# Composition and morphology of Atlantic salmon (Salmo salar L.) as affected by dietary oil

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Master Thesis 30 credits 2013

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**UMB**

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Saeid Dadras

## Abstract

A feeding experiment was conducted to examine the effect of diets high or low in rapeseed and additionally the effect of protein supplementation of diets rich in rapeseed oil in feed for farmed Atlantic salmon (*Salmo salar* L.). The aim was to investigate the impact on growth performance, slaughter parameters, total lipid content and fatty acid profile in skeletal muscle, intestine, liver and heart, together with the impact on health related parameters such as organ morphology, heart fat, and also liver color and patches. The salmon were fed extruded dry feed containing 35.6% fat and 41.4% protein during August-December, and 37.7% fat and 34.6% protein during December-March. The lipid source was either 70% marine and 30% rapeseed oil in the Marine+ group or 30% marine and 70% rapeseed oil in Control group (commercial standard). The third diet, Protein+ was same as Control diet added an extra protein (2%) which was extracted from fish skin (triplicate net-pens per treatment). Fish weights and lengths were measured in October, December and March. Initial weight of the salmon in August was 2.5 kg.

There were no significant differences in final weight (mean weight of 6.5 kg), TGC and FCR due to the dietary treatments. However, the Protein+ and Control group had higher condition factor compared to the Marine+ group. Significantly lower CF of the Marine+ group coincided with higher fork length and lower fillet yield. The Control group showed significantly highest viscera-somatic index, visual visceral fat and visual heart fat, whereas these were lowest in the Protein+ group. It was possible to stimulate increased muscle building by providing extra protein into commercial salmon feed, since the slaughter and fillet yield of the Protein+ group increased significantly by 0.9% and 1.6%, respectively. The fatty acid profile of all organ tissues examined was significantly altered due to the dietary treatment, where 18:1n-9 changed most significantly. There were higher levels of n-3 fatty acids, such as of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the Marine+ group. On the other hand, n-6 fatty acids and C18 fatty acids, such as 18:1n-9 and 18:2n-6 were presented in a higher amount in the Control group. Consequently, the n-3/n-6 fatty acids ratio was higher in the Marine+ group and fish in the Control group had the lower ratio.

In conclusion, the present study suggests that addition of 2% extra protein from fish skin to salmon diets rich in rapeseed oil is beneficial in terms of stimulating muscle growth and counteracting fat accumulation cost by high levels of rapeseed oil.


Keywords: Atlantic salmon, fish oil, rapeseed oil, EPA and DHA

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# 1.0 Introduction

Aquaculture is one of the fastest growing food production areas in the world and contributes to the global solution to environmentally sustainable food production for a growing population (FAO, 2012). To improve in farming of Atlantic salmon (*Salmo salar* L.) it is essential to manage a high production efficiency and at the same time ability to produce and control the fillet quality according to market demands. In a maximum catch year, wild salmon can fulfill about one-third of the worldwide demand for salmon. While wild fisheries have seasonal accessibility, as well as restrictions on the amount of fish caught, salmon cage culture produce large amounts of steady, high quality salmon year-round (Purser & Forteath, 2003).

Marine fish ingredients have been the major sources of energy and protein in salmon feeds. However, because of the imminent lack of fish oil for the rapidly extending aquaculture industry and its upward cost trend, oils of plant origin gained huge interest in fish feed production (Hardy, 2001; FAO, 2007). Therefore, investigations of substitute lipid and protein sources are vital to ensure a sustainable exploitation of marine resources and to view at the impacts on product yield and quality. Soy oil is a widely available supply among the alternative lipids that can keep fast growth and efficient feed conversion, provided that the dietary requirement for essential fatty acids is met (Storebakken et al., 2000). Previous observations on Atlantic salmon have documented that the fatty acid composition of the muscle is principally a result of the dietary fatty acid composition (Hardy et al., 1987; Thomassen & Røsjø, 1989; Greene & Selivonchick, 1990; Guillou et al., 1995; Bell et al., 2001, 2002, 2003; Caballero et al., 2002). In addition, the former showed that vegetable oils could replace fish oil to a certain extent without comprising growth and feed utilization. However, plant oils are absent from the natural diet of salmonids and other carnivorous fish. Their fatty acid profile varies from that of fish oils by chain length (not more than 18 carbons) and rate of unsaturation (not more than 3 double bonds) (Geurden et al., 2009). This involvement of plant oils in fish diets also modifies body fatty acid composition and may significantly influence fish flesh quality and sensory characteristics (Guillou et al., 1995; Morris et al., 1995). Hence, useful human health promoting fatty acids such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) and the high n-3/n-6 ratio in marine oils need to be examined when replacing it with vegetable oils (Ackman, 2001; Izquierdo et al., 2003). Feeding the last period of on-growing with 100% fish oil diets which is called “washout period” may enable a recovery of the n-3 HUFA levels in fillets of fish fed plant oils earlier.

Atlantic salmon is a high value carnivorous fish species mostly farmed in intensive systems and fed high-energy commercial feeds including high-quality protein. Traditionally, marine fish meals (FM) obtained from industrial and reduction fisheries has been the protein source

of feed for farmed salmon (Hardy, 1996; Sargent & Tacon, 1999; Pike, 2005). It is obvious that fish meal and fish oil resources from these finite fisheries are strictly inadequate and, if aquaculture continues to elevate, the need for FM and FO will exceed global supplies shortly (FAO, 2007). Salmon is the largest consumer of fish oil among all farmed fish species with an estimated consumption of 40-43% on dry feed basis (Tacon & Metian, 2008). The pressure that utilization of these marine products enforces has led to increasing researches into alternative protein and oil sources in fish feed to maintain aquaculture development.

Many studies have evaluated the substitution of fish meal in diets with a variety of vegetable protein at different amounts of inclusion for a range of fish including Atlantic salmon (Storebakken et al., 1998a, b; Carter & Hauler, 2000; Refstie et al., 2000, 2001; Opstvedt et al., 2003; Mundheim et al., 2004; Dias et al., 2005). Alteration of fish meal with soybean protein concentrate up to 80% or 100% in feeds for halibut (Berge et al., 1999) and rainbow trout *Oncorhynchus mykiss* (Kaushik et al., 1995) revealed no unfavorable impacts on growth performance or nutrient utilization. However, total substitution of fish meal with plant protein affected growth performance of rainbow trout (Gomes et al., 1995) and Atlantic salmon (Espe et al., 2006); although, replacement of fish meal in feeds just about 100% was possible in salmon with no adverse effect on growth if the amino acid profile was well balanced (Espe et al., 2007). Several experiments have revealed that the utilization of vegetable oil in aquafeeds at levels of >50% substitution for all species, or indeed total replacement in the case of salmon, is now achievable in practical feeds without compromising growth rate of fish, but had enormously impact on tissue fatty acid profile and metabolism (Bransden et al., 2003; Torstensen et al., 2004; Izquierdo et al., 2005; Pratoomyot et al., 2008; Petropoulos et al., 2009). Consequently, substituting fish meal and fish oil with non-marine ingredients can influence not only production items such as growth, but also nutritional index including fillet fatty acid profile. Additionally it is important to monitor dietary effects on welfare, fish health, product yield and flesh quality parameters including fat content and level of EPA, DHA, and n-3/n-6 fatty acids ratio.

The main aim of this thesis was to investigate the impact of rapeseed oil in diets for farmed salmon with emphasis on fatty acid profile in fillet, intestine, liver and heart together with the influence on total fat content of muscle and all of the mentioned organs. This also included examination of biometric traits and health related parameters.

## 2.0 Background

Fish and fish oils have many protective features against several diseases such as cardiovascular diseases (Kris-Etherton et al., 2002), rheumatoid arthritis (Rennie et al., 2003), depression (Nemets et al., 2006), cognitive decline (Morris et al., 2005) and neurological disorders (Lukiw & Bazan, 2008). Due to the n-3 fatty acids (FA) which have anti-thrombotic (Din et al., 2004) and anti-inflammatory characteristics (Rennie et al., 2003) and also because of micro constituents with anti-thrombotic properties (Kristensen et al., 2001; Nasopoulou et al., 2007) seafood is considered a vital component of human's meal.

One of the important dietary ingredients in extruded fish feed for carnivorous species is fish oil, due to its high digestibility and enough content of indispensable fatty acids, in particular n-3 PUFA. Fish oil replacement in fish diets has reached to the forefront just recently, especially when fish feed production consumed 75% (0.96 million tons) of the world fish oil production in 2010 (Barlow, 2000). Nowadays, the aquaculture industry utilizes nearly 40% and 60% of the global production of fish meal and fish oil, respectively. Fish oil production might not secure all the necessary quantity for fish farming in the next ten years (Kaushik, 2004; Tacon, 2005). Fish meal production has remained stable from the late 1980s at almost 6 million metric tons/annum (FAO, 2004), declaring that fisheries providing fish oil and fish meal may have approached their limit of sustainability (Pike & Barlow, 2003; Shepherd et al., 2005).

### 2.1 Chemical composition

Individual fish species differ remarkably in chemical composition. Factors such as fish species, fish age and size, maturation phase, and swimming activity together with the environmental conditions establish the chemical composition of the fish body (Dunajski, 1979). The main flesh components are proteins and water, with a small quantity of carbohydrates, vitamins, minerals and non-protein-nitrogen (Lynum, 1997). The chemical composition of salmon fillets contain 16-21% protein, 0.2-25% lipid, <0.5% carbohydrates and 1.2-1.5% ash (Murray & Burt, 2001).

#### Protein

Proteins in fish muscle are classified into functional, sarcoplasmic and connective tissue proteins. The functional proteins such as myosin and actin, are vital structures for the ability of the muscle to contract, while the sarcoplasmic proteins like globulin, myoalbumin and enzymes are situated in the sarcoplasma. The connective tissue, which is less represented in fish compared to mammals, consists primarily of collagen, but also elastin and reticulin are found, i.e. around the muscle fibers. The content of connective tissue is generally increasing from the head towards the tail, that way showing more connective tissue in the tale area (Sikorski & Borderias, 1994). The protein content in salmon muscle is relatively constant,

but may vary with season and fish size. In wild salmon, higher levels of protein were found in the feeding season and less around the spawning season (Belitz et al., 2009).

## Fat

Lipids are high energy nutrients that can be fractionally replaced instead of protein in the fish diet. In this way, protein can be consumed for building of new tissue (Wilson, 1989; Pickering & Black, 1998). The energy produced of lipids (9.4 Kcal of $GE^{-1}$) is almost two-fold higher than of proteins (5.6 Kcal of $GE^{-1}$) and carbohydrates (4.1 Kcal of $GE^{-1}$). Lipids have various important roles in the body, besides from being a source of energy; for example they supply the body with essential fatty acids, they are used as structural constituents and they also have several crucial controlling functions (intracellular signaling, local hormonal regulation etc.) (Christie, 2010).

Lipids consist of fatty acid and triacylglycerols. Concerning the number of carbon and double bonds, a fatty acid is named saturated (SFA, no double bonds), mono unsaturated (MUFA, one double bond), polyunsaturated (PUFA, > 2 double bonds), or highly unsaturated (HUFA, > 4 double bonds). Marine fish oils have usually high levels of omega 3 HUFA and they are determined as the best source of lipid in fish diets (EPA or eicosapentaenoic acid, 20:5n-3 and DHA or docosahexaenoic acid, 22:6n-3 are two important essential fatty acid of this group) (Lim & Webster, 2002). Fish require dietary lipids to cover essential fatty acid demands including especially EPA and DHA, to cause normal growth and improvement of cells and tissues. However, fatty acid requirement varies among fish species. It is obvious that cold water fish species need highly unsaturated fatty acids of the n-3 type, whereas warm water fish species require HUFA from either n-3 or n-6 classes or a combination of them. The main signs of shortage of essential fatty acids are reduction of growth, high mortality, lower essential fatty acids in blood and liver phospholipids (Ruyter et al., 2000).

Phospholipids (PL), also named polar lipids because of the substitution of phosphate on the glycerol molecule, are seen in cell membranes, whereas triacylglycerols (TAG) are neutral lipids devoted for the storage of fat and transportation of fatty acids. Lipids can be somehow manipulated by diet quantity and quality. The fatty acid composition of the fish, particularly TAG reflects the fatty acid profile of the diet. Therefore, a fish fed a diet with soy oil will have more C18:1n-9 and C18:2n-6, and less C20:5, C22:5 and C22:6n-2 than if the feed just contains fish oils. This result has been announced for a variety of fish species (Sargent et al., 1995).

Fat content in salmon fillets is essential for the texture, flavor and color. The fat content in farmed adult salmon shows a high variation between and within the same population of fish (Mørkøre et al., 2001). In sexually immature, healthy fish, the fat and water contents normally add up to about 80% of the muscle weight (Haard, 1992). The fat level in muscle of adult salmon depends on feed composition, feeding intensity as well as season. Mørkøre & Rørvik, (2001) reported that salmon accumulate substantial amounts of fat during the autumn, whereas the fillet fat content dropped slightly (by 1.5% units) during the winter. Certain reports stated that farmed salmon are fatter today than for ten years ago (Stead &

Laird, 2001). Higher standards of fish health and husbandry, improvements in diets and feeding regimes have led to higher growth rates in farmed stocks. The feeding regime is influencing the fat content in salmon fillets, and is negatively correlated to feed ration level (Einen et al., 1999). The fat content increases with increasing the body size of fish (Shearer et al., 1994), but the relationship between fish size and fillet fat content is less pronounced for fish larger than 2 kg (Mørkøre & Rørvik, 2001).

### Glycogen

Carbohydrates represent only a few proportion within the fish body (~ 0.3% of the body weight), principally accumulated as glycogen in the liver and the muscle (Lynum, 1997; Jobling, 2001). After slaughtering, the carbohydrates are broken down to lactic acid, which in turn will impose a decline in the muscle pH. The muscle pH is known to be a major factor influencing the fillet and water binding capacity (Dunajski, 1979; Love, 1980; Rustad et al., 1993). The consumption of carbohydrates, which are the first nutrient to be used when starving, is dependent on fish species (Love, 1980). Cold-water species have generally lower ability to utilize carbohydrates compared to warm-water species (Morris, 2001).

### Water and Dry matter content

Overall amount of lipid and water together is about 80% of the fish body composition (Jobling, 2001). Consumption of body lipid in fatty species makes a rise in the water content of the muscle; inducing increase to a fat-water line that is an inverse dynamic correlation between those two components, whereas non-fatty species have a protein-water line of similar pattern (Love, 1980). As said earlier, carbohydrates forms a small amount of the fish body and the greater part of the weight gain in fish are water and approximately 25% of dry substances in general. It was reported that dry matter content of salmon is 25-40% depending on the fat content in muscle and viscera (Jobling, 2001). Hemre et al. (2002) showed an almost linearly decrease of dry mater content as the gonads increased, without any relation towards total body lipid, but highly correlated to reduced whole body protein levels.

## 2.2 Fatty acids

The lipids utilized by fish can be oxidized to produce energy (also called beta-oxidation), and accumulated or applied as structural lipids (Torstensen et al., 2001). The beta-oxidation occurs both in the mitochondria and in the peroxisomes where active cells such as heart cells, liver cells and kidney cells are especially rich in mitochondria. White muscle seems to be responsible for total fatty acid oxidation capacity in fish, hence mitochondrial beta-oxidation dominate over peroxisomal oxidation in this tissue (Frøyland et al., 2000). It was reported that Eicosapentaenoic acid (EPA) is primarily oxidized by mitochondria, while

Docosahexaenoic acid (DHA) appears to be oxidized by the peroxisomes and to a lower degree than EPA (Madsen et al., 1998).

Besides providing energy, the dietary lipid content must render the essential fatty acids required for normal growth and development (Torstensen et al., 2001). The fatty acids Linoleic acid (C18:2n-6) and Linolenic acid (C18:3n-3) are believed to be essential because the fish do not have ability to produce them. Supplementation of these fatty acids in the diet is therefore necessary due to their importance in the elongation and desaturation process of the PUFAs (Torstensen et al., 2001). However, the demand for n-3 fatty acids in freshwater fish can be gained by C18:3n-3, while it seems to only be obtained by EPA and DHA in marine species.

Many plant oils such as soybean oil contain high amount of C16:0, C18:1n-9 and C18:2n-6, whereas Northern Hemisphere fish oils like capelin oil have more long-chain monoenes, C20:1n-9 and C22:1n-11. Southern Hemisphere fish oils like anchovy have generally high content of C16:0 and omega-3 HUFA (Table 2.1).

Table 2.1 The fatty acid composition of marine oils and vegetable oils.

| | Marine oils | | Vegetable oils |
|---|---|---|---|
| | Anchovy oil | Capelin oil | Soybean oil |
| C14:0 | 1.1 | 6.3 | - |
| C15:0 | - | 0.4 | - |
| C16:0 | 17.7 | 11.0 | 10.9 |
| C18:0 | 2.1 | 0.9 | 3.9 |
| C20:0 | - | - | 0.3 |
| C22:0 | - | - | 0.5 |
| C16:1 n-7 | 14.2 | 6.7 | - |
| C18:1 n-7 | - | 1.9 | 1.3 |
| C18:1 n-9 | 15.2 | 6.7 | 21.9 |
| C20:1 n-7 | - | 0.8 | - |
| C20:1 n-9 | 3.4 | 15.9 | - |
| C20:1 n-11 | - | 0.9 | - |
| C22:1 n-9 | - | 2.7 | - |
| C22:1 n-11 | - | 20.2 | - |
| C18:2 n-6 | 12.4 | 1.3 | 54.3 |
| C20:4 n-6 | 3.2 | - | - |
| Sum n-6 | 15.6 | 1.3 | 54.3 |
| C18:3 n-3 | 5.1 | 0.7 | 6.5 |
| C18:4 n-3 | - | 4.8 | - |
| C20:5 n-3 | 11.4 | 7.5 | - |
| C22:5 n-3 | 4.1 | 0.6 | - |
| C22:6 n-3 | 5.7 | 5.7 | - |
| Total n-3 | 26.3 | 19.3 | 6.5 |
| Ratio n-3/n-6 | 1.7 | 14.8 | 0.1 |

### Dietary plant sources

Considerable research has been performed recently to reveal the potential of decreasing the relying on fish oil by vegetable oil supplementation. Several experiments have been done to assess certain plant oils as feasible sustainable substitution for fish oils in commercial fish feeds. By replacing feeds with plant oils, total feed costs reduced. The most frequent plant oils applied for fish feed industry have been rapeseed, linseed, soybean, sunflower, palm and olive oil.

Rapeseed and soybean oil are recognized as possible substitute lipid sources for salmonids as well as others; fresh water and marine fish since they are rich in PUFAs, particularly linoleic (18:2n-6) and oleic acid (18:1n-9), but lacking of n-3 PUFA (Caballero et al., 2002; Izquierdo et al., 2005; Mourente & Bell, 2006). However, in some trials fish oil alteration by 60% rapeseed oil reported to decline European sea bass (*Dicentrarchus labrax*) growth (Montero et al., 2005). Soybean oil seems to be a better vegetable lipid source concerning gilthead sea bream (*Sparus aurata*) growth while significant savings in feed costs would be gained if it could be used as a partial dietary replacement for fish oil within extruded feeds. A similar achievement is true of rapeseed oil and linseed oil, although to a lesser extent (El-Kerdawy & Salama, 1997; Wassef et al., 2009). Moreover, the use of palm oil in diets of salmon and rainbow trout resulted in growth and feed utilization efficiency comparable to fish fed with same amount of fish oil (Torstensen et al., 2000; Rosenlund, 2001; Caballero et al., 2002). Olive oil could also be applied as a partial alteration for dietary fish oil in salmon (Torstensen et al., 2004), European sea bass farming (Mourente et al., 2005) and rainbow trout (Caballero et al., 2002), with data indicating equivalent growth performance to the ones when fish was fed on 100% fish oil diet. Olive pomace (OP) and olive pomace oil (OPO) are natural by-products of olive oil industry, which include micro components with atheroprotective activity (Karantonis et al., 2008) and phenolic/polyphenolic molecules with antioxidant responsibility. Considerable research has been carried out on olive oil by-products and the possibility of partially replacing fish oil in gilthead sea bream and sea bass grow-out diet that resulted in an increased ability to prohibit atherogenesis and consequently heart diseases (Nasopoulou et al., 2011).

Partial substitution of fish oil by plant oils would be possible when fatty acids are presented in the diets in sufficient quantities to fulfill their essential fatty acid demands. Replacement of fish oil with plant lipid sources up to 50-60% can produce similar outcomes to diets consisting of 100% fish oil during the grow-out period of Atlantic salmon in sea (El-Kerdawy & Salama, 1997; Rosenlund, 2001; Figueiredo-Silva et al., 2005). A level of 60% alteration was considered to be the preferable percentage by many researchers, in order not to compromise growth rate or feed utilization efficiency of fish (Alexis, 1997; Izquierdo et al., 2003, 2005; Caballero et al., 2004; Montero et al., 2005; Mourente et al., 2005; Mourente & Bell, 2006; Wassef et al., 2009). However, these high amounts of fish oil replacement are not always required. Substituting fish oil by olive pomace at a lower level (8%) in gilthead sea bream diet revealed similar growth performance in comparison to the one fed on 100% fish oil diet (Nasopoulou et al., 2011). Furthermore, partial substitution of dietary fish oil with plant oil in diets for salmonids, where plant oil and fish oil were used in feed formulations with levels of dietary lipids between 14% and 19%, did not impact growth performance (Guillou et al., 1995; Tocher et al., 2000). On the other hand, scientists determined that it is

possible to replace up to 69% fish oil by plant oils such as rapeseed and soybean oil in long-term trials, without influencing the growth and feed utilization of gilthead sea bream (Fountoulaki et al., 2009). Higher levels of dietary fish oil replacement up to 80% in gilthead sea bream feeds (Montero et al., 2003) caused a considerable reduction in growth rate (Izquierdo et al., 2005) and a decline in feed utilization efficiency. Additionally, liver structure appeared to be altered (Caballero et al., 2004; Wassef et al., 2009) as well as the fish immune system (Montero et al., 2003). However, huge levels of various vegetable oils (up to 80-90% of the supplemented oil) can be applied in high-energy diets for trout without affecting growth performance (Caballero et al., 2002). More recent experiments exhibited that even complete replacement (100%) of fish oil with plant oil (rapeseed, linseed and palm oil) in plant protein-rich diets for gilthead sea bream showed favorable growth rates (Benedito-Palos et al., 2008). Moreover, total fish oil substitution with plant oil (sunflower and palm oil) in diets of salmon indicated that the growth, feed efficiency and protein utilization were not significantly influenced by dietary fatty acid composition during the trial (Torstensen et al., 2000). The observed trends, however, showed that the experimental diets, fed over longer time periods and in life phases with higher growth rates, would probably result in significant differences (Torstensen et al., 2000).

Plenty of researches reported that soybean oil can partially replace the fish oils in salmonid species (Hardy et al., 1987; Thomassen & Røsjø, 1989; Greene & Selivonchick, 1990; Guillou et al., 1995; Caballero et al., 2002) without affecting growth performance and feed efficiency. Results from these examinations declared that substitution of fish oil with plant oils caused lower levels of long chain n-3 fatty acids, EPA and DHA, and higher levels of the C18 fatty acids, oleic acid (C18:1n-9), linoleic acid and linolenic acid in the fish tissue (Figure 2.2). Results clarified that replacement with plant oils up to 60% of fish oil in diets for gilthead sea bream did not affect growth rate and feed utilization even after a long feeding period. However, 80% alteration of fish oil significantly decreased growth (Izquierdo et al., 2005). In addition, the fatty acid profile of the fish tissue resembled those of the dietary lipids (Bell et al., 2001, 2002, 2003). Soy oil has high levels of n-6 fatty acids that are protected with natural antioxidants in fish feeds. However, the high proportion of C18:2n-6 may provoke competition between the n-3 and the n-6 fatty acids that might compromise performance and health in fish with a high demand for n-3 fatty acids (Storebakken et al., 2000). Besides soy phospholipids might stimulate growth and enhance whole body triacylglycerol content in turbot (Geurden et al., 1998) and promoting stress-tolerance with extra C22:6n-3, in larval red sea bream (*Pagrus major*) and marbled sole (*Limanda yokohamae*) (Kanazawa, 1997). Furthermore, Geurden et al. (2009) reported that feed oil history did not influence the triacylglycerol/phosphatidylcholine ratio (TAG/PC) of the newly synthesized lipids in the segments of intestinal integrity. The fish oil-feeding history reduced permeability and improved transepithelial resistance of the intestinal sections. Transepithelial passage rate of 18:3n-3 was higher when pre-fed linseed oil compared to rapeseed or fish oil. Similarly, pre-feeding linseed oil enhanced apparent lipid and fatty acid digestibility in comparison to rapeseed or fish oil. These outcomes showed that the absorptive intestinal functions in fish can be changed by the feed oil history and that the effect lasts after a return to a standard fish oil diet.

| | | |
|---|---|---|
| **Oleic acid** | **C18:1n-9** | **$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$** |
| **Linoleic acid** | **C18:2n-6** | **$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$** |
| **Eicosapentaenoic acid** | **C20:5n-3** | **$CH_3(CH_2CH=CH)_5(CH_2)_3COOH$** |
| **Docosahexaenoic acid** | **C22:6n-3** | **$CH_3(CH_2CH=CH)_6(CH_2)_2COOH$** |

Figure 2.2 Name and structure of the fatty acids: Oleic acid, Linoleic acid, EPA and DHA.

Although results demonstrated that salmon can grow normally on diets rich in plant oils and with their ability to convert C18:3n-3 and C18:2n-6 to their PUFA products such as EPA, DHA and Arachidonic acid (C20:4n-6) (Bell et al., 1997; Torstensen et al., 2000), it is possible that the levels of these endogenous PUFA production may not satisfy the optimal demands, hence adding some dietary EPA and DHA for optimal growth and well-being of the fish, will be needed (Bell et al., 2001). Monitoring production efficiency in fish fed diets with inclusion of various plant oils should be supplemented with health related parameters and flesh quality.

Still, items other than the dietary fatty acid content can influence on the tissue fatty acid profile. Factors including digestibility (Sigurgisladottir et al., 1992), fatty acid transfer and uptake (Torstensen et al., 2000), elongation and desaturation processes (Bell et al., 2001, 2002) and Beta-oxidation of fatty acids (Frøyland et al., 2000) showed to effect on membrane and deposit lipid composition. In vitro experiments carried out to evaluate mitochondrial beta-oxidation in fish showed substrate preferences, whereof saturated and monounsaturated fatty acids were preferred over PUFAs, hence C16:0, C16:1, C18:1n-9 and C18:2n-6 has been favored and mobilized during starvation, whereas DHA realized to be oxidized at low rates (Kiessling & Kiessling, 1993; Schulz, 1996). Lie (1991) stated that the long chain monoenoic fatty acids such as C20:1 and C22:1 appeared to be preferentially catabolized. Results from the same trials demonstrated that lower water temperature induced the relative amounts of both saturated and monoenes fatty acids to decline, whereas the amounts of PUFAs increased.

### EPA and DHA

Marine lipids, EPA and DHA are recommended for human health due to cardiovascular and anti-inflammatory properties, especially when concerning the development in the western diet indicating insufficient consumption of these fatty acids (Williams, 2000). Marine fish species known to contain high percentages of n-3 PUFAs and low percentages of n-6 fatty acids, and thought to be a health-promoting product for human consumption by decreasing n-6 and increasing n-3 input (Torstensen et al., 2004b).

Hemre et al. (2004) found high direct relationship between dietary lipid composition and muscle EPA, whereas this was not found for muscle DHA concentrations. The former authors proposed that temperature is a more important parameter than day length related to utilization and retention of fatty acids. Robin et al. (2003) reported that DHA displayed lower changes than predicted in contrast to other fatty acids. Feeding plant oils in diets for gilthead sea bream caused a reduction in muscle contents of docosahexaenoic acid (DHA) and arachidonic acid (ARA) (Izquierdo et al., 2005). Although re-feeding with a fish oil diet for 60 days effectively retrieved muscle DHA and ARA contents, but EPA were not recovered even after 90 days. Linoleic acid was strongly retained even after a "wash out" period. It was demonstrated that the degree of n-3 fatty acid composition in fish is possible to adjust right before slaughtering, by supplementing high levels of n-3 into a finishing diet (Espe & Lie, 2001).

### Lipid classes

The phospholipids in the fillet appear to be more affected by the fatty acid content in the diet compared to phospholipids in the eye and brain. Tissues with high metabolism such as receptors in the eye and brain naturally have a higher amount of DHA in both fish and humans (Torstensen et al., 2001). A shortage of this fatty acid is stated to influence the sight ability in marine larvae. Thomassen & Røsjø (1989) reported that the n-3/n-6 ratio of both heart and muscle lipids decreased in salmon given plant oil based diets which were related to dietary changes in the level of C18:2n-6 and EPA, while there was a little change for DHA. A soy oil diet in the aforementioned experiment resulted in a reduction of almost 60% of the EPA and DHA as compared to a pure fish oil diet.

### Dietary effects on fatty acid profile

The evidence that the fatty acid profile of the fish tissues usually expresses that of the diet (Bell et al., 2003) creates a basis for the use of lipids as biomarkers in food chain researches (Kirsch et al., 1998). There has been few studies evaluating the time course of a probable change in fatty acid profile when substituting the fat and oil sources in feeds, but it was suggested that a change in the fatty acid profile can be remarkable within 2-6 weeks (dos Santos et al., 1993; Kirsch et al., 1998), even though it is hard to define the exact time needed for fatty acid profile of the fish tissue to balance after a dietary change. It is important to gain more knowledge on the time course of a shift in the fatty acid composition when restoring a "marine profile" after a plant oil diet.

Partial substitution of fish oil by plant oils would be functional if the diet contains sufficient quantities of essential fatty acids. The demand for essential fatty acid requirements vary between species; for example the lowest requirements of gilthead bream for eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are about 0.9% of the diet

(Kalogeropoulos et al., 1992). In addition, linoleic and α-linolenic (18:3n-3) acid can fulfill the essential fatty acid requirements of fresh water fish, whereas marine fish need long-chain n-3 and n-6 PUFA for optimum growth and health aspects (Watanabe, 1982). Confirming this, the process of fatty acid desaturation and elongation of linoleic acid and α-linolenic acid are well established in fresh water anadromous species (Sargent et al., 2002), but marine fish such as sea bass (Mourente et al., 2005) and gilthead sea bream (Mourente & Tocher, 1994; Seiliez et al., 2003) have low capability to convert linoleic acid and α-linolenic acid into arachidonic (ARA, 20:4n-6), EPA and DHA which are necessary for marine fish.

The substitution of 60% plant oils changed the nutritional quality of European sea bass and gilthead sea bream muscles, decreased the percentages of n-3 PUFA, EPA and DHA (Izquierdo et al., 2003, 2005; Montero et al., 2005; Mourente et al., 2005; Mourente & Bell, 2006) and increased percentages of C18 fatty acids: linoleic, α-linolenic and oleic fatty acid (Izquierdo et al., 2003; Montero et al., 2005; Mourente et al., 2005; Mourente & Bell, 2006), which was expected since most plant oils contain high amounts of unsaturated 18C fatty acids (linoleic, α-linolenic and oleic), but are poor sources of n-3 PUFAs. On the contrary, Izquierdo et al. (2005) reported increased linoleic acid in gilthead sea bream muscle but reduced degrees of α-linolenic acid in muscle of fish fed with diets containing 60-80% plant oil. According to Wassef et al. (2009), lower amounts of linoleic and α-linolenic acid in the muscle of gilthead sea bream fed with the plant oil diets (60%) is due to utilization of these fatty acids for oxidation. Elevated level of EPA in gilthead sea bream fed 60% soybean oil support possible chain elongation and desaturation of α-linolenic acid. Therefore, soybean oil and to a lesser amount linseed oil give potential for utilization as a source in aquafeeds for gilthead sea bream (El-Kerdawy & Salama, 1997).

In some experiments re-feeding gilthead sea bream for a period of 90 (Izquierdo et al., 2005) and 120 days (Fountoulaki et al., 2009) with a fish oil finishing diet was not enough to compensate DHA and EPA level in fish muscle. Regarding sea bass re-feeding on a fish oil diet during finishing phase for 20 weeks, the rate of DHA and EPA in the fish muscle did not restore to the levels examined in fish when fed with fish oil diet (Mourente et al., 2005). The amounts of DHA in sea bass muscle restored at the end of the re-feeding phase with 100% fish oil indicated equivalent level of DHA to those fish constantly fed on fish oil diet (Montero et al., 2005). Similar results were found for Atlantic salmon, demonstrating that the levels of DHA and EPA in fish muscle recovered to a value of 90% to those observed in fish fed with fish oil diet (Bell et al., 2003, 2004). Such contrasts are probably due to the different lipid storage capacity in muscle and also to preferences in selective retention and mobilization of particular fatty acids between the aforementioned fish species (Mourente & Bell, 2006). The fat level also seems to be influenced by the dietary oil source, as enhanced fat accumulation in the liver of gilthead sea bream (Caballero et al., 2004) and Atlantic salmon (Nanton et al., 2007) was reported in fish fed high levels of plant oil. Additionally lipid deposition pattern seems to be effected by dietary oil source. Histologically, a supranuclear amount of lipid droplets was noticed in the intestinal cells of the groups fed diets replaced by plant oils. In the same manner, livers from these groups included large degree of lipid droplets within the hepatocytes (Caballero et al., 2004; Nanton et al., 2007).

Plant oil inclusion 50-60% in Atlantic salmon feeds, resulted in increased 18:2n-6 and 18:3n-3 levels in the fillet, whereas amount of DHA and EPA resulted in similar values to those fish fed with fish oil diet (Rosenlund, 2001). Complete substitution of fish oil by plant oil (rapeseed oil) in Atlantic salmon showed changes in 3.7-fold increase of 18:2n-6 and 1.9-fold decrease of 22:6 levels in white muscle compared to salmon fed 100% fish oil (Torstensen et al., 2004). Similar results were found for 18:2n-6 levels in muscle of rainbow trout. The high amount of this fatty acid could be related to direct absorption and esterification, and also to the good affinity of the acyltransferases synthesizing phospholipids containing this fatty acid (Caballero et al., 2002).

The insertion of plant oils in fish feeds can lead to changes of the fatty acid profile, and in some cases may significantly affect fish fillet quality and sensory traits (Guillou et al., 1995; Martínez-Llorens et al., 2007). Additionally, some impact on odor compounds is also possible (Sérot et al., 2002). Alteration of fish oils with plant oils in the dietary feed of farmed fish requires to be assessed not only to bring lipids at the sufficient level with the exact balance of essential fatty acids (EFA) for optimum growth, but also to enhance the proper immune function in fish (Montero et al., 2003) which contributes to the long term sustainability of fish farming industry (Hardy, 2010).

## 3.0 Materials and Methods

A feeding trial was carried out at Nofima research station in Averøy, Norway, over a period of seven months from August 15th 2011 to March 22nd 2012. The fish used were 1800 farmed Atlantic salmon (*Salmo salar* L.) with an average weight of 2.5 kg that were randomly distributed into twelve net pens (volume of 125 $m^3$), giving 150 salmon in each net pen.

The feeding trial of the project was divided into two phases.
**Phase 1:** 08.2011-12.2011. Feeding with three different diets: Marine+, Protein+ and Control; four net pens per dietary treatment.
**Phase 2:** 12.2011-03.2012. The salmon was fed by the same diets as in Phase 1; three net pens per dietary treatment.

### Phase 1 (August 2011 – December 2011)

**Marine+:** Marine oil = high level (70% marine and 30% rapeseed oil) Protein = standard (41.4%)
**Protein+:** Marine oil = standard (30% marine and 70% rapeseed oil) Protein = high (43.5%)
**Control:** Marine oil = standard (30% marine and 70% rapeseed oil) Protein = standard (41.4%)

### Phase 2 (December 2011 – March 2012)

**Marine+:** Marine oil = high level (70% marine and 30% rapeseed oil) Protein = standard (34.5%)
**Protein+:** Marine oil = standard (30% marine and 70% rapeseed oil) Protein = high (37%)
**Control:** Marine oil = standard (30% marine and 70% rapeseed oil) Protein = standard (34.5%)

The Control feed reflected a standard feed for adult salmon according to season.

Salmon was collected for analysis in August, October, December and March. Table 3.1 shows the dates of samplings and number of fish analyzed at each sampling time.

The water temperature at 3 meters depth averaged 8.8 ˚C during the trial, with a minimum of 3.9 ˚C on 20th of February and a maximum of 14.9 ˚C on 10th of September (Figure 3.1).

Table 3.1 Overview of the sampling times and number of fish analyzed at each of the samplings. All fish in each net pen were weighed and the lengths were recorded.

| Sampling | August | October | December | March |
|---|---|---|---|---|
| **Date** | 9-11 | 18-19 | 6-9 | 20-22 |
| **Number of nets the fish were taken from** | 3 | 6 | 12 | 9 |
| **Number of fish for Growth measurement** | 1620 | 900 | 1620 | |
| **Number of slaughtered fish** | 60 | 180 | 360 | 270 |

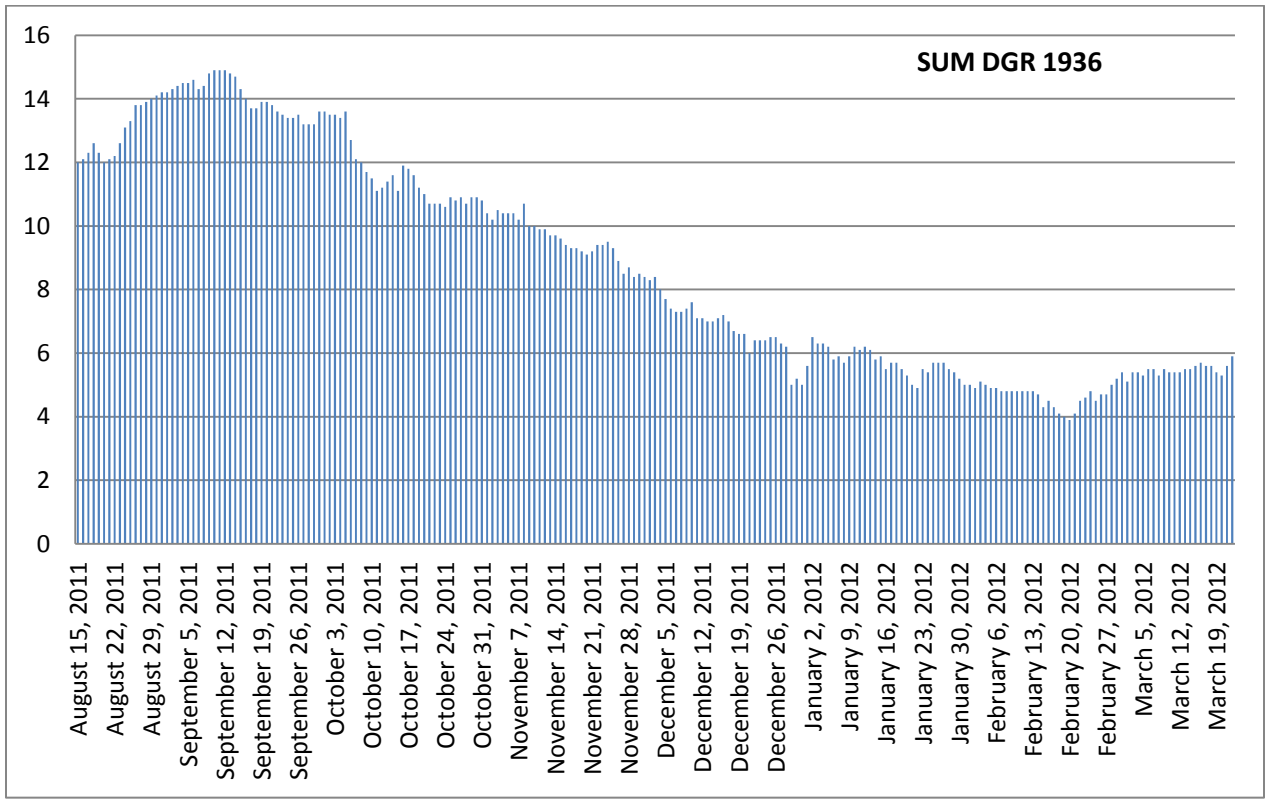



Figure 3.1 Sea water temperatures during the experiment from August 15th 2011 to March 22nd 2012.

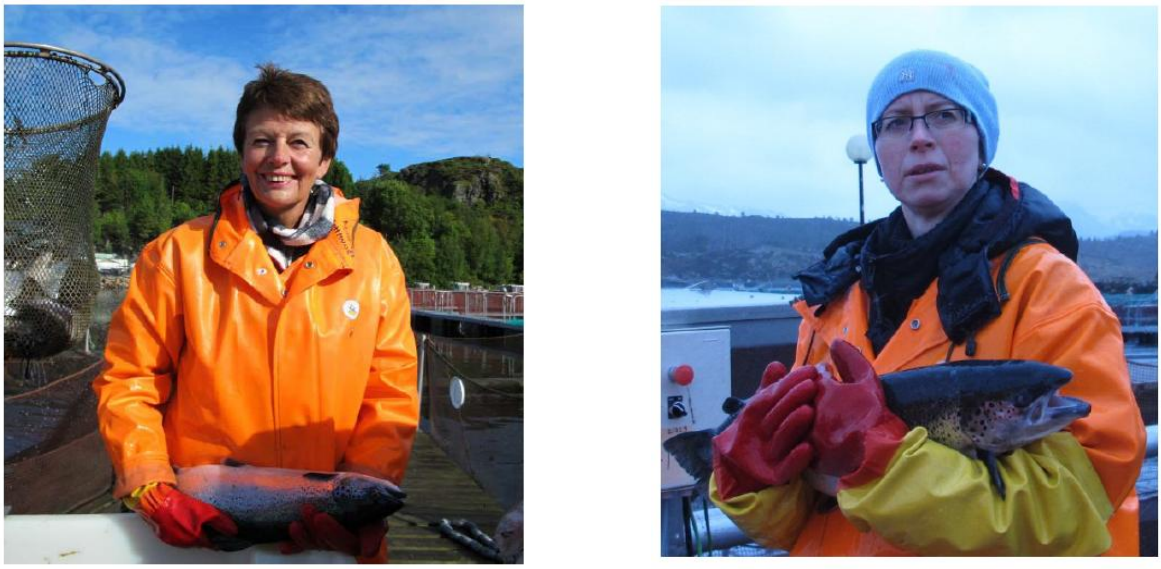


Figure 3.2 Pictures taken at Nofima research station, Averøy, showing the changes in fish size throughout the experiment from August (average of 2.5 kg) to March (average of 6.5 kg). Photo: T. Mørkøre.

## 3.1 Experimental design, Phase 1: August 2011-December 2011

In August 2011, 1800 farmed Atlantic salmon (*Salmo salar* L.) were randomly distributed into twelve net pens (volume of 125 $m^3$), giving 150 salmon in each net pen. Phase 1 of the dietary experiment was carried out during the period August 2011-December 2011. The feeds used were:

**Marine+:** High level of marine oil (70% marine & 30% rapeseed oil) and standard protein
**Protein+:** Standard level of marine oil (30% marine & 70% rapeseed oil) and high protein
**Control:** Standard level of marine oil (30% marine & 70% rapeseed oil) and standard protein

The figure below shows the distribution of nets that were devoted to the various feed (Block design). All nets were attached to the same pier with such a distribution that the environmental effects were minimal (Figure 3.3). The nets were divided into two blocks with equal number of net pens from each dietary treatment within each block. All salmon from the "Block 1" were weighed and length measured in October. Salmon from "Block 1" and "Block 2" were analyzed in December. Fish from each net pen were transferred in batches into a fiberglass tank for anesthetization (MS 222 0.1 g/l, Alpharma, Animal Health Ltd, Hampshire, UK) before weighing and length measurements. After that the fish were transferred back to the cages or sampled for analyses (n=30 per net pen, a total of 180 salmon in October and 360 salmon in December). Weight and length measurements were recorded to monitor the growth rate of individual fish and the change in condition factor. The

technical employees at the research station have long experience of such operations, and the time it took from the fish was taken out of the anesthetic tank until it was back in the cage was about two minutes. All handling was done as gently as possible, but still handling causes some stress and subsequently growth stagnation in a shorter or longer period after sampling. To prevent growth stagnation caused by stress, only salmon from "Block 1" was analyzed in October.

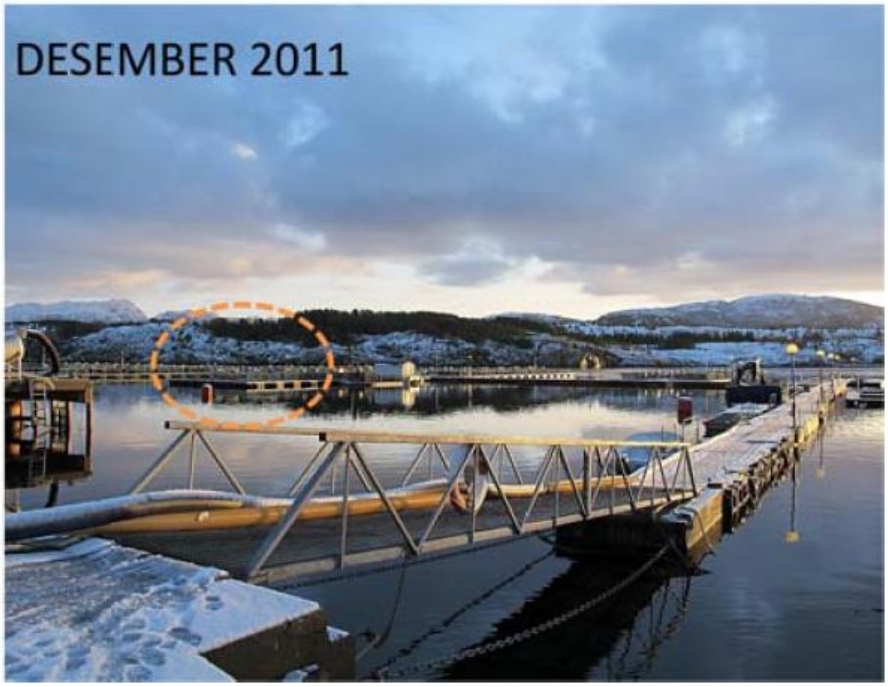



Photo: Jacob Torgersen

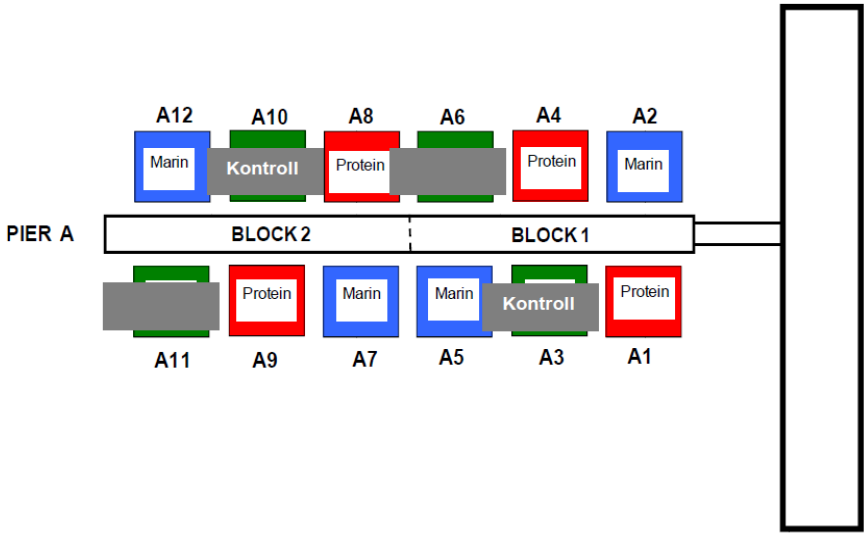



Figure 3.3 Distribution of the dietary treatments in the 12 nets at the Nofima research station in Averøy, during the period August-December 2011. During the period December 2011-March 2012, the three net pens A9, A11 and A12 were removed from the experiment.

## 3.2 Phase 2: December 2011-March 2012

In Phase 2, we used three net pens per each of the dietary treatments. It means that one net pen was taken out from each of the dietary groups: Marine+, Protein+ and Control. 75 Atlantic salmon were transferred from phase 1 to each of the nine net pens. Salmon in all of the nets were fed to satiation and there was 3 days starvation before slaughtering in March.

The crude composition and level of EPA+DHA of the experimental Control feed is shown in table 3.2.

Table 3.2 Ingredient composition of the experimental diets during Phase 1 (7 mm pellets) and Phase 2 (9 mm pellets).

| | Control feed 7 mm | Control feed 9 mm |
|---|---|---|
| **Dry matter (DM, % diet)** | 94 | 93.9 |
| **Crude protein (% DM), (N*6.25)** | 41.4<br>(+2% in Protein+ feed) | 34.6<br>(+2% in Protein+ feed) |
| **Ash (% DM)** | 4.8 | 5.1 |
| **Crude fat (% DM)** | 35.6 | 37.7 |
| **Total starch (% DM)** | 6.1 | 6.8 |
| **EPA+DHA** | 2.9<br>(+5.5 in Marine+ feed) | 2.8<br>(+7 in Marine+ feed) |

Table 3.3 The amino acid composition of the Control diet and protein source which was used in the Protein+ diet.

| Amino acid | Control diet | Protein source in Protein+ diet g/100g |
|---|---|---|
| **Essential amino acid** | | |
| **Histidine** | 0.86 | 0.75 |
| **Leucine** | 2.51 | 2.64 |
| **Isoleucine** | 1.53 | 1.24 |
| **Lysine** | 2.18 | 3.30 |
| **Methionine** | 0.85 | 1.11 |
| **Phenylalanine** | 1.60 | 1.86 |
| **Threonine** | 1.26 | 2.13 |
| **Tryptophan** | 0.27 | <0.05 |
| **Valine** | 1.64 | 2.04 |
| **Non-essential amino acid** | | |
| **Alanine** | 1.60 | 9.31 |
| **Arginine** | 2.25 | 7.79 |
| **Aspartate** | 3.33 | 5.66 |
| **Cysteine** | 0.42 | 0.05 |
| **Glutamate** | 6.57 | 9.74 |
| **Glycine** | 1.61 | 22.35 |
| **Hydroxylysine** | 0.06 | 1.46 |
| **Hydroxyproline** | | 9.0 |
| **Proline** | 1.64 | 11.56 |
| **Serine** | 1.58 | 3.17 |
| **Tyrosine** | 0.99 | 0.42 |

# Rousselot® 100 FG 8

## Fish gelatine

## Product description

Rousselot® 100 FG 8 is an acid process gelatine extracted from fish skin for edible applications.

Gelatine is used in confectionery, water jellies and desserts, dairy products, aspics or functional food, for its versatility. Its functionalities include gelling, binding, stabilizing, thickening, whipping, emulsifying, sticking and foaming power, syneresis prevention and thermo-reversibility.

Rousselot® 100 FG 8 fish gelatine complies with most international edible regulations, including the European Regulations (EC) N°853/2004 and N°2073/2005, and their latest modifications in force at the date of issue of this datasheet.

However, we recommend that the customer ensures that this product is in compliance with local regulation in force, particularly in the countries where the finished product is to be consumed.

Rousselot® 100 FG 8 fish gelatine is available in the following particle size: 8 mesh ASTM.

## Physical/Chemical/Microbial Limits

| Standard parameters | Specifications | Test Method referenced (*) |
|---|---|---|
| Gel strength | 90 - 110 g | GME, GMIA |
| Viscosity | 3.0 - 4.0 mPa.s | GME, GMIA |
| pH | 4.9 - 5.8 | GME, GMIA |
| Loss on drying | ≤ 13 | GME, GMIA |
| Residue on ignition | ≤ 2 | GME, GMIA |
| Residue limits | | |
| Arsenic | ≤ 1.0 ppm | GME |
| Cadmium | ≤ 0.5 ppm | GME |
| Chromium | ≤ 10 ppm | GME |
| Copper | ≤ 30 ppm | GME |
| Mercury | ≤ 0.15 ppm | GME |
| Lead | ≤ 5 ppm | GME |
| Zinc | ≤ 50 ppm | GME |
| Sulfites ($SO_2$) | ≤ 10 ppm | GME, GMIA |
| Peroxides | ≤ 10 ppm | GME |

| Microbial limits | | |
|---|---|---|
| Total Bacterial count | < 1000 CFU/g | GME, GMIA |
| E. Coli | Absence in 10 g | GME, GMIA |
| Salmonella | Absence in 25 g | GME, GMIA |
| Anaerobic sulphite-reducing bacteria | < 10 CFU/g | GME |

*(*) Test method used depends on the country of production of the gelatine*

Figure 3.4 Description of the protein source used in the Protein+ diet.

Table 3.4 Fatty acid composition of the different dietary treatments (% total fatty acids).

| | Feed 7 mm | | Feed 9 mm | |
|---|---|---|---|---|
| Fatty acid | Control diet Protein+ diet | Marine+ diet | Control diet Protein+ diet | Marine+ diet |
| **14:0** | 2.4 | 4.9 | 2.4 | 5.4 |
| **15:0** | 0.3 | 0.3 | 0.3 | 0.4 |
| **16:0** | 8.5 | 12.7 | 9.3 | 14.3 |
| **16:1n-7** | 2.9 | 6.0 | 2.7 | 5.9 |
| **17:0** | 0.4 | 0.9 | 0.3 | 0.3 |
| **16:2n-6** | 0.0 | 0.0 | 0.3 | 0.8 |
| **16:3n-4** | 0.5 | 1.0 | 0.4 | 0.9 |
| **18:0** | 2.7 | 3.3 | 2.8 | 3.7 |
| **18:1n-11** | 0.0 | 0.0 | 0.3 | 1.5 |
| **18:1n-9** | 41.7 | 26.6 | 42.2 | 23.5 |
| **18:1n-7** | 0.2 | 0.1 | 2.3 | 2.5 |
| **18:2n-6** | 13.8 | 8.1 | 14.0 | 7.4 |
| **18:3n-3** | 6.4 | 3.4 | 6.0 | 2.9 |
| **20:0** | 0.0 | 0.0 | 0.5 | 0.4 |
| **20:1n-11** | 0.8 | 0.5 | 0.9 | 0.8 |
| **20:4n-3** | 0.0 | 1.8 | 0.1 | 0.0 |
| **20:1n-9** | 1.5 | 1.4 | 1.8 | 1.5 |
| **20:4n-6** | 0.4 | 0.8 | 0.0 | 0.1 |
| **20:3n-3** | 0.0 | 0.0 | 0.0 | 0.0 |
| **22:0** | 0.9 | 0.8 | 0.2 | 0.1 |
| **22:1n-7** | 0.0 | 0.0 | 0.3 | 0.2 |
| **22:1n-11** | 0.9 | 1.4 | 1.3 | 0.5 |
| **22:1n-9** | 0.5 | 0.3 | 0.3 | 0.8 |
| **20:5n-3** | 4.6 | 10.2 | 4.2 | 11.0 |
| **24:1n-9** | 0.3 | 0.3 | 0.3 | 0.3 |
| **22:5n-3** | 0.6 | 1.3 | 0.5 | 1.4 |
| **22:6n-3** | 3.4 | 7.3 | 3.7 | 7.7 |
| **Sum EPA/DHA** | 8.0 | 17.5 | 7.9 | 18.7 |
| **Sum n-3** | 15.1 | 24.2 | 14.6 | 23.1 |
| **Sum n-6** | 14.5 | 9.3 | 14.5 | 8.8 |
| **Sum n-0** | 15.1 | 22.8 | 16.1 | 24.9 |

Fatty acids < 0.3 are not shown in the table.

## 3.3 Registration of fish and organs

The fish was gutted after bleeding for 25 minutes in seawater. Then the following registrations were done: Gender, amount of fat around the intestine (score 1-5), liver color (score 1-3) and fat accumulation on the surface of heart. Aberrant appearance of the organs was also noted. The weight of liver, heart and intestines (viscera apart from liver and heart) were recorded. The fish were filleted by hand and fillet weights were registered. The left side fillets were individually wrapped in plastic bags, placed on ice and transported to the laboratory at Nofima in Ås for quality analysis. Samples of muscle and organs were also frozen in liquid nitrogen for possible future analyses.

### Intestine fat

At each slaughtering time, there was a large number of fish which were registered within a short time. Therefore, it was important that each recording to be both efficient and informative. As a result, a new scale for the assessment of the amount of visceral fat was developed that requires no tools, but just a quick observation (Figure 3.5).

Score 1: Pyloric ceaca are visible clearly
Score 2: Pyloric ceaca are visible
Score 3: Pyloric ceaca are visible through cracks in intestine fat
Score 4: Pyloric ceaca are visible through the fat (the strips of intestine fat)
Score 5: Pyloric ceaca are not visible

This scale worked very well, and has been utilized in several F&U projects.

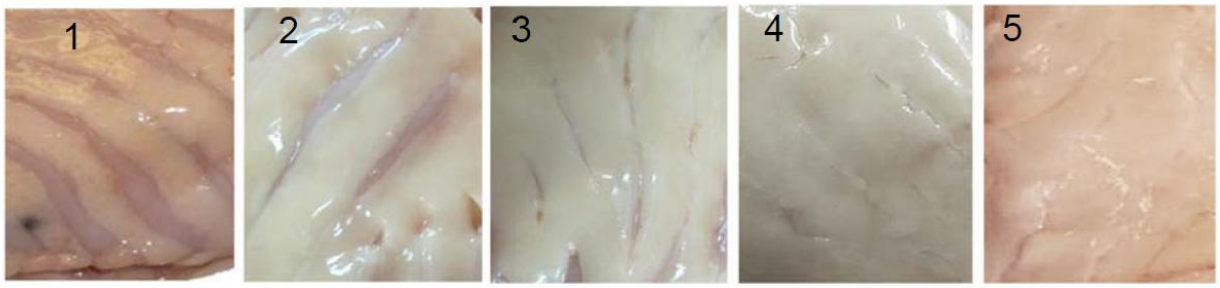



Figure 3.5 The score which was used for assessment of the intestine fat.

### Liver color

The focus on the relationship between health status and liver condition has increased significantly in recent years. Nofima also in previous FHF-projects demonstrated a close relationship between liver status and fillet quality. Since there was not any scale for liver

color registration, a scoring system was developed, ranging from 1-3; where score 1 is pale and score 3 is dark brown (Figure 3.6).

Figure 3.6 Liver colors in salmon from March (end of the project). The bright liver (equivalent to score 1) is of salmon from the Control group and the dark liver (equivalent to score 3) is of salmon from the Marine+ group.

### Heart

Visible fat deposition of the surface of heart was carefully considered as a key characteristic. The weight of the heart was registered after removing the bulbous and atrium (Figure 3.7).

Evaluation - heart size: % of the body weight (ventricle weight)

- appearance: visible fat deposition on the surface of heart

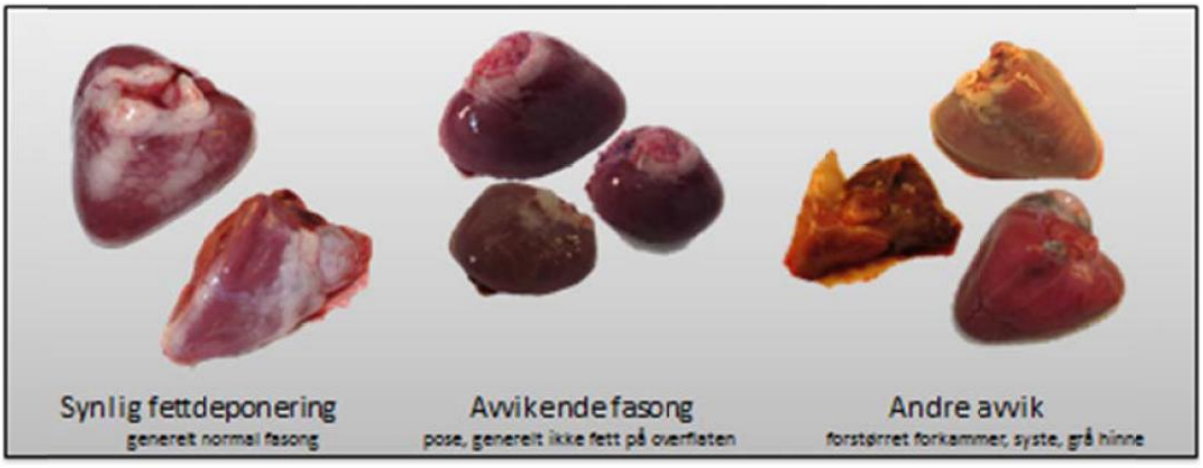



Figure 3.7 Pictures illustrating the accumulation of fat on the surface of heart, hearts with deformity and other abnormalities. The pictures are of the salmon from the project (December and March samplings).

## 3.4 Chemical analysis

Analyses of fat in the fillet were performed by chemical analysis (muscle below the dorsal fin). Chemical analyses in addition to fillets also performed in the intestines, liver and heart (Folch et al., 1957). Protein and amino acid composition were analyzed as explained by www.nofima.no/ingredients.

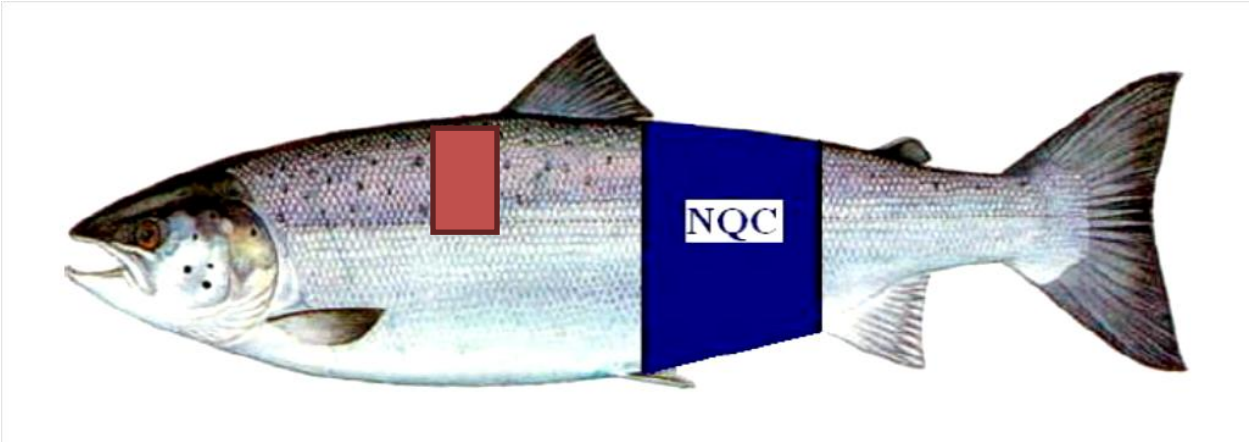



Figure 3.8 Fillet fat content and fatty acid composition were analyzed in the dorsal fillet part (red square), whereas the protein content and amino acid composition were analyzed in Norwegian quality cut (NQC) (blue square).

## 3.5 Statistical analysis and calculations

The results were analyzed using the statistical program SAS. In most cases, analysis of variance was used to investigate differences between treatments. Correlation analysis (Pearsons) was used to investigate the relationship between registered features. The statistical models were adjusted for possible gender differences. Student's t-test was used to determine differences between groups/dietary treatments.

- For growth, TGC = $[(FBW^{1/3} – IBW^{1/3}) / (T*D)] * 1000$, where FBW and IBW refer to final and initial mean body weight, respectively. T and D show average of temperature and number of days.
- Condition factor is calculated as, CF = live weight (g) / length $(cm)^3 * 100$.
- Organ index: organ weight (g) / body weight (g) * 100.
- Fillet Yield: fillet weight * 2 (g) / body weight (g) * 100.
- Slaughter Yield: gutted weight (g) / body weight (g) * 100.

# 4.0 Results

This chapter begins with growth performance and FCR results. Then, results of the fillet, intestine, liver and heart parameters are given. The fatty acid profile of all the mentioned tissues is shown, but the main focus is on specific fatty acids such as Oleic acid (18:1n-9), Linoleic acid (18:2n-6), EPA (20:5n-3) and DHA (22:6n-3), along with EPA+DHA and n-3/n-6 fatty acids ratio. Finally, chemical analysis of total fat content in muscle and organs is covered.

## 4.1 Growth performance and FCR

The growth and FCR for the whole trial from August 2011 to March 2012 were calculated and in addition periodic growth and FCR was calculated for the period August-October, October-December and December to March.

### Body weight

The growth rate during the whole seven months trial showed no significant variation between dietary treatments. Numerically, the final body weight for the Protein+ group was lowest with 6.4 kg, whereas the Control group had highest final body weight with 6.6 kg as shown in Table 4.1.

Comparison of the dietary treatments within each sampling period demonstrated that the body weight of the Protein+ group was significantly lowest compared to the Marine+ and Control group at the sampling in October. In other sampling times, no significant differences were recorded between dietary treatments.

### Thermal growth coefficient (TGC)

In October, the Marine+ group represented significantly higher TGC with 3.6 than the Protein+ group with 3.4. Otherwise, no significant differences were observed among the dietary treatments. The TGC for the whole trial was 3.3 for dietary treatments (Table 4.1).

### Feed conversion ratio (FCR)

The overall FCR showed no significant differences between dietary treatments (FCR equal to 1.1). At the sampling in October, however, the Protein+ group had significantly highest feed conversion ratio with 1.1 in comparison with the Marine+ and Control group with 1.06 and 1.08, respectively (Table 4.1).

### Condition factor (CF)

The average condition factor ranged from 1.41 to 1.45 for the dietary fish groups. The Marine+ group had significantly lower condition factor compared to the Protein+ and Control group (Table 4.1).

### Fork length

The fork length of the Marine+ group with 77.2 cm was significantly longer than the Control group with 76.5 cm (Table 4.1).

### Gutted weight

The average gutted weight ranged from 5.8 kg to 5.9 kg at the end of the trial. Significant differences were found among the dietary treatments, where the Marine+ and Protein+ group showed higher gutted weight in comparison with the Control group (Table 4.1).

### Slaughter yield (%)

The overall slaughter yield ranged from 88.8% to 89.6%, where the Protein+ group displayed significantly higher slaughter yield than the Control group (Figure 4.1).

Table 4.1 Body weight, Thermal growth coefficient (TGC), feed conversion ratio (FCR), condition factor (CF), fork length and gutted weight of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012.

| Parameters | Marine+ | Protein+ | Control |
|---|---|---|---|
| **Body weight (g)** | 6519 ± 54 | 6448 ± 273 | 6645 ± 91 |
| **TGC (% day$^{-1}$)** [1] | 3.3 ± 0.0 | 3.3 ± 0.1 | 3.3 ± 0.1 |
| **FCR** [2] | 1.1 ± 0.0 | 1.1 ± 0.0 | 1.1 ± 0.0 |
| **CF** [3] | 1.41 ± 0.0 b | 1.44 ± 0.0 a | 1.45 ± 0.0 a |
| **Fork length (cm)** | 77.2 ± 0.2 a | 76.7 ± 0.2 ab | 76.5 ± 0.2 b |
| **Gutted weight (g)** | 5820 ± 13 a | 5855 ± 13 a | 5784 ± 13 b |

1. TGC = [(FBW$^{1/3}$ – IBW$^{1/3}$) / (T*D)] * 1000, where FBW and IBW refer to final and initial mean body weight, respectively. T and D show average of temperature and number of days.
2. FCR = eaten feed (kg) / biomass increased (kg), where biomass increased calculated as;
final biomass (kg) + mortality biomass (kg) – initial biomass (kg)
3. CF = BW (g) * 100 / FL$^3$ (cm), where FL indicates fork length
Different letters within the same row indicate significant differences between dietary treatments.

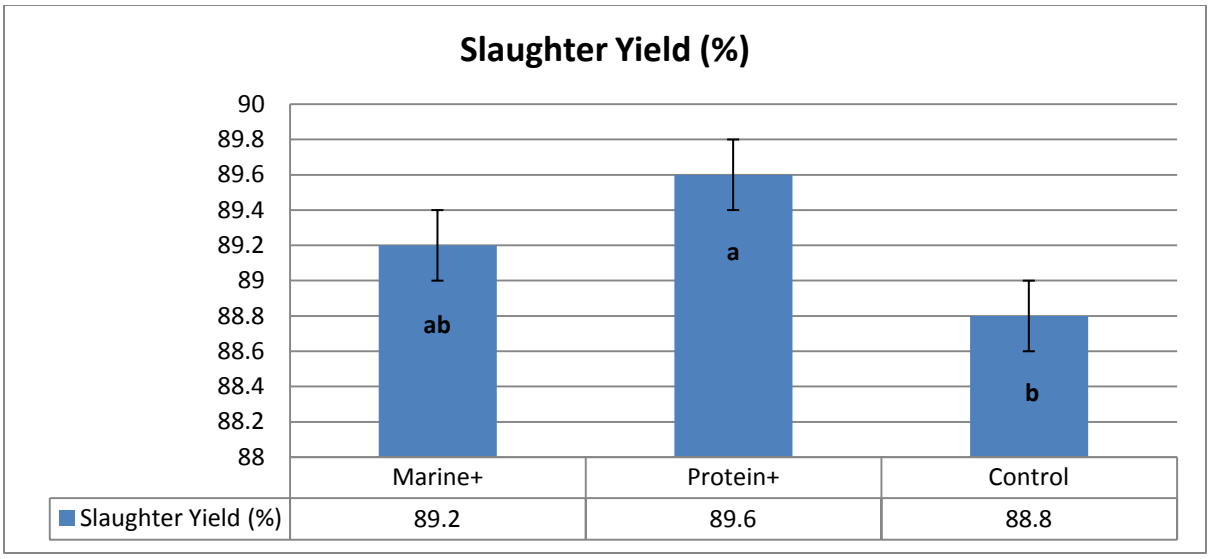



Figure 4.1 Slaughter yield (%) of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error.
Different letters indicate significant differences between dietary treatments.
Slaughter yield (%) = gutted weight (g) * 100 / body weight (g)

## 4.2 Fillet parameters

### Fillet weight

The fillet weight varied significantly between dietary treatments, ranging from 2059 g to 2112 g. The Protein+ group showed significantly highest fillet weight compared to the Marine+ and Control group.

### Fillet yield (%)

The overall fillet yield ranged from 63% to 64.6%. The Protein+ group demonstrated significantly highest fillet yield in comparison with the Marine+ and Control group (Figure 4.2).

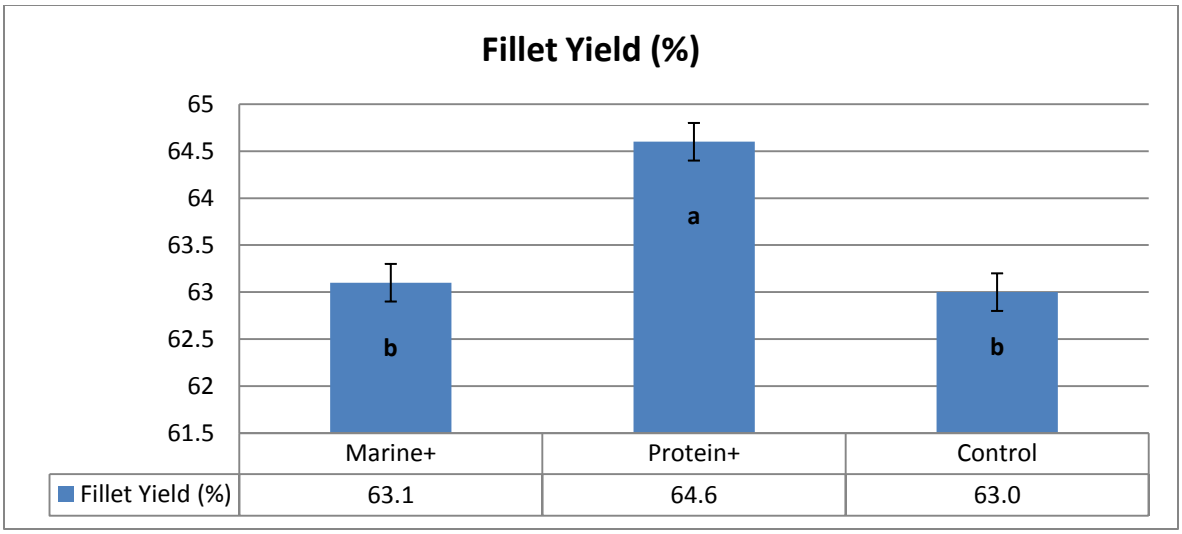



Figure 4.2 Fillet yield (%) of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error.
Different letters indicate significant differences between dietary treatments.
Fillet yield (%) = [fillet weight * 2 (g) * 100] / body weight (g)

## 4.3 Intestine parameters

### Viscera-somatic index (VSI)

The average of viscera-somatic index ranged from 9.2% to 10.2%. The Control group showed significantly highest VSI compared to the Protein+ group (Table 4.2).

### Intestine fat score

Intestine fat score was evaluated as the amount of visible fat (score 1-5). Significant differences were found between dietary treatments. The Control group showed highest level of intestine fat with score 2.9, whereas the Marine+ and Protein+ group had lowest level with score 2.4 and 2.5, respectively (Table 4.2).

## 4.4 Liver parameters

### Liver weight

The overall liver weight ranged from 70 g to 74 g. Significant differences were observed among the dietary treatments where the Marine+ group demonstrated lowest liver weight in comparison with the Protein+ and Control group (Table 4.2).

### Hepatosomatic index (HSI)

The hepatosomatic index ranged from 1.06% to 1.13%. The Marine+ group had significantly lowest HSI (Table 4.2).

### Liver color

Liver color was scored visually from 1 to 3 (score 1 shows palest and score 3 darkest). There were significant differences between dietary treatments. The Marine+ group with score 2.3 displayed darkest color compared to the Protein+ and Control group with score 1.6 (Table 4.2).

### Liver patches

Liver patches (%) evaluated as frequency of fish with uneven color of the liver, ranged from 3% to 42%. There were significant differences among the dietary treatments, where the Control group showed highest frequency and Marine+ lowest (Table 4.2).

## 4.5 Heart parameters

### Heart weight

There were no significant differences between dietary treatments, although the Marine+ and Control group had numerically highest heart weight with 6.4 g compared with 6.3 g (Table 4.2).

### Cardio somatic index (CSI)

The cardio somatic index with 0.1% demonstrated no overall significant differences among the dietary treatments (Table 4.2).

### Visible heart fat (%)

Heart fat (%) was evaluated as visible fat on the surface of the heart. The frequency of visible fat on the surface of the heart ranged from 21% to 52%. The Protein+ group showed significantly lowest frequency in comparison with the Marine+ and Control group (Table 4.2).

Table 4.2 Intestine, liver and heart parameters (LSmeans ± SE) of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012.

| Parameters | | Marine+ | Protein+ | Control |
|---|---|---|---|---|
| | | | | |
| **Intestine** | **Percentage** [1] | 9.7 ± 0.2 ab | 9.2 ± 0.2 b | 10.2 ± 0.2 a |
| | **Fat score** [2] | 2.4 ± 0.1 b | 2.5 ± 0.1 b | 2.9 ± 0.1 a |
| | | | | |
| **Liver** | **Weight (g)** | 70 ± 0.1 b | 74 ± 0.1 a | 74 ± 0.1 a |
| | **Percentage** [1] | 1.06 ± 0.01 b | 1.13 ± 0.01 a | 1.12 ± 0.01 a |
| | **Color score** [3] | 2.3 ± 0.1 a | 1.6 ± 0.1 b | 1.6 ± 0.1 b |
| | **Patches (%)** [4] | 3 ± 4 c | 29 ± 4 b | 42 ± 4 a |
| | | | | |
| **Heart** | **Weight (g)** | 6.4 ± 0.1 | 6.3 ± 0.1 | 6.4 ± 0.1 |
| | **Percentage** [1] | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| | **Visible fat (%)** [5] | 39 ± 5 a | 21 ± 5 b | 52 ± 5 a |

1. Organ index = weight of organ (g) * 100 / whole body weight (g)
2. Intestine fat score was evaluated as the amount of visible fat, score 1-5.
3. Liver color was scored visually from 1 to 3, 1 shows palest and 3 darkest.
4. Liver patches (%) evaluated as frequency of fish with uneven color of the liver. Results are given as frequency; i.e. 100 * number of fish with uneven color of the liver.
5. Heart fat (%) evaluated as visible fat on the surface of the heart. Results are given as frequency; i.e. 100 * number of fish with visible fat on the heart surface.

Different letters within the same row indicate significant differences between dietary treatments.

## 4.6 Fatty acid composition

The replacement of fish oil with rapeseed oil resulted in marked increases in 18:1n-9, 18:2n-6 and 18:3n-3, and decreases in 16:0, 18:0, 20:5n-3 and 22:6n-3 in the diets. This was reflected in the fatty acid contents of the muscle, intestine, liver and heart at the end of the experiment, where there were significant increases in 18:1n-9 and 18:2n-6, and significant decreases in 18:0, 20:5n-3 and 22:6n-3 in the muscle and all of the mentioned organs from fish fed the rapeseed oil diet (Control) compared to those fed the fish oil diet (Marine+).

## Fatty acid profile of muscle

Table 4.3 Fatty acid profile (% total fatty acids) for muscle of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012.

| Fatty acid | Marine+ | Control | *P-value* |
|---|---|---|---|
| 14:0 | 3.9 ± 0.1 | 2.4 ± - | 0.000 |
| 15:0 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.922 |
| 16:0 | 11.8 ± 0.2 | 9.6 ± 0.1 | 0.000 |
| 17:0 | 0.5 ± 0.1 | 0.3 ± - | 0.008 |
| 18:0 | 2.7 ± 0.1 | 2.5 ± - | 0.008 |
| 20:0 | 0.2 ± - | 0.3 ± - | 0.000 |
| 22:0 | 0.1 ± - | 0.2 ± 0.1 | 0.094 |
| 24:0 | 0.2 ± - | 0.2 ± - | 0.148 |
| Σ SAT | 19.6 ± 0.2 | 15.6 ± 0.3 | 0.000 |
| 16:1n-7 | 5.5 ± 0.1 | 3.1 ± - | 0.000 |
| 17:1n-7 | 0.3 ± 0.3 | 0.2 ± - | 0.569 |
| 18:1n-9 | 31.1 ± 0.4 | 41.7 ± 0.1 | 0.000 |
| 18:1n-7 | 0.2 ± - | 0.1 ± - | 0.007 |
| 20:1n-11 | 0.8 ± 0.1 | 0.6 ± 0.1 | 0.016 |
| 20:1n-9 | 2.9 ± 0.2 | 3.1 ± 0.2 | 0.300 |
| 20:1n-7 | 0.4 ± 0.2 | 0.3 ± 0.2 | 0.845 |
| 22:1n-11 | 1.7 ± 0.1 | 1.4 ± - | 0.010 |
| 22:1n-9 | 0.3 ± 0.1 | 0.4 ± - | 0.223 |
| 22:1n-7 | 1.0 ± - | 0.7 ± - | 0.000 |
| 24:1n-9 | 0.3 ± - | 0.3 ± - | 0.265 |
| 18:2n-6 | 9.0 ± 0.2 | 12.7 ± 0.1 | 0.000 |
| 18:3n-6 | 0.2 ± - | 0.1 ± 0.1 | 0.094 |
| 20:2n-6 | 0.6 ± 0.1 | 0.9 ± 0.1 | 0.020 |
| 20:3n-6 | 0.2 ± - | 0.3 ± - | 0.126 |
| 20:4n-6 | 0.6 ± - | 0.3 ± - | 0.000 |
| Σ n-6 | 10.9 ± 0.2 | 14.5 ± 0.2 | 0.000 |
| 16:3n-4 | 0.6 ± - | 0.2 ± - | 0.000 |
| 18:3n-4 | 0.3 ± - | 0.1 ± - | 0.000 |
| 18:3n-3 | 3.3 ± 0.1 | 5.1 ± - | 0.000 |
| 20:3n-3 | 0.3 ± - | 0.3 ± 0.2 | 0.725 |
| 20:4n-3 | 0.4 ± - | 0.3 ± - | 0.013 |
| 20:5n-3 | 6.7 ± 0.4 | 3.5 ± 0.1 | 0.000 |
| 22:5n-3 | 2.8 ± 0.1 | 1.5 ± - | 0.000 |
| 22:6n-3 | 8.4 ± 0.1 | 5.3 ± 0.1 | 0.000 |
| Σ n-3 | 22.0 ± 0.4 | 16.1 ± 0.2 | 0.000 |
| Σ EPA/DHA | 15.0 ± 0.4 | 8.8 ± 0.2 | 0.000 |
| n-3/n-6 | 2.0 ± 0.1 | 1.1 ± - | 0.000 |

When P-value < 0.05, the differences are significant.

## Fatty acid profile of intestine

Table 4.4 Fatty acid profile (% total fatty acids) for intestine of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012.

| Fatty acid | Marine+ | Control | *P-value* |
|---|---|---|---|
| 14:0 | 4.1 ± 0.1 | 2.4 ± - | 0.000 |
| 15:0 | 0.3 ± - | 0.2 ± - | 0.004 |
| 16:0 | 10.9 ± 0.1 | 8.6 ± 0.1 | 0.000 |
| 17:0 | 0.6 ± - | 0.3 ± 0.1 | 0.002 |
| 18:0 | 2.6 ± - | 2.4 ± - | 0.003 |
| 19:0 | 0.3 ± - | 0.2 ± - | 0.000 |
| 20:0 | 0.2 ± - | 0.3 ± - | 0.002 |
| 22:0 | 1.1 ± - | 0.8 ± - | 0.000 |
| 24:0 | 0.2 ± 0.1 | 0.1 ± - | 0.040 |
| Σ SAT | 20.1 ± 0.3 | 15.1 ± 0.1 | 0.000 |
| 14:1n-5 | 0.2 ± - | 0.1 ± 0.1 | 0.169 |
| 16:1n-7 | 5.6 ± 0.1 | 3.2 ± 0.1 | 0.000 |
| 16:1n-5 | 0.3 ± - | 0.2 ± 0.1 | 0.342 |
| 18:1n-9 | 28.0 ± - | 40.1 ± 0.2 | 0.000 |
| 18:1n-7 | 3.5 ± 0.1 | 3.3 ± - | 0.068 |
| 20:1n-11 | 1.1 ± - | 0.6 ± - | 0.000 |
| 20:1n-9 | 2.7 ± - | 3.1 ± - | 0.000 |
| 20:1n-7 | 0.2 ± - | 0.2 ± - | 0.013 |
| 22:1n-11 | 1.7 ± 0.1 | 1.3 ± - | 0.000 |
| 22:1n-9 | 0.3 ± 0.1 | 0.4 ± - | 0.124 |
| 24:1n-9 | 0.3 ± - | 0.3 ± - | 0.002 |
| 16:2n-6 | 0.2 ± - | 0.2 ± - | 0.274 |
| 18:2n-6 | 9.5 ± 0.1 | 13.5 ± 0.1 | 0.000 |
| 18:3n-6 | 0.2 ± - | 0.1 ± - | 0.172 |
| 20:2n-6 | 0.2 ± - | 0.2 ± - | 0.209 |
| 20:3n-6 | 0.6 ± 0.1 | 0.2 ± 0.1 | 0.009 |
| 20:4n-6 | 0.3 ± - | 0.4 ± - | 0.000 |
| 22:4n-6 | 0.4 ± 0.1 | 0.2 ± - | 0.152 |
| Σ n-6 | 11.4 ± - | 14.9 ± 0.1 | 0.000 |
| 16:3n-4 | 0.5 ± 0.1 | 0.2 ± 0.1 | 0.016 |
| 18:3n-3 | 3.4 ± - | 5.3 ± - | 0.000 |
| 18:4n-3 | 0.7 ± - | 1.0 ± - | 0.000 |
| 20:3n-3 | 0.1 ± - | 0.2 ± - | 0.302 |
| 20:4n-3 | 0.5 ± 0.1 | 0.3 ± 0.1 | 0.042 |
| 20:5n-3 | 6.7 ± 0.2 | 3.1 ± - | 0.000 |
| 22:5n-3 | 2.9 ± 0.1 | 1.5 ± - | 0.000 |
| 22:6n-3 | 7.1 ± 0.2 | 3.9 ± - | 0.000 |
| Σ n-3 | 21.4 ± 0.4 | 15.2 ± 0.1 | 0.000 |
| Σ EPA/DHA | 13.8 ± 0.4 | 7.0 ± 0.1 | 0.000 |
| n-3/n-6 | 1.9 ± - | 1.0 ± - | 0.000 |

When P-value < 0.05, the differences are significant.

## Fatty acid profile of liver

Table 4.5 Fatty acid profile (% total fatty acids) for liver of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012.

| Fatty acid | Marine+ | Control | *P-value* |
|---|---|---|---|
| 14:0 | 1.9 ± - | 1.2 ± - | 0.000 |
| 15:0 | 0.2 ± 0.2 | 0.1 ± - | 0.901 |
| 16:0 | 11.4 ± 0.7 | 6.9 ± 0.7 | 0.001 |
| 17:0 | 0.3 ± - | 0.2 ± - | 0.031 |
| 18:0 | 5.8 ± 0.1 | 3.6 ± 0.3 | 0.000 |
| 20:0 | 0.2 ± - | 0.2 ± - | 0.848 |
| 22:0 | - ± - | - ± - | 0.092 |
| 24:0 | 0.2 ± - | 0.2 ± - | 0.065 |
| Σ SAT | 20.0 ± 0.9 | 12.5 ± 1.0 | 0.001 |
| 14:1n-5 | 0.1 ± 0.1 | 0.1 ± - | 0.404 |
| 16:1n-7 | 3.2 ± 0.2 | 2.4 ± 0.1 | 0.004 |
| 17:1n-7 | 0.2 ± - | 0.2 ± 0.1 | 0.616 |
| 18:1n-9 | 24.4 ± 1.5 | 41.6 ± 1.6 | 0.000 |
| 18:1n-7 | 0.2 ± - | 0.1 ± - | 0.078 |
| 20:1n-11 | 0.9 ± 0.7 | 1.2 ± 1.7 | 0.973 |
| 20:1n-9 | 1.6 ± 0.2 | 3.4 ± 2.6 | 0.417 |
| 20:1n-7 | 0.6 ± 0.3 | 0.2 ± 0.1 | 0.128 |
| 22:1n-11 | 0.3 ± 0.1 | 0.4 ± - | 0.220 |
| 22:1n-9 | 0.1 ± 0.1 | 0.2 ± - | 0.111 |
| 22:1n-7 | 1.2 ± - | 1.1 ± - | 0.054 |
| 24:1n-9 | 0.2 ± - | 0.2 ± - | 0.191 |
| 18:2n-6 | 4.8 ± 0.1 | 9.3 ± 0.2 | 0.000 |
| 18:3n-6 | 0.2 ± 0.1 | 0.1 ± - | 0.475 |
| 20:2n-6 | 1.2 ± 0.1 | 2.3 ± 0.1 | 0.000 |
| 20:3n-6 | 0.2 ± 0.1 | 0.5 ± 0.1 | 0.011 |
| 20:4n-6 | 2.8 ± 0.1 | 1.2 ± 0.2 | 0.000 |
| 22:4n-6 | 0.3 ± - | 0.1 ± - | 0.011 |
| Σ n-6 | 9.5 ± 0.2 | 13.5 ± 0.1 | 0.000 |
| 16:3n-4 | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.379 |
| 18:3n-4 | 0.3 ± - | 0.2 ± - | 0.010 |
| 16:2n-3 | 0.2 ± 0.2 | 0.2 ± 0.2 | 0.843 |
| 18:3n-3 | 1.6 ± 0.1 | 3.3 ± 0.1 | 0.000 |
| 18:4n-3 | 0.2 ± - | 0.1 ± - | 0.116 |
| 20:3n-3 | 0.4 ± - | 0.9 ± 0.1 | 0.001 |
| 20:5n-3 | 10.1 ± 0.1 | 4.9 ± 0.5 | 0.000 |
| 22:5n-3 | 4.6 ± 0.2 | 2.2 ± 0.2 | 0.000 |
| 22:6n-3 | 17.9 ± 1.0 | 9.5 ± 1.0 | 0.000 |
| Σ n-3 | 34.9 ± 1.5 | 21.0 ± 1.1 | 0.000 |
| Σ EPA/DHA | 28.0 ± 1.1 | 14.4 ± 1.5 | 0.000 |
| n-3/n-6 | 3.7 ± 0.1 | 1.6 ± 0.1 | 0.000 |

When P-value < 0.05, the differences are significant.

## Fatty acid profile of heart

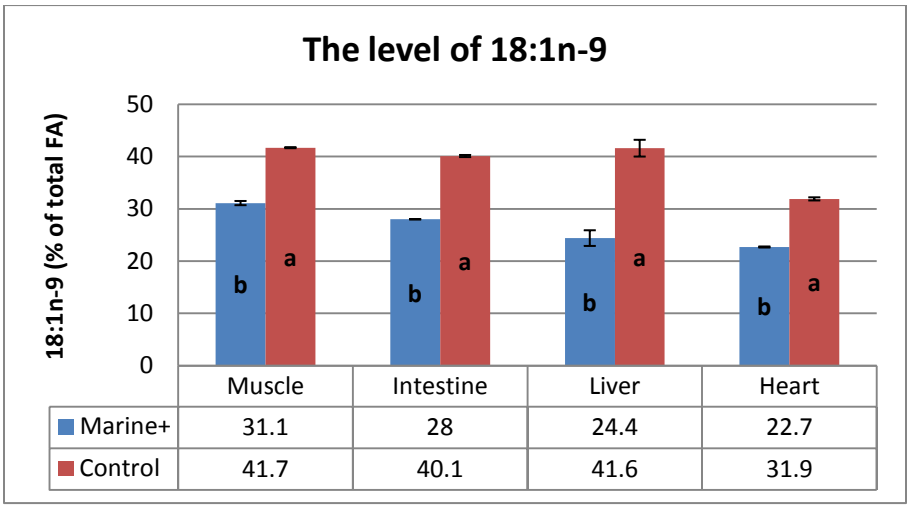
Table 4.6 Fatty acid profile (% total fatty acids) for heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012.

| Fatty acid | Marine+ | Control | *P-value* |
|---|---|---|---|
| 14:0 | 3.4 ± 0.1 | 2.1 ± 0.1 | 0.000 |
| 15:0 | 0.3 ± - | 0.2 ± 0.1 | 0.125 |
| 16:0 | 13.7 ± - | 11.5 ± 0.2 | 0.000 |
| 17:0 | 0.3 ± - | 0.2 ± - | 0.015 |
| 18:0 | 3.7 ± - | 3.4 ± 0.1 | 0.002 |
| 20:0 | 0.7 ± 0.1 | 0.4 ± 0.1 | 0.036 |
| 22:0 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.615 |
| Σ SAT | 22.4 ± 0.1 | 18.0 ± 0.3 | 0.000 |
| 14:1n-5 | 0.2 ± - | 0.1 ± - | 0.186 |
| 16:1n-9 | 0.2 ± - | 0.2 ± - | 0.002 |
| 16:1n-7 | 4.2 ± 0.1 | 2.5 ± 0.1 | 0.000 |
| 17:1n-7 | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.587 |
| 18:1n-11 | 0.5 ± 0.1 | 0.2 ± 0.0 | 0.002 |
| 18:1n-9 | 22.7 ± 0.1 | 31.9 ± 0.3 | 0.000 |
| 18:1n-7 | 3.2 ± - | 3.0 ± - | 0.001 |
| 20:1n-11 | 0.3 ± - | 0.3 ± 0.2 | 0.603 |
| 20:1n-9 | 2.0 ± - | 2.5 ± - | 0.000 |
| 20:1n-7 | 0.2 ± - | 0.2 ± - | 0.000 |
| 22:1n-11 | 0.9 ± - | 0.7 ± - | 0.000 |
| 22:1n-9 | 1.1 ± 0.1 | 1.0 ± 0.1 | 0.223 |
| 24:1n-9 | 0.4 ± - | 0.4 ± - | 0.227 |
| 16:2n-6 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.191 |
| 18:2n-6 | 7.4 ± 0.1 | 10.7 ± 0.1 | 0.000 |
| 18:3n-6 | 0.2 ± 0.1 | 0.1 ± - | 0.390 |
| 20:2n-6 | 0.6 ± - | 0.9 ± - | 0.000 |
| 20:3n-6 | 0.2 ± - | 0.3 ± - | 0.001 |
| 20:4n-6 | 1.1 ± - | 0.8 ± 0.1 | 0.004 |
| 22:4n-6 | 0.2 ± - | 0.2 ± - | 0.419 |
| Σ n-6 | 10.0 ± 0.1 | 13.1 ± 0.2 | 0.000 |
| 16:3n-4 | 0.4 ± - | 0.4 ± 0.3 | 0.483 |
| 18:3n-4 | 0.2 ± - | 0.1 ± - | 0.006 |
| 16:2n-3 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.714 |
| 18:3n-3 | 2.8 ± 0.1 | 4.4 ± - | 0.000 |
| 18:4n-3 | - ± 0.1 | - ± 0.1 | 0.718 |
| 20:3n-3 | 0.2 ± 0.1 | 0.2 ± 0.2 | 0.766 |
| 20:4n-3 | 0.2 ± - | 0.2 ± - | 0.004 |
| 20:5n-3 | 8.2 ± 0.1 | 5.2 ± 0.1 | 0.000 |
| 22:5n-3 | 2.9 ± 0.1 | 1.8 ± 0.1 | 0.000 |
| 22:6n-3 | 14.4 ± 0.2 | 11.5 ± 0.5 | 0.001 |
| Σ n-3 | 28.8 ± 0.3 | 23.3 ± 0.7 | 0.000 |
| Σ EPA/DHA | 22.6 ± 0.2 | 16.7 ± 0.4 | 0.000 |
| n-3/n-6 | 2.9 ± - | 1.8 ± 0.1 | 0.000 |

When P-value < 0.05, the differences are significant.

### C18:1n-9

There were significant differences between dietary treatments on the level of 18:1n-9 in the tissues analyzed. The Control group had highest level of that fatty acid (31.9 - 41.7%), while the Marine+ group ranged from 22.7% to 31.1%. A drop in the level of 18:1n-9 was recorded within the heart with 22.7% and 31.9% for the Marine+ and Control group, respectively. Greater differences were recorded within the liver with 24.4% and 41.6% for the Marine+ and Control group, respectively (Figure 4.3).




| | Muscle | Intestine | Liver | Heart |
|---|---|---|---|---|
| Marine+ | 31.1 | 28 | 24.4 | 22.7 |
| Control | 41.7 | 40.1 | 41.6 | 31.9 |

Figure 4.3 The level of 18:1n-9 in the muscle, intestine, liver and heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error. Different letters indicate significant differences between dietary treatments.

### C18:2n-6

The Control group showed significantly higher content of 18:2n-6 for the muscle (ranged 9% and 12.7%, Marine+ and Control respectively), intestine (ranged 9.5% and 13.5%, Marine+ and Control respectively), liver (ranged 4.8% and 9.3%, Marine+ and Control respectively) and heart (ranged 7.4% and 10.7%, Marine+ and Control respectively). The level of 18:2n-6 within the liver showed greatest differences between the dietary treatments (Figure 4.4).

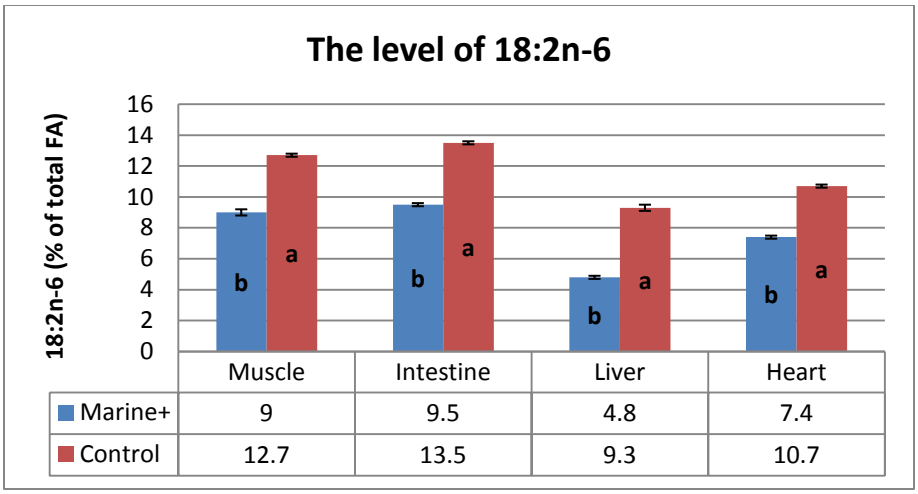



Figure 4.4 The level of 18:2n-6 in the muscle, intestine, liver and heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error. Different letters indicate significant differences between dietary treatments.

### C20:5n-3

The level of 20:5n-3 was significantly highest for the Marine+ group for all tissues analyzed. The greatest differences between dietary treatments on the level of 20:5n-3 was related to the liver, averaging 10.1% and 4.9 % for the Marine+ and Control group, respectively. The heart averaging 8.2% and 5.2%, Marine+ and Control group respectively had lowest difference in the level of 20:5n-3 between the dietary fish groups (Figure 4.5).

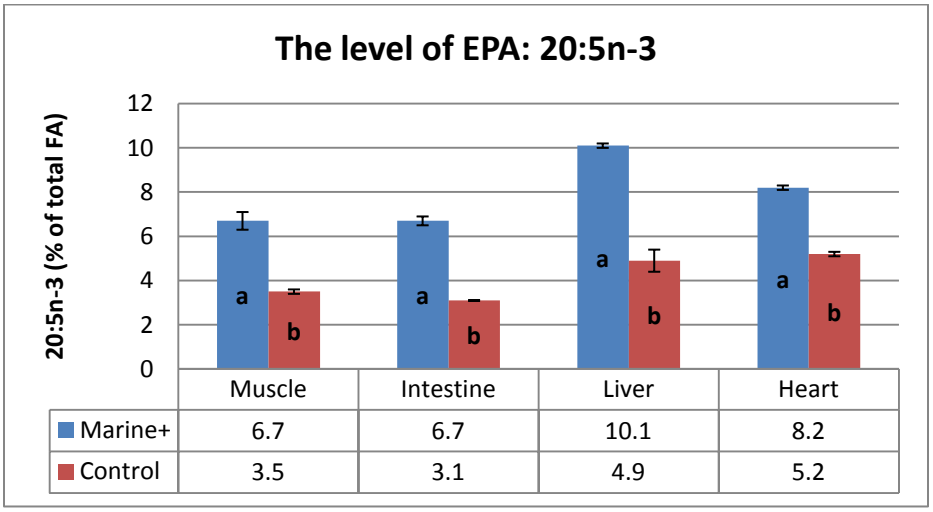



Figure 4.5 The level of 20:5n-3 in the muscle, intestine, liver and heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error. Different letters indicate significant differences between dietary treatments.

### C22:6n-3

This fatty acid showed significant differences between dietary treatments for the muscle, intestine, liver and heart. The level of 22:6n-3 ranged from 7.1% to 17.9% and 3.9% to 11.5%, for the Marine+ and Control group respectively. The liver had highest content of 22:6n-3 relative to the total fatty acid profile with 17.9% for the Marine+ and 9.5% for the Control group. In contrast to the liver, the intestine had lowest level of that same fatty acid averaging 7.1% and 3.9% for the Marine+ and Control group, respectively (Figure 4.6).

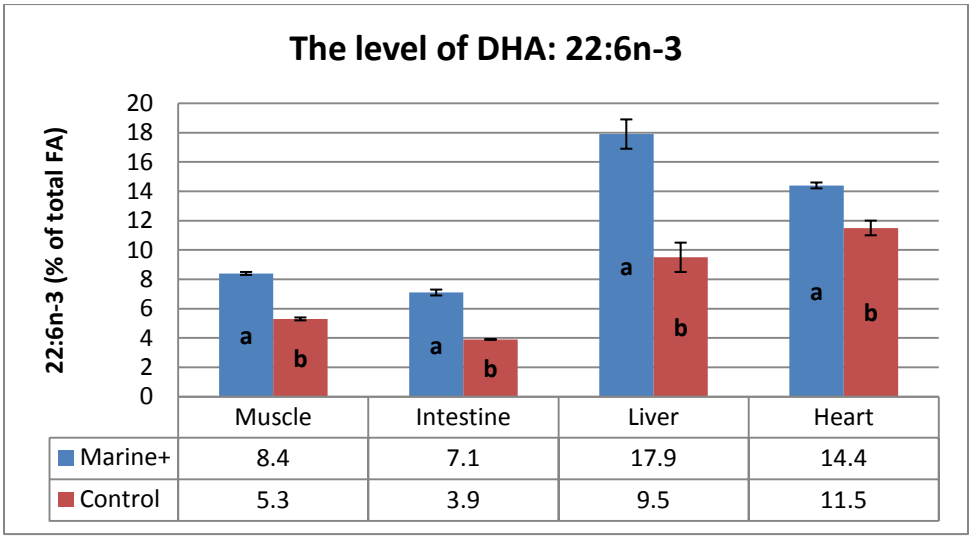



Figure 4.6 The level of 22:6n-3 in the muscle, intestine, liver and heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error. Different letters indicate significant differences between dietary treatments.

### EPA+DHA

The sum of EPA+DHA showed significant differences between dietary treatments for all tissues analyzed. The Marine+ group had higher level of EPA+DHA (ranged 13.8 - 28%) compared to the Control group (ranged 7 - 16.7%). Lowest level of EPA+DHA was recorded within the intestine with 13.8% and 7% for the Marine+ and Control group, respectively. The greatest difference in the level of EPA+DHA between the dietary treatments was recorded within the liver with 28% and 14.4% for the Marine+ and Control group, respectively. The highest level of EPA+DHA was shown for the liver and the heart (Figure 4.7).

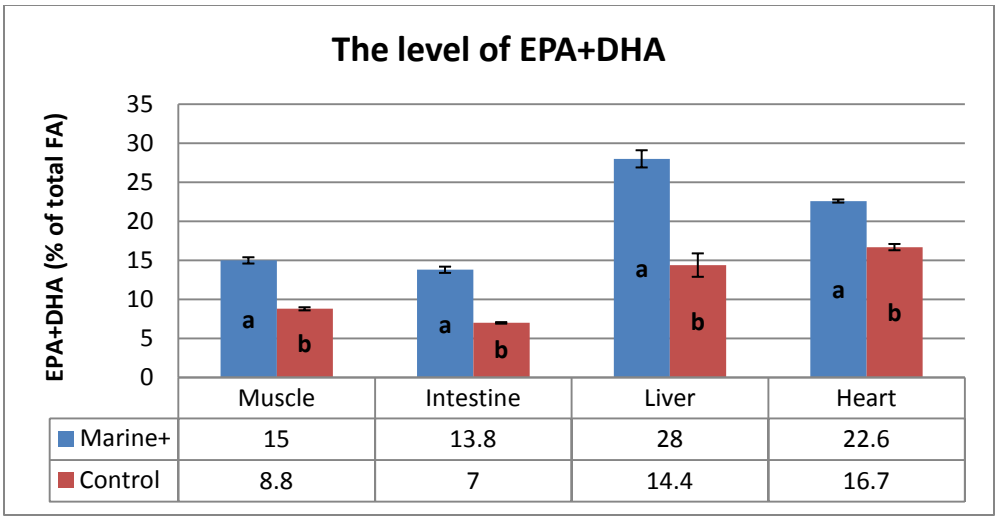



Figure 4.7 The sum of EPA+DHA in the muscle, intestine, liver and heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error. Different letters indicate significant differences between dietary treatments.

### n-3 to n-6 fatty acids ratio

Significant differences on the n-3/n-6 ratio were found between dietary treatments for the muscle (averaging 2 and 1.1 for Marine+ and Control group, respectively), intestine (averaging 1.9 and 1 for Marine+ and Control group, respectively), liver (averaging 3.7 and 1.6 for Marine+ and Control group, respectively) and heart (averaging 2.9 and 1.8 for Marine+ and Control group, respectively). The n-3/n-6 ratio was higher in the Marine+ group than Control group in all analyzed tissues (Figure 4.8).

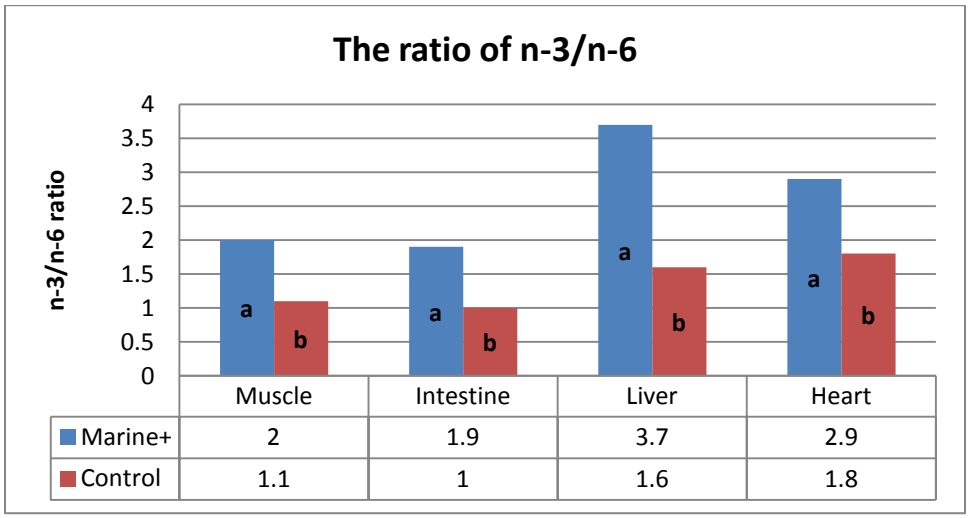



Figure 4.8 The ratio of n-3/n-6 in the muscle, intestine, liver and heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error. Different letters indicate significant differences between dietary treatments.

## 4.7 Total fat content

In addition to visual evaluation of fat on the surface of the tissues, chemical analysis of total fat content were conducted for the muscle, intestine, liver and heart.

### Total fat content of muscle

The chemical analysis of the total fat content of the muscle was 11.4% and 11.1% on average for Marine+ and Control group, respectively. Thus, no significant differences due to dietary treatments were found (Figure 4.9).

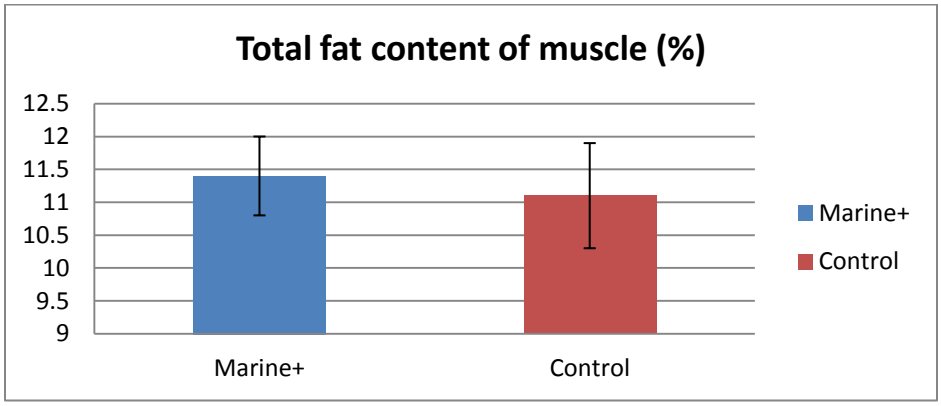



Figure 4.9 Chemical analysis of total fat content in the muscle of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error.

### Total fat content of intestine

The chemical analysis of the total fat content of the intestine showed no significant differences due to the dietary treatments, averaging 56.5% and 55.3% Marine+ and Control, respectively (Figure 4.10).

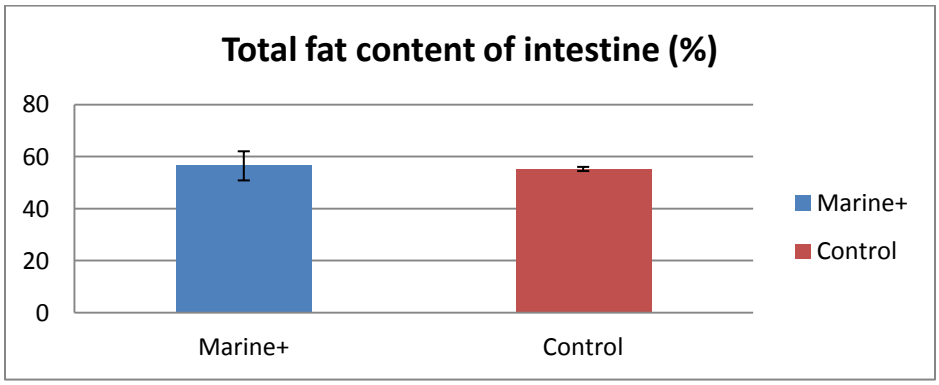



Figure 4.10 Chemical analysis of total fat content in the intestine of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error.

### Total fat content of liver

The liver lipid differed significantly between the dietary treatments, averaging 5.6% and 9.7% for Marine+ and Control group, respectively (Figure 4.11).

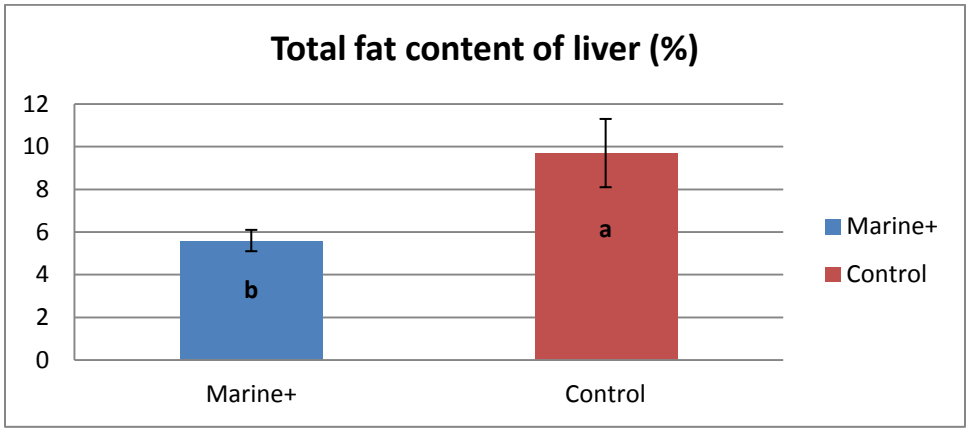



Figure 4.11 Chemical analysis of total fat content in the liver of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error. Different letters indicate significant differences between dietary treatments.

### Total fat content of heart

Total lipids in hearts were not significantly different between the Marine+ (6.9% on average) and Control group (7.1% on average) (Figure 4.12).

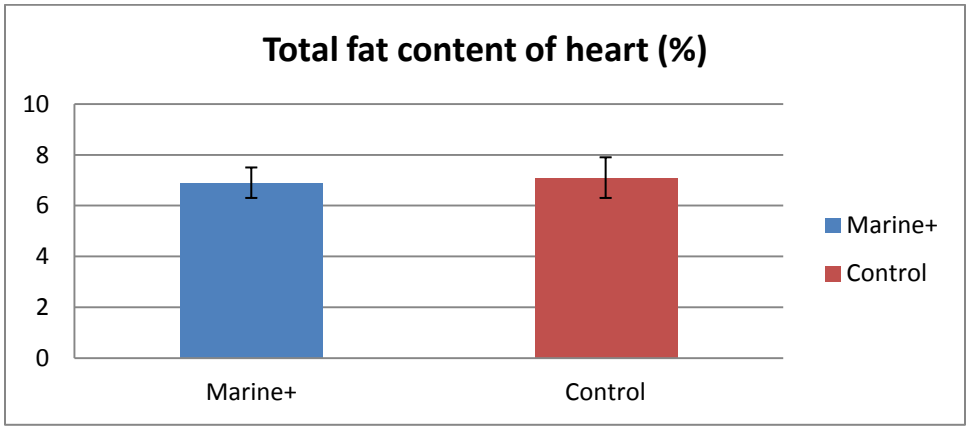



Figure 4.12 Chemical analysis of total fat content in the heart of farmed Atlantic salmon (*Salmo salar* L.) fed different diets during the period August 2011 – March 2012. Results are given as LSmeans ± standard error.

# 5.0 Discussion

## 5.1 Growth performance and FCR

The present study showed that feeding on Marine+, Protein+, and Control diet did not significantly affect weight gain and specific growth rate of salmon (Table 4.1). The non-significant negative effects of feeding with rapeseed oil diet on growth performance in this experiment coincide with studies in salmon (Thomassen and Røsjø, 1989; Bell et al., 2001, 2003; Rosenlund, 2001; Regost et al., 2004; Thomassen et al., 2012), rainbow trout (Geurden et al., 2009), gilthead sea bream (Izquierdo et al., 2005; Fountoulaki et al., 2009), and European sea bass (Montero et al., 2005). However, the numerical values displayed that the final body weight of the Marine+ and Protein+ groups was 126 g and 197 g lower in comparison with the Control group. Scientists have determined that it is possible to replace up to 69% fish oil by plant oils such as rapeseed and soybean oil in long-term trials, without influencing the growth and feed utilization of gilthead sea bream (Fountoulaki et al., 2009). The level of rapeseed oil was therefore within the range of earlier studies. Bendiksen et al. (2011), showed that replacement of fishmeal and fish oil, in which 50% of the dietary oil was of vegetable origin (rapeseed), and further, half of the dietary fish oil was originated from fish processing waste (herring offal silage oil); no significant differences were observed between feed treatments with respect to growth and feed utilization. Feed conversion ratio (FCR) with the same value of 1.1 did not show any significant differences among the dietary treatments. This is similar to previous studies in which dietary fish oil was replaced by rapeseed oil in salmon (Bell et al., 2000, 2003; Torstensen et al., 2008; Bendiksen et al., 2011; Thomassen et al., 2012) and gilthead sea bream (Izquierdo et al., 2005; Fountoulaki et al., 2009 ). Fork length showed significant differences between dietary treatments where the Marine+ group with 77.2 cm was significantly longer than the Control group with 76.5 cm (Table 4.1). These results are in line with an earlier study where salmon fed the menhaden oil had significantly higher final length than fish fed a diet supplemented with soybean oil (Hardy et al., 1987).

## 5.2 Condition factor and Yield

The condition factor (ranging from 1.41 to 1.45) was significantly different due to the dietary treatments. The Protein+ and Control groups had the highest CF compared to the Marine+ group. The normal variation in condition factor for salmon is from 0.7 to 1.9 (Einen & Thomassen, 1998). Significantly lower CF of the Marine+ group coincided with higher fork length. Generally, a higher CF coincides with high visceral fat (slaughter yield) and/or higher

muscularity (fillet yield). In the present study a lower CF of the Marine+ group coincided with significantly lower fillet yield compared with the Protein+ group. Hence, results from present study indicate stimulated length growth but impaired ability of the Marine+ group to synthesize new muscle tissue relative to the growth in body length when compared to the other experimental diets.

The gutted weight ranged from 5.8 kg to 5.9 kg. The gutted weight differed significantly between the dietary treatments where the Marine+ and Protein+ group showed higher gutted weight compared to the Control group, although the whole body weight was similar. Consequently, the overall slaughter yield (range from 88.8% to 89.6%) was significantly higher of the Protein+ group as compared with the Control group. The results therefore indicate that the Control group had lower ability to build new muscle tissue of the ingested feed as compared to the other dietary treatments. In a previous study, the slaughter yield of salmon dropped significantly as the protein content of the diets decreased from 42% to 37% and 35% (Hillestad et al., 1998). In the present study, the protein supplementation was considerably lower (2.5%) than that in the aforementioned study, but the source of protein and processing of the protein ingredient used (acid process gelatin extracted from fish skin) might have contributed to the positive results observed regarding the slaughter yield. Also the protein level in the Control group feed was relatively low (35%) during the last 3-4 months of the trial (Phase 2).

The fillet weight varied significantly between dietary treatments, ranging from 2059 g to 2112 g. The Protein+ group showed the significantly highest fillet weight in comparison with the Marine+ and Control groups. Consequently, the Protein+ group had the highest fillet yield (64.6%) compared to the Marine+ and Control groups with 63.1% and 63%, respectively. The fillet yield of salmon in the present study was higher when compared to Bendiksen et al. (2011) who reported a filet yield of 58% for salmon fed vegetable based diets supplemented with processing by-products. However, Regost et al. (2004) reported that the slaughter yield was significantly higher for salmon fed soybean oil diet than for other treatments, whereas no significant differences were observed on fillet yield. The fillet yield has high economic importance, as fillets are the main and most valuable products of salmon (Gjedrem, 2008).

## 5.3 Organs parameters

Except for the heart, other organs showed differences in index measurements between dietary treatments. The viscera-somatic index (VSI) ranged from 9.2% to 10.2% of the body weight. The Control group showed significantly highest VSI compared to the Protein+ group. The VSI normally comprise 6-12% of the body weight of salmon (Rørå et al, 2001). Panserat et

al. (2009) indicated that the viscera-somatic index was significantly higher with a higher level of perivisceral tissue in rainbow trout fed plant oil diet. This is also in accordance with the previous studies in salmon fed vegetable oil based diet (Nanton et al., 2007; Pratoomyot et al., 2010). On the contrary, the VSI of salmon, averaging 12% was unaffected by diet in a study performed by Pratoomyot et al. (2011). Hepatosomatic index (HSI) and Cardio somatic index (CSI) are defined as the ratio of liver and heart weight to body weight. In this study, significant differences were observed for HSI. The Protein+ and Control groups had significantly higher HSI with 1.13 and 1.12 respectively, compared to the Marine+ group with 1.06. Fountoulaki et al. (2009) reported that hepatosomatic index was significantly lower in gilthead sea bream fed a fish oil based diet than those fed a diet high in rapeseed oil.

The visual scoring of liver color showed significant differences between dietary treatments. The Marine+ group with score 2.3 displayed the darkest color in comparison with the two other groups (score 1.6). Consequently, the frequency of livers with uneven color (liver patches) in the Control group with 42% was significantly higher than the Marine+ group with only 3%. Discolored livers may be an indication of a metabolic imbalance related to dietary oil composition. Higher degrees of dietary fish oil replacement up to 80% in gilthead sea bream feeds appeared to cause altered liver structure (Caballero et al., 2004; Wassef et al., 2009). In summary, the present study suggests that supplementing the salmon diets high in rapeseed oil with gelatin extracted from fish skin permitted similar growth rates and feed efficiency, but stimulated protein growth (less visceral fat and higher muscularity of fillets) and improved fish health related parameters. Replacement of marine proteins and oils with processing by-products and plant alternatives would improve the sustainability of farmed carnivorous fish species, such as salmon (Gatlin et al., 2007; Miller et al., 2008; Tacon & Metian, 2008; Naylor et al., 2009; Turchini et al., 2009, 2011; Crampton et al., 2010; Hardy, 2010; Welch et al., 2010; Stone et al., 2011a, b).

## 5.4 Total fat content

The total fat content of the muscle (range 11.1-11.4%) showed no significant differences due to dietary treatments. This is supported by previous studies that showed no effect on muscle lipid deposition in gilthead sea bream (Fountoulaki et al., 2009) or European sea bass (*Dicentrarchus labrax* L.) (Montero et al., 2005) fed vegetable oil. According to Hillestad et al. (1998), fillet fat content increased as dietary protein decreased.

Visual scoring of visceral fat revealed that the Control group showed significantly highest level of visceral fat with score 2.9, whereas the Marine+ and Protein+ groups had the lowest level with scores 2.4 and 2.5, respectively. These results demonstrated that the apparent lipid in fish can be altered by the feed oil profile. Visual assessment of the visceral fat by Hillestad et al. (1998) on salmon showed an increased level of visceral fat as dietary protein decreased. However, the chemical analysis of the total fat content of the intestine in the present study showed no significant differences due to the dietary treatments (56.5% and

55.3%, Marine+ and Control respectively). The high visceral level of fat is supported by Nanton et al. (2007) who reported that the visceral contained the highest levels of total lipid compared with other tissues. Fountoulaki et al. (2009) reported that dietary vegetable oils increased the level of intestine total fat (perivisceral and peritoneal). This was also confirmed by the presence of lipid droplet accumulations in enterocytes of fish fed vegetable oils, indicating a higher uptake than export rate (Olsen et al., 1999, 2000; Caballero et al., 2002). In addition, this can be explained by the differences in the apparent digestibility of certain fatty acids, partly depending on the nature of the feed oil.

The liver lipid content differed significantly between the dietary treatments (range 5.6% and 9.7%, Marine+ and Control group respectively). According to Bell et al. (2001), the highest lipid levels in liver were found in salmon fed 100% rapeseed oil. This is in accordance with a number of studies which showed that diets did not affect the chemical analysis of intestine fat content, but rather to what was found in the liver (Caballero et al., 2002; Mourente et al., 2005; Pratoomyot et al., 2008). Ruyter et al. (2006) reported that fish fed 100% soybean oil diet had higher accumulation of fat in the liver than fish fed 100% fish oil diet. The authors suggested that, higher fat accumulation in the liver was caused by a selective accumulation of 18:2n-6 and 18:1n-9.

When total lipids in hearts were analyzed chemically, there seemed to be somewhat higher values in the Control group given high rapeseed oil; these differences were, however, not statistically significant (range 6.9-7.1%, Marine+ and Control respectively). This is consistent with the study in salmon (Tomassen & Røsjø, 1989). Nanton et al. (2003) found that the heart of haddock (*Melanogrammus aeglefinus* L.) consisted of 2.5% fat and was not significantly affected by an increase of dietary lipid level.

## 5.5 Fatty acid composition

The replacement of fish oil with rapeseed oil resulted in marked increases in 18:1n-9 and 18:2n-6, and decreases in 20:5n-3 and 22:6n-3 in the diets. This was reflected in the fatty acid contents of the fillets and the intestine, liver, and heart where there were significant increases in 18:1n-9 and 18:2n-6, and significant decreases in 20:5n-3 and 22:6n-3 in the fillets and the organs from fish fed the rapeseed oil diet compared to those fed the fish oil diet. The results coincide with previously observation in fish fed vegetable oil based diets (Caballero et al., 2002; Izquierdo et al., 2003, 2005; Montero et al., 2005; Mourente et al., 2005; Mourente & Bell, 2006; Drew et al., 2007; Pratoomyot et al., 2011).

### Oleic acid (18:1n-9)

In a study performed by Bell et al. (2001), the typical fatty acid representing rapeseed oil, 18:1n-9 was the most noticeable fatty acid dividing salmon fed marine oil from rapeseed oil in muscle and all the organs (Bell et al., 2001). In the present study, the heart had the lowest level of 18:1n-9, while the muscle had the highest. The intestine and liver had values in a range between those of the muscle and heart. The Control group showed the highest level of that fatty acid (31.9-41.7%) while the Marine+ group ranged from 22.7% to 31.1%. Significant differences between dietary fish groups did occur in the muscle, intestine, liver and heart. The Control group did always show the highest level of 18:1n-9 as it was confirmed by earlier studies when fish fed plant oil based diets (Izquierdo et al., 2003; Montero et al., 2005; Mourente et al., 2005; Mourente & Bell, 2006; Ruyter et al., 2006; Torstensen et al., 2008; Fountoulaki et al., 2009). Therefore, the higher 18:1n-9 content in the Control diet was to some extent reflected in the fish. Rapeseed oil and olive oil have moderate levels of 18:2n-6, low levels of 18:3n-3, and high levels of 18:1n-9 which is considered a preferred substrate for energy production (Kiessling & Kiessling, 1993; Henderson, 1996; Caballero et al., 2002; Izquierdo et al., 2005; Mourente & Bell, 2006). Lie (1991) proposed that the relative high level of 18:1n-9, at least when compared to the levels of 18:2n-6, 20:5n-3, and 22:6n-3 might indicate that 18:1n-9 is the end product of the endogenous fatty acid synthesis in cod.

### Linoleic acid (18:2n-6)

The amount of 18:2n-6 was significantly highest in salmon fed the Control diet. Heart and liver showed a lower incorporation of 18:2n-6 compared with the muscle and intestine. In addition, the level of 18:2n-6 within the liver changed more significantly. Pratoomyot et al. (2008) reported that essentially all diets supplemented with vegetable oils will increase dietary 18:2n-6 in comparison to fish oil based diets, and this has been a widely reported observation in salmon tissues, especially liver. In general, fatty acid composition of muscle showed significant increases in 18:1n-9 and 18:2n-6 with inclusion of rapeseed oil (Bell et al., 2001, 2003; Izquierdo et al., 2003; Montero et al., 2005; Mourente et al., 2005; Mourente & Bell, 2006; Ruyter et al., 2006, Torstensen et al., 2008).

### Eicosapentaenoic acid (20:5n-3)

Significant differences between dietary fish groups were seen in the muscle and all the organs. The Marine+ group ranged from 6.7% - 10.1%, while the Control group ranged from 3.1% - 5.2%. The percentage of 20:5n-3 was significantly reduced in the Control group compared to the Marine+ group, which is in accordance with several previous studies (Bell et al., 2001, 2003; Jobling et al., 2002; Izquierdo et al., 2003; Regost et al., 2004; Torstensen et

al., 2004b, 2008; Fountoulaki et al., 2009). The liver (range 10.1% and 4.9%, for the Marine+ and Control respectively) and the heart (range 8.2% and 5.2%, for the Marine+ and Control respectively) stood out compared to the muscle (range 6.7% and 3.5%, for the Marine+ and Control respectively) and the intestine (range 6.7% and 3.1%, for the Marine+ and Control respectively). In line with the current results, Izquierdo et al. (2005), when was examining gilthead sea bream (*Sparus aurata*) recorded that the muscle content of EPA was reduced to a lower level than their reduction in the diet, denoting their importance. The greatest significant difference between dietary treatments on the level of 20:5n-3 was related to the liver (Montero et al., 2005). It seems that 20:5n-3 metabolized in muscle and retained in liver (Izquierdo et al., 2005). This tendency has been also observed in an experiment where salmon was fed increasing dietary inclusion of rapeseed oil (Torstensen et al., 2004a). Moreover, a higher reduction of EPA and lower incorporation may be related to a preferential oxidation of EPA over DHA in those organs (Frøyland et al., 2000; Bell et al., 2001; Montero et al., 2005). This has been indicated in rats as well (Madsen et al., 1998).

### Docosahexaenoic acid (22:6n-3)

This fatty acid showed the same pattern as the EPA between the muscle and the organs. The liver had the highest content of 22:6n-3, ranging from 17.9% to 9.5%, for the Marine+ and Control respectively. In contrast to the liver, the intestine had lowest level of that same fatty acid, ranging from 7.1% to 3.9%, for the Marine+ and Control respectively. A general non-specific retention of fatty acids has been observed in the liver of cod (Bell et al., 1986) and haddock (Nanton et al., 2001) fed oils of varying fatty acid composition. The level of 22:6n-3 was significantly higher for the Marine+ group (ranging from 7.1% to 17.9%) than the Control group (ranging from 3.9% to 11.5%). In the present study, a much greater share was recorded of DHA in the fish compared to the level of EPA. This is similar to experiments performed on gilthead sea bream showing that feeding rapeseed oil reduced muscle contents of DHA and EPA, but reduction of EPA in muscle being more pronounced (Izquierdo et al., 2005). As also it was previously reported, replacement of fish oil with vegetable oil give only modest decrease in muscle DHA (Bell et al., 2001, 2002; Pratoomyot et al., 2010). Therefore, 22:6n-3 was selectively retained in all the analyzed tissues, and particularly with higher retention in the liver, as supported by pervious experiments (Caballero et al., 2002; Izquierdo et al., 2003; Regost et al., 2003; Torstensen et al., 2004b). A high retention of DHA in salmon muscle when reducing the level of that fatty acid in the diet (Bell et al., 2001; Torstensen et al., 2004a) might suggest a protection from a metabolic breakdown. Thomassen et al. (2012) reported that salmon fed rapeseed oil converted more of the EPA to DHA, suggesting an efficient C20 fatty acid elongase activity.

### EPA+DHA

There was significant difference between dietary treatments on the sum of EPA+DHA in the muscle, intestine, liver and heart. The Marine+ group had the highest level of EPA+DHA (13.8-28%), while the Control group had lower content (7-16.7%). The highest level of EPA+DHA was shown for the liver and heart, while the contrary was registered for the muscle and intestine. In general, the proportions of monoenes were significantly higher and those of polyunsaturated fatty acids (PUFA) significantly lower in flesh and the organs of fish fed diets with reduced levels of fish oil, which is consistent with other studies (Izquierdo et al., 2003; Montero et al., 2005; Mourente et al., 2005; Mourente & Bell, 2006; Pratoomyot et al., 2011). The intestine contained a higher proportion of monounsaturated fatty acids as well as a lower proportion of EPA+ DHA polyunsaturated fatty acids compared to the muscle, liver, and heart. This was confirmed in salmon fed vegetable oil based diet from start-feeding until harvest size (Ruyter et al., 2006; Nanton et al., 2007). Several studies have demonstrated that salmon can grow normally on diets containing high levels of plant oils and that they are able to convert 18:3n-3 and 18:2n-6 to their longer chain, highly unsaturated fatty acid (HUFA) products, including EPA, DHA, and 20:4n-6 (Bell et al., 1997; Tocher et al., 2000; Torstensen et al., 2000; Pratoomyot et al., 2008). However, it is possible that the capacity for endogenous production of these HUFA may not fulfill optimal requirements. So that for optimal growth and well being of the fish, some dietary EPA and DHA will be required (Bell et al., 2001).

### n-3 to n-6 fatty acids ratio

The n-3/n-6 PUFA ratio was significantly higher in the Marine+ group compared with the Control group, ranging from 1.9% to 3.7% and 1-1.8% in the Marine+ and Control group, respectively. This is in line with previous studies (Tomassen & Røsjø, 1989; Bell et al., 2001, 2002, 2003; Regost et al., 2004; Torstensen et al., 2004b, 2008). The n-3/n-6 ratio is a good indicator of the nutritional value of fillets for human health. In general, the concerns with plant oil based diets is the low n-3/n-6 ratio, due the increased levels of linoleic acid, the presence of monoene fatty acids, and the low levels of n-3 PUFAs or with more than 18 carbons in the chain (Tomassen & Røsjø, 1989; Bell et al., 2002; Nanton et al., 2007; Torstensen et al., 2008). Montero et al. (2005) reported similar results when the relationship between n-3 and n-6 fatty acids decreased from 4.9 in fish fed fish oil diet to 1.8 in rapeseed oil containing diet. Overall, the effect that substitution of fish oil with vegetable oil has on tissue lipid contents and compositions is dependent upon a number of factors including the specific fish oil or vegetable oil blends used plus other factors including the specific tissue itself and possibly growth stage and/or season as well as the genetic origin of the stock (Pratoomyot et al., 2008).

## 6.0 Conclusion

There were no significant differences in final weight, TGC or FCR due to the dietary treatments. However, the Protein+ and Control group had higher CF compared to the Marine+ group. Significantly lower CF of the Marine+ group coincided with higher fork length and lower fillet yield.

The Protein+ group had significant positive effects on slaughter parameters like gutted weight, slaughter and fillet yield. It was demonstrated that salmon fed the rapeseed oil based diet (Control group) showed significantly highest viscera-somatic index, visual visceral fat, and visual heart fat, whereas these were lowest in the Protein+ group. Frequency of liver patches was higher of the Control group compared with the Protein+ group, and lowest of the Marine+ group.
In summary, supplementation of the rapeseed oil based diet with gelatin extracted from fish skin rendered improved ability to build new muscle tissues rather than stimulating to fat accumulation of the ingested feed. Future studies may reveal whether the positive effects observed for the Protein+ diet were related to the elevated protein level per se, or the nature of the protein source (amino acid composition/availability).

There were higher levels of n-3 fatty acids, such as of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) in the Marine+ group. On the other hand, n-6 fatty acids and C18 fatty acids, such as 18:1n-9 and 18:2n-6 were presented in higher amount in fish feed with the rapeseed oil based diet (Control group). As a result, the n-3/n-6 ratio was higher in the Marine+ group.

To sum up, with the increased use of fishery by-products, for instance as it was shown in the present study, dietary rapeseed oil supplemented with gelatin extracted from fish skin, the aquaculture becomes more sustainable, the prices of fish food decrease, the demand of fish catch to fishmeal is reduced and the fish quality to the consumer is still good.

*The main differences between the experimental diets and the Control feed*

**Marine+ vs. Control**

- Altered fatty acid profile
- Lower condition factor
- Smaller livers
- Darker and more uniform appearance of livers

**Protein+ vs. Control**

- Higher slaughter yield
- Higher fillet yield
- Less fat accumulation in viscera
- Less fat accumulated on the surface of the heart
- More uniforms appearance of livers

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# 8.0 Appendix