the n-3/n-6 ratio when FO in the fish diet was replaced by vegetable oils, which may be extremely negative from a nutritional point of view. However, comparing the n-3/n-6 ratio between fish fed 50% RO (2:1) and

the average Western diet today (1:10-1:25), fish fed vegetable oil can still be considered a healthy food product.

6 Main findings

- Growth of rainbow trout and Arctic charr is not affected when they are fed rapeseed oil.

- Total lipid in white muscle of rainbow trout and Arctic charr is not affected by rapeseed oil inclusion in the diet.

- Total lipid increases in liver of Arctic charr fed 75% RO.

- Shifts in the fatty acid composition of different tissues are correlated to the diet:

    - White and red muscle are more affected than liver in rainbow trout
    - Phospholipids are not as affected as triacylglycerols
    - Lower levels of highly unsaturated fatty acids (EPA, DHA) are found in muscle and liver of rainbow trout and Arctic charr fed rapeseed oil → lower n-3/n-6 ratio

- Vitamin E content in muscle of rainbow trout increases with increasing levels of rapeseed oil in the diet.

- Significant differences in cholesterol content occur in muscle and liver of Arctic charr fed rapeseed oil diets.

- Rainbow trout prefer fish oil to rapeseed oil but do not discriminate between different levels of rapeseed oil inclusions in the diet.

- Arctic charr can be fed a blend of rapeseed oil and palm oil without compromising growth.

- Feeding Arctic charr a blend of rapeseed oil and palm oil significantly impeded swimming performance at 4 °C.

- Wild Arctic charr differ in their fatty acid composition from farmed Arctic charr, especially in their n-3 and n-6 fatty acid content.

7 Future prospects

In general, since there is a great need for alternative lipid sources in feed for farmed fish in order to make fish farming a sustainable and beneficial practice, more studies are necessary to gain an overall understanding of feed optimization and lipid metabolism in fish.

Some future research interests:

- Further investigations are needed on the effects of vegetable oils on lipid content during longer periods (juvenile to slaughter weight).

- The effects of vegetable oils should be studied on a molecular level including gene expression.

- Studies are needed to identify other lipid sources, such as single cell oils, as alternatives to FO, which can provide n-3 PUFA without any negative impact on wild fish stocks.

- Commercial feed ingredients should be optimized to satisfy the nutritional requirements of freshwater salmonids and other farmed species.

- Further studies are needed on the use of alternative oils in diets tailored to the specific requirements of Arctic charr in addition to enhancing the nutritional benefits from consuming fish.

- Additional investigations are needed on the effects of arachidonic acid on reproductive success and hatching rate of Arctic charr.

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