Háskólinn á Akureyri

University of Akureyri
Department of Business and Science

Replacing fish oil in Arctic charr diets.

Effect on growth, feed utilization and product quality

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“If the facts don't fit the theory, change the facts.”

Albert Einstein

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Abstract

The experiment was conducted to investigate if Arctic charr (*Salvelinus alpinus*) would express satisfactory growth on diets where fish oil had been substituted for plant oils. It was then monitored how long time it would take to regain acceptable levels of *n* – 3 polyunsaturated fatty acids (PUFA) in fish muscle by feeding the fish diets containing fish oil.

Arctic charr with a mean weight of 550 ±83 g was reared for 13 weeks with a mean water temperature of 6°C and fed diets containing fish oil (FO), soybean oil (SO) or palm oil (PO). Different lipid sources in the diets did not significantly affect fish growth, hepatosomatic index or product quality. However, palm oil inclusion in diets significantly affected the feed conversion ratio, protein efficiency ratio and apparent digestibility. Different fatty acid (FA) profiles of the diets were reflected in the fish muscle with significantly lower levels of *n* – 3 PUFA in groups fed diets containing PO or SO. Due to increasing levels of 18:2*n* – 6 FAs, considerably lower *n* – 3 to *n* – 6 ratios was observed in the muscle of fish fed PO or SO. After the ´recovery feeding´, some alterations in the profiles of the major fatty acids were observed, the regain of *n* – 3 FAs was acceptable but 18:2*n* – 6 had a slow flush out from the fish muscle. Our suggestions are, that acceptable growth of Arctic charr can be obtained on diets containing 100% substitution of FO, but not without diminishing the nutritional value with respect to the consumers.

The Second part of this study was to examine if Arctic charr fingerlings could be used to hunt for acceptable ingredients for grower feeds. It would be cost beneficial since fingerlings do not require as much volume and multiple ingredients can be studied simultaneously. Fingerlings (0.246 g) were fed diets containing various combinations of different lipid sources from the 24th of May-14th of August 2006. The inclusions of different lipid sources were found to significantly affect the growth of the fingerlings.

Keywords: Arctic charr, palm oil, soybean oil, fatty acids

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Útdráttur

Þessi rannsóknin var framkvæmd til að kanna hvort að bleikja (*Salvelinus alpinus*), gæti vaxið vel á fóðri sem inniheldur plöntuolíur í stað lýsis. Eftir fóðrun með tilraunafóðrum var kannað hve langan tíma það tæki að endurheimta ásættanlegt hlutfalli af fjölómettuðum *n* – 3 fitusýrum (PUFA) í fiskholdið til að hámarka næringarlegt gildi fyrir neitendur.

Bleikja með meðalþyngd 550 ±83 g var alin í 13 vikur við 6°C og fóðruð með fóðri sem innihélt lýsi (FO), soya- olíu (SO) eða pálmolíu (PO). Ólíkir fitugjafar höfðu ekki áhrif á vöxt, lifrarstuðul eða afurðargæði bleikjunnar. Samt sem áður hafði pálmolía í bleikjufóðri marktæk áhrif á fóðurstuðul, próteinnýtingu og meltanleika fóðursins. Mismunandi fitusýrur í fóðri endurspegluðust í ólíkum fitusýrusamsetningum fiskholds. Magn *n* – 3 PUFA fitusýra í holdi í hópum sem fóðraðir voru með jurtaolíum var marktækt lægra. Óhagstæðari hlutföll af *n* – 3 og *n* – 6 fitusýrum komu fram í öllum hópum fóðruðum á plöntuolíum vegna umtalsverðs magns af línólsýru (18:2*n* – 6) sem kemur úr plöntuolíum. Eftir bakfóðrun með lýsisfóðri hafði hold endurheimt umtalsvert magn af *n* – 3 fitusýrum en línólsýra, 18:2*n* – 6 virðist sitja lengi í holdi. Hlutfall milli *n* – 3 og *n* – 6 var mun ásættanlegra í holdi bleikju sem fóðruð var á pálmolíu, borið saman við soyaolíu. Þessar niðurstöður benda til þess að bleikja geti vaxið vel á fóðri sem inniheldur plöntuolíur í stað lýsis.

Einnig var kannað hvort smáseiði gætu komið að notum við að spá fyrir um heppileg hráefni í fóður fyrir stærri fisk. Tilraunir í slíkum stærðarskala myndu spara mikinn kostnað þar sem eldið er ekki jafn umfangsmikið. Fóður með 5 mismunandi fitugjöfum í mismunandi samsetningum voru gefin smáseiðium (0.246 g) frá 24. maí til 14. ágúst 2006. Mismunandi fitugjafar höfðu marktæk áhrif á vöxt smáseiðanna.

Lykilorð: Bleikja, pálmaolía, soyaolía, fitusýrur

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1. Introduction

1.1. The use of fish oil in Aquaculture

World wide aquaculture production has increased rapidly in recent years, with fish produced by farming currently accounting for almost half of total fish consumptions by humans (ARD, 2006). This has increased the pressure on the world fisheries since fish feed, especially feed for carnivorous fishes, has mainly been produced from fishmeal and oil coming from small bony pelagic fishes (Tacon et al., 2006). Constant increase in demand for fishmeal and oil for animal feeds has led to the predictions that, within few years, fish oil production may not meet the increasing demand (Barlow, 2000). As a result, there is increasing pressure from environmentalist lobby to reduce fishing. Moreover, the possibility of global warming and natural climatic events such as ´El Nino´ have further emphasised the vulnerability of these resources (Sargent et al., 1999[a]). Furthermore, there are increasing concerns regarding the ethics of using potentially food-grade fishery resources for animal feeding rather than for direct human consumption. Already, increased proportions of pelagic fish landings are being processed for human consumption, mainly in products being sold to African countries (Tacon et al., 2006).

The world production of fishmeal has been similar for the last 20 years, and with fish oil production at its limits there is an increased pressure to replace these ingredients (FAO, 2003). Plant protein and plant oils are the most obvious protein and lipid sources that could be used in fish feeds for aquaculture (Naylor et al., 2000). Suggested substitutes include protein-rich oilseed and grain meal products, including soybean, rapeseed, corn gluten, wheat gluten, pea and lupine meals, palm oil, soybean oil, maize oil, rapeseed oil, canola oil coconut oil, sunflower oil, linseed oil and olive oil. These products are all highly commercial, sustainable, rich in nutrients and low in price compared with fishmeal and oil (Tacon et al., 2006).

It is important for the aquaculture sector not to be entirely dependent on vulnerable resources and, with increasing consumers awareness of environmental quality, the use of plant proteins and oils is a step in the right direction.

1.2. Fishmeal and fish oil

Carnivorous fishes, such as Arctic charr, are primarily fed on diets rich in protein and fat. Even though carbohydrates are a good source of energy, they are not the principal source of energy or carbon for fish and elevated levels of carbohydrates can lead to reduced growth (Hemre et al., 1995). On a dry-weight basis, fish tissue consists primarily of protein (65- 75%). Fish do not have a true requirement for specific proteins but rather for a well balanced mixture of essential amino acids (Wilson, 2002). Fish meal is rich in nutrients and with excellent blend of minerals and amino acids which have suitable for carnivorous fish without supplementing any amino acids to the diet (Refstie, 2000). It is generally accepted, that plant proteins can be substituted for fish meal as a protein source for carnivorous (Refstie, et al., 1998; Burrells, et al., 1999; Kissil, et al., 2000). The extent to which plant proteins can be substituted in fish feed is primarily due to lack of certain amino acid in plant proteins and the high contents of anti nutritious factors such as protein inhibitors (Refstie, 2000).

The major role of fatty acids in fish, as in most other organisms, is to produce metabolic energy through β- oxidation. Lipids also play an important role in membrane structure and reproductive functions of fish. Fish oil is a rich source of easily digestible energy and is very well suited to supply all the essential fatty acids needed for normal development of fish, including reproductive functions (Sargent et al., 2002)..

In resent years, an increasing amount of fish oil has been added to diets of salmonid fish in order to increase growth and reduce more expensive protein. These high energy diets are know to have a protein sparing effect, where dietary protein is converted to muscle proteins rather then being used as energy (Grisdale-Helland et al., 1997). Fish oil is a cheap source of energy compared with protein and, therefore, the high energy diets are less expensive than diets higher in protein.

Many studies have demonstrated that fish oil can be substituted with plant oil in diets for carnivorous fish, including salmonids. Feed formulations where fish oil is entirely substituted with plant oil give acceptable growth in Atlantic salmon (Bell et al., 2003), rainbow trout (Drew et al., 2007) and Arctic charr (Tocher et al., 2001).

Using plant oils in fish diets creates certain problems that must be solved. (1) It is well established, that the fatty acid profiles of salmonid diets are reflected in their tissues (Olsen et al., 1997). Fish oil contains high levels of long chain polyunsaturated fatty acids of the *n* – 3 family (sometimes referred to as *n* – 3 HUFA) known for their beneficial effects on human health (Steffens, 1997). Feeding the fish on conventional fish oil based diets will, therefore, produce a healthy food for fish consumers. Changes in the lipid sources used in the fish diets may, therefore, compromise the nutritional value of farmed fish (Bureau et al., 2008). (2) Certain fatty acids are essential for animals (Tocher, 2003). These fatty acids can not be synthesized by the animal and they have to be provided through the diets. (3) Fatty acids are selectively oxidized with different rates and some fatty acids are spared as precursors for essential fatty acids (Kiessling et al., 1992). Thus, both energy availability and the content of essential fatty acid content must be considered.

1.3. Fatty acid requirements of freshwater fish

Generally, freshwater fish can synthesize their own long chain polyunsaturated fatty acids (PUFA) from 18 carbon precursors (linoleic acid; 18:2*n* – 6 and linolenic acid; 18:3*n* – 3) that are considered essential (EFAs) (Tocher, 2003). By elongation and the use of Δ6 and Δ5 desaturases (enzymes), fresh water fish can synthesize AA (arachidonic acid; 20:4*n* – 6) from 18:2*n* – 6 (LA) and EPA (eicosapentaenoic acid; 20:5*n* – 3) and DHA (docosahexaenoic acid 22:6n – 3) from 18:3n – 3 (LNA) (Manabu et al., 2004). Thus, freshwater fish species require all these long chain polyunsaturated FAs but only LA and LNA are generally considered to be essential (Sargent et al., 2002).

This is, however, different for marine teleost fish which lack the ability to synthesize the HUFA fatty acids (EPA, DHA and AA) which are, therefore, all considered to be essential for most marine species (Bruce et al., 1999). Microalgae are at the bottom of the food chain of freshwater and marine fish species. Freshwater microalgae, unlike marine microalgae, are generally rich in LA rather than EPA and DHA. Furthermore, while containing high levels of EPA and DHA, the marine microalgae contain relatively low levels of LNA (Tocher, 2003). Therefore, it has been suggested that the marine fish have primarily adopted

carnivorous lifestyles. Some authors have suggested that marine fish, consuming diet generally rich in HUFA fatty acids, have through evolution down regulated the activities of elongation and desaturases required to convert the 18 carbon precursors to the longer chain HUFAs (Henderson, 1996).

The biochemical, cellular and physiological functions of EPA, DHA and AA can be divided into two categories: (a) maintaining the structural and functional integrity of cell membranes; (b) a more specific role as precursors of the group of highly biologically active paracrine hormones known collectively as eicosanoids (Sargent et al., 1999). An imbalance and interactions of these FAs may therefore disturb steroidogenesis in fish (Mercure et al., 1995), reduce incorporation of lipids to oocytes (Bruce et al., 1999), affect the immune system via eicosanoids and prostaglandin irregularities (Ganga et al., 2005), reduce fecundity (Rainuzzo et al., 1997) and disturb membrane phase behaviour which is important in e.g. controlling fusion of the sperm plasma membrane with the egg (Bell et al., 1996). Moreover, fatty acids are, in addition to other nutrients, essential for normal development of the embryo and their optimum levels in diets will increase hatching rates and egg morphology (Sargent et al., 2002). Fish eggs are generally rich in n-3 HUFA and fish appear to selectively store HUFAs in eggs. Changes in dietary lipids will thus have greater effects on tissues than on the eggs (Sargent et al., 2002).

In freshwater fish, the activity of the desaturases involved in the conversions of the 18 carbon LA and LNA to the HUFAs are known to be under nutritional regulation. The activities of the desaturation/elongation pathway is depressed when animals, including fish, are fed fish oils rich in HUFA compared with animals fed vegetable oils rich in 18 carbon EFAs (Tocher et al., 2003). This indicates, that feeding oil sources rich in 18 carbon EFAs to Arctic charr, may activate desaturation/elongation pathways. This is important physiologically and indicates that these species could be reared on vegetable oils although this requires further study. Furthermore, the fatty acid requirement of fish may change between life stages due to the differences in natural prey selection (Sargent et al., 1999).

Compared with Atlantic salmon and rainbow trout, there is a paucity of information on the EFA requirement for Arctic charr. According to Sargent (2002), Arctic charr only require 18:3n – 3, which should be supplemented as 1-

2% of dry diets. Yang et al. (1994), investigated the effect of dietary 18:3*n* – 3 and 18:2*n* – 6 on the growth and EFA requirement of Arctic charr. Their results suggest that increased levels of 18:3*n* – 3 in the diet did not compensate for the lack of dietary HUFA resulting in low levels of EPA and DHA in tissues. Furthermore, considerable interactions between 18:3*n* – 3 and 18:2*n* – 6 were observed where conversion of 18:3*n* – 3 to long chain PUFA was dominating. This further indicates that due to competition for Δ6 desaturases, the ratio of these FAs could affect muscle FA profile. The authors suggested that 18:3*n* – 3 should be supplemented in 1.6 g/100 g diet and considered 18:2*n* – 6 as essential for Arctic charr.

The hepatocyte fatty acid desaturation in Arctic charr is quite different from that of brown trout and Atlantic salmon (Tocher et al. 2001). Arctic charr fed diets rich in vegetable oil showed significant differences in hepatic PUFA metabolism compared with the rainbow trout and the Atlantic salmon. The results indicated that compared with the other species, Arctic charr had the lowest levels of liver 18:2*n* – 6, 18:3*n* - 3 and no decrease in liver DHA when fed diets rich in the 18 carbon FAs (Tocher et al. 2001). The authors suggested that this could have important consequences, physiologically as well as with respect to the possibility to feed diets containing high inclusion of vegetable oils with less effect on muscle *n* – 3 levels in Arctic charr.

1.4. Alternative lipid sources in aquaculture feeds

The Northern hemisphere fish oil generally used in Arctic charr farming is rich in 16:0, 18:1*n* – 9, 20:1*n* – 9, and 22:1*n* – 11 fatty acids that are all heavily catabolised by fish (Sargent et al., 2002). Furthermore, the oil contains considerable amounts of *n* – 3 HUFA, both DHA and EPA. Digestibility and absorption of fatty acids decreases with increasing chain length and increases with increasing saturation (Sigurgísladóttir et al., 1992). It has been suggested, that this is dependent on the melting point of different FAs and the tendencies of some monoenes and long chain saturates to form insoluble soaps in the gut (Olsen et al., 1998). When choosing the right plant oils for substitution of fish oil, especially for cold water species, both energy availability and PUFA content must be considered (Bell et al., 2002).

Palm oil (PO) is rich in both 16:0 and 18:1*n* – 9 and therefore considered a potential candidate to substitute FO in diets for salmonid fish and is currently second behind soy oil in the world seed oil production tonnage (Bell et al., 2002). In addition, PO is low in *n* – 6 fatty acids compared with many commercially available plant oils (Sargent, et al., 2002). Substitution of FO with PO in fish diets has been studied in various fish species including, Atlantic salmon (Torstensen et al., 2000), rainbow trout (Fonseca-Madregal et al., 2005), gilthead seabream (Benedito-Palos et al., 2007) and Tilapia (Bahurmiz et al., 2007). A complete substitution of FO with PO in diets for rainbow trout (Fonseca-Madregal et al., 2005, OO et al., 2007) and Atlantic salmon (Bell et al., 2002) had no or minimum effects on growth and feed utilization. However, in these experiments, the FA profiles of fish muscle where changed considerably, with lower levels of *n* – 3 HUFA and increasing levels of 18:2*n* – 6 and 16:0 (palmitic acid). Another possible drawback of PO inclusion in salmonid diets, is the reduced digestibility (Ng et al., 2004, Torstensen et al., 2000) which has been found to be further reduced by lower temperatures (Ng et al., 2003). This is because PO contains about 50% saturates and 40% monoenes that may reduce the fatty acid digestibility and energy availability, especially in fish reared at low temperatures (Ng et al., 2003).

Soybean oil (SO) has been used in diets for several salmonid species including rainbow trout (Liu et al., 2004) and Atlantic salmon (Grisdale-Helland et al., 2002) resulting in growth comparable with FO diets. Soybean oil contains high levels of PUFA which are known to be efficiently utilized by fish (Olsen et al., 1999). Compared with FO, SO is not as rich in 16:0 but contains substantially higher levels of 18:1*n* – 9 (Sargent et al., 2002). One of the major disadvantages of SO in diets for salmonids is that due to high levels of *n* – 6, primarily 18:2*n* – 6, the *n* – 3/*n* – 6 ratio in the fish muscle has been greatly decreased by SO inclusion resulting in reduced nutritional values of the fish (Olsen et al., 2003, Liu et al., 2004, Ruyter et al., 2005). Furthermore, some studies indicate that higher levels of 18:2*n* – 6 may inhibit the conversion of 18:3*n* – 3 to EPA and DHA, causing EFA deficiency. However, the mechanism behind this is not completely understood (Ruyter et al., 2003). Some authors have reported accumulations of lipid droplets in the intestines (Olsen et al., 2003) and also in the liver (Ruyter et al., 2005) of salmonids fed diets containing high proportions of SO. It has been

suggested that this may be caused by temporal storage of lipid due to insufficient lipoprotein synthesis and could, therefore, be ameliorated by supplementation of phospholipids (Olsen et al., 1999).

Rapeseed oil (RO) is another candidate for use in salmonid diets, an abundant source of 18:1*n* – 9 and lower levels of 18:2*n* – 6 than in SO. The ratio of 18:2*n* – 6 to 18:3*n* – 3 is 2.1 compared with 6.75 in SO (Sargent et al., 2002). Results from experiments indicate that salmonids, including Atlantic salmon, can show acceptable growth reared on diets with complete substitution of FO with RO (Bell et al., 2001, Bell et al., 2003). However, although the capacity of hepatocytes to produce 22:6*n* – 3 increases when Atlantic salmon are fed diets containing RO, the concentration of this important FA is reduced (Moya-Falcón, et al., 2005). Thus, RO, as PO and SO inclusion in diets will result in a muscle composition of fatty acids less desirable for the consumer, i.e. lower levels of EPA, DHA and higher levels of 18:1*n* – 9 and 18:2*n* – 6 (Drew et al., 2007, Torstensen et al., 2004b, Bell et al., 2001). After feeding Atlantic salmon post smolts (150- 250 g) diets containing RO for 16 weeks, Bell and co-workers (2003) studied the time needed to convert muscle FA profile to those normally obtained with FO diet feeding. Their findings indicated that after 12 weeks of feeding FO diets, muscle DHA was restored. However, muscle *n* – 3/*n* – 6 ratios were lower (63%) than in fish fed marine FO throughout the experiment.

In the present work, we had the opportunity to study the effects of an ingredient called sterin in diets for Arctic charr fingerlings. This substance was offered by Lýsi h/f (Reykjavík, Iceland), a company involved in research and development in addition to processing and marketing of fish oils. This substance is a residual from the fish oil production and is formed when the fish oil is chilled. The FA composition of this product may be suitable for use in salmonid diets (19% 16:0, 20% 18:1*n* – 9, 11% 22:1*n* – 9+11, 6.2% EPA and 8.2% DHA). There appear to be no existing studies on sterin as an ingredient in fish feed.

1.5. Healthy food, consumer's perspective

It is well established that consumption of diets and supplements rich in *n* – 3 fatty acids has cardioprotective effects on humans and has also been found to play important role in prevention of hypertension, diabetes, cancer and arthritis as

well as other inflammatory and autoimmune disorders (Simopoulos, 2000). Salmonids, for example Atlantic salmon and Arctic charr, accumulate substantial amounts of lipids from the diet into the tissues for physiological functions, such as reproduction (Sargent et al., 2002). These species are generally reared on diets containing fish oils and have thus been a great source of *n* – 3 PUFA for humans. Since plant oils are generally deficient in *n* – 3 PUFA fatty acids, the use of plant oils in aquaculture diets would profoundly affect the nutritional value of fish. Over the last 100 - 150 years there has been an enormous increase in human consumption of *n* – 6 fatty acids, due to increased intake of vegetable oils from corn, sunflower seeds, safflower seed, cottonseed and soybeans (Simopoulos, 1999). Because of the increased amount of *n* – 6 fatty acids in the Western world diets, the eicosanoids metabolic products from arachidonic acid (20:4*n* – 6; AA), are formed in larger quantities and are involved in inflammation and numerous homeostatic biological functions (Simopoulos, 2000). Not only is it important to maintain high levels of *n* – 3 PUFA in fish muscle, the intake of these fatty acids must also be kept in balance. Increased levels of *n* – 6 in diets for salmonids would therefore be undesirable. Salmon fillets, rich in *n* – 3 PUFA fatty acids, have positive effects on patients suffering from coronary heart diseases compared with fillets containing low levels of marine *n* – 3 PUFA when the fish oil was replaced with rapeseed oil (Seierstad et al., 2005).

Previous studies indicate that it is possible to grow fish on diets rich in plant oils during the growth phase but change to diets rich in FO to regain *n* – 3 PUFA levels in muscle before slaughtering. However, some of these studies indicate that even though the *n* – 3 PUFA levels can be increased to acceptable levels, the wash out of *n* – 6 FAs was a slow process (Torstensen et al., 2004, Izquierdo et al., 2005, Bahurmiz et al., 2007).This further indicates, that presentable substitution for fish oil should ideally contain low levels of *n* – 6 fatty acids.

2. Research objectives

The objective of this research was to study if fish oil could be partially or completely replaced by plant oil ingredients in diets for Arctic charr (*Salvelinus alpinus*). Due to increasing demand for fish oil and the continuing expansion of the aquaculture industry, the prices of this ingredient have increased markedly in resent years. This will affect the aquaculture industry and the proportion of fish oil will be reduced in fish feeds in the nearest future at the expense of plant oils. Experiments in this field are therefore receiving increased attention. The main goal of this study was to search for high quality diet for Arctic charr that will result in similar growth as obtained by conventional diets based on fish oil, without affecting the health of the fish and the product quality.

3. Materials and methods

3.1. Experimental diets (slaughtering size)

Six experimental diets containing identical amounts of protein (~44%) and lipid (~22%) but variable concentrations of different lipid sources were prepared at Laxá Feed Mill in Akureyri, Iceland. The oils used in this experiment were Northern hemisphere fish oil (FO), soy oil (SO) and palm oil (PO). Three of the diets contained 100% of each oil (FO, SO, PO) and other 3 diets contained various combinations and ratios of the same oils (P75, P50 and P50/S25) (Table 3.1).

Table 3.1. Formulation and chemical analysis of the experimental diets.

Ingredients	Diet					
(%)	FO	SO	PO	P75	P50	P50/S25
Fish oil	100			25	50	25
Soy oil		100				25
Palm oil			100	75	50	50
Fish meal	53.41	53.41	53.41	53.41	53.41	53.41
Wheat flour	27.46	27.46	27.46	27.46	27.46	27.46
Marker (celite)	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix	1.00	1.00	1.00	1.00	1.00	1.00
Astaxanthin	0.03	0.03	0.03	0.03	0.03	0.03
Cantaxanthin	0.03	0.03	0.03	0.03	0.03	0.03
Total Lipid	17.07	17.07	17.07	17.07	17.07	17.07
Chemical analyses*						
Moisture	6.0	5.3	5.3	5.1	5.5	5.6
Crude ash	7.8	7.7	7.7	7.7	7.6	7.6
Crude protein	44.4	44.5	44.4	44.1	43.8	44.1
Crude lipid	21.3	21.7	21.8	21.9	22.0	22.1
True celite value	0.91±0.01	0.87±0.04	0.95±0.04	0.88±0.07	0.90±0.02	0.94±0.04
Gross energy (MJ)	24.2	24.2	24.3	24.1	24.4	24.3
Sinking time**	12.4±0.54^{a}	12.7±0.58^{a}	22.0±2.48^{c}	17.7±3.97^{b}	13.4±1.22^{a}	15.6±0.85^{ab}

**Chemical analyses have fixed standard deviation values of ±0.4 for crude protein, lipid and moisture. Standard deviation for crude ash is ±0.5.*

***Sinking time is expressed as the time in seconds needed for 5 of 10 feed pellets to sink 1 meter. Different letters in the same line denote significant differences (P<0.05).*

The feed ingredients were weighed on a gravimetric scale and then transferred to a pre-blender together with approximately 1% celite (silica) added as an inert marker. The feed mash was then transferred to a hammer mill and grinded with a 1.25 mm sieve before entering a conventional ribbon mixer. The feed mash was extruded in a Clextral BC 105 Twin screw Extruder (France) with 6mm die holes and finally dried in an belt dryer with air temperature of 85°C. The feed pellets (6 mm) were allowed to cool down and then drum coated with

different oils and in various combinations. To accomplish proper lipid coating for the PO diets, the palm oil was warmed up to ca. 40°C to when the oil becomes fluid. To assure good absorption of palm oil in to the feed pellets, the diets containing palm oil were warmed up to approximately 40°C as well. When warm, fluted palm oil was introduced to cold feed pellets, formation of crust occurred, preventing proper absorption of oil in to the feed pellets.

The sinking rate of the experimental diets was measured at Laxá Feedmill, Akureyri. A total of 10 feed pellets were dropped into a 1.5 meter long plastic cylinder (~10 cm diameter) filled with fresh water. The time from the release of the feed pellets, until they had sunk 1 meter was measured. This was repeated 5 times for each type of experimental diet. Pellets that did not sink were counted and recorded.

3.2. Experimental diets (fingerlings)

Experimental diets for the fingerlings were prepared as described above (3.1.) but with pellet size of 3 mm. The pellets were crusted to form a normal size fingerling diet (~1 mm) before drum coating with various lipid sources, one at a time or in various 50/50 combinations. A total of fifteen different experimental diets were prepared with fixed inclusion of all factors except lipids (Table 3.3). After the growth trial, and based on the results, 9 of the diets were selected and analysed for crude protein, lipid, ash and moisture (Table 3.2). A commercial Ewos Micro® (Ewos©, Norway) starter diet was used as a control diet.

Table 3.2. Chemical analyses of selected fingerling diets.

		Diet								
	±sd	FO	PO	RO	SO	ST	FO/PO	FO/SO	FO/ST	SO/PO
Moisture	0.4	10.6	10.9	10.7	10.0	10.6	10.6	9.8	10.9	10.6
Crude protein	0.4	47.1	47.1	47.0	48.0	46.9	47.1	47.5	46.9	47.1
Crude fat	0.4	16.3	16.0	15.9	16.1	15.7	15.9	16.1	15.8	15.6
Crude ash	0.5	8.5	8.7	8.6	8.7	8.6	8.7	8.7	8.5	8.6

Table 3.3. Formulation of the experimental diets for the fingerlings.

Ingredients	*%values*	*Lipids used*	*Acronym*
Fish meal (special)*	65.381	Fish oil	FO
Wheat (binder)	20.053	Soy oil	SO
Lipid	11.926	Palm oil	PO
Fish meal (super)**	1.597	Rapeseed oil	RO
Fish premix	1.000	Sterin	ST
C vitamin	0.043		
Chemical analyses†		Ewos††	
Moisture	10.5±0.38	8.0	
Crude protein	47.2±0.35	55.0	
Crude fat	15.9±0.21	14.0	
Crude ash	8.6±0.08	10.0	

** Capelin fish meal containing 68.0% protein.*
***Capelin fish meal containing 73.3% protein.*
†Mean (±SD)of 9 selected diets.
††Commercial Ewos values

3.3. Experimental setup, facilities and fish maintenance (slaughtering size)

The experiment was carried out in the research facilities at Hólar University College, Hólalax Ltd, Iceland (4^{th} of January to 11^{th} of April, 2007). Arctic Charr (*Salvelinus alpinus*) obtained from Hólalax fish farm, with a mean body weight of 550±83 g were randomly distributed into 18 fibreglass tanks, with 90 fish per tank (N=1620). The volume of the tanks was 1.6 m^3 (2×2×0.4 m). The tanks were supplied with fresh water (6.0 °C ±0.4) with adequate flow to maintain oxygen saturation at 100%. Each treatment was applied in triplicate tanks.

Different treatments were randomly assigned to the tanks to avoid tank effects. A feed collector was place on the outflow on each tank to measure the feed consumption and determine the feed conversion ratios. The daily quantity of feed presented in each tank with conventional belt feeders. Uneaten pellets were counted every morning to determine the amount of leftover feed. The fish were reared in continuous throughout the experiment (24 h light : 0 h dark).

3.3.1. Sampling (slaughtering size)

At the end of the feeding trial (94 days), 4 fish were sampled were from each tank and the fish killed with a blow to the head. The fish were filleted and samples from fillets were analysed for chemical and fatty acid composition. Fresh fillets were sent for sensory and quality evaluation and livers were weighed for hepatosomatic index. Faeces were collected from the intestines for digestibility analyses with stripping. Both sides of the abdominal area were given a firm pressure towards the anus and faeces collected in a plastic cups. Pooled samples of faeces from all tanks were collected for digestibility analyses. Samples of faeces were collected from all fish, or until sufficient amount for marker analyses was obtained (40 g wet weight of faeces per tank).

All data are based on individual fish weight and length. Before all measurements, including stripping, all fish was starved for 24 hours and anesthetized using 3-aminobenzoic acid ethyl ester (MS222; 100 μg/ml) to assure acceptable treatment and easy handling of the fish. In between the first and the last measurement fish was sampled the 6th of February and 6th of Mars.

At the end of the growth trial with the experimental diets, the fish in all treatments were fed for 76 days with the FO diet (containing only fish oil) to study the recovery of fatty acids (FA) composition and the n–3/n–6 ratio in the fillets. Pooled samples of fish were randomly collected (21st of May and 26th of June) from each treatment and analysed for FA composition and fresh fillets were stored at 4°C for 24 hours before they were evaluated by a trained sensory evaluation panel (Table 3.4).

Table 3.4. Overview of the sample analyses.

	Growth	Chemical analyses	FA-analyses	Sensory evaluation	Hepasomatic index	Collection of faeces	FCR/count of pellets
January 4th	x						
February 6th	x						x
Mars 6th	x						x
April 11th	x	x	x	x	x	x	
*May 21st**			x	x			
June 26th			x	x			

* *Dates presented with italic letters represents the recovery period for FA profiles.*

3.4. Experimental setup (fingerlings)

The experiment was conducted in the research facilities at Hólar University College in Sauðárkrókur. Arctic charr fingerlings obtained from the Hólar breeding program, with a mean weight of 0.246g ±0.078, were randomly distributed into 64 plastic tanks with a volume of 20 L. A biomass of approximately 50 grams (~200 individuals) were held in each tank. The mean water temperature was 12±0.3 °C and photoperiod was continuous light (24 Light : 0 Dark) from the 24th of May to the 14th of August 2006. Different dietary treatments were arbitrarily assigned to the tanks to avoid possible tank effects and each treatment carried out in 4 tanks (N total=12.800 fish). The fish was fed in excess every 10th minute at all hours throughout the period. Dead fish from individual tanks were counted daily and recorded. One month into the experiment, the numbers of fingerlings were reduced to 150 individual's tank^{-1} to adjust the density.

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3.4.1. Sampling (fingerlings)

Growth calculations for the first two sampling periods (22nd of June and 18th of July) are based on group weight using the total biomass in each tank divided by the number of fish. For the last measurement (14th of August) all fish were measured individually for weight and length. The fish were anaesthetised using MS222 (100 μg/ml) during all samplings.

Pooled samples of fingerlings where collected in the beginning of the experiment for analyses of the initial FA composition. After the final weighing, pooled samples of approximately 100 fingerlings were collected and stored at -80°C. Based on data obtained from the growth trial, nine treatments of interest were then selected for diet and whole body FA and chemical analyses of crude protein, fat, moisture and ash.

3.5. Feed conversion ratio

Feed collectors were placed under the water outlets of each tank to measure the actual intake of feed. The collector boxes were made of laminated plywood with fine gridded polyester net. When water, faeces and feed pellets followed the outlet through the outlet pipe, the residue hit the net of the collector box which had ca. 45° angle. Water had clear access through the net but feed pellets slide down the slope to a pellet collector.

Pellets collected from all tanks were counted every morning and the mean weight of diet samples then used to convert the number of pellets into weight values for use in calculations of the feed conversion ratio (number of pellets/100 g feed). Pellet count was not performed for the first period of the growth trial (4^{th} of January to 6^{th} of February) while the fish were adapting to the experimental diets.

3.6. Apparent digestibility

Faeces were dried at 104°C for 24 h for measuring the dry matter content. Approximately 3 g of the dried faeces were then weighed accurately in a small beaker and placed in an oven at 550°C for 24 h.

The remaining ash was then boiled for 5 minutes in a 100 ml solution of 5N Hydrochloric Acid (HCl) to completely dissolve the ash. The only solid material left in the liquid phase was the feed marker (celite). The small beakers were then removed from the acid solution with pliers; and the celite carefully flushed of the beakers with boiling distilled water. The solution was then filtered trough an ash free filter paper, the paper folded and then placed in the beaker again and put in to the oven at 600°C for 16 h. The beakers were then removed from the oven, cooled down and weighed again to calculate the mass of the inert marker.

3.6.1. Lipid analyses of faeces

Soxhlet extraction was used to measure the total lipid content of faeces samples.

Ceramic bowls with approximately one tablespoon of sea sand and a glass stick were heated at 104°C for 4 hours. The bowls were then cooled down in a desiccator and weighed accurately up to 4 decimal places. Faeces were weighed with difference, accurately 5 g, added to the bowls and carefully mixed with the sand using the glass stick and the bowls weighed again before heating at 104°C for 4 hours. The samples were then cooled down in the desiccator and weighed again for dry matter analyses of faeces.

Samples were transferred to cylindrical thimbles of filter paper, using a cotton wad and carefully removing all sample residues from the bowls. The samples were covered with cotton and the thimbles then placed in the main chamber of the Soxhlet extractor unit. Boiling flasks (250 ml) were accurately weighed with 6-8 boiling stones each and 200 ml of diethyl ether then added to each flask which was then placed beneath the cylinders and on the hotplates resting under the distillation unit. The hotplates were then turned on 40°C causing the diethyl ether to evaporate to the water cooled condenser at the top of the unit. The cooling causes the solvent to drip down to the thimble chamber containing the sample and extracting the lipid from the sample. When the thimble chamber is full, the solvent which now contains lipids extracted from the sample travels back down to the boiling flask through a siphon side arm.

The solvent was kept in a continuous circulation for 6 hours and the lipids remains in the boiling flask, since they do not evaporate together with the solvent. The solvent was then removed from the boiling flasks using a rotary vacuum distillation unit. The flasks were then accurately weighed for calculation of the lipid content of samples.

3.6.2. Calorimetric analyses

Gross energy (GE) of faeces and diets was determined using an adiabatic oxygen bomb calorimeter (PARR model 1241, Parr Instrument Company, Illinois). The amount of heat, measured in calories, that is released when a substance is completely oxidized in a bomb colorimeter, is called the gross energy (GE) of the substance. Materials are weighed in to a combustion capsule and placed in an oxygen bomb containing 25-30 atmosphere of oxygen. The oxygen bomb is then covered with 2000 g of water. After the bomb and the calorimeter have been adjusted to the same temperature, the sample is ignited with a fuse wire and the temperature rise measured under adiabatic conditions. The calorie content can then be calculated from hydrothermal equivalent of the calorimeter multiplied with the temperature rise with the following formula:

$$GE\ (\frac{cal}{g}) = \frac{(final\ temp - init.temp)x(hydro\ thermal\ equiv.) - (wire\ cal. + acid\ cal.)}{sample\ weight}$$

where wire cal. are the calories expended in the ignition of the fuse wire and acid cal., stands for calories that remains in the bomb and are incidental oxidations of nitrogen and sulphur compounds. A detailed transcription of these methods is detailed in Appendix I.

(Parr Instrument Company, 1966).

3.7. Chemical analyses

Analyses of protein, fat, moisture and ash were carried out at Matís ohf. (Icelandic food research LTD) in Reykjavík, Iceland, using methods according to the ISO 17025 standard.

Analysis of total fat content in fish and meal (AM-00901a) with standard deviation of ±0,4 is according to AOCS, Official Method BA 38 and application noteTecador no. AN 301. 1997.

Analysis of crude protein in fish or fishmeal using the Kjeldahl method (AM-00903), according to the ISO 5983-2:2005 (SD ±0.4).

Analysis of the water content of fishmeal or fish (AM-00904) with standard deviation of 0.4 according to the ISO 6496 (1999).

Analysis of the ash content of fishmeal and feed (AM-00905) according to the ISO 5984-2002 (E) (SD±0,5).

The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%. All methods are guarantied by the divisional manager of chemical analyses (Matís ohf.).

3.7.1. Fatty acid analyses

10 g of sample were mixed with a handheld mixer (Janke&Kunkel, IKA®, Labarotecnic) with a solvent of chloroform and methanol (80:20) for 2 minutes. The samples were kept cold on ice during the process to avoid oxidation of fatty acids. A sample of minced flesh or experimental diets were poured into a plastic separator cup and placed in a separator (Sorvall Legend Mach 1.6 R) for 10 minutes at 5000 rpm and 15°C. The liquid separated from the flesh during the process was then removed and placed in a 50 ml boiling flask. The solvent was then removed from the flask by distillation in a vacuum unit.

70 mg of lipid were then transferred from the boiling flask to a 10 ml test tube with a screwed on hermetical cover. 1.5 ml of 0.5N NaOH diluted in MeOH was added to each test tube which were then sealed and placed in a 100°C water bath for 7 minutes. The tubes were then cooled down and 2 ml of approximately 1.3 M ([~10%) Boron trifluoride (BF_3) diluted in 12% methanol added before putting the tubes back to 100°C water bath for 30 minutes.

The tubes were then cooled down and 1 ml of isooctane and 5 ml of saturated salt solution added to each tube and mixed up in a vortex vibrating shaker (Heidolph, ReaxTop) for 0.5 minutes. The isooctane layer was then transferred to a clean test tube and the procedure repeated using isooctane and finally transferring the isooctane layer to a clean tube. The solution was dried by adding 5 mg of anhydrous sodium sulphate (Na_2SO_4) and the solution then transferred to a sealed medicine vials. The samples were stored at -80°C until analysed of fatty acid composition using Perkin Elmer gas chromatography (Auto System XL, Perking Elmer®). with a Supelco capillary column (Omegawax™ 320, Supelco®).

3.8. Sensory evaluation

Fresh fillets from a pooled sample of 5 fish from each dietary treatment were analysed using sensory evaluation. The samples were evaluated using the picturesque test or the QDA method (quantitative descriptive analyses), where defined factors were used to describe the characteristic look, taste, smell and texture (Hootman, 1992, Stone et al., 1985). A panel of 9-12, specially trained judges, all familiar with sensory evaluation tests (ISO, 1993), participated in the testing.

Most of the characteristics evaluated had previously been described by other evaluation panels (Ginés et al., 2004) and were presented for the judges on a training course before the evaluation. The evaluation panel decided to add features regarding the texture of fish fillets, “adherent”, additional smell and additional taste. A total of 23 evaluation characteristics were described in the test (Table 3.5.). Each organoleptic characteristic of fish fillets was evaluated according to strength and attributes on an unscaled line ranging between 0-100.

Before testing, the samples were steam boiled in an aluminium server at 98°C for 5-6 minutes and 30- 40 g assigned for each judge. The samples were evaluated when still hot, four samples at a time. All samples were encrypted and evaluated in duplicates. Fizz, a computer program for sensory evaluation, was used for experimental setup, organisation and data analyses.

3.8.1. Flesh colour

Flesh colour was measured with a Minolta light meter were the L-value (light value) ranged between 0-100, were 1 is black and 100 is white, indicating how dark or light/bright the fish fillet was. Other values measured were, a*-value (a+ =red, a- =green) which indicates how green or red the fillet was and b*-value (b+ =yellow, b- = blue) illustrating how blue or yellow the fish fillet was. Five fillets from each treatment were measured (FO, SO, PO, P75, P50 and P50/S25) and 3 dots were spotted on each fillet.

Table 3.5. Definitions and segments for sensory evaluation (QDA)

	Factor	Scale (0-100%)		Definition
Smell				
	Sweet/representative	none	significant	representative smell for Arctic Charr
	Metal	none	significant	
	Fresh oil	none	significant	fresh, oil
	Mould/mustiness	none	significant	mould/mustiness/earth smell
	Sour smell	none	significant	indication of impairment
	Rancidity	none	significant	indication of impairment
	Taint	none	significant	taint, plaster, disinfecting agent
Appearance				
	Colour	white	orange	how white/orange is surface
	Heterogeneous	none	significant	how heterogeneous is sample
	Yellow fluid	colourless	yellow	how yellow is the liquid
	Lipid drops in water	small and few	large and many	how are the drops of lipid
	White precipitations	none	significant	white precipitations on or between lamellas
Taste				
	Sweet/representative	none	significant	representative taste for Arctic Charr
	Metal	none	significant	
	Oil/fat	none	significant	fresh oil
	Mould or mustiness	none	significant	
	Sour	none	significant	indication of impairment
	Rancidity	none	significant	indication of impairment
	Additional	none	significant	additional taste
Texture				
	Softness	stiff	soft	rated from first bite
	Juiciness	dry	juicy	rated when chewed
	Tenderness	viscous	tender	rated when chewed
	Viscosity	none	significant	teeth sticks together

3.8.2. Data analyses for sensory evaluation

The QDA data were corrected for different applications on a scale, according to methods described by Thybo & Martens (2000). ANOVA and Duncan´s test were performed on corrected values in NCSS 2000 (NCSS, Utah, USA) to analyse if the test groups were different with respect to the topic of the evaluation characteristics (significant difference if $p<0,05$). Evaluation characteristics of different treatments were analysed using Principal Component Analyses-PCA and the statistical software Unscrambler® (Version 8.0, CAMO, Trondheim, Norway).

Light values for different treatment groups, recorded with the Minolta light meter, were compared using the statistical software SigmaStat from Systat Software (CA 94804-2028, USA). ANOVA and Tukey´s test were performed to check for significant differences in L-values (significant if $p<0.05$).

All descriptions of the implementation of the sensory evaluations as well as the results presented in graphs, tables and bar charts were carried out by Kolbrún Sveinsdóttir, scientist and PhD student at the Department of Consumers and Sensory Evaluation at Matís ofh.

3.9. Calculations

Specific growth rate (SGR) was calculated as % daily weight gain using the formula:

$$SGR = (\ln BW_2 - \ln BW_1 \times Days\ of\ experiment^{-1}) \times 100$$

where BW_2 stands for final body weight and BW_1 is the initial body weight. Values for SGR are based on calculations between all individual samplings.

Feed conversion ratio (FCR) was calculated as:

$$FCR = \frac{Feed\ consumed\ (kg)}{Biomass\ weight\ gain(kg) - dead\ fish(kg)}$$

ΔG% (Percentage weight gain) was calculated as a % increase in biomass for fish within each treatment, using the following formula:

$$\Delta G = \left(\frac{Weight\ gain\ (g)}{Initial\ weight\ (g)}\right) \times 100$$

Condition factor (CF) was calculated as:

$$CF = \left(BW(g) \times FL^{-3}(cm)\right) \times 100$$

Where BW stands for body weight and FL stands for fork length measured as the length from the fork of the fin to the nose tip.

Hepatosomatic index (HSI) was calculated as the % liver of whole body weight:

$$HSI = (liver\ weight\ x\ BW^{-1}) * 100$$

Protein efficiency ratio (PER) was calculated as the weight gain (g) for each gram of protein consumed, using the formula:

$$PER = \frac{Weight\ gain\ (g)}{Protein\ consumed\ (g; dry\ weight\ basis)}$$

Thermal growth coefficient (TGC) was calculated using the formula:

$$TGC = 100 \times \left(\frac{Final\ weight^{1/3} - Initial\ weight^{1/3}}{Sum\ degree\ days\ (C°)}\right)$$

Where the third root of the final minus the initial weight are divided by the sum of degree days (degrees C° x time in days).

Apparent digestibility coefficient (ADC) was calculated using the formula:

$$ADC = \left(1 - \left(\frac{Feed\ marker\ content\ \times Faecal\ nutrient\ content}{Feed\ nutrient\ content\ \times Faecal\ marker\ content}\right)\right) \times 100$$

ADC was calculated as digestibility for dry matter, lipid and gross energy.

Survival (%) was calculated as:

$$Survival\ (\%)\ = \frac{(Initial\ number\ of\ fish - number\ of\ dead\ fish)}{Initial\ number\ of\ fish} \times 100$$

3.10. Statistical analyses and graph designs

Mean values are presented with the standard deviation of the mean (±SD)[1]. Values for mean weight, fork length and condition factor in both experiments were statistically analysed using the Systat software (Version 11.0, Systat software, inc. 2004, Bangalore India). Data were subjected to two-way ANOVA using the general linear model of the Systat program. Dietary treatment and tank numbers were nested as independent treatment variables. If significant differences occurred, the analyses were followed by Bonferroni multiple comparison hypothesis test with $\alpha = 0.05$.

Effects of palm oil inclusion on feed conversion ratios (FCR) and physical quality (sinking time) were tested using regression analyses in Minitab 14.1 statistical software (MINITAB®, inc. 2003, Coventry, England). Differences in means between chemical compositions of the experimental diets and whole body analyses of fingerlings was checked with a paired T-test ($\alpha = 0.05$).

All other data, including values for SGR, ΔG, HSI, PER, ADC, TGC, chemical analyses for large fish and fatty acid composition of the diets and fish muscle was treated with one-way analyses of variance (ANOVA). If significant differences occurred between different treatments, the test was followed by either Tukey-Kramer HSD or Fisher´s LSD multiple comparisons with a level of significance = 0.05. One-way ANOVA analyses and all tests for normality of data were performed using the Minitab software.

Sigmaplot 9.01 graphical software (Systat software, inc. 2004, Bangalore, India) was used to plot graphs and construct bar charts. Microsoft Office Excel (Microsoft©, 2006) was used for constructing radar plots for effects of different fatty acids on flesh FA compositions.

[1] In figure 4.1, mean values are presented plus or minus standard error of the mean (SEM)

4. Results (fingerlings)

4.1. Growth

Feeding diets containing different lipid sources significantly affected the weight, fork length, specific growth rates (SGR) and thermal growth coefficients (TGC) of the fingerlings (Table 4.1. and Figure 4.1.). The best growth was obtained in fingerlings fed the FO diet, which resulted in a final weight of 5.2 g and a SGR value of 3.87 during the feeding trial. The values were, however, not significantly different from the values obtained by feeding either the Ewos (control diet) or a diet containing fish oil with a 50% inclusion of sterin (ST). In contrast, rather poor growth was observed by feeding ST only compared with the FO based diets.

The lowest final weight was observed in the group fed a mixture of fish oil and soy oil (FO/SO). The final weight of fish from this group was 4.3 g compared with a mean weight of 4.5 g of fingerlings fed pure soy oil diet (SO). The best growth of fingerlings fed pure plant oil based diets was achieved in the group fed the PO diet, with a mean weight of 4.7 g and a SGR value of 3.75 at the end of the experimental period.

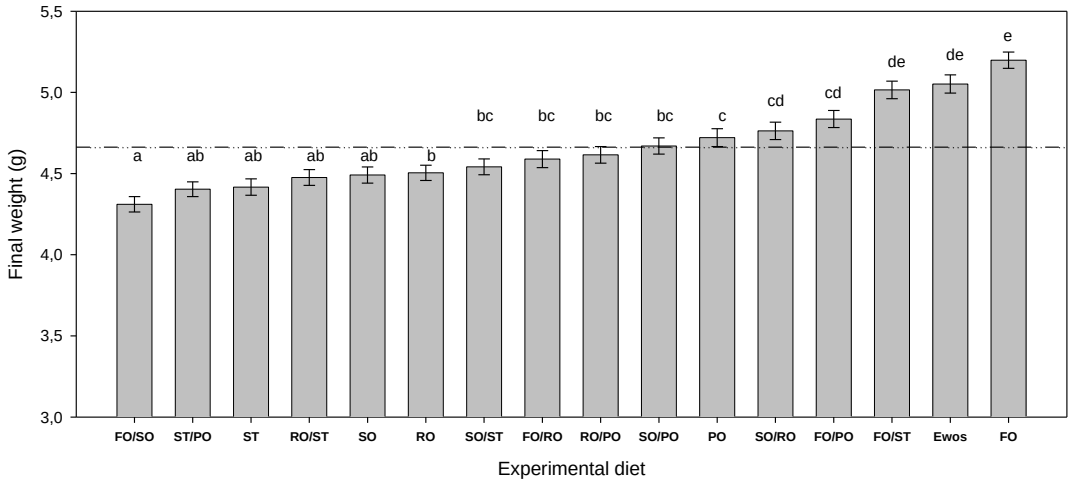


Figure 4.1. Mean final weight of fingerlings fed different experimental diets. Values are expressed as mean of 4 tanks ±SEM and dashed line is the line for mean fingerling weight. (FO= fish oil, PO = palm oil, RO = rapeseed oil, SO = soy oil, ST = sterin)

Feeding the fingerlings diet with a mixture of palm oil (FO/PO) resulted in a final weight (4.8 g) that was compatible with feeding the control (Ewos) and FO/ST diets. Lower final weight values were observed in fingerlings fed diet containing a mixture of rapeseed oil (RO) and FO compared to the FO/PO diets (4.6 g), however not significant. Feeding SO/RO gave similar growth as observed with the FO/PO diet. The fingerlings from the SO/PO group gained significantly ($P<0.05$) lower values of lipid compared to all treatments except the FO/SO treatment (Figure 4.2.). SO/PO group as well gained significantly higher values of protein compared to all groups except the FO and FO/ST groups. Survival in the experimental groups was ranging from 86% in the FO/SO group and no mortalities were recorded in the RO/PO group.

Table 4.1. Growth and survival of fingerlings fed diets containing various lipids and combinations of lipids.

Diet	FL	CF	SGR	TGC	Survival%
FO	8.05 ± 0.61^{a}	0.98 ± 0.08	$3,87\pm0,06^{a}$	1.16 ± 0.02^{a}	98.0
Ewos	8.00 ± 0.68^{ab}	0.96 ± 0.06	$3,84\pm0,08^{ab}$	1.15 ± 0.05^{ab}	97.5
FO/ST	7.95 ± 0.68^{bc}	0.97 ± 0.06	$3,82\pm0,05^{ab}$	1.15 ± 0.03^{ab}	99.0
FO/PO	7.88 ± 0.69^{cd}	0.97 ± 0.09	$3,78\pm0,10^{abc}$	1.13 ± 0.04^{ab}	96.0
SO/RO	7.78 ± 0.68^{de}	0.99 ± 0.06	$3,76\pm0,09^{abc}$	1.12 ± 0.05^{abc}	97.0
PO	7.83 ± 0.70^{ef}	0.96 ± 0.08	$3,75\pm0,10^{abcd}$	1.11 ± 0.04^{abc}	98.5
SO/PO	7.80 ± 0.63^{efg}	0.96 ± 0.07	$3,73\pm0,09^{bcd}$	1.11 ± 0.05^{bc}	97.5
RO/PO	7.73 ± 0.64^{fgh}	0.98 ± 0.09	$3,72\pm0,09^{bcd}$	1.10 ± 0.05^{bc}	100.0
FO/RO	7.69 ± 0.67^{fghi}	0.98 ± 0.06	$3,71\pm0,10^{bcd}$	1.10 ± 0.06^{bc}	98.0
SO/ST	7.75 ± 0.65^{ghij}	0.95 ± 0.06	$3,70\pm0,05^{cd}$	1.09 ± 0.01^{bc}	97.5
RO	7.67 ± 0.63^{hij}	0.98 ± 0.07	$3,69\pm0,04^{cd}$	1.08 ± 0.02^{c}	99.0
SO	7.65 ± 0.64^{hij}	0.98 ± 0.15	$3,68\pm0,14^{cd}$	1.08 ± 0.08^{c}	96.5
RO/ST	7.72 ± 0.65^{hij}	0.95 ± 0.06	$3,68\pm0,08^{cd}$	1.08 ± 0.03^{c}	97.5
ST	7.70 ± 0.68^{hij}	0.95 ± 0.06	$3,67\pm0,06^{cd}$	1.07 ± 0.04^{c}	99.0
ST/PO	7.70 ± 0.63^{jk}	0.94 ± 0.07	$3,66\pm0,04^{cd}$	1.07 ± 0.03^{c}	97.0
FO/SO	7.61 ± 0.63^{k}	0.95 ± 0.06	$3,63\pm0,14^{d}$	1.06 ± 0.07^{c}	86.0

Values are presented as mean of fork length (FL), condition factor (CF), specific growth rates (SGR) and thermal growth coefficients (TGC). ± SD. Different letters in a row represent significant different values ($P<0.05$). (FO= fish oil, PO = palm oil, RO = rapeseed oil, SO = soy oil, ST = sterin)

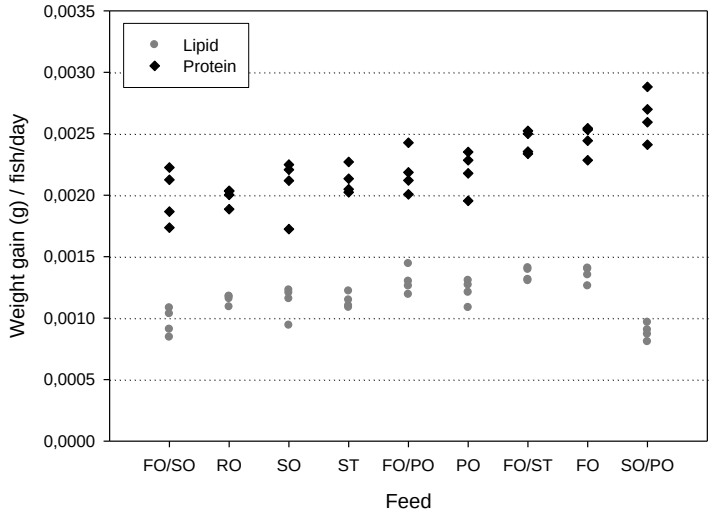


Figure 4.2. Weight gain of lipids and protein of fingerlings (g/day).

4.2. Chemical analyses

Fingerlings from the SO/PO group had significantly (P<0.05) higher levels of protein, lower levels of lipids and lower concentrations of ash in whole body after the experimental feeding trial (Table 4.2.).

Table 4.2. Whole body chemical analyses of selected fingerling groups fed different experimental diets.

		Diet								
*Hole body analyses**	±SD	FO	PO	RO	SO	ST	FO/PO	FO/SO	FO/ST	SO/PO
Moisture	0,4	74,3	74,7	74,9	74,6	74,9	74,6	74,9	73,9	74,5
Crude protein	0,4	15,2	15,3	14,7	15,2	16,0	14,8	15,4	15,4	18,5[a]
Crude fat	0,4	8,4	8,5	8,5	8,3	8,6	8,8	7,5	8,6	6,2[b]
Crude ash	0,5	2,4	2,3	2,2	2,2	2,2	2,2	2,4	2,4	1,5[c]

Values are presented as mean ± SD. Values have fixed SD for different chemical components. Letters in SO/PO row represents significant differences (P<0.05).

4.3. Fatty acid analyses

The different dietary lipid compositions of the experimental diets were reflected in the FA profiles of the diets with higher levels of palmitic acid (16:0) and Σ SFA detected in diets containing palm oil (Table 4.3.). The highest levels of Σ MUFA were detected in the RO diet (54%). This diet was rich in oleic acid (18:1*n* – 9), representing 33.5% of the total fatty acid content (FA). The PO diet was also rich in oleic acid, representing 26.8% of the total FAs. The FO diet was rich in MUFAs (52.3%), containing prominent levels of long chain 20:1 and 22:1

monoenes. The predominant *n* – 6 FA was the LA (18:2*n* – 6) which represented 30.7% of the total FAs in the SO diet, 20.1% in the SO/PO diet, 17.7% in the FO/SO diet and 14.2% in the RO diet. Additionally, these diets had the lowest n - 3/n - 6 ratio values observed 0.4, 0.6, 0.9 and 1.0, respectively. Furthermore, these diets contained elevated proportions of LNA (18:3*n* – 3), ranging from 2.1% in the SO/PO diet to 5.3% in the RO diets, compared to 0.3% in the PO diet and 0.6% in the FO diet.

Table 4.3. Fatty acid analysis of selected fingerling diets.

	Diet								
FA	FO	PO	RO	SO	ST	FO/PO	FO/SO	FO/ST	SO/PO
14:00	4.8	2.4	1.9	1.8	4.2	3.8	3.3	4.5	2.1
16:00	12.6	31.0	9.3	12.3	14.9	22.1	12.4	13.6	22.4
18:00	1.4	3.4	1.8	3.1	2.5	2.3	2.3	2.0	3.3
Σ SFA	**19.5**	**37.4**	**14.0**	**18.0**	**22.5**	**28.9**	**18.7**	**20.8**	**28.6**
16:1*n* – 7	6.3	2.8	3.0	2.8	5.9	5.1	4.4	6.1	2.8
18:1*n* – 9	10.9	26.8	33.5	17.0	13.4	18.5	13.9	12.2	21.3
18:1*n* – 7	2.9	1.5	3.0	2.1	3.9	2.3	2.4	3.4	2.8
20:1*n* – *7* + 20:1*n* – 9	13.8	4.4	5.3	4.4	10.7	9.3	9.0	12.3	4.1
22:1*n* – *9* + 22:1*n* – 11	17.1	4.7	5.1	4.6	10.0	11.1	10.7	13.5	4.6
24:1	0.8	0.4	0.5	0.4	0.7	0.6	0.6	0.8	0.4
Σ MUFA	**52.3**	**41.1**	**53.9**	**31.7**	**45.2**	**47.6**	**41.7**	**48.7**	**36.2**
18:2*n* – 6 (LA)	2.4	7.1	14.2	30.7	2.3	4.7	17.7	2.7	20.1
18:3*n* – 6	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1
20:2*n* – 6	0.3	0.2	0.3	0.2	0.4	0.2	0.3	0.2	0.1
20:3*n* – 6	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0
20:4*n* – 6	0.4	0.2	0.3	0.2	0.5	0.3	0.3	0.5	0.3
Σ *n* - 6	**3.2**	**7.6**	**14.9**	**31.2**	**3.4**	**5.3**	**18.4**	**3.6**	**20.6**
18:3*n* – 3 (LNA)	0.6	0.3	5.3	3.9	0.7	0.4	2.2	0.7	2.1
18:4*n* – 3	1.9	0.6	0.6	0.6	1.8	1.1	1.2	1.8	0.6
20:3*n* – 3	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1
20:4*n* – 3	0.4	0.2	0.2	0.2	0.5	0.3	0.3	0.5	0.3
20:5*n* – 3 (EPA)	6.9	3.8	3.8	3.7	8.2	5.0	5.2	7.5	3.9
22:5*n* – 3 (DPA)	0.6	0.4	0.4	0.4	0.9	0.5	0.5	0.8	0.4
22:6*n* – 3 (DHA)	8.1	4.8	5.1	4.8	9.8	6.0	6.4	9.0	4.8
Σ *n* - 3	***18.7***	***10.1***	***15.5***	***13.6***	***22.0***	***13.3***	***15.9***	***20.4***	***12.2***
Total	93.8	96.3	95.3	94.5	93.1	95.1	94.8	93.6	94.9
Unknown	6.2	3.7	4.7	5.5	6.9	4.9	5.2	6.4	5.1
Σ PUFA	**22.4**	**18.1**	**30.7**	**45.2**	**25.8**	**19.3**	**34.9**	**24.3**	**33.1**
***n* - 3/*n* - 6 ratio**	***5.8***	***1.3***	***1.0***	***0.4***	***6.5***	***2.5***	***0.9***	***5.6***	***0.6***
Σ EPA +DPA +DHA	**15.6**	**8.9**	**9.2**	**8.9**	**18.9**	**11.5**	**12.0**	**17.2**	**9.1**

The proportions of AA (20:4*n* – 6) were highest in the ST and the FO/ST diets (0.5%). The lowest values observed for AA were 0.2% in the PO and SO diets. The Σ PUFA were found to be affected by the dietary lipid sources. The highest proportions of PUFA were observed in diets containing either SO or RO. SO diet had PUFA proportions of 45.2%, FO/SO 34.9% proportions, RO proportions of 30.7% and lowest proportions were 18.1% in PO diet.

The FO diet contained high proportions of Σ *n* – 3 (18.7%) where the most dominant FAs were DHA (8.1%), EPA (6.9%) and a *n* - 3/*n* - 6 ratio of 5.8. However, the values detected were not higher than found in the ST diet. The ST diet contained 8.2% proportion of EPA and 9.8% proportion of DHA, the highest levels of Σ *n* – 3 (22%) and a *n* - 3/*n* - 6 ratio of 6.5.

5. Results (slaughtering size)

5.1. Physical quality of the experimental diets

The inclusion of palm oil in the experimental diets had significant effects on the sinking time (Figure 5.1.). Diets containing soy oil (SO) or fish oil (FO) had similar sinking times. The longest sinking times, 22±0.58 sec/m, were for the pure palm oil diet (PO) and 75% palm oil inclusion (P75) resulted in significantly longer sinking time compared with all the other diets except the diets containing palm oil (PO and P50/S25).

Feed pellets with high palm oil inclusion levels had a tendency to float, with a number of pellets remaining in the water surface 2 minutes after they were released. All pellets of the P50 and P50/S25 diets sunk but 2% of the FO and SO pellets did not sink. A total of 10% of the PO pellets did not sink and 20% of the P75 pellets remained in the water surface 2 minutes after they were released.

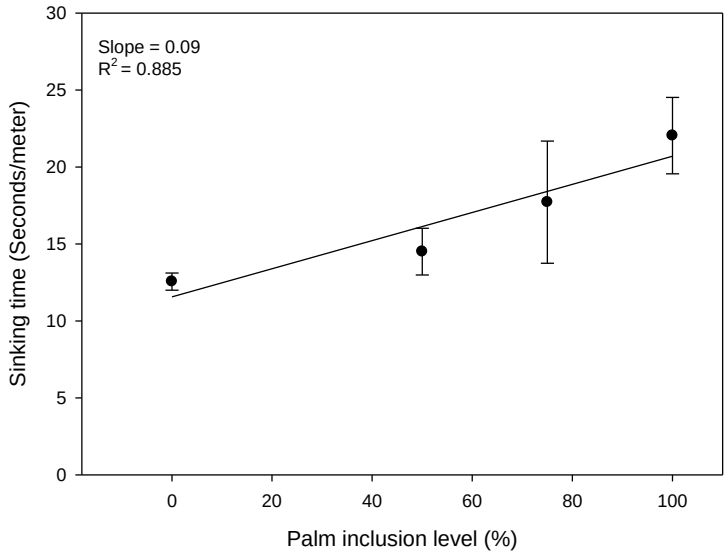


Figure 5.1. Sinking velocity plotted against palm inclusion level of the experimental diets. Values are presented as mean of 5 tests ±SD.

5.2. Growth and FCR

No significant difference was observed when the mean initial weight, final weight, fork length, condition factor, growth increase (ΔG), specific growth rates or hepatosomatic index of fish fed various experimental diets were compared (Table 5.1. Figure 5.3.). The growth of the fish was acceptable, with SGR values ranging from 0.70% in the PO group to 0.75% in the FO group. Up to 100% gain in biomass was achieved in fish fed the experimental diets during the 94 day feeding trial, despite the low water temperature (6°C). The protein efficiency ratio (PER) was, however, significantly different in the various groups, with the highest PER in the FO diet (2.41) and the lowest in the PO group (2.11).

Table 5.1. Growth of the experimental fish. Final fork length (FL) within each treatment, growth increase (ΔG) as a percent increase of biomass, condition factor (CF), feed conversion ratio (FCR), specific growth rate (SGR), hepatosomatic index (HSI) and protein efficiency ratio (PER).

	Diet					
	FO	SO	PO	P75	P50	P50/S25
ΔG	102.8 ±5.1	95.5±1.2	92.7±3.5	92.7±10	99.6±9.0	98.4±1.4
FL	40.9±1.58	40.3±1.7	40.5±1.7	40.6±1.7	40.8±1.5	40.8±1.5
CF	1.62±0.11	1.62±0.13	1.57±0.12	1.57±0.12	1.61±0.11	1.62±0.11
FCR*	1.06 ± 0.02^{a}	1.09 ± 0.01^{ab}	1.21 ± 0.00^{c}	1.14 ± 0.06^{bc}	1.11 ± 0.03^{ab}	1.13 ± 0.03^{ab}
SGR	0.75±0.09	0.71±0.12	0.70±0.05	0.70±0.14	0.73±0.05	0.73±0.05
HSI	1.99±0.20	1.92±0.27	1.97±0.33	1.95±0.22	1.82±0.25	1.89±0.19
PER	2.41 ± 0.04^{a}	2.35 ± 0.03^{a}	2.11 ± 0.00^{b}	2.24 ± 0.14^{ab}	2.36 ± 0.07^{a}	2.28 ± 0.06^{ab}

Values are presented as mean ±SD and different letters denote significant differences (P<0.05)

Feed conversion ratios (FCR) were calculated for the period from the 6th of February to the 11th of April. Introduction of palm oil into the experimental diets resulted in significantly higher (P<0.05) feed conversion ratios (Figure 5.2.). The lowest FCR values were observed in groups fed the FO diet (1.06) and the highest values in groups fed the PO diet (1.21). Significantly lower FCR values were observed in the FO group compared with groups fed either PO or P75 diets. The PO group had significantly higher FCR values compared with all groups except the P75 group. Furthermore, there was a considerable variance in the FCR values in different tanks within the P75 treatment, ranging from 1.18 to 1.07.

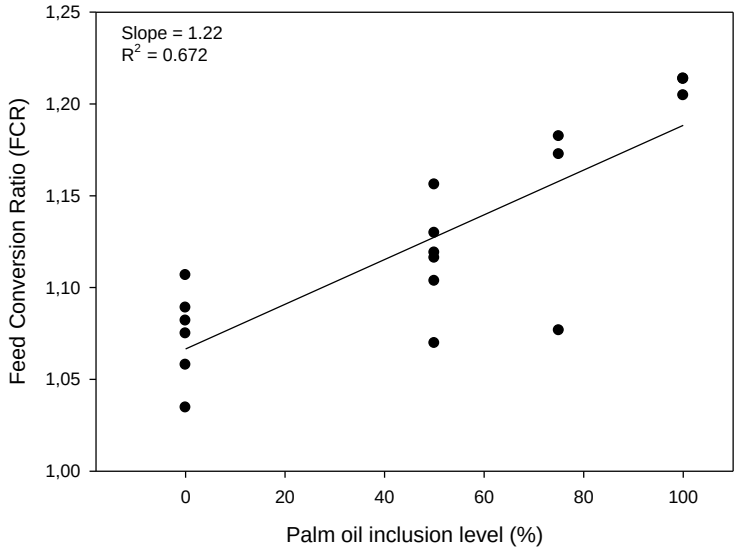


Figure 5.2. Feed of groups fed diets with different inclusion levels of palm oil. Each point represents FCR for one tank.

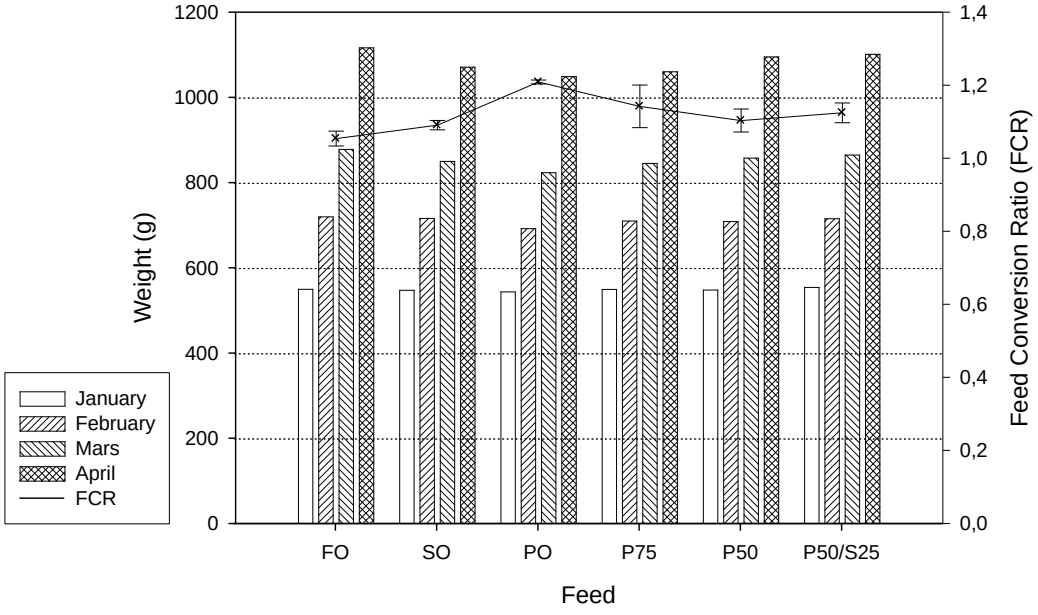


Figure 5.3. Mean values of weight and FCR. The FCR values are presented as mean of three tanks ±SD.

5.3. Apparent digestibility

The apparent digestibility coefficients were, in general, affected by inclusion of palm oil and soy oil (Table 5.2.). The lowest values of the dry matter (DM) digestibility were in groups fed either the PO diet (90.3%) or the P75 diet (92.2%). The SO diet gave the highest DM digestibility (95.7%), which was significantly higher than in all groups except for fish fed P50/S25.

As for DM digestibility, the lowest lipid digestibility was in groups fed either the PO or the P75 diets (88.6 and 89.7% respectively). The highest coefficients for lipid digestibility was in fish fed soy oil based diets even though it was not significantly different from FO, P50 and P50/S25 treatments.

Apparent digestibility for gross energy (GE) was highest in the SO group, significantly higher than in the PO, P75 and P50 groups. Lowest ADC of gross energy was in the group fed PO diet (92.5%).

Table 5.2. Apparent digestibility coefficients for the experimental diets.

	Diet*					
ADC**	FO	SO	PO	P75	P50	P50/S25
DM	93.1±1.3^{b}	95.7±0.3^{a}	90.3±1.8^{c}	92.2±1.4^{bc}	92.6±1.2^{bc}	93.7±1.8^{ab}
Lipid	96.9±0.6^{a}	97.4±0.2^{a}	88.6±2.13^{b}	89.7±1.9^{b}	94.6±0.8^{a}	95.7±1.2^{a}
GE	95.5±0.9^{ab}	97.2±0.2^{a}	92.5±1.4^{c}	94.6±1.0^{bd}	93.9±1.0^{bc}	95.7±1.2^{ad}

**Values are presented as a mean (±SD) of 12 replicas pooled from 3 tanks in each treatment. Different letters denote significant differences (P<0.05)*
***Apparent digestibility coefficient is presented for total dry matter (DM), total lipid digestibility and for gross energy (GE).*

5.4. Chemical analyses

There was no significant difference in the chemical composition of muscle of fish fed different the experimental diets (Table 5.3). The amounts of crude protein and lipid in the fish fillets were not affected by different dietary treatments. The lowest protein value obtained was for the FO group and the highest value in the group fed the PO diet, 20.6% and 21.1%, respectively.

Table 5.3. Chemical analyses of muscle from fish fed the various experimental diets.

	Diet*					
	FO	SO	PO	P75	P50	P50/S25
Moisture	73.6±0.6	73.5±0.0	73.8±0.7	73.1±0.4	73.27±0.2	72.5±0.8
Crude protein	20.6±0.3	20.7±0.2	21.1±0.4	20.8±0.3	21.03±0.3	21.00±0.1
Crude lipid	4.83±0.5	4.63±0.1	4.03±0.6	4.90±0.3	4.60±0.6	5.43±0.7
Crude ash	1.60±0.1	1.77±0.4	1.73±0.1	1.67±0.3	1.93±0.6	2.20±0.3

**Chemical analyses are presented as mean values (±SD) of a pool of fish from 3 tanks after 94 days feeding the experimental diets.*

5.5. Fatty acid analyses

The different dietary lipid compositions were reflected in the fatty acid (FA) composition of the experimental diets (Table 5.4.). Compared to the other diets, the FO diet contained considerably higher concentrations of monounsaturated (Σ MUFA) and omega 3 (Σ *n* – 3) fatty acids, including eicosapentaenoic acid (20:5*n* – 3; EPA), docosahexaenoic acid (22:6*n* – 3; DHA) and docosapentaenoic acid (22:5*n* – 3; DPA). The proportions of EPA ranged from 1.6% in the PO diet up to 7.8% in the FO diet, with a good correlation to the amount of fish oil included in the diets. Similarly, levels of DHA where highest in the FO diet (9.1%) and lowest in the PO diet (2.6%). The high proportions of MUFAs in the FO diet were due to noticeably higher levels of the long chain monoenes 20:1 and 22:1 compared to other diets. In diets with palm oil or soy oil inclusion, the most dominant MUFA was oleic acid (18:1*n* – 9). Total saturated fatty acids (Σ SFA) ranged from 16.6% in the SO diet (12% palmitic acid; 16:0) and up to 46.6% in the PO diet (with 38.3% palmitic acid). The concentration of linoleic acid (18:2*n* – 6; LA) increased with soy oil addition, with values ranging from 2.2% in the FO diet up to 40.2% in the SO diet. Total amounts of omega 6 fatty acids (Σ *n* – 6) were in good correlation with LA which was the predominant *n* – 6 fatty acid of the diets. Similarly, soy oil addition lead to increased concentrations of linolenic acid (18:3*n* – 3; LNA) in the diets, with highest values of 4.5% found in the SO diet and the lowest values in the PO diet (0.3%). The concentration of arachidonic acid (20:4*n* – 6; AA) was 0.3% in the FO diet and between 0.1- 0.2% in the other diets. The high levels of 18:2*n* – 6 in the SO diet gave the lowest *n* -3/*n* -6 ratio compared to the other, 0.2 compared to 0.9 for the PO diet and 7.1 for the FO diet. The SO diet contained prominent levels of total polyunsaturated fatty acids (Σ PUFA) compared to all other diets. In the SO diet,

51% of all fatty acid were PUFAs, compared to only 10.7% in the PO diet, 24.3% in the P50/S25 diet and 25.1% in the FO diet.

Table 5.4. Fatty acid analyses of the experimental diets.

	Diet					
FA	FO	SO	PO	P75	P50	P50/S25
14:00	6.7	1.5	2.3	4.0	4.2	3.4
16:00	11.8	12.0	38.3	25.1	18.5	24.1
18:00	1.1	3.1	6.0	4.3	4.0	4.1
Σ SFA	**19.6**	**16.6**	**46.6**	**33.4**	**26.7**	**31.6**
16:1*n* – 9	0.1	0.0	0.1	0.1	0.1	0.1
16:1*n* – 7	4.2	0.9	0.9	2.2	2.5	1.8
18:1*n* – 9	8.8	19.5	30.7	21.9	19.7	21.7
18:1*n* – 7	1.4	1.5	1.0	2.5	3.3	2.2
20:1*n* – 11 + 20:1*n* – 9	12.9	2.6	2.3	6.2	7.7	4.8
22:1*n* – 11 + 20:1*n* – 13	17.9	3.3	2.9	8.6	10.7	6.3
22:1*n* – 9	1.2	0.2	0.2	0.6	0.7	0.4
24:1	0.7	0.2	0.2	0.4	0.5	0.3
Σ MUFA	**48.0**	**28.5**	**38.3**	**42.9**	**45.8**	**38.0**
18:2*n* – 6 (LA)	2.2	40.2	5.1	4.4	4.3	12.9
20:2*n* – 6	0.2	0.1	0.0	0.1	0.1	0.1
20:3*n* – 6	0.1	0.0	0.0	0.0	0.0	0.0
20:4*n* – 6 (AA)	0.3	0.1	0.1	0.2	0.2	0.1
Σ *n* - 6	**2.7**	**40.4**	**5.3**	**4.6**	**4.6**	**13.1**
18:3*n* – 3 (LNA)	1.0	4.5	0.3	0.7	0.8	1.6
18:4*n* – 3	3.2	0.7	0.6	1.6	1.9	1.3
20:3*n* – 3	0.1	0.0	0.0	0.1	0.1	0.0
20:4*n* – 3	0.5	0.1	0.1	0.3	0.3	0.2
20:5*n* – 3 (EPA)	7.8	1.8	1.6	4.0	4.8	3.4
22:5*n* – 3 (DPA)	0.6	0.2	0.1	0.3	0.4	0.3
22:6*n* – 3 (DHA)	9.1	3.2	2.6	5.4	6.2	4.3
Σ *n* - 3	***19.2***	***9.9***	***4.8***	***10.7***	***12.6***	***9.8***
Total	92.7	96.0	95.6	93.3	91.6	93.8
Unknown	7.3	4.0	4.4	6.7	8.4	6.2
Σ PUFA	**25.1**	**51.0**	**10.7**	**17.0**	**19.1**	**24.3**
***n* - 3/*n* - 6 ratio**	***7.1***	***0.2***	***0.9***	***2.3***	***2.7***	***0.7***
ΣEPA +DPA +DHA	**17.5**	**5.2**	**4.4**	**9.7**	**11.4**	**8.0**

There was a significant ($p<0.05$) correlation between some of the major PUFAs in the experimental diets and the fatty acid composition of the fish tissue (Figure 5.4.). However, there was no significant relationship between AA diet and in tissue.

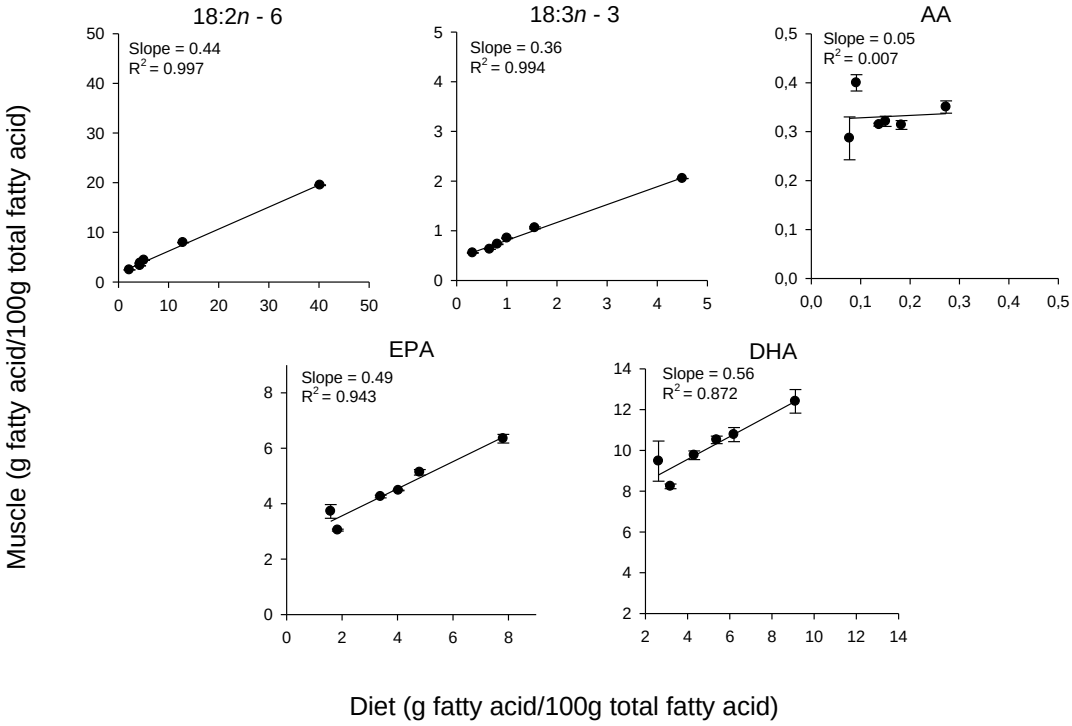


Figure 5.4. Concentration of individual fatty acids in diets and in muscle. Values are presented as a mean of 3 replicas ±SD.

Significantly higher levels of palmitic acid (16:0) were found in the muscle of fish from the PO group compared with other groups. Significantly higher Σ SFA were also found in this group compared with the FO and the SO group (Table 5.5.). In the FO group, the levels of long chain, 20 and 22 carbonic monoenes were considerably lower in the fish muscle compared with the diet, and the concentration of 18:1*n* – 9 were higher to the same extent. In all other treatments, the levels of 20:1*n* – 11 and 20:1*n* – 9 were higher in the fish muscle compared to the diets. The two long chain fatty acids, 22:1n – 11 and 20:1n – 13, were found in higher levels in fish muscle compared to the diets in the groups fed diets devoid of fish oil. Similarly, diets rich in 20:1*n* – 11 and 20:1*n* – 9 resulted in lower levels of these FAs in the fish tissue compared to the diets.

There was a significant difference in muscle Σ MUFAs in fish from the various groups, with 38% in the SO group, 46% in the FO group and 50% in fish fed the PO diet. As in the diets, LA (18:2*n* – 6) was the primary *n* – 6 fatty acid appearing in the fish muscle. Diets containing soy oil (SO and P50/S25), resulted in significantly higher levels of LA and lower *n* – 3/*n* – 6 ratios in muscle compared with all other treatments, with total levels of 19.5% LA and *n* – 3/*n* – 6 ratio of 0.7. The overall ratios of *n* – 3/*n* – 6 were corresponded well to the dietary

lipids, with highest values found in the FO group (7.1, the same value as found in the diet) and values ranging from 3.0 to 4.8 in diets containing palm oil. The highest levels of AA were found in muscle from fish fed either the SO or the FO diets (0.4%). These values were significantly higher than in all other groups where the measured levels of AA were 0.3%.

Table 5.5. Fatty acid composition of fish muscle post 94 day feeding with the experimental diets.

FA*	FO	SO	PO	P75	P50	P50/S25
14:00	4.6±0.4^{a}	2.5±0.1^{c}	3.4±0.5^{bc}	3.3±0.1^{bc}	3.8±0.2^{ab}	3.2±0.2^{bc}
16:00	14.0±0.4^{c}	14.1±0.2^{bc}	18.0±1.4^{a}	15.8±0.1^{b}	15.3±0.2^{bc}	15.0±0.2^{bc}
18:00	1.7±0.0^{d}	2.5±0.0^{a}	2.3±0.0^{b}	2.1±0.0^{c}	2.1±0.0^{c}	2.2±0.0^{bc}
Σ SFA	**20.2±0.8^{b}**	**19.2±0.4^{b}**	**23.6±1.9^{a}**	**21.2±0.2^{ab}**	**21.2±0.4^{ab}**	**20.4±0.4^{b}**
16:1*n* – 9	0.3±0.0^{b}	0.3±0.0^{ab}	0.4±0.0^{a}	0.3±0.0^{ab}	0.3±0.0^{ab}	0.3±0.0^{ab}
16:1*n* – 7	7.3±0.3^{a}	4.7±0.1^{c}	7.3±0.5^{a}	6.7±0.1^{ab}	6.7±0.1^{ab}	6.3±0.2^{b}
18:1*n* – 9	16.9±0.1^{f}	20.9±0.1^{e}	28.1±0.2^{a}	25.2±0.1^{b}	22.1±0.1^{d}	23.5±0.2^{c}
18:1*n* – 7	2.3±0.0^{d}	2.2±0.0^{e}	2.7±0.0^{b}	2.7±0.0^{b}	2.8±0.0^{a}	2.5±0.0^{c}
20:1*n* – 11 + 9	9.1±0.3^{a}	5.0±0.1^{e}	5.9±0.3^{d}	7.2±0.0^{c}	7.8±0.1^{b}	6.7±0.2^{c}
22:1*n* – 11 + 20:1*n* – 13	8.1±0.4^{a}	4.0±0.1^{d}	4.5±0.4^{d}	5.9±0.1^{c}	6.8±0.2^{b}	5.6±0.3^{c}
22:1*n* – 9	0.8±0.0^{a}	0.5±0.0^{d}	0.5±0.1^{d}	0.7±0.0^{b}	0.7±0.0^{b}	0.6±0.0^{c}
24:1	0.5±0.1^{a}	0.3±0.0^{b}	0.3±0.1^{bc}	0.4±0.0^{abc}	0.4±0.0^{ac}	0.4±0.0^{abc}
Σ MUFA	**45.9±0.4^{b}**	**38.3±0.3^{d}**	**50.2±0.1^{a}**	**49.6±0.0^{a}**	**48.3±0.4^{c}**	**46.6±0.5^{b}**
18:2*n* – 6 (LA)	2.4±0.0^{f}	19.5±0.1^{a}	4.4±0.0^{c}	3.8±0.0^{d}	3.3±0.0^{e}	7.9±0.0^{b}
20:2*n* – 6	0.2±0.0^{c}	0.8±0.0^{a}	0.3±0.0^{c}	0.2±0.0^{c}	0.2±0.0^{c}	0.4±0.0^{b}
20:3*n* – 6	0.1±0.0^{c}	0.7±0.0^{a}	0.2±0.0^{c}	0.2±0.0^{c}	0.1±0.0^{c}	0.3±0.0^{b}
20:4*n* – 6 (AA)	0.4±0.0^{a}	0.4±0.0^{a}	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}
Σ *n* - 6	**3.1±0.0^{f}**	**21.3±0.1^{a}**	**5.1±0.1^{c}**	**4.5±0.0^{d}**	**3.9±0.0^{e}**	**8.9±0.0^{b}**
18:3*n* – 3 (LNA)	0.9±0.0^{c}	2.1±0.0^{a}	0.6±0.0^{e}	0.6±0.0^{de}	0.7±0.0^{d}	1.1±0.0^{b}
18:4*n* – 3	2.3±0.0^{a}	1.2±0.0^{d}	1.1±0.0^{d}	1.4±0.0^{c}	1.7±0.0^{b}	1.5±0.0^{c}
20:3*n* – 3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:4*n* – 3	1.0±0.0^{a}	0.5±0.0^{d}	0.6±0.0^{c}	0.8±0.0^{b}	0.8±0.0^{b}	0.7±0.0^{c}
20:5*n* – 3 (EPA)	6.3±0.2^{a}	3.0±0.0^{e}	3.7±0.2^{d}	4.5±0.0^{c}	5.1±0.1^{b}	4.3±0.0^{c}
22:5*n* – 3 (DPA)	1.3±0.0^{a}	0.7±0.0^{cd}	0.8±0.1^{c}	1.0±0.0^{b}	1.1±0.0^{b}	1.0±0.0^{bcd}
22:6*n* – 3 (DHA)	12.4±0.6^{a}	8.2±0.1^{c}	9.5±1.0^{b}	10.5±0.2^{b}	10.8±0.3^{b}	9.8±0.2^{b}
Σ *n* - 3	***22.0±0.8^{a}***	***14.7±0.2^{c}***	***15.3±1.4^{c}***	***17.5±0.2^{b}***	***18.6±0.5^{b}***	***16.9±0.2^{b}***
Total	**93.7±0.3**	**94.7±0.3**	**95.4±0.4**	**94.4±0.1**	**93.9±0.2**	**94.3±0.3**
Unknown	6.3±0.3	5.3±0.3	4.6±0.4	5.6±0.1	6.1±0.2	5.7±0.3
Σ PUFA	**27.5±0.8^{b}**	**37.3±0.3^{a}**	**21.6±1.5^{d}**	**23.5±0.3^{c}**	**24.4±0.5^{c}**	**27.3±0.2^{b}**
***n* - 3/*n* - 6 ratio**	***7.1±0.2^{a}***	***0.7±0.0^{f}***	***3.0±0.2^{d}***	***3.9±0.0^{c}***	***4.8±0.1^{b}***	***1.9±0.0^{e}***
Σ EPA +DPA +DHA	**20.1±0.8^{a}**	**12.0±0.2^{e}**	**14.0±1.3^{d}**	**16.0±0.2^{bc}**	**17.0±0.5^{b}**	**15.0±0.2^{dc}**

**The concentrations of fatty acids are presented as percentage (g fatty acid/100 total fatty acids). Different letters denote significant difference between dietary treatments (n=3 ±SD using 95% level of significance (P<0.05).*

The FO diet resulted in significantly higher levels of EPA, DPA, DHA and Σ *n* – 3 in the fish muscle. DHA was the key *n* – 3 fatty acid found in the fish muscle, with values ranging from 12.4% in the FO group, 10.8% in the P50 and down to 8.2% found in the SO group. In all groups, the DHA levels were higher in the fish tissue compared to the diets. The same was true for EPA with the lowest value of 3.0% found in fish fed the SO diet and highest value of 6.3% in

the FO group. The values of EPA were higher in the fish tissue compared with the diet in all groups with the exception of the FO group.

After 39 days of recovery feeding with the FO diet, a considerable alteration was observed in the FA profiles of the fish tissue. The levels of palmitic acid (16:0) in the fillets of fish from the PO group declined from 18.0 to 15.3%, with levels comparable to the values from other palm oil treatments (Table 5.6). The values of total saturated fatty acids in the PO group had declined from 23.6% to 20.9%. The lowest values of Σ SFA were found in groups fed the FO and the SO diets, 19.6 and 19.4%, respectively.

Table 5.6. Fatty acid composition of the fish muscle post 39 day recovery feeding with fish oil based diet.

FA	FO	SO	PO	P75	P50	P50/S25
14:00	4.4±0.2^{a}	3.4±0.0^{c}	3.5±0.1^{bc}	3.7±0.1^{bc}	3.9±0.0^{b}	3.7±0.2^{bc}
16:00	13.7±0.2^{c}	14.0±0.1^{bc}	15.3±0.3^{a}	15.8±0.2^{a}	14.4±0.1^{b}	15.4±0.2^{a}
18:00	1.6±0.0^{d}	2.1±0.0^{a}	2.1±0.0^{a}	2.0±0.0^{b}	1.8±0.0^{c}	1.9±0.0^{b}
Σ SFA	**19.6±0.3^{b}**	**19.4±0.1^{b}**	**20.9±0.4^{ac}**	**21.5±0.2^{c}**	**20.1±0.1^{b}**	**21.0±0.4^{c}**
16:1*n* – 9	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
16:1*n* – 7	6.9±0.1^{a}	6.5±0.0^{b}	6.7±0.1^{a}	6.6±0.0^{b}	6.9±0.0^{a}	6.3±0.1^{b}
18:1*n* – 9	16.3±0.0^{d}	20.3±0.0^{c}	23.6±0.0^{a}	23.2±0.0^{b}	20.2±0.1^{c}	20.7±0.1^{b}
18:1*n* – 7	2.2±0.0^{c}	2.2±0.0^{cd}	2.6±0.0^{a}	2.4±0.0^{b}	2.6±0.0^{a}	2.3±0.0^{d}
20:1*n* – 11 + 9	9.3±0.1^{a}	5.9±0.4^{c}	7.2±0.6^{b}	7.4±0.1^{b}	7.8±0.0^{b}	7.1±0.1^{b}
22:1*n* – 11 + 20:1*n* – 13	8.3±0.1^{a}	4.9±0.0^{e}	5.9±0.1^{d}	6.2±0.1^{c}	6.6±0.0^{b}	6.1±0.1^{c}
22:1*n* – 9	0.8±0.0^{a}	0.6±0.0^{c}	0.7±0.0^{b}	0.7±0.0^{b}	0.7±0.0^{b}	0.6±0.0^{c}
24:1	0.5±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{b}	0.4±0.0^{b}	0.4±0.0^{b}	0.4±0.0^{b}
Σ MUFA	**45.2±0.0^{c}**	**41.7±0.3^{d}**	**48.1±0.6^{a}**	**47.8±0.1^{a}**	**46.2±0.1^{b}**	**44.4±0.2^{c}**
18:2*n* – 6	2.4±0.0^{f}	11.3±0.0^{a}	3.7±0.0^{c}	3.5±0.0^{d}	3.0±0.0^{e}	6.6±0.0^{b}
20:2*n* – 6	0.2±0.0^{c}	0.6±0.0^{a}	0.3±0.0^{b}	0.2±0.0^{c}	0.2±0.0^{c}	0.3±0.0^{b}
20:3*n* – 6	0.1±0.0^{d}	0.5±0.0^{a}	0.2±0.0^{c}	0.1±0.0^{d}	0.1±0.0^{cd}	0.2±0.0^{c}
20:4*n* – 6	0.3±0.0^{b}	0.4±0.0^{a}	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}
Σ *n* - 6	**3.1±0.0^{f}**	**12.7±0.0^{a}**	**4.5±0.0^{c}**	**4.1±0.0^{d}**	**3.7±0.0^{e}**	**7.5±0.1^{b}**
18:3*n* – 3	0.9±0.0^{c}	1.5±0.0^{a}	0.7±0.0^{e}	0.7±0.0^{e}	0.8±0.0^{d}	1.0±0.0^{b}
18:4*n* – 3	2.3±0.0^{a}	1.8±0.0^{c}	1.6±0.0^{e}	1.6±0.0^{e}	2.0±0.0^{b}	1.7±0.0^{d}
20:3*n* – 3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.0
20:4*n* – 3	1.1±0.0^{a}	0.8±0.0^{b}	0.8±0.0^{b}	0.8±0.0^{b}	1.0±0.0^{a}	0.8±0.0^{b}
20:5*n* – 3	6.7±0.1^{a}	4.6±0.0^{d}	5.0±0.1^{c}	4.9±0.0^{c}	5.8±0.0^{b}	5.0±0.1^{c}
22:5*n* – 3	1.7±0.6^{a}	1.0±0.0^{b}	1.1±0.0^{a}	1.1±0.0^{a}	1.3±0.0^{ab}	1.0±0.0^{b}
22:6*n* – 3	13.2±0.4^{a}	10.2±0.1^{c}	11.3±0.2^{b}	11.1±0.1^{b}	12.8±0.0^{a}	11.1±0.3^{b}
***Σ n* - 3**	***23.7±0.4^{a}***	***18.1±0.1^{d}***	***18.9±0.3^{c}***	***18.7±0.2^{cd}***	***21.7±0.0^{b}***	***19.1±0.4^{c}***
Total	**94.1±0.5**	**93.9±0.3**	**94.0±0.5**	**93.9±0.1**	**93.7±0.2**	**93.7±0.2**
Unknown	5.9±0.5	6.1±0.3	6.0±0.5	6.1±0.1	6.3±0.2	6.3±0.2
Σ PUFA	**29.2±0.4^{b}**	**32.7±0.1^{a}**	**25.1±0.3^{d}**	**24.6±0.2^{d}**	**27.5±0.0^{c}**	**28.3±0.4^{c}**
***n* - 3/*n* - 6 ratio**	***7.7±0.1^{a}***	***1.4±0.0^{f}***	***4.2±0.0^{d}***	***4.5±0.0^{c}***	***5.8±0.0^{b}***	***2.6±0.0^{e}***
Σ EPA +DPA +DHA	**21.7±0.4^{a}**	**15.8±0.1^{d}**	**17.3±0.3^{c}**	**17.1±0.2^{c}**	**19.8±0.0^{b}**	**17.1±0.3^{c}**

**Fatty acids are presented as percentage (g fatty acid/100 total fatty acids). Different letters denote significant difference between the dietary treatment (n=3 ±SD) with 95% level of significance (P<0.05).*

A decline in Σ MUFA during the 39 days of recovery feeding was observed in all palm oil treatments (~4.5%). In contrast, an 8.9% increase in Σ MUFA was detected in the SO group (from 38.3- 41.7%). The dominating MUFA in all treatments was oleic acid (18:1n – 9), with values ranging from 16.3% in the FO group to a muscle level of 23.6% in the PO group which was significantly higher compared with all groups. LA (18:2n – 6) was reduced by 73% in the SO group and by 19.7% in the P50/S25 group during the 39 days recovery feeding. However, the LA values of 11.3% for the SO group and 6.6% for the P50/S25 group were significantly higher than in the other groups (2.4% in the FO group, 3.0% in the P50 group, 3.5% in the P75 group and 3.7% in the PO group).

The Σ *n* – 3 (including EPA, DPA and DHA) in fish tissue was significantly higher in the FO group compared to other groups.. LNA (18:3*n* – 3) was the only *n* – 3 FA that occurred in higher levels in tissue of fish from other groups and LNA was found in significantly higher proportions in tissue of fish from the soy oil groups (SO and P50/S25). A noticeable recovery of Σ *n* – 3 was observed in all groups previously fed diets containing the various plant oils. A 23.5% increase in Σ *n* – 3 was observed in fillets of fish from the PO group, 23.1% in the SO group, 16.7% in the P50 group, 6.9% in the P75 group and 13.0% in the P50/S25 group. However, the *n* – 3/*n* – 6 ratios in fish from all groups were generally low compared to the 7.7 ratio in fish muscles from the FO group. The lowest ratios observed were 1.4 (increase from 0.7) in the SO group and 2.6 in the P50/S25 group (increase from 1.9). In other treatments, the *n* – 3/*n* – 6 ratios were 4.2 in the PO group, 4.5 in the P75 group and 5.8 in the P50 group.

After 76 days recovery feeding with the FO diet, no statistical difference ($P>0.05$) in tissue Σ SFA was observed when the various groups were compared (Table 5.7.). The levels of monounsaturated fatty acids ranged from 42% in the SO group to 49.8% in fillets from the group primarily fed the P75 diet. The SO and P50/S25 groups, primarily fed diets containing soy oil, resulted in fish flesh with lower values of 16:1*n* – 7 compared to the other diets. Oleic acid (18:1*n* – 9) was the primary MUFA in all treatments, with highest value found in the PO fish muscles (22.7%).

Despite the 76 day recovery period, fish from the SO and P50/S25 groups contained considerable higher muscle Σ *n* – 6 compared to the other groups. LA

measured in fish fillets from the SO group was 12.8% compared to the lowest value of 2.4% found in the FO group. No statistical difference in AA levels was detected between treatments at this point.

Table 5.7. Fatty acid analyses of fish muscle after 76 days recovery feeding with fish oil based diet.

FA	FO	SO	PO	P75	P50	P50/S25
14:00	4.8±0.8^{a}	3.7±0.3^{ab}	3.6±0.2^{b}	4.1±0.3^{ab}	4.2±0.2^{ab}	4.0±0.4^{ab}
16:00	14.0±0.9^{b}	13.7±0.4^{b}	15.9±0.3^{a}	14.9±0.4^{ab}	14.2±0.3^{b}	15.9±0.6^{a}
18:00	1.8±0.0^{b}	2.1±0.0^{c}	2.0±0.0^{ac}	1.9±0.0^{a}	1.9±0.0^{ab}	2.0±0.0^{a}
Σ SFA	**20.5±1.7**	**19.5±0.7**	**21.5±0.5**	**21.0±0.7**	**20.3±0.5**	**21.8±1.0**
16:1*n* – 9	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
16:1*n* – 7	7.5±0.6^{a}	5.6±0.2^{c}	7.0±0.2^{ab}	7.1±0.2^{ab}	7.2±0.1^{ab}	6.4±0.3^{b}
18:1*n* – 9	17.3±0.1^{e}	18.8±0.0^{d}	22.7±0.0^{a}	21.5±0.1^{b}	20.1±0.1^{c}	18.6±0.1^{c}
18:1*n* – 7	2.4±0.0^{d}	2.2±0.0^{e}	2.5±0.0^{bc}	2.5±0.0^{c}	2.7±0.0^{a}	2.5±0.0^{b}
20:1*n* – 11 + 9	9.5±0.5^{a}	7.3±0.2^{c}	7.6±0.1^{c}	8.6±0.2^{b}	9.2±0.1^{ab}	7.4±0.2^{c}
22:1*n* – 11 + 20:1*n* – 13	8.3±0.7^{a}	6.2±0.3^{b}	6.5±0.2^{b}	7.6±0.3^{a}	8.2±0.2^{a}	6.2±0.3^{b}
22:1*n* – 9	0.8±0.1^{a}	0.6±0.0^{b}	0.7±0.0^{b}	0.8±0.0^{a}	0.8±0.0^{a}	0.7±0.0^{b}
24:1	0.5±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{b}	0.5±0.0^{a}	0.5±0.0^{a}	0.4±0.0^{b}
Σ MUFA	**47.4±0.8^{c}**	**42.0±0.3^{e}**	**48.4±0.2^{b}**	**49.6±0.4^{a}**	**49.8±0.2^{ab}**	**43.3±0.4^{d}**
18:2*n* – 6 (LA)	2.4±0.0^{f}	12.8±0.0^{a}	3.3±0.0^{c}	3.2±0.0^{d}	2.9±0.0^{e}	4.8±0.0^{b}
20:2*n* – 6	0.2±0.0^{c}	0.6±0.0^{a}	0.3±0.0^{b}	0.3±0.0^{b}	0.2±0.0^{c}	0.3±0.0^{b}
20:3*n* – 6	0.1±0.0^{c}	0.5±0.0^{a}	0.2±0.0^{b}	0.1±0.0^{c}	0.1±0.0^{c}	0.2±0.0^{b}
20:4*n* – 6 (AA)	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0
Σ *n* - 6	**3.1±0.0^{f}**	**14.2±0.0^{a}**	**4.1±0.0^{c}**	**3.9±0.0^{d}**	**3.6±0.0^{e}**	**5.7±0.0^{b}**
18:3*n* – 3 (LNA)	0.9±0.0^{b}	1.6±0.0^{a}	0.6±0.0^{f}	0.7±0.0^{d}	0.8±0.0^{e}	0.9±0.0^{c}
18:4*n* – 3	2.4±0.0^{a}	1.7±0.0^{c}	1.6±0.0^{d}	1.8±0.0^{e}	2.0±0.0^{b}	1.6±0.0^{dc}
20:3*n* – 3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:4*n* – 3	1.1±0.0^{a}	0.8±0.0^{c}	0.8±0.0^{c}	0.9±0.0^{b}	0.9±0.0^{b}	0.8±0.0^{c}
20:5*n* – 3 (EPA)	5.9±0.3^{a}	4.3±0.1^{c}	4.9±0.1^{b}	4.9±0.1^{b}	5.2±0.1^{bd}	5.6±0.2^{ad}
22:5*n* – 3 (DPA)	1.3±0.1^{a}	1.0±0.0^{c}	1.1±0.0^{bc}	1.1±0.0^{b}	1.2±0.0^{ab}	1.2±0.1^{ab}
22:6*n* – 3 (DHA)	10.6±0.9^{ab}	8.8±0.4^{d}	11.2±0.2^{a}	9.9±0.3^{abd}	9.6±0.2^{bd}	12.9±0.7^{c}
Σ *n* - 3	***19.8±1.3^{ab}***	***16.6±0.5^{c}***	***18.7±0.3^{bd}***	***17.6±0.5^{cd}***	***17.9±0.3^{bc}***	***21.5±0.9^{a}***
Total	**93.4±0.5**	**94.2±0.2**	**94.3±0.1**	**94.0±0.2**	**93.8±0.1**	**94.0±0.3**
Unknown	6.6±0.5	5.8±0.2	5.7±0.1	6.0±0.2	6.2±0.1	6.0±0.3
Σ PUFA	**25.5±1.3^{c}**	**32.7±0.6^{a}**	**24.4±0.3^{bc}**	**23.4±0.5^{b}**	**23.7±0.3^{bc}**	**28.9±0.9^{d}**
***n* - 3/*n* - 6 ratio**	***6.3±0.4^{a}***	***1.2±0.0^{d}***	***4.6±0.1^{b}***	***4.55±0.1^{b}***	***4.9±0.0^{b}***	***3.7±0.2^{c}***
Σ EPA +DPA +DHA	**17.8±1.3^{ab}**	**14.1±0.5^{c}**	**17.2±0.3^{b}**	**15.9±0.5^{bc}**	**16.1±0.3^{b}**	**19.7±0.9^{a}**

**Fatty acids are presented as percentage (g fatty acid/100 total fatty acids) and different letters denotes significant difference between dietary treatment (n=3 ±SD).Level of significance is P<0.05.*

The recovery of *n* – 3 fatty acids was lowest in the SO and P75 groups, with significantly lower values of Σ *n* – 3 compared to the FO group. Total tissue *n* – 3 of the P50/S25 group was however measured 21.5%, which was higher compared to the FO group even though the difference was not significant. The LNA levels in tissue from the SO group were significantly higher than in the other treatments with levels of 1.6% compared to 0.9% in the FO group and 0.6 in the PO group. Other *n* – 3 FAs, including 18:4*n* – 3, EPA and DPA were found in

higher concentrations in the FO group compared to the other groups. Nonetheless, the highest value of DHA was measured 12.9% in the P50/S25 group compared to 10.6% in fish from the FO and 8.8% in the SO group. Despite considerably good recovery of *n* – 3 HUFAs, the *n* – 3/*n* – 6 ratios of fish from groups primarily fed diets containing soy oil appeared to be significantly lower compared to the other treatments. The high levels of LA resulted in an *n* – 3/*n* – 6 ratio of 1.2 in the SO group and 3.7 in the P50/S25 group. The highest *n* – 3/*n* – 6 ratio occurred in tissue of fish from the FO group (6.3) and the P50 group (4.9).

Between the first sampling and last sampling of the recovery feeding period (day 39 – day 76) there was an significant increase in muscle LA (18:2*n* – 6) and LNA (18:3*n* – 3) of fish previously fed the SO diet (Figure 5.5. and 5.6.). A table showing the FA values for all treatments during different time periods are shown in Appendix II.

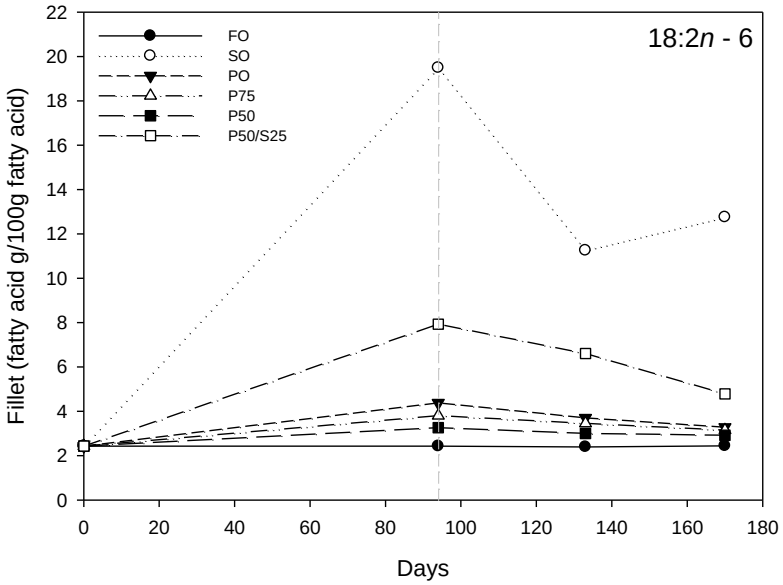


Figure 5.5. Linoleic acid (18:2n – 6) profiles of fish fillets from groups fed various experimental diets. Values are presented from day 0 (beginning of experimental feeding) throughout the 76 days period of recovery feeding. Slashed line indicates the beginning of the recovery feeding period.

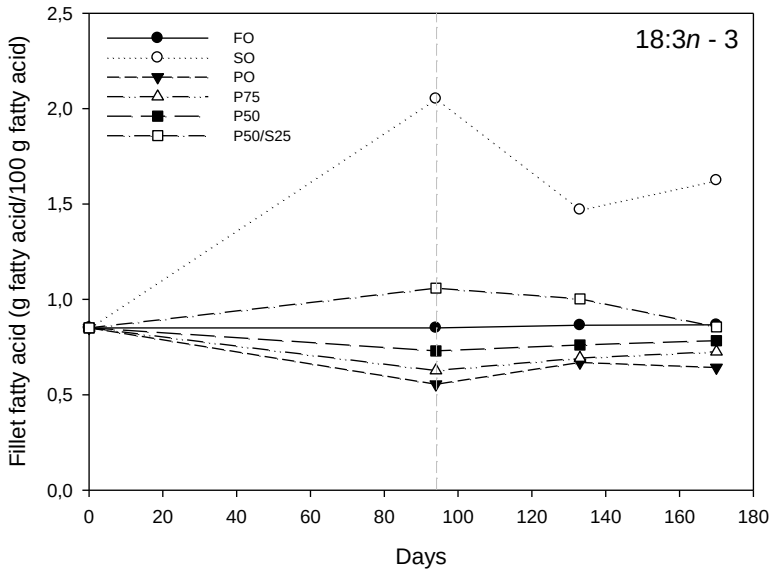


Figure 5.6. Linolenic acid (18:3n – 3) profiles of fish fillets from groups fed the various experimental diets. Values are presented from day 0 (beginning of experimental feeding) throughout the "wash out" period. Slashed line presents the beginning of wash out period.

To get a clear perspective of the effects of various lipid sources on the FA profiles of the fish flesh and their retention, the FA values were plotted in radar graphs. Figure 5.7 shows the levels of muscle 18:2*n* – 6 (LA), 18:3*n* – 3 (LNA), 20:4*n* – 6 (AA), 20:5*n* – 6 (EPA) and 22:6*n* –3 (DHA), compared with a fixed index number of 100 that was calculated from the values observed in fish from the FO group after 94 days feeding the fish oil diets. By this method, it is easy to compare values of the major PUFAs to the FO treatment, both after the 94 day experimental feeding and after 76 day feeding the FO diet.

Fish muscles from the SO group contained eight times higher levels of LA and double the levels of LNA after feeding the experimental diets (Figure 5.7.). After the recovery period, the tissues levels of LA were still significantly higher fish from the SO group. The AA levels remained stable, with same values found in both SO treatments during the experiment. Levels of both EPA and DHA were significantly affected and did not reach the control values of the FO group during the 76 days of recovery feeding.

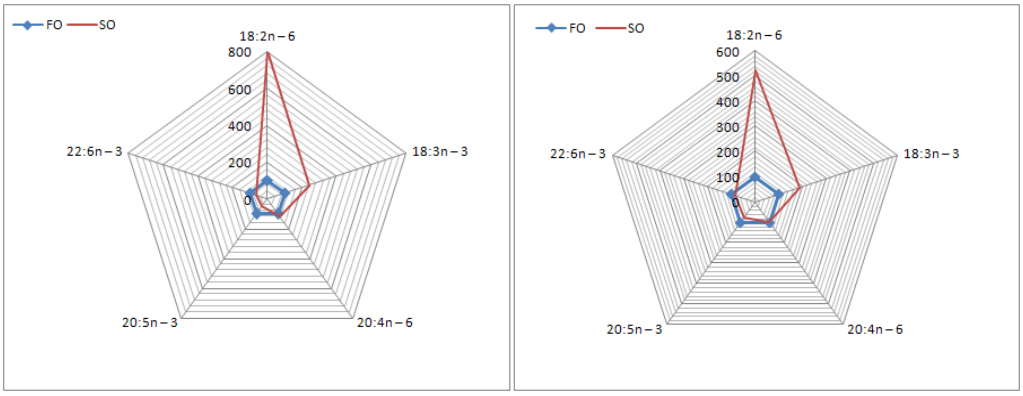


Figure 5.7. Fatty acid profiles of fish muscle from the group fed SO diet compared to the group fed the FO diet. The FO group was designated a fixed index number of 100. The figure to the left represents values post 94 day feeding with the experimental diets and the figure to the right, values after 76 day recovery feeding with the FO diet.

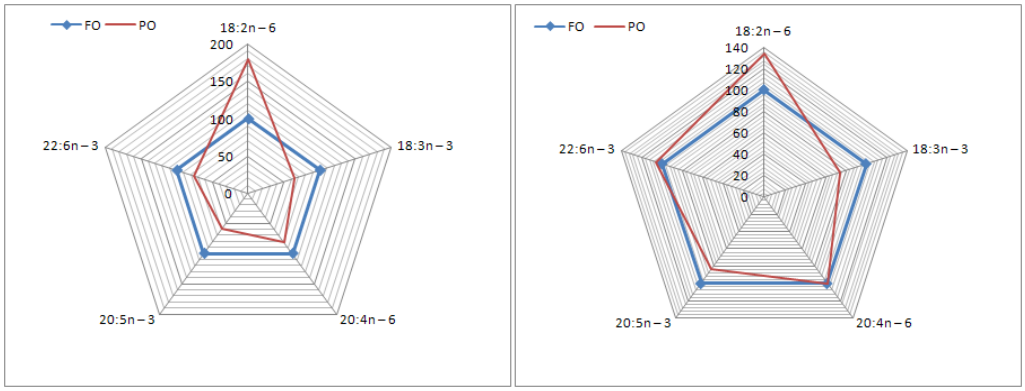


Figure 5.8. Fatty acid profiles of fish muscles from the group fed PO diet compared to the group fed the FO diet. The FO group was designated a fixed index number of 100. The figure to the left represents values post 94 day feeding with the experimental diets and the figure to the right values after 76 day recovery feeding with the FO diet.

Feeding the PO diet resulted in alterations of the major PUFAs in the fish muscles. Significantly lower levels of DHA, EPA, LNA and AA were observed in the fish muscles after feeding the experimental diets for 94 days. The levels of LA in muscle were significantly increased with palm oil diets compared with the FO diets (Figure 5.8.),. After recovery feeding for 76 days, the levels of DHA and AA were in the range of values observed in the FO group, with the EPA and LNA still significantly lower and higher levels of LA compared to the FO group.

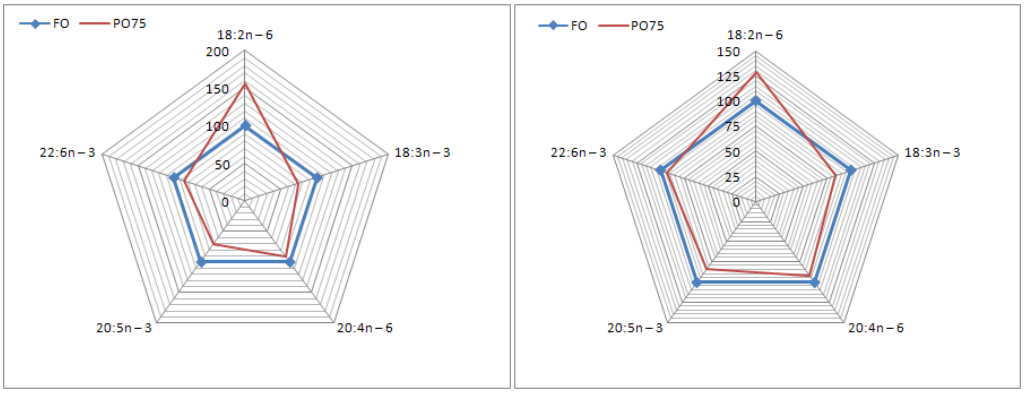


Figure 5.9. Fatty acid profiles of fish muscles from the group fed P75 diet compared to the group fed the FO diet. The FO group was designated a fixed index number of 100. The figure to the left represents values post 94 day feeding with the experimental diets and the figure to the right, values after 76 day recovery feeding with the FO diet.

The fatty acid ratios of fish from the P75 group followed the same trend observed in the PO group with significantly lower levels of LNA, AA, DHA and EPA after feeding the experimental diets (Figure 5.9). The DHA, EPA, AA and LNA levels were significantly different from the values observed in the FO after 76 days recovery feeding using the FO diet. Like in the PO treatment, the LA levels were higher compared to the FO treated fish.

Tissue of fish from the P50 group had significantly lower values of DHA, EPA, AA and LNA compared to FO group after 94 days of experimental diet feeding (Figure 5.10.). After 76 days of recovery feeding using the FO diets, fish from the P50 group contained significantly lower levels of DHA, EPA, AA and LNA compared to the FO control.

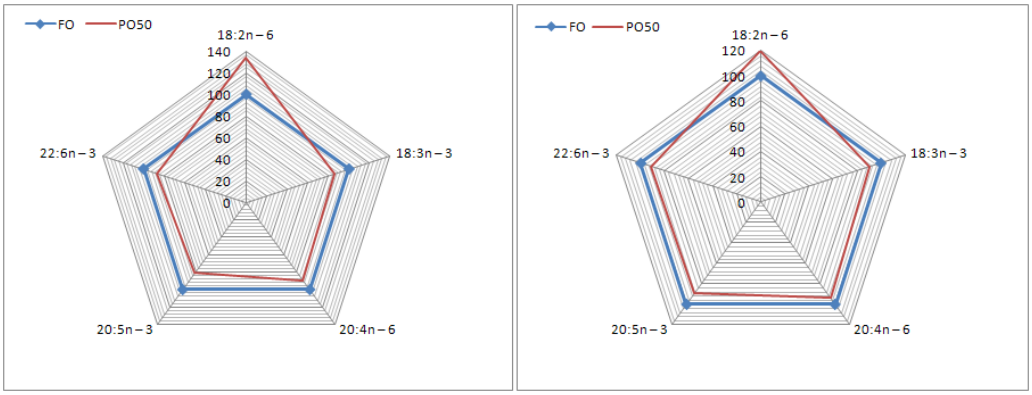


Figure 5.10. Fatty acid profiles of fish muscles from the group fed P50 diet compared to the group fed the FO diet. The FO group was designated a fixed index number of 100. The figure to the left represents values post 94 day feeding with the experimental diets and the figure to the right, values after 76 day recovery feeding with the FO diet.

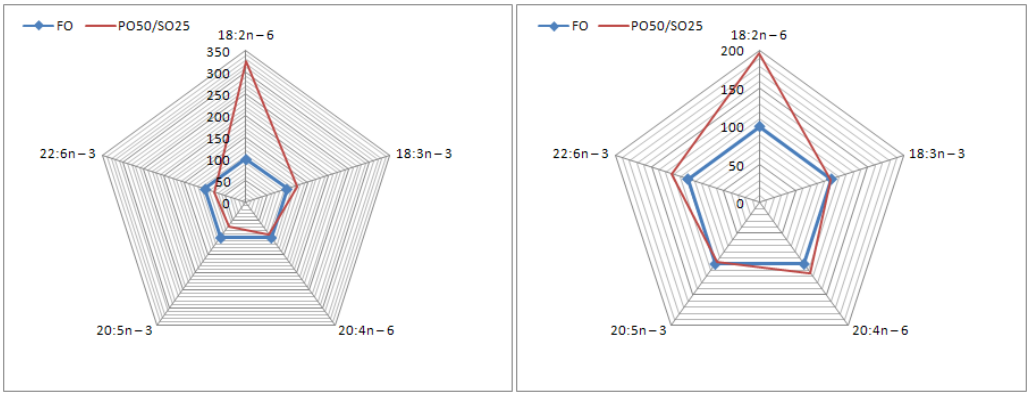


Figure 5.11. Fatty acid profiles of fish muscles from the group fed P50/S25 diet compared to the group fed the FO diet. The FO group was designated a fixed index number of 100. The figure to the left represents values post 94 day feeding with the experimental diets and the figure to the right, values after 76 day recovery feeding with the FO diet

A 25 % inclusion of soy oil in the P50/S25 group significantly affected the tissue LA (18:2*n* – 6) and LNA (18:3*n* – 3) with higher levels compared with muscles of fish from the FO group (Figure 5.11). The EPA, DHA and AA were found in lower levels post 94 day experimental diet feeding compared to fish from the FO group. After 76 days of recovery feeding with the FO diet, fish in the FO and the P50/S25 groups contained similar levels of LNA and EPA but LA was still significantly higher in tissue of fish from the P50/S25 group. At this point, the P50/S25 group had advantage in both DHA and AA with significant differences ($P<0.05$).

5.6. Sensory evaluation

In general, fish from all groups had sweet/representative smell, and a smell of metal and fresh oil (Table 5.8. and Figure 5.12.) The taste of the fish fillets was generally sweet and representative, and the colour was declared orange (Figure 5.13.).

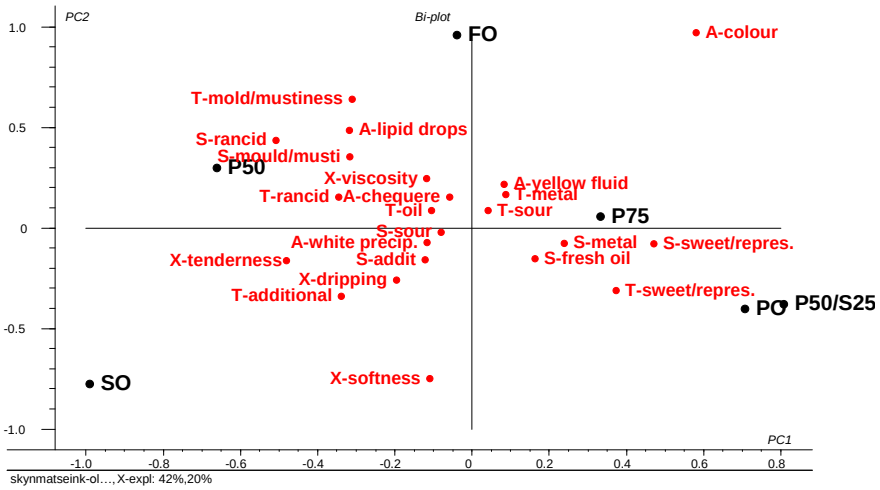
Table 5.8. Sensory evaluation of fish fillets after 94 days feeding the experimental diets. The table shows mean values for 12 panel members

			Diet					
Smell			FO	SO	PO	P75	P50	P50/S25
	Sweet/representative	ns	49	45	52	50	44	52
	Metal	ns	31	30	34	32	27	30
	Fresh oil	ns	29	29	30	27	27	30
	Mould/mustiness	*	21	22^{a}	12^{b}	22^{a}	19^{a}	14
	Sour	ns	6	7	4	5	3	3
	Rancidity	*	12	14	5^{b}	13	14^{a}	4^{b}
	Taint	*	5	7^{a}	5	2^{b}	3	3
Appearance								
	Colour	***	64^{a}	47^{b}	58	64^{a}	57	60^{a}
	Heterogeneous	ns	48	45	44	39	44	45
	Yellow fluid	ns	40	36	35	31	35	39
	Lipid drops in water	ns	53	52	48	49	55	46
	White precipitations	*	30	33	28	36	40	38
Taste								
	Sweet/representative	ns	50	50	57	53	49	54
	Metal	ns	41	38	38	34	34	38
	Oil/fat	ns	35	35	38	32	36	30
	Mould or mustiness	**	23^{a}	18	17^{bc}	16	21^{ab}	13^{cd}
	Sour	ns	5	4	5	5	5	4
	Rancidity	ms (p = 0,12)	11	12	7	8	13	7
	Additional	ms (p = 0,07)	5	11	5	3	6	3
Texture								
	Softness	ns	43	53	48	50	47	48
	Juiciness	ns	38	43	40	44	39	35
	Tenderness	ms (p = 0,12)	40	46	36	36	43	39
	Viscosity	ns	54	51	52	47	51	48

ns = not significant
ms = marginal significance
** p<0.05*
***p<0.01*
****p<0.001*

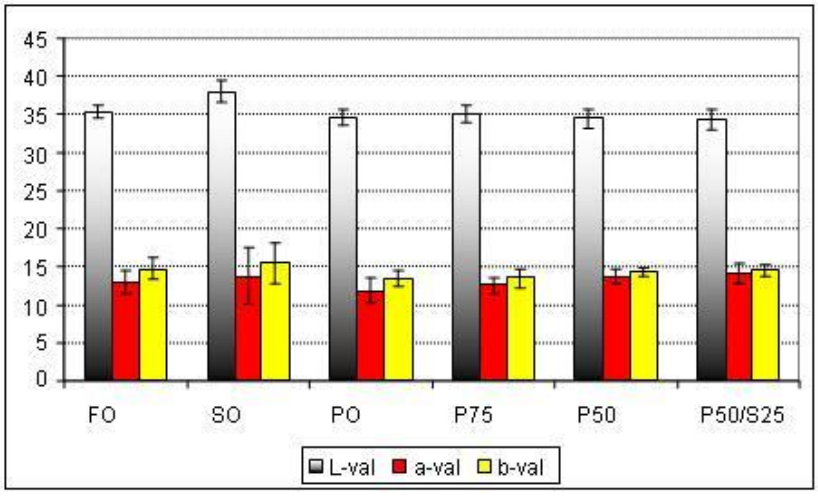
Some differences in individual evaluation parameters were, however, detected when the different dietary treatments were compared. After feeding the experimental diets for 97 days, a smell of mould/mustiness was detected in the FO, SO and P75 groups. The PO group had significantly less smell of mould/mustiness compared with the SO, P75 and P50/S25 groups. The texture of the fillets was not different when the various groups were compared. However,

fish fillets from SO group had the highest values for tenderness on the QDA scale (most tender), but fish from the PO and P75 groups had the lowest values (most firm). Apart from that, the fish fillets were in general evaluated as rather soft, dry and firm and with rather high values of viscosity.



(K.Sveinsdóttir)

Figure 5.12. A Bi-plot of the sensory evaluation scores after 94 days of feeding the experimental diets (T=taste, S=smell, A=appearances, X=texture).



(K.Sveinsdóttir)

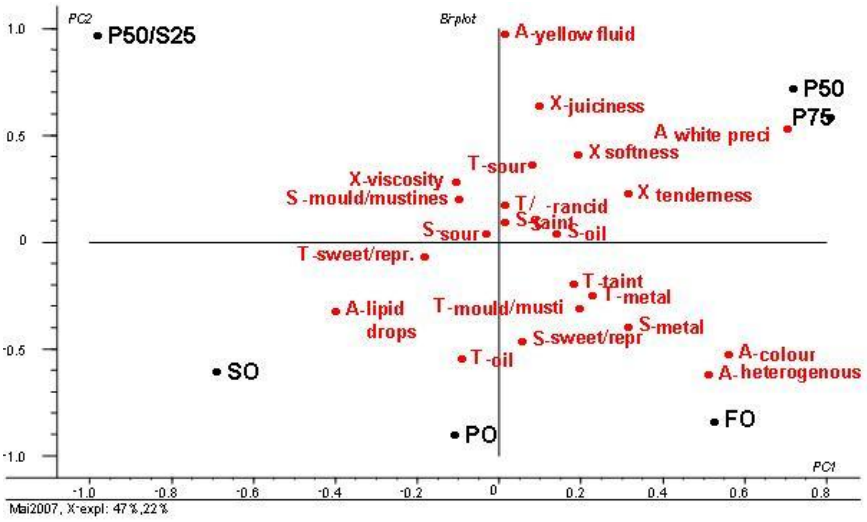
Figure 5.13. Colour values for fish fillets after 94 days of feeding the experimental diets (L-value = dark/bright, a-value = red, b-value = yellow).

After 39 days of recovery feeding, a smell of mould/mustiness was detected in fish fillets from the SO group but this smell was barely recognized in fish from other groups (Table 5.9., Figure 5.14.). Sour, rancid or tainted smell was barely detected in fish fillets. The colour of fillets from the P50/S25 group was significantly whiter compared to the FO and the P75 group. Minor variations in fillet colour were detected within all dietary treatments; water phase was still somewhat yellow and as well with large lipid drops observed in the water. White precipitations were detected in fillets from all groups, but these were more prominent in the P75 group. A taste of mould/mustiness was identified in fish fillets from the FO, PO and P75 groups but barely detectable in other groups. Sour taste was only slightly detected in the P50 group and not in other groups. Rancid or additional taste was not detected in fish from any of the groups and no differences were observed regarding the texture.

Table 5.9. Sensory evaluation of fish fillets after 39 days of recovery feeding the FO diets. The table shows mean values for 9 panel members

			Diet					
Smell			FO	SO	PO	P75	P50	P50/S25
	Sweet/representative	ns	45	49	51	49	46	44
	Metal	ns	40	34	37	34	41	34
	Fresh oil	ms (p=0.07)	35	30	34	30	37	32
	Mould/mustiness	ns	18	20	14	18	16	17
	Sour smell	ns	5	4	5	4	5	6
	Rancidity	ns	6	5	2	5	6	3
	Tainted	ns	2	3	2	3	3	2
Appearance								
	Colour	*	56^{a}	48	51	55^{a}	51	44^{b}
	Heterogeneous	*	42^{a}	38^{a}	41^{a}	41^{a}	44^{a}	32^{b}
	Yellow fluid	ns	28	28	29	36	31	35
	Lipid drops in water	ns	49	50	48	42	44	52
	White precipitations	**	27	20^{b}	23^{b}	35^{a}	31	22^{b}
Taste								
	Sweet/representative	ns	46	49	49	48	47	50
	Metal	ns	49	40	47	45	44	44
	Oil/fat	ns	42	46	38	36	39	38
	Mould or mustiness	ns	22	18	23	20	19	19
	Sour	*	5	4	6	7	9	8
	Rancidity	ns	6	6	5	5	7	6
	Additional	ns	5	4	6	8	3	2
Texture								
	Softness	ns	47	45	50	51	51	50
	Juiciness	ns	43	46	46	48	53	48
	Tenderness	ns	45	40	40	43	49	43
	Viscosity	ns	49	52	48	50	52	53

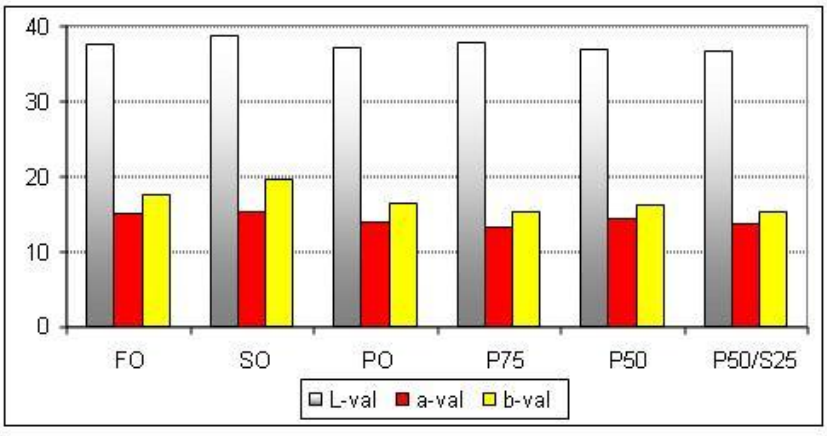
ns = not significant
ms = marginal significance
** p<0.05*
***p<0.01*



(K.Sveinsdóttir)

Figure 5.14. A Bi-plot of the sensory evaluation scores after 39 days of recovery feeding the FO diets (T=taste, S=smell, A=appearances, X=texture).

After 76 days of recovery feeding using the FO diets, a trace of mould/mustiness smell was found in fillets from the FO, SO, PO and P50/S25 groups (Table 5.10., Figure 5.16). Sour and rancid smell was not detected for any group at that time. However, a trace of tainted smell was detected in fillets from the P50 group and was described as the smell of fish meal by particular panel members.



(K.Sveinsdóttir)

Figure 5.15. Colour values for fish fillets after 76 days of recovery feeding the FO diets (L-value = dark/bright, a-value = red, b-value = yellow).

The colour of fillets from the P50/S25 groups was still significantly whiter compared to the FO and P75 groups as well as the P50 group (Figure 5.15.). A slight variation in fillet colour was still found as prior to the recovery feeding, but with the P50/S25 group now revealing less variability in colour compared to the other treatments. The fluid was yellowish in all groups and large as well as many lipid drops found in the water phase. White precipitations were detected in the fillets of fish from all groups, but were more visible in the P75 group and significantly higher compared to P50 and P50/S25.

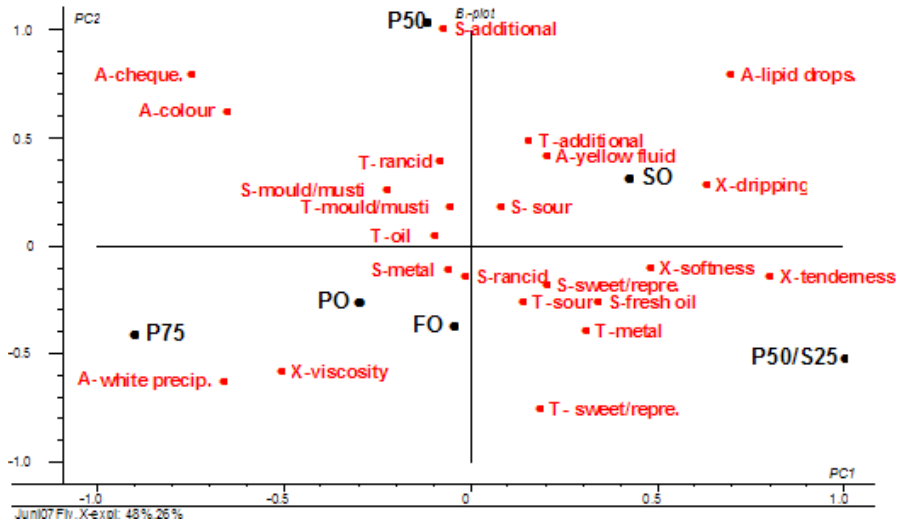
Table 5.10. Sensory evaluation of fish tissue after 76 days of recovery feeding the FO diets. The table shows mean values for 9 panel members

			Diet					
Smell			FO	SO	PO	P75	P50	P50/S25
	Sweet/representative	ns	53	57	53	58	55	60
	Metal	ns	29	29	33	32	32	32
	Fresh oil	ns	33	33	35	33	35	40
	Mould/mustiness	ns	18	19	18	21	21	16
	Sour smell	ns	5	6	5	5	8	6
	Rancidity	ns	10	6	9	8	8	8
	Additional smell	***	3^{b}	8^{b}	5^{b}	5^{b}	15^{a}	3^{b}
Appearance								
	Colour	**	56^{a}	48	45	53^{a}	55^{a}	38^{a}
	Chequered	**	36^{a}	42^{a}	46^{a}	46^{a}	45^{a}	28^{b}
	Yellow fluid	ns	37	40	36	37	43	41
	Lipid drops in water	ms (p = 0.07)	34	42	33	28	41	41
	White precipitations	**	33	33	43	47^{a}	30^{b}	30^{b}
Taste								
	Sweet/representative	*	58^{a}	47^{b}	53	55	52	60^{a}
	Metal taste	ns	32	30	32	31	32	40
	Oil/fat taste	ns	36	31	37	34	36	33
	Mould or mustiness taste	ns	16	18	20	30	21	18
	Sour taste	ns	9	8	9	9	8	11
	Rancidity taste	ns	8	6	10	10	15	8
	Additional taste	ns	4	8	7	6	13	9
Texture								
	Softness	ns	50	55	52	42	46	52
	Dripping	ns	43	45	38	35	44	47
	Tenderness	**	48^{a}	50^{a}	38	31^{b}	38	48^{a}
	Viscosity	**	49	39^{b}	45	54^{a}	41^{b}	40^{b}

ns = not significant
ms = marginal significance
** p<0.05*
***p<0.01*

The taste of metal and fresh oil was considerable in all groups. A taste of mould/mustiness was barely detectable in fish fillets from the PO, P75 and P50/S25 groups but slightly more prominent in other groups. A sour taste was negligible in all treatments. Traces of rancid taste were detected in fillets from fish primarily fed the P50 diet.

Fish from the FO, SO and P50/S25 groups had more tender fillets compared to the P75 group. However, the fillets of fish from the P75 group had significantly higher viscosity compared to the SO, P50 and P50/S25 groups.



(K.Sveinsdóttir)

Figure 5.16. A Bi-plot of the sensory evaluation scores after 76 days of recovery feeding the FO diets (T=taste, S=smell, A=appearances, X=texture).

6. Discussion

Studies of essential fatty acid requirements of Arctic charr indicate the need for 1 – 2% (dry weight) of 18:3*n* – 3 in the diet (Sargent, et al., 2002, Yang et al., 1993, Yang et al., 1994). Though not strictly defined, Yang and co-workers (1993) observed that Arctic charr possibly had requirements for 18:2*n* – 6 as approximately 0.7% of the diet (dry weight). However, it has been indicated that most salmonids can satisfy their requirements with lower levels of *n* – 3 HUFA (Sargent et al., 2002). In the present study, fingerling diets contained high proportion of fish meal which could compensate for the lack of *n* – 3 HUFA in some of the oils used in the diets. No diet was deficient in monoenes and saturates that are highly β- oxidised by fish (Kiessling et al., 1992). Best growth was achieved in groups fed pure FO diet, Ewos commercial fingerling diet or diets containing a mixture of FO and sterin (FO/ST). The poorest growth was observed in the group fed a mixture of soy oil and fish oil. Due to technical difficulties, we could not accomplish whole body FA analyses of the fingerlings. As a consequence, the utilization of the various lipid sources on FA composition in fingerling could not be studied. No correlation was found when comparing the diet Σ *n* – 3 and growth performance of the fish. The FO/SO and SO diets contained Σ *n* – 3 proportions of 15.9% and 13.6%, respectively, final weight of these groups was however significantly lower compared with the FO/PO group fed diets with 13.3% Σ *n* – 3. Furthermore, a diet containing pure rapeseed oil and Σ *n* – 3 levels of 15% resulted in significantly lower growth compared to the PO group fed diets containing 10% Σ *n* – 3. Sterin was found to be a good substitute if blended with FO but resulted in poor growth as a primary lipid source in diet, despite the high levels of *n* – 3 HUFA. No further chemical analyses and limited information are to be found of this product. Apart from the diet containing sterin, the diets resulting in the poorest growth all contained high levels of PUFA and 18:2*n* – 6 fatty acids. It has been reported that lipids containing high proportions of PUFA may paradoxically be detriment to fish health due to peroxidation and forming of free radicals (Olsen et at., 1997). Furthermore lipid accumulations have been found in the intestines of Arctic charr fed diets containing high proportions of PUFA, due to insufficient lipoprotein synthesis (Olsen et al., 1998).

Some authors have suggested that high levels of 18:2*n* – 6 may inhibit the conversion of 18:3*n* – 3 to EPA and DHA, causing EFA deficiency (Ruyter et al., 2003). However, the SO/PO diets, containing considerable amounts of 18:2*n* – 6 and PUFA, gave similar growth compared to diets containing lower levels of these FA groups. Other studies have indicated that rainbow trout can discriminate between diets containing different lipid sources and the fish seems to prefer FO based diets and be less prone to diets including vegetable oils (Geurden, et al., 2005). We however did not observe any obvious suggestions supporting this theory in the present study as feed intake could not be estimated.

This part of the study was partly conducted to examine if Arctic charr fingerlings could be used to hunt for acceptable ingredients for grower feeds. It would be cost beneficial since fingerlings do not require as much volume and multiple ingredients can be studied simultaneously. However, we found it hard to find a certain explanation of different growth rates between different dietary treatments in this trial. Due to the lack of information from the FA analyses we do not know how different FAs were utilized in the fingerlings.

Numerous studies have confirmed that fish oil can be replaced up to 100% with various plant oils in diets for salmonids without negatively affecting the growth (Brandsen et al., 2003, Torstensen et al., 2004, Fonseca-Madregal et al., 2005, Miller et al., 2006, OO et al., 2007). In our study, Arctic charr were fed diets containing up to 100% of palm or soy oil for 94 days without significant effects on growth but had significantly lower PER values. Fonseca-Madregal and co-workers (2005) experienced slightly lower weight gain for rainbow trout fed diets containing 100% palm oil compared to FO based diets, but the difference was not significant. This could possibly be explained by lower digestibility of saturates, especially oleic acid (16:0) which was found in 38.3% proportions in our PO diet (Ng et al., 2004). Ng and co-workers (2003) observed significant interactions between the temperature and digestibility of fatty acids, where lower water temperatures lead to reduced digestibility of saturates in rainbow trout. The temperature in our experiment was 6.0°C during the growth trial, which is lower than the temperature ranges covered by Ng and co-workers (2003).

Mortalities were negligible throughout the experiment and not related to different diets. The hepatosomatic index was not affected by different lipid sources in the diets and the livers showed no signs of changes in colour and looked fresh and healthy. Some authors have reported formations of lipid droplets in the intestines of salmonids fed diets containing high proportions of plant oils containing considerable amounts of polyunsaturated fatty acids (Caballero et al., 2002). It has been suggested that this may be caused by temporal storage of lipid, due to insufficient lipoprotein synthesis (Olsen et al., 1999). It is nevertheless not clear if this should be considered as a pathological problem. Ruyter and his group (2005) and Grisdale-Helland and co-workers (2002) observed no accumulation of lipid in the intestines of Atlantic salmon fed 100% SO diet for approximately 950 day degrees. When feeding Arctic charr diets containing either linseed oil or soybean lecithin oil for 420 day degrees, Olsen and his group (1999) discovered lipid droplets in the intestines of fish in both treatments. However, fish fed linseed oil had serious epithelial damage with fat vacuoles and cell debris in the intestinal lumen but fish fed the SO diet did not. The authors suggested that this damage is likely to be pathological and detrimental to the health of the fish. In our experiment, no histological analyses of the fish intestines were performed. We fed Arctic charr for approximately 565 day degrees on a SO diet rich in PUFA (51%) with acceptable growth and lipid digestibility with no signs of liver damages. However, this would need further investigation if Arctic charr are to be fed for extended periods on SO diets.

Good utilization of diets containing 100% soybean oil has been reported for Atlantic salmon (Grisdale-Helland et al., 2002). In our experiment, similar FCR values were observed for the SO and the FO group. However, palm oil inclusion significantly affected the FCR. These findings are in disagreement with the findings of Fonseca-Madregal and his group (2005) as well as OO and his group (2007) from experiments with PO diets for rainbow trout. Torstensen and co-workers (2000) did, however, obtain higher FCR values for salmon fed diets containing 100% palm oil compared to other diets but the difference was not statistically significant.

An inert marker, used for studying the apparent digestibility of various nutrients should ideally fulfil the following criteria: (1) homogeneously incorporated into the feed and easily and accurately analysed, even at low

concentrations; (2) indigestible and does not affect the metabolism of the animal; (3) pass through the gastro-intestinal tract at the same rate as the dietary nutrient; and (4) hygienic and harmless to people and the environment (Austreng et al., 2000). The most widely used markers for evaluation of digestibility in fish have been chromic oxide (Cr_2O_3) according to the method described by Morales et al., (1999), the acid insoluble ash method (AIA) (Atkinson et al., 1984) and more recently the use of trivalent oxides (Austreng et al., 2000). The AIA method was used in the present, as described by Atkinson and co-workers (1984).

In the present study, the apparent digestibility coefficients of dry matter (DM), gross energy and lipid appeared to be relatively high and were affected by palm oil inclusion. Soy oil has been reported to be readily utilized in salmonids with even higher digestibility compared to fish oil (Refstie, 2000, Grisdale-Helland et al., 2002). In our study, the SO diet had the highest values of lipid and DM digestibility. The lipid digestibility was, however, affected by 75-100% palm oil inclusion. Ng and his group (2003) observed significant reduction in apparent digestibility coefficence of DM but not for lipid when feeding 25% palm oil diet to salmon reared at 6°C. However, the authors found significant differences in the digestibility of saturate which was reduced by lowering temperatures. This group later reported that the digestibility of saturates in rainbow trout was reduced by lowering the temperature, regardless of the palm oil inclusion (Ng et al. 2004). It is interesting that in our experiment, the lipid digestibility of the PO and P75 diet was lower than the DM. This strongly suggests that the high level of saturates in these diets together with the low temperature could be responsible for reduced digestibility, slightly less growth and hence higher FCR.

Some authors have suggested that the AIA method has a tendency to overestimate the ADC values (De Silva et al., 1983, Morales et al., 1999). We have a reason to believe that in our experiment, the fish did absorb sand or other ash insoluble substances which may have resulted in exaggerated ADC values. After dissolving the ash from the faeces, by boiling in 5N HCl, small particles became visible in the solution. While working on another project (results not published) stones with a size of 8 mm in diameter were found in faeces from Arctic charr after burning and boiling in ash for 5 minutes. When measuring the celite content of faeces, values ranging from 0.04 – 0.1 g were obtained in the present study. Minor changes in these values could, however, considerably affect

the ADC values causing overestimation. However, the ADC values obtained in various experiments and studying different fish species should be compared with caution. The different methods used for ADC analyses can not be compared due to the relative differences between different methods (Morales et al., 1999). It is, however, our conclusion that the results are in agreement with other studies on the same subject and are valuable in order to compare the digestibility for different ingredients in Arctic charr. Different methods for ADC measurements should, however, be considered when studying the apparent digestibility of various nutrients in Arctic charr.

Several factors such as different ingredients and processing methods may affect the physical quality of feed pellets (Aarseth et al., 2006). Due to differences in current rearing techniques of Arctic charr compared to e.g. Atlantic salmon, diets for Arctic charr are not as dependent on durability requirements and no durability tests were performed for the diets in our experiment. Nevertheless, the sinking rate was examined and the results indicate that palm oil significantly affects the time it takes the feed pellets to sink in fresh water. This could possibly be a drawback, resulting in increased feed loss out of tanks.

The chemical composition of the experimental diets was not affected by different lipid compositions and no statistically significant differences were observed when the fillets of the experimental fish were compared with respect to the content of crude lipid, protein, moisture or ash. However, fish fed the PO diet had the lowest concentration of lipids in the flesh which is in agreement with results of Bell and his group (2002) where palm oil inclusion significantly reduced the whole body gross lipid in Atlantic salmon and leading the authors to suggest that different vegetable oils may affect adiposity. Conversely, these were not the findings of Ng and co-workers (2004) in an experiment with Atlantic salmon or OO and group (2007) with rainbow trout fed PO diets. In rainbow trout, increased dietary lipid levels have been found to result in higher lipids deposit in the fish fillet (Gélineau et al., 2002). However, if reduced lipid digestibility would result in lower flesh lipid composition like observed in the PO group, this will not explain the higher flesh lipid observed in the P75 group compared to FO and SO groups.

PER was significantly reduced by palm oil inclusion. Tocher and co-workers (2004) discovered that enterocyte β- oxidation was reduced by

temperature and dependent on palm oil inclusion in an experiment with rainbow trout. Similar findings were published by Torstensen and co-workers (2000) experimenting with Atlantic salmon, suggesting that this could affect protein utilization. The authors suggested that high levels of dietary lipids increase fish growth, spare dietary protein and increase feed efficiency. If dietary fatty acid composition will induce β- oxidation this will further increase the protein utilization by selectively providing energy through lipid oxidation and there for sparing protein for muscle growth. The authors did, however, not obtain significant differences related to palm oil inclusion but concluded that it would probably have occurred feeding diets containing more than 50% palm oil for longer periods. It should be noted though, that our protein efficiency ratios are based solely on weight gain/consumed protein, not on whole body protein retention.

It is well established that the fatty acid composition of salmonid diets will be reflected in the fish muscle, liver and whole body compositions (Olsen et al., 1997, Bell et al., 2001, Bell et al., 2003, Bell et al., 2002, Torstenson et al., 2004[b], Tocher et al., 2003, Ruyter et al., 2003). In our experiment, different dietary lipids significantly affected total saturates, monoenes, *n* – 6, *n* – 3, polyunsaturated fatty acids and the *n* – 3/*n* – 6 ratio in fish fillets. Recently, there has been an increase in the consumer's awareness and interest in the nutritional aspects of health. Governments have started developing dietary guidelines in addition to their traditionally recommended dietary allowances, and various scientific societies and non-governmental organizations have issued their dietary advice to combat chronic diseases and obesity (Simopolous, 2001). The beneficial effects of *n* – 3 fatty acids, including cardio protective effects, are well recognised in humans (Seierstad et al., 2005) and in resent years, more attention has been paid to the ratio of the *n* – 3 and *n* – 6 fatty acids. The increased ingestion of *n* – 6 fatty acids in the current Western world diets, has been found to induce the formation of AA eicosanoids which in large quantities have been found to contribute to the formation of thrombi and atheromas resulting in increasing development of allergic and inflammatory disorders (Simopoulos, 2000). Thus, to maximize the nutritional values for humans, the ratio of *n* – 3 and *n* – 6 fatty acids should be considered, not only the amount of *n* – 3 FAs.

After feeding the different experimental diets to Arctic charr for 94 days (565 day degrees) we obtained the acceptable *n* – 3 to *n* – 6 ratio of 7.1 in fillets from fish fed the FO diets, and significantly higher compared to all other treatments. According to Simopoulos (2001) the recommended intake of DHA+EPA for humans is 0.65 g per day. A 200 g portion of fish fillet from the SO group, containing the lowest levels of EPA and DHA in our experiment, would represent double the recommended daily intake. However, the fish fed the SO diet contained the lowest muscle *n* – 3 to *n* – 6 ratio (0.7), decreasing the nutritional value of the fish fillets for human consumption. Furthermore, the recommendations for daily maximum intake of 18:2*n* – 6 is 6.67 g/day and a 200 g portion of fish fillet from the SO group therefore contains ~34% of the recommended daily intake of this FA. Due to the high human consumption of *n* – 6 fatty acids, e.g. from vegetable oils, it would be desirable to maintain higher *n* – 3 to *n* – 6 ratio than obtained in fish muscle from the SO group (Simopoulos, 1999).

Other treatments, including the P50/S25 group, all contained acceptable *n* – 3 to *n* – 6 ratios according to Simopoulos (2001), with a value of 3.0 in PO group, 3.9 in P75 group, 4.9 in the P50 group and 1.9 in the P50/S25 group.

A 39 days recovery feeding using the FO diets resulted in considerable alterations in the Σ *n* – 3 and as well the *n* – 3 to *n* – 6 ratios in the muscle of fish from the various dietary treatment groups. However, fish muscle from the FO group contained significantly higher levels of Σ *n* – 3 and a higher ratio of *n* – 3/*n* – 6 compared to all other groups. After 76 days of recovery feeding using the FO diets, the levels of muscle Σ *n* – 3 were within the range of the values measured in the FO group in all groups except the SO and P75. The SO group still contained an *n* – 3/*n* – 6 ratio representing only 20% of the ratio observed in muscle of fish from the FO group. These findings are in agreement with previous studies, where the recovery of *n* – 3 is gained faster than the flush out of 18:2*n* – 6 (Izquierdo et al., 2005, Bell et al., 2003, Torstensen et al., 2004).

Between day 39 and 76 of recovery feeding, muscle levels of Σ *n* – 3 had declined in all groups except the P50/S25. Muscle levels of Σ PUFA followed the same trend but were stable in both groups previously fed SO diets. The decline in Σ *n* – 3 and a slight increase in Σ MUFA resulted in lower *n* – 3/*n* – 6 ratios in the FO and P50 groups between 39 and 76 days of recovery feeding. At the same time

there was a considerable increase in muscle LA in the SO group muscle, despite the FO feeding. Seasonal changes and increased unsaturation activities with lower water temperatures have been well documented in fish (Olsen et al, 1997, Farkas et al. 2001). Other physiological factors, such as mobilisation of lipids to gonads and liver PUFA and MUFA levels appear to be related to environmental factors such as temperature (Schwame et al., 1993). This alteration of $\Sigma\ n-3$ and Σ PUFA between the recovery feeding periods may therefore be explained by seasonal changes rather than nutritional since all groups were fed the same FO diets. However, the increase in LA in the SO group and increased levels of $\Sigma\ n-3$ in the P50/S25 are somewhat contradictory.

Previous studies show that different lipid sources can affect the sensory and quality parameters of fish fillets, such as colour, odour, taste, colour shade and colour intensity (Thomassen, et al., 1989). Feeding diets containing different lipid sources resulted in significant differences for some of the evaluated parameters. The results, however, indicate that the inclusion of SO or PO does not negatively affect the quality or taste of the final product. Furthermore, pure palm oil in diets for Arctic charr result in fish fillets even more desirable compared to fillets from the FO group.

7. Conclusion

In conclusion, we found that Arctic charr express satisfactory growth on diets where fish oil has been replaced by either soybean or palm oil. However, the substitution will result in some alterations of the fatty acid profiles of the fish muscle. Even though fillets from fish fed diets containing soybean or palm oil contained acceptable values of dietary $n-3$ polyunsaturated fatty acids, the higher levels of the 18:2$n-6$ fatty acid are undesirable. This further indicates that vegetable oils used as a substitution for fish oil in aquaculture diets should ideally contain low levels of 18:2$n-6$. Mixing palm oil with other lipid sources seems to have a promising potential for use in diets for Arctic charr but digestibility should be further investigated in relation to temperature and the different methods used for studying digestibility. Fish fed diets with palm inclusion had higher feed conversion ratios compared with other fish in this experiment. Due to the rapid changes within the world markets of oil sources, it is hard to estimate the cost beneficial aspects of our findings. However, the fish oil prices may be expected to continue to rise in the future and it is urgent to continue our search for alternatives that maximize growth with minimum effects on dietary values for the consumer. The Icelandic Arctic charr industry will have to define their policies regarding the nutritional value of their products and marketing strategies. In the end, it will be up to them and the feed industry to take the step.

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9. Appendices

9.1 Appendix I (detailed calorimetric method)

The samples were weighed by difference and approximately 1.0 g placed in an empty combustion capsule. A 10 cm fuse wire was placed between the electrodes of the oxygen bomb and the combustion capsule then placed in the loop electrode. The wire was attached so that it was in touch with the sample but without touching the capsule itself. Approximately 1 ml of distilled water was placed in the bomb cylinder and swirled around in order to wet the sides. The bomb was then assembled, the screws caps tightened and the pressure valve closed before the cylinders were filled with 25-30 atmosphere of oxygen.

The bomb was then placed in an oval in the colorimeter unit and connected to a clip terminal. Approximately 2000 g of distilled water was accurately weighed and poured into the oval/bucket. The initial temperature was recorded to 0.0002°C accuracy and the sample then ignited. The outer jacket temperature was kept stable by running hot or cold water rotationally with automatic circulation. Temperatures were recorded every second minute and the value recorded when the same temperature was observed for three times in a .

The calorimeter was then opened, the bomb removed and residual pressure released. The sample was then carefully removed and remaining pieces of fuse wire from the electrodes were straightened and the length measured. The inner surface of the bomb was then cleaned using deionised water that was collected into a clean beaker. The washings were titrated with s standard solution of sodium carbonate using methyl orange as an indicator to determine the amount of acid formed during the incidental oxidation of nitrogen and sulphur compounds.

(Parr Instrument Company, 1966).

9.2 Appendix II (FA analyses, complete data)

		Diet																	
	Initial	FO			SO			PO			P75			P50			P50/S25		
FA	Fish	Day 94	Day 133	Day 170	Day 94	Day 133	Day 170	Day 94	Day 133	Day 170	Day 94	Day 133	Day 170	Day 94	Day 133	Day 170	Day 94	Day 133	Day 170
14:00		4.6±0.4	4.4±0.2	4.8±0.8	2.5±0.1	3.4±0.0	3.7±0.3	3.4±0.5	3.5±0.1	3.6±0.2	3.3±0.1	3.7±0.1	4.1±0.3	3.8±0.2	3.9±0.0	4.2±0.2	3.2±0.2	3.7±0.2	4.0±0.4
16:00		14.0±0.4	13.7±0.2	14.0±0.9	14.1±0.2	14.0±0.1	13.7±0.4	18.0±1.4	15.3±0.3	15.9±0.3	15.8±0.1	15.8±0.2	14.9±0.4	15.3±0.2	14.4±0.1	14.2±0.3	15.0±0.2	15.4±0.2	15.9±0.6
18:00		1.7±0.0	1.6±0.0	1.8±0.0	2.5±0.0	2.1±0.0	2.1±0.0	2.3±0.0	2.1±0.0	2.0±0.0	2.1±0.0	2.0±0.0	1.9±0.0	2.1±0.0	1.8±0.0	1.9±0.0	2.2±0.0	1.9±0.0	2.0±0.0
Σ SFA		20.2±0.8	19.6±0.3	20.5±1.7	19.2±0.4	19.4±0.1	19.5±0.7	23.6±1.9	20.9±0.4	21.5±0.5	21.2±0.2	21.5±0.2	21.0±0.7	21.2±0.4	20.1±0.1	20.3±0.5	20.4±0.4	21.0±0.4	21.8±1.0
16:1*n* – 9		0.3±0.0	0.2±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
16:1*n* – 7		7.3±0.3	6.9±0.1	7.5±0.6	4.7±0.1	6.5±0.0	5.6±0.2	7.3±0.5	6.7±0.1	7.0±0.2	6.7±0.1	6.6±0.0	7.1±0.2	6.7±0.1	6.9±0.0	7.2±0.1	6.3±0.2	6.3±0.1	6.4±0.3
18:1*n* – 9		16.9±0.1	16.3±0.0	17.3±0.1	20.9±0.1	20.3±0.0	18.8±0.0	28.1±0.2	23.6±0.0	22.7±0.0	25.2±0.1	23.2±0.0	21.5±0.1	22.1±0.1	20.2±0.1	20.1±0.1	23.5±0.2	20.7±0.1	18.6±0.1
18:1*n* – 7		2.3±0.0	2.2±0.0	2.4±0.0	2.2±0.0	2.2±0.0	2.2±0.0	2.7±0.0	2.6±0.0	2.5±0.0	2.7±0.0	2.4±0.0	2.5±0.0	2.8±0.0	2.6±0.0	2.7±0.0	2.5±0.0	2.3±0.0	2.5±0.0
20:1*n* – 11 + 20:1*n* – 9		9.1±0.3	9.3±0.1	9.5±0.5	5.0±0.1	5.9±0.4	7.3±0.2	5.9±0.3	7.2±0.6	7.6±0.1	7.2±0.0	7.4±0.1	8.6±0.2	7.8±0.1	7.8±0.0	9.2±0.1	6.7±0.2	7.1±0.1	7.4±0.2
22:1*n* – 11 + 20:1*n* – 13		8.1±0.4	8.3±0.1	8.3±0.7	4.0±0.1	4.9±0.0	6.2±0.3	4.5±0.4	5.9±0.1	6.5±0.2	5.9±0.1	6.2±0.1	7.6±0.3	6.8±0.2	6.6±0.0	8.2±0.2	5.6±0.3	6.1±0.1	6.2±0.3
22:1*n* – 9		0.8±0.0	0.8±0.0	0.8±0.1	0.5±0.0	0.6±0.0	0.6±0.0	0.5±0.1	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.8±0.0	0.7±0.0	0.7±0.0	0.8±0.0	0.6±0.0	0.6±0.0	0.7±0.0
24:1		0.5±0.1	0.5±0.0	0.5±0.0	0.3±0.0	0.4±0.0	0.4±0.0	0.3±0.1	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.5±0.0	0.4±0.0	0.4±0.0	0.5±0.0	0.4±0.0	0.4±0.0	0.4±0.0
Σ MUFA		45.9±0.4	45.2±0.0	47.4±0.8	38.3±0.3	41.7±0.3	42.0±0.3	50.2±0.1	48.1±0.6	48.4±0.2	49.6±0.0	47.8±0.1	49.6±0.4	48.3±0.4	46.2±0.1	49.8±0.2	46.6±0.5	44.4±0.2	43.3±0.4
18:2*n* – 6		2.4±0.0	2.4±0.0	2.4±0.0	19.5±0.1	11.3±0.0	12.8±0.0	4.4±0.0	3.7±0.0	3.3±0.0	3.8±0.0	3.5±0.0	3.2±0.0	3.3±0.0	3.0±0.0	2.9±0.0	7.9±0.0	6.6±0.0	4.8±0.0
20:2*n* – 6		0.2±0.0	0.2±0.0	0.2±0.0	0.8±0.0	0.6±0.0	0.6±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.4±0.0	0.3±0.0	0.3±0.0
20:3*n* – 6		0.1±0.0	0.1±0.0	0.1±0.0	0.7±0.0	0.5±0.0	0.5±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.3±0.0	0.2±0.0	0.2±0.0
20:4*n* – 6		0.4±0.0	0.3±0.0	0.3±0.0	0.4±0.0	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0
Σ *n* - 6		3.1±0.0	3.1±0.0	3.1±0.0	21.3±0.1	12.7±0.0	14.2±0.0	5.1±0.1	4.5±0.0	4.1±0.0	4.5±0.0	4.1±0.0	3.9±0.0	3.9±0.0	3.7±0.0	3.6±0.0	8.9±0.0	7.5±0.1	5.7±0.0
18:3*n* – 3		0.9±0.0	0.9±0.0	0.9±0.0	2.1±0.0	1.5±0.0	1.6±0.0	0.6±0.0	0.7±0.0	0.6±0.0	0.6±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.8±0.0	0.8±0.0	1.1±0.0	1.0±0.0	0.9±0.0
18:4*n* – 3		2.3±0.0	2.3±0.0	2.4±0.0	1.2±0.0	1.8±0.0	1.7±0.0	1.1±0.0	1.6±0.0	1.6±0.0	1.4±0.0	1.6±0.0	1.8±0.0	1.7±0.0	2.0±0.0	2.0±0.0	1.5±0.0	1.7±0.0	1.6±0.0
20:3*n* – 3		0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
20:4*n* – 3		1.0±0.0	1.1±0.0	1.1±0.0	0.5±0.0	0.8±0.0	0.8±0.0	0.6±0.0	0.8±0.0	0.8±0.0	0.8±0.0	0.8±0.0	0.9±0.0	0.8±0.0	1.0±0.0	0.9±0.0	0.7±0.0	0.8±0.0	0.8±0.0
20:5*n* – 3		6.3±0.2	6.7±0.1	5.9±0.3	3.0±0.0	4.6±0.0	4.3±0.1	3.7±0.2	5.0±0.1	4.9±0.1	4.5±0.0	4.9±0.0	4.9±0.1	5.1±0.1	5.8±0.0	5.2±0.1	4.3±0.0	5.0±0.1	5.6±0.2
22:5*n* – 3		1.3±0.0	1.7±0.6	1.3±0.1	0.7±0.0	1.0±0.0	1.0±0.0	0.8±0.1	1.1±0.0	1.1±0.0	1.0±0.0	1.1±0.0	1.1±0.0	1.1±0.0	1.3±0.0	1.2±0.0	1.0±0.0	1.0±0.0	1.2±0.1
22:6*n* – 3		12.4±0.6	13.2±0.4	10.6±0.9	8.2±0.1	10.2±0.1	8.8±0.4	9.5±1.0	11.3±0.2	11.2±0.2	10.5±0.2	11.1±0.1	9.9±0.3	10.8±0.3	12.8±0.0	9.6±0.2	9.8±0.2	11.1±0.3	12.9±0.7
Σ *n* - 3		22.0±0.8	23.7±0.4	19.8±1.3	14.7±0.2	18.1±0.1	16.6±0.5	15.3±1.4	18.9±0.3	18.7±0.3	17.5±0.2	18.7±0.2	17.6±0.5	18.6±0.5	21.7±0.0	17.9±0.3	16.9±0.2	19.1±0.4	21.5±0.9
Total		93.7±0.3	94.1±0.5	93.4±0.5	94.7±0.3	93.9±0.3	94.2±0.2	95.4±0.4	94.0±0.5	94.3±0.1	94.4±0.1	93.9±0.1	94.0±0.2	93.9±0.2	93.7±0.2	93.8±0.1	94.3±0.3	93.7±0.2	94.0±0.3
Unknown		6.3±0.3	5.9±0.5	6.6±0.5	5.3±0.3	6.1±0.3	5.8±0.2	4.6±0.4	6.0±0.5	5.7±0.1	5.6±0.1	6.1±0.1	6.0±0.2	6.1±0.2	6.3±0.2	6.2±0.1	5.7±0.3	6.3±0.2	6.0±0.3
Σ PUFA		27.5±0.8	29.2±0.4	25.5±1.3	37.3±0.3	32.7±0.1	32.7±0.6	21.6±1.5	25.1±0.3	24.4±0.3	23.5±0.3	24.6±0.2	23.4±0.5	24.4±0.5	27.5±0.0	23.7±0.3	27.3±0.2	28.3±0.4	28.9±0.9
***n* - 3/*n* - 6 ratio**		7.1±0.2	7.7±0.1	6.3±0.4	0.7±0.0	1.4±0.0	1.2±0.0	3.0±0.2	4.2±0.0	4.6±0.1	3.9±0.0	4.5±0.0	4.55±0.1	4.8±0.1	5.8±0.0	4.9±0.0	1.9±0.0	2.6±0.0	3.7±0.2
Σ EPA +DPA +DHA		20.1±0.8	21.7±0.4	17.8±1.3	12.0±0.2	15.8±0.1	14.1±0.5	14.0±1.3	17.3±0.3	17.2±0.3	16.0±0.2	17.1±0.2	15.9±0.5	17.0±0.5	19.8±0.0	16.1±0.3	15.0±0.2	17.1±0.3	19.7±0.9