MINIMIZING MARINE RESOURCE UTILIZATION IN DIETS OF FARMED ATLANTIC SALMON *(SALMO SALAR)*: EFFECTS ON GROWTH PERFORMANCE AND MUSCLE, LIVER, AND HEAD KIDNEY CHEMICAL COMPOSITION

by:

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ABSTRACT

Due to the limited availability of fish meal and fish oil resources and their high costs in producing aquafeeds for the aquaculture industry, it is important to conduct research on possible replacements that are sustainable sources of protein and lipids. In this study, seven different diets containing various protein and lipid sources were formulated to feed farmed Atlantic salmon, and their effects on growth performance, tissue lipid class, fatty acid, and elemental composition were examined. Growth performance results showed that the diet containing the lowest level of fish meal and fish oil led to the lowest weight gain, followed by the diet with the highest content of animal by-products. The lipid class analysis for the three examined tissues (muscle, liver, and head kidney) showed no statistical difference in the total lipid content using the seven dietary treatments. However, there was a statistical difference between the main lipid classes; triacylglycerols, phospholipids, and sterols. The elemental analysis of the three tissues revealed a higher ratio of carbon to nitrogen (C/N) in head kidney tissues compared to muscle and liver tissues, which consequently resulted in the highest level of total lipids in head kidney tissues. The carbon and nitrogen analysis of the liver tissues showed that diet with 1.4% ω3 long chain fatty acids (ω3LC1.4) resulted in a higher nitrogen concentration and a lower C/N ratio than when other dietary treatments were used. In terms of essential fatty acids, liver and head kidney tissues of fish fed the diet with lowest amount of fish meal and fish oil had the lowest EPA and DHA and the highest ARA levels. Diets with low levels of fish meal and a medium level of fish oil, resulted in as high a level of ω3 fatty acids in the examined tissue as when diets containing high levels of fish meal and fish oil were used.

This study suggests that fish meal can be reduced to 5% without affecting growth as long as there is a minimum of 5% fish oil, and animal by-products do not exceed 26% of diet. It was concluded that reducing fish meal and fish oil to less than 10% in diets of farmed Atlantic salmon

affects growth performance, as well as elemental, lipid, and fatty acid compositions of the muscle, liver, and head kidney tissues.

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ADC: apparent digestibility coefficients

AFI: apparent feed intake

ai: *anteiso*

ALA: alpha linolenic acid

ANOVA: analysis of variance

ARA: arachidonic acid

C/N: carbon to nitrogen ratio

C: carbon

CF: condition factor

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

FAME: fatty acid methyl ester(s)

FCR: feed conversion ratio

hr: hour

HSI: hepatosomatic index

i: *iso*

LC: long chain

LNA: linoleic acid

MUFA: monounsaturated fatty acid

N: nitrogen

NQC: Norwegian quality cut

PIT: passive integrated transponder

ppm: parts per million

PUFA: polyunsaturated fatty acid(s)

SDA: stearidonic acid

SGR: specific growth rate

VSI: viscerosomatic index

Δ: delta

ω: omega

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1. INTRODUCTION AND OVERVIEW

The aquaculture industry is the fastest growing food production sector around the world. Due to the increase in the world population, the demand for seafood has increased, so that aquaculture production is expected to increase in the future as marine capture production has remained almost constant since 1990 at about 90 million tons annually (FAO, 2014). Increasing demand for seafood product means higher consumption of aquafeeds and aquafeed production has been growing exponentially, up to 30% annually. Aquafeeds are the major cost for aquaculture production (50 to 80% of the total cost) (Turchini et al., 2011).

1.1 Primary aquafeeds

Fish meal and fish oil have been the most used ingredients in aquafeed manufacturing as they are excellent sources of lipids and proteins, respectively. From 1995 to 2004, the global consumption of fish meal and fish oil approximately doubled increasing from 1,728,000 to 3,452,000 tons for fish meal and from 494,000 to 893,000 tons for fish oil (Hasan and Halwart, 2009).

The production of fish meal has not changed significantly in the past 30 years. On average, 6.07 million tons of fish meal per year was produced with the lowest production of 4.57 million tons in 1977 and the highest figure of 7.48 million tons in 1994. Fish oil has been produced at much lower levels. The world production of fish oil fluctuated remarkably in recent years with an average of 1.25 million tons. The highest production was in 1987 and 1990 at around 1.6 million metric tons (Tacon et al., 2006).

The aquaculture industry consumes about 68.2% and 88.5% of the world production of fish meal and fish oil, respectively (Tacon and Metian, 2008). While salmonids dominate the consumption of fish oil at 64% (49.7% by salmon and 14.8% by trout), the main consumers of fish meal are marine shrimp (22.4%), marine fish (18.3%), salmon (18%), carp (13.1%), trout (6.6%), freshwater crustaceans (5.3%), and eels (5.1%) (Huntington and Hasan, 2009).

1.1.1 Fish meal

Fish meal is the best diet used for fish providing the required protein and oil source for carnivorous fish species as it contains superior amino acid profile (Hasan and Halwart, 2009). Fish meal contains valuable protein with high digestibility, and is an excellent source of eicosapentaenoic acid (EPA, 20:5ω3), and docosahexaenoic acid (DHA, 22:6ω3) as well as essential vitamins and minerals (IFOMA, 2001). High quality fish meals contain high levels of crude protein (more than 66%) and have a lipid content of 8 to 11% (Kaushik, 2010). Fish meal is a healthy nutritional diet for both fish and terrestrial livestock, which can improve productivity and reduce the use of antibiotics. Fish meal has anti-inflammatory effects, which make fish resistant to diseases (Hasan and Halwart, 2009).

1.1.2 Fish oil

One of the most important factors in the sustainability and development of the aquaculture industry has been the use and availability of fish oil. Fish oil is a valuable primary resource of lipid providing the required and beneficial omega (ω)-3 polyunsaturated fatty acids (PUFA). Aquaculture's high reliance on fish oil has recently

made researchers study alternative lipid sources to reduce the pressure on the consumption of fish oil (Turchini et al., 2010). However, finding sustainable ingredients that could provide the required nutrition for fish have been found to be difficult as these alternative sources are not as nutritious as fish oil (Hixson, 2013).

1.2 Importance of lipids and proteins in aquafeeds

Cultured fish need lipid, protein, energy, vitamins and minerals in their diet for growth, reproduction, and other normal physiological functions. Lipid contributes to the structure of biomembranes (Higgs and Dong, 2000) and has a critical role in providing energy for animal tissues since it is the source of essential fatty acids. In addition, it has an important role in carrying fat-soluble vitamins such as A, D, and K. Studies on essential fatty acids in fish have shown that the need for essential fatty acids differs between species. Lipid is also an important source of energy in carnivorous fish like rainbow trout (*Oncorhynchus mykiss*), eel (Anguilliformes), yellowtail (*Seriola quinqueradiata*) and plaice (*Pleuronectes platessa*) which have limited ability to utilize carbohydrates (Watanabe, 1982). However, it has been reported that excess dietary lipid has negative effects on protein intake and growth and may cause feed pellets to become rancid during storage (Lochmann 1994, 2004).

Marine fish and salmonids require arachidonic acid (ARA, 20:4ω6) and high levels of essential omega-3 fatty acids (20:5ω3, 22:6ω3) as they cannot biosynthesize them easily. However, fish have some ability to supply these essential fatty acids by synthesizing them from shorter chain polyunsaturated fatty acids (PUFA) (e.g. 18:3ω3

and 18:2ω6). Therefore, it is important that high levels of PUFAs are available in the diet (Sargent et al., 2002).

Fish are a great source of ω3 in the human diet. EPA and DHA play an important role in different functions, including nervous system, photoreception, reproduction, as well as in reducing the risk of cardiovascular and inflammatory diseases (Siriwardhana et al. 2012). Therefore, it is important to ensure that these essential fatty acids are provided in fish diets as they contribute to the health of fish and the human consumer. The required level of EPA and DHA in the diet of marine fish is 1 – 2% each (NRC, 2011). The growth, flesh quality, and health of fish are improved as the level of EPA and DHA in the diet increases (Sargent et al., 2002).

Proteins constitute the major part of the organic material in fish tissues (70% of dry weight). Hence, protein content and quality in fish diets play a very important role in producing a high quality fish. For feeds with 20-40% crude protein, the growth performance of fish is directly dependent on the level of protein in the diet as it provides essential amino acids. The optimum dietary protein level depends on several factors, such as the type of species, life stage, water temperature, food consumption level, feeding frequency, and amino acid composition. Fish use amino acids to build the proteins necessary for muscle formation and enzymatic function. However, minimizing the usage of protein is important as it is one of the most expensive components of the feeds.

1.2.1 Lipid biosynthesis

Desaturase and elongase enzymes play an important role in forming longer and more unsaturated fatty acids. The delta (Δ) 9 desaturase enzyme is involved in converting

saturated fatty acids like 16:0 and 18:0 to 16:1ω7 and 18:1ω9 in fish. However, alpha-linolenic acid (ALA) and linoleic acid (LNA) are formed by Δ12 and Δ15 desaturases, the amounts of which are limited in vertebrates. That is why these fatty acids are known as essential fatty acids (Sargent et al., 2002). The essential PUFA (e.g. EPA, DHA, and ARA) are formed through desaturating and elongating ALA and LNA. The more active these enzymes are, the more ability animals have to synthesize essential fatty acids from ALA and LNA (Tocher, 2003). The activity level of the desaturase and elongase enzymes depends on the availability of EPA, DHA, and ARA in their natural diet. Due to the low content of EPA and DHA in the natural diet of freshwater fish, their ability to biosynthesize EPA and DHA from ALA is superior to carnivorous marine fish. This is due to the fact that the natural diet of marine fish already contains high level of EPA and DHA, so they do not need to convert ALA and LNA to DHA and EPA (Sargent et al., 2002). Consequently, since anadromous fish such as Atlantic salmon spend only a period of their life in fresh water, their ability to synthesize EPA and DHA is restricted (Sargent et al., 2002). However, C_{18} PUFA, ALA or LA are essential fatty acids required by freshwater and diadromous fish species (Tocher, 2010).

1.3 Alternative lipid sources in aquaculture feeds

The increasing need of fish meal and fish oil has resulted in higher prices of these sources. The high costs as well as the unsustainability of these sources have brought a need to use alternative sustainable sources. In order to reduce the cost of producing aquafeeds, fish meal or fish oil have been replaced with less expensive sources of lipid and protein such as terrestrial plants and animals.

1.3.1 Terrestrial plant lipid sources

Aquafeeds contain oil and fat to meet the essential fatty acid and energy requirements of farmed marine species. While fish oil has been the main raw material used to provide the required lipid (Sargent et al., 2002), there has recently been an interest to replace fish oil with alternative sustainable sources such as plant oils. While the production of fish oil has not changed significantly over the past few decades, plant oil production has been growing remarkably. Due to this expanding production, reduced availability of marine feed raw material, and relatively high prices of alternative lipid sources rich in ω3 PUFAs, fish oils are increasingly being replaced by ingredients from terrestrial plant sources in the feeds for farmed animals (Turchini et al., 2009).

Plant oils, like fish oil, can provide energy for fish growth (Bell et al. 2001, Stubhaug et al. 2007). Therefore, some plant oils could be great alternatives to reduce the consumption of fish oil as they are sustainable sources that are readily available at a lower price. However, one problem that limits the full replacement of fish oil with plant oils is the poor level of ω3 fatty acids. In general, plant oils contain high levels of ω6 and ω9 fatty acids. Plant oils can provide the same relative ratios of saturated fatty acids, monounsaturated fatty acids (MUFA) and PUFA as found in fish oil, but not the level of highly unsaturated fatty acids (HUFA) in fish oil (Torstensen et al. 2005; Francis et al. 2007b). Nonetheless plant oils can replace a major amount of fish oil in the diets (Jobling et al., 2008; Bell et al., 2010; Torstensen et al., 2008; Turchini et al., 2011) with no significant influence on the fish growth as long as sufficient levels of essential fatty acids are provided in the diet (Turchini et al., 2009).

Depending on fatty acid composition, terrestrial plant sources can be divided into four groups, including oils with high level of saturated fatty acids (palm and coconut), ω6 PUFA (soybean, sunflower, and corn), MUFA (rapeseed, canola, peanut, and olive), and ω3 PUFA (linseed and camelina).

Studies investigating mitochondrial-oxidation suggested that saturated fatty acids and MUFAs are preferred over PUFA for energy production in fish (Henderson, 1996). When selecting potential plant oils to substitute for fish oil in fish diets, energy availability as well as PUFA content must be considered. To minimize any reduction in growth rate, and nutritional quality in terms of the health benefits of farmed fish to human consumers, potential substitutes for fish oil should avoid excessive deposition of 18:2ω6, retain high levels of ω3 HUFA and provide sufficient energy in the form of saturated and MUFAs (Bell et al. 2002).

1.3.1.1 Palm oil as a plant oil source rich in saturated fatty acid

One of the most cost-effective and sustainable sources of plant oil is palm oil as it provides a rich source of antioxidants such as vitamin E, consisting of tocopherols and tocoterienols. Palm oil contains 48% saturated fatty acids and low concentrations of PUFAs, which make them resistant to oxidation. However, there are disadvantages in using palm oil in fish diets. For example, due to the high melting point of palm oil because of its high saturated fatty acid content, the fatty acid digestibility and subsequent energy availability are low in fish fed diets containing palm oil. This problem becomes more severe in winter due to the low water temperature.

Using palm oil in the diets of herbivorous species which need lower amounts of ω3 LC-PUFA is feasible (Turchini et al., 2009). However, the use of palm oil in the diets of marine carnivorous fish species like Atlantic salmon and humpback grouper (*Cromileptes altivelis*) has also great potential as long as the fish are fed a fish meal based diet as it contains higher level of ω3.

Inclusion of a high level of palm oil in the diet can result in a lower concentration of LC-PUFA in fish fillets. Bell et al. (2002) revealed that increased use of palm oil in fish diets significantly reduced concentrations of EPA in Atlantic salmon. However, DHA concentrations reduced markedly only when 100% of fish oil in the diet was replaced with palm oil. Therefore, Bell et al. (2002) suggested diets could be formulated with up to 50% replacement of fish oil with palm oil, to avoid a marked decrease in EPA and DHA levels.

Crude palm oil is a potential candidate for aquafeeds in that it has relatively low levels of 18:2ω6 and an abundance of 16:0 and 18:1ω9 (Ng 2002a, b). The use of crude palm oil in the diets of Atlantic salmon and rainbow trout successfully provided growth and a feed utilization efficiency comparable with fish fed equivalent levels of marine fish oil (Torstensen et al., 2000; Rosenlund et al., 2001; Bell et al., 2002; Caballero et al., 2002).

1.3.1.2 Soybean as a plant oil source rich in ω6 PUFA

Soybean, corn, safflower, cottonseed, and sunflower oils are known as ω6 polyunsaturated fatty acid-rich plant oils since they contain high level of 18:2ω6, but low levels of 18:3ω3. Soybean is categorized as an oilseed as it contains a high level of lipid

(Lim et al., 2008). More than 57% of oilseed production is from soybean. In addition, 84 and 86% of global oilseed exports and imports are for soybean (soyatech, 2009). The 18:2ω6 and 18:1ω9 are the most abundant fatty acids in soybean with concentrations of 48-58 and 19-30%, respectively; however, 18:3ω3 is only 4-10% of fatty acids (Spencer et al., 1976). Although oils rich in ω6 PUFA have less ω3 PUFA, when replaced with fish meal or fish oil at a concentration below 80-100% in fish diets, the results showed no change in animal weight gain in most of the cases (Lim et al., 2008).

1.3.1.2.1 Utilization of soybean oil by salmonids

Soybean oil is rich in ω6 fatty acids and relatively rich in ALA. Due to its fatty acid composition, researchers have studied the use of soybean in diets of salmonids such as rainbow trout (Cho et al., 1974; Reinitz and Yu, 1981). Hardy et al. (1987) partially replaced fish oil with soybean oil in the diet of Atlantic salmon and found no change in growth performance, fatty acid composition, or sensory attributes. Grisdale-Helland (2002) concluded that full replacement of fish oil with soybean oil resulted in good growth with high feed efficiency in Atlantic salmon. The replacement results did not show any detrimental effect on the fish health.

Greene and Selivonchick (1990) suggested that using soybean in the diet of rainbow trout did not change the growth performance or feed efficiency, however; the fatty acid profiles in fish flesh were modified. In another study, Regost (2001) replaced fish oil with soybean oil in the diet of brown trout (*Salmo trutta*) and studied the effects of replacement. The results showed no change in growth or feed efficiency, whereas the replacement changed fish flesh fatty acid profiles. These changes in fatty acid profiles

can be compensated for through feeding a finishing control (fish oil-based) diet. This is due to the fact that fatty acid composition of fish normally shows the fatty acid content of the diet.

1.3.1.3 Rapeseed (Canola) oil as a plant oil source rich in MUFA

It is claimed that as long as essential fatty acid requirements are met, fish oil can be substantially replaced by MUFA-rich alternative sources. Rapeseed oil is one of the best candidates to replace fish oil because it contains a high portion of MUFA, varying between 55 and 72% of total fatty acids (Turchini and Mailer, 2011). Among MUFAs, oleic acid (18:1ω9) is easy to digest, a good source of energy, and resistant to oxidation. In addition, MUFAs have some health benefits to humans, such as reducing low-density lipoproteins, progression of atherosclerosis, and blood pressure. Other MUFA-rich plant oils are olive oil, avocado oil, peanut oil, and rice bran oil. MUFAs seem to be the next suitable alternatives to ω3 PUFAs since they have some favourable characteristics such as being liquid at room temperature, being resistant to thermal treatments, and not prone to oxidation. MUFAs cannot be bioconverted to essential fatty acids like LA, ALA, and ω3 long-chain PUFAs, so the main reason for using plant oils rich in MUFAs is their ability to provide the required energy (Turchini and Mailer, 2011).

Bell et al. (2001) evaluated the effects of replacing fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) on tissue lipid compositions and hepatocyte fatty acid metabolism. The experiments were conducted over a 17-week period based on five different levels of fish oil replacement in diets, including 0% (control diet), 10, 25, 50, and 100%. The results showed no effect on growth performance, feed efficiency, and

histopathological lesions in the samples of liver, heart, muscle, or kidney. Fish oil diets resulted in high concentrations of lipids and low levels of protein in the muscle. However, high lipid levels occurred in the liver in fish fed 100% rapeseed oil. The result of muscle lipid fatty acid composition analysis revealed that 18:1ω9, 18:2ω6, and 18:3ω3 all increased by the increase in the replacement level. Increase of rapeseed oil in the diet, however, considerably reduced total saturated fatty acids and 20:5ω3 and 22:6ω3. The increase in replacement level also increased the liver fatty acid desaturation and elongation activities. Bell et al. (2001) suggested that rapeseed oil can be a great candidate for fish for replacing fish oil in the diet of Atlantic salmon. However, replacement at 50% or higher significantly reduces the concentrations of 20:5ω3, 22:6ω3, and the ratio of ω3 to ω6 in the muscle making it less beneficial for humans.

1.3.1.4 Plant oils rich in ω3 PUFA

Plant oils such as linseed, camelina, perilla, and echium oils are PUFA-rich sources which are good candidates to replace fish oil in aquafeed but the total production of these oils is low in comparison with other terrestrial oil sources. They are great sources of high 18:3ω3 and/or stearidonic acid (SDA, 18:4ω3) the ω3 to ω6 ratio is high, and there can be subsequent bioconversion to ω3 LC-PUFA in farmed aquatic species. It has been possible to replace 100 and 60-70% of fish oil for freshwater and marine species with minimum effects on growth performance. However, reduction in the level of health-promoting EPA and DHA was noticed because of the replacement of fish oil with ω3-rich plant oils /or plant oil blends (Turchini et al., 2010)

1.3.1.4.1 Utilization of the ω3 PUFA-rich plant oils in aquafeeds

Replacement of fish oil with plant oil has negative impacts on fatty acid composition and nutritional quality (Sargent et al., 2002; Bell and Waagb, 2008) unless it is rich in ω3 PUFA which are required for EPA and DHA synthesis in freshwater and diadromous species.

1.3.1.4.1.1 Linseed oil (LO)

Linseed oil is one of the major plant oils capable to replace fish oil in aquaculture fish diets. Some of the characteristics which make linseed oil unique include its high level of 18:3ω3 (Popa et al., 2012) and its lack of ω3 long-chain PUFAs such as EPA and DHA.

In Atlantic salmon parr fed diet in which 100% of fish oil was replaced with linseed oil for 19 weeks prior to seawater transfer, there were no effects on growth (Tocher et al., 2000). However, after seawater transfer, when fish were fed standard fish oil diets, the fish previously fed linseed oil were slightly but significantly smaller after 15 weeks in seawater. In Atlantic salmon smolts (initial weight, 127 g) in seawater fed graded increases in linseed oil up to 100% replacement of fish oil for 40 weeks, there was no significant effect of linseed oil substitution on final weights (1.8-1.9 kg), specific growth rate (SGR, 0.94-0.98), feed conversion ratio (FCR, 1.3-1.4), flesh lipid, or astaxanthin levels (Bell et al., 2004). In a very similar trial, graded increases in LO for 12 weeks had no significant effect on final weight, condition factor, hepatosomatic index (HSI), viscerosomatic index (VSI), or muscle and liver lipid contents in Atlantic salmon (Menoyo et al., 2005). In a trial using Atlantic salmon smolts of 130 g initial weight and grown for 16 weeks on 100% linseed oil, slightly but significantly lower final weights

were obtained with fish fed linseed oil compared to fish fed fish oil, but this was not reflected in SGR obtained for individually passive integrated transponder (PIT) tagged fish (Leaver et al. 2008). Dietary linseed oil had no effect on FCR, HSI, VSI, or condition factor. The linseed oil diet significantly increased the relative percentage of protein, reduced whole-body lipid, and increased the protein: lipid ratio. Similarly, rainbow trout fed semi-purified diets containing 100% linseed oil as the lipid source for 72 days had a slight but nonsignificant decrease in growth performance in comparison to fish fed the control diet, with no effect on SGR and FCR (Turchini and Francis, 2009). In Nile tilapia *(Oreochromis niloticus*) fed a diet with linseed oil replacing 100% of the dietary fish oil for 20 weeks, there were no significant effects on growth performance, with no differences in final weights, SGR, and FCR, and no effect on flesh lipid content (Karapanagiotidis et al., 2007). No significant differences in final weights, SGR, or FCR were observed in Eurasian perch (*Perca fluviatilis*) fed 33% LO for 11 weeks compared to freshwater fish, Murray cod (*Maccullochella peelii peelii*), feeding 100% linseed oil in combination with semi-purified diets for 12 weeks resulted in lower growth as determined by lower final weights and SGR (Francis et al., 2006). Likewise, a later study on juvenile Murray cod by the same authors demonstrated similar negative effects of graded dietary linseed oil inclusion (25, 50, 75, 100%) on fish growth when data were subjected to linear regression analysis (Francis et al,. 2007a).

Regost et al. (2003) studied the effects of replacing fish oil with soybean or linseed oil with a return to fish oil on growth performance and lipid metabolism of turbot (*Psetta maxima*). Three experimental fish meal-based diets were formulated, which

contained either 9% of added control diet (fish oil) soybean oil, or linseed oil. Turbot grown in seawater was fed these experimental diets once per day over a 13-week growth trial period. The fish were then fed fish oil for 8 weeks. Although the growth was high, the results of the experiments conducted by Regost et al. showed a small decrease in the growth of turbot fed plant-based diets, compared to the ones fed control diets. The use of different dietary lipid sources did not affect turbot body composition, feed efficiency (wet weight gain/dry matter intake) and protein efficiency (wet weight gain/crude protein intake). The results also revealed low levels of total lipid content in the muscle of turbot (less than 2%), where ventral muscle was fatter than dorsal muscle. The analysis of liver and muscle showed high levels of 18:2ω6 in the fish fed soybean oil diets, and high levels of 18:3ω3 in the ones fed linseed oil diets. Compared to the fish fed control diet, the use of soybean oil and linseed oil in the diets resulted in lower 20:5ω3 and 22:6ω3 levels in liver and muscle samples. Fatty acid profiles in liver and muscle of turbot reflected fatty acid profiles of the experimental diets. In terms of fatty acid profiles, the effects of plant-based diets initially used in the 13-week growth period were still present, even after two months. Finally, after 8 weeks of feeding fish oil, the levels of 18:2ω6 and 18:3ω3 fatty acids decreased significantly, whereas EPA and DHA levels increased remarkably. Regost et al. concluded that a final 8-week period of feeding fish oil is not enough to result in the same fatty acid profile as the turbot fed fish oil over the whole experimental span.

Bell et al. (2004) replaced fish oil with increasing levels of linseed oil to modify the flesh fatty acid composition in Atlantic salmon. To conduct the experiments, five

groups of Atlantic salmon were fed diets containing a blend of capelin oil (control diet) and different levels of linseed oil. These diets were fed to fish for 40 weeks, after which a finishing diet consisting of only fish oil as the added oil was fed for a 24-week period. The results of the dietary treatment showed no change in flesh lipid content and fish growth. However, the fatty acid compositions of flesh lipids were linearly correlated with the fatty acid composition in the diets. Using 50% linseed oil in the diet reduced DHA and EPA concentrations in fish flesh, compared to when the control diet was fed. The complete replacement of fish oil with linseed oil reduced DHA and EPA levels even further. The results also revealed that after 16 weeks of using control diet as a finishing diet, DHA and EPA concentrations were boosted up to 80% of the values when the diet consisted only of fish oil throughout. Up to 50% replacement of added fish oil with linseed oil in the diet resulted in flesh DHA and EPA levels above the recommended values. Bell et al. (2004) suggested that the replacement of fish oil with linseed oil in the diet of Atlantic salmon was feasible. However, the resulted reduction in DHA and EPA values should be largely compensated by using a finishing diet consisted of only fish oil.

1.3.1.4.1.2 Camelina oil

Among the plant oils, camelina oil is distinguished by its high level of total lipid (around 40%) and 18:3ω3, around 30% of the total fatty acid (Eidhin et al., 2003). The high content of MUFAs and ω3 PUFA in camelina oil, as well as its high level of tocopherol (a potent antioxidant) makes it unique compared to other plant oils and a good candidate for the replacement of fish oil (Eidhin et al., 2003).

Hixson et al. (2013) evaluated the effect of replacing fish oil with camelina (*Camelina sativa*) oil on growth performance, lipid class, and fatty acid composition of farmed juvenile Atlantic cod (*Gadus morhua*). Fish was fed with three different diets for a 9-week period, and the liver and muscle samples were collected for the experiments. The diets included one control formulation, and two different levels of replacement of fish oil with camelina oil (40 and 80%). In terms of growth performance, no effect was seen as up to 80% of fish oil was replaced with camelina oil in the diet. However, 80% replacement of fish oil with camelina oil increased the lipid amount, including both triacylglycerol and total neutral lipid. The 80% replacement level in the diet also reduced the total PUFAs and increased the level of MUFAs in the muscle of Atlantic cod, compared to the control diet and 40% replacement level.

The results also revealed lower levels of ω3 fatty acids and higher levels of ω6 fatty acids in the fish fed diets in which 80% of fish oil was replaced with camelina oil, in comparison to when 40% of fish oil was replaced with camelina oil as well as when control fish oil diet was used. In terms of lipid requirements, Hixson et al. (2013) observed promising results with the replacement of fish oil with camelina oil. The replacement, however, reduced the essential LC-PUFAs in both liver and muscle samples.

To expand their previous study, Hixson et al. (2014) fully replaced fish oil with camelina oil and compared the growth and lipid composition of Atlantic salmon, rainbow trout, and Atlantic cod. The experimental diets were used for a 16-week period for Atlantic salmon, a 12-week period for rainbow trout and a 13-week span for Atlantic cod.

While the growth performance of Atlantic cod was significantly affected by the replacement of fish oil with camelina oil, there was almost no change in growth performance of Atlantic salmon. In addition, the fatty acid profiles of Atlantic salmon fed camelina oil were different from the profiles of Atlantic cod fed camelina oil. The results of regression analysis showed linear relationships between the tissue concentration and dietary concentrations of DHA, EPA, ALA and LA. To estimate the biosynthesis capacities of the experimental fish fed camelina oil-based diets, a fatty acid mass balance method was used. The results of the method revealed 25 and 23% desaturation and elongation ability for Atlantic salmon and rainbow trout, respectively, of ALA to long chain ω3 PUFA. However, Atlantic cod showed a considerably weaker ability (6.1%) to elongate dietary ALA. Using transgenic camelina oil with high levels of DHA and EPA may make it a more eligible candidate for fish oil replacement for fish (Betancor et al., 2015, 2016).

1.3.2 Terrestrial animal lipid sources

Terrestrial animal fats are less expensive than plant and fish oil and rich in saturated fatty acids. However, some of them are rich in MUFAs and PUFAs. One benefit of animal fats over plant oils is that they contain higher levels of ω3 HUFA (Moretti and Corino 2008). The extent of digestibility in fish depends on the melting point of the lipid sources used in the diet; the lower the melting point, the higher the digestibility (Turchini et al., 2009). Studies in the past showed that animal fats are poorly digested and growth performance of fish fed diets containing animal fat was inferior (Cho and Kaushik, 1990);

however, inclusion of animal fats at levels of 50% or less does not affect growth performance significantly if the essential fatty acids are provided (Turchini et al. 2003).

Digestibility of animal fats depends on different fatty acid profiles and different water temperature. Studies have suggested that the digestibility of diets containing fish and plant oils which have higher levels of ω3 and ω6 PUFA is 6% better than diets with rendered animal fats which contain saturated fatty acids. The apparent digestibility coefficients (ADC) of fish oil and plants oils (rapeseed, soybean, and linseed) remained approximately unchanged but high over the water temperature range of 5 to 15˚C (Cho and Kaushik 1990,) while the ADC of animal fats (lard and tallow) were significantly lower, but increased as water temperature increased. ADC of animal-derived fats is more affected by water temperature in comparison with fish oil and plant oils. In a similar study, Austrenge et al. (1979) indicated that there were no significant differences in the lipid digestibility of diets with soybean oil and fish oil which contain high amounts of PUFA at 3 to 11˚C to rainbow trout. These studies indicated that low water temperature significantly reduced digestibility of lipid sources rich in MUFA and PUFA (Olsen et al., 1998; Ng et al., 2003).

Several studies have shown that replacing fish oil with terrestrial animal fats did not have any significant effect on carcass yield and proximate composition of fish (Hardy et al., 1987; Greene and Slivonchik, 1990; Craig and Gatlin, 1995; Reigh and Ellis, 2000; Luzzana et al., 2003; Xue et al., 2006; Bureau et al., 2008). There is a direct relationship between diets rich in saturated fatty acids (animal fats) and the amount of saturated fatty acids in fish bodies (Greene and Selivonchick, 1990; Hoffman and Prinsloo, 1995).

Replacement of fish oil with rendered animal fats may result in products with different fatty acid profiles in fish tissues. The replacement also decreased the amount of ω3 LC-PUFA (Hoffman and Prinsloo, 1995). In some cases, inclusion of animal fats in fish diets affected the taste and other sensory attributes of the fish flesh which is another concern about replacement of fish oil with animal fats (Turchini et al., 2003, 2004). In order to successfully use terrestrial animal fats as alternative sources of lipid, fish diets should contain no more than 30-50% terrestrial animal fats. There should also be a sufficient level of ω3 and/or ω6 PUFA to meet essential fatty acid requirements of fish. Significant inclusion of MUFA for better digestibility of lipids has also been recommended (Turchini, 2010).

1.4 Alternative protein sources in aquaculture feeds

A major challenge for aquaculture is reducing consumption of fish meal, as well as increasing ingredient diversity, due to the limited sources and high cost of fish meal, and more focus is now being placed on production of plant-protein feeds (e.g., Glencross et al., 2005; Gatlin et al., 2007; Naylor et al., 2009). Lim et al. (2008) found that plant-protein concentrates are better alternatives than plant-protein meals for the replacement of fish meal. Several plants including canola, soy, pea, barley, and rice have been examined with different results (Thiesses et al. 2003; Barrows et al., 2007; Lim et al., 2008; Gaylord and Barrows, 2009).

1.4.1 Terrestrial plant protein sources

Protein ingredients are defined as those feedstuffs used in animal diets whose levels of crude protein are equal to or higher than 20% (NRC, 1969). Different plant

ingredients are grouped in this category and have been commonly used as main or complementary sources of protein in animal diets. Many of these protein sources are by-products of other industries, such as the oilseed meals obtained after extracting oils from the seeds. The leaves or seeds of plants can be processed directly as plant protein meals. Protein concentrates are also used and are obtained after removing some of the non-protein components from protein meals (Li et al., 2000; Hertrampf & Piedad-Pascual, 2000).

Torstensen et al. (2008) replaced fish meal and fish oil with plant meal and vegetable oil blends to make a sustainable diet for Atlantic salmon, meeting the nutritional requirements. Atlantic salmon smolts were fed either a control diet or one of the three plant-based diets during a 12-month period. At the highest level of replacement, 80% of the fish meal was replaced with a blend of plant proteins and krill meal, and 70% of the fish oil was replaced with a blend of vegetable oils. This was followed by feeding moderate plant-based diets, where half of the fish meal was replaced with plant proteins and fish oil was replaced with vegetable oils at a maximum level, or *vice versa*. They assessed Atlantic salmon performance by measuring mortality, feed intake, and digestibility. Among all experimental groups, the growth rate for combined high level replacement was significantly lower. Also, the final weights of fish were 17% lower for combined high level replacement and 9% lower in high plant protein and moderate vegetable oil replacement, compared to the control diet groups and moderate plant protein groups. At maximum replacement levels, 2 kg salmon protein were produced per

kg fish meal protein fed. This suggested four times greater efficiency in terms of fish meal consumption at 80% replacement level with plant proteins.

Rapeseed not only is well known for its oil but also is a rich source of protein. The seed meal left after the oil extraction is almost 40% by weight. Rapeseed products are also some of the richest sources of amino acids among plant alternatives. Rapeseed meals and concentrates both have better protein than soybean meal in terms of quality. The protein quality of rapeseed meals and concentrates is comparable to that of fish meal. High levels of methionine and cysteine make rapeseed products a good source of sulfur-containing amino acids. However, Higgs et al. (1996) found that rapeseed proteins show similar quality to soybean products due to minimal levels of lysine and sulfur-containing amino acids.

Studies on fish fed rapeseed products-based diets show that the reduction in growth performance is associated with different kinds of fish species due to the decrease in diet intake or feed efficiency. Burel et al. (2000) studied trout and turbot fed diets with rapeseed meal at 30-50 and 30-46%, respectively. Trout growth was affected by high levels of rapeseed meal due to reduction in feed efficiency. Reduction in turbot growth was related to reduction in diet intake. The reduction in growth performance of fish fed high levels of rapeseed meals (40%) is mostly related to a decrease in feed efficiency rather than diet intake. However, Kissil et al. (2000) evaluated the effects of diet with 75% rapeseed meal on gilthead seabream (*Sparus aurata*) and observed a reduction in growth performance as a result of reduction in diet intake probably due to palatability.

1.4.2 Terrestrial animal protein sources

Generally, carnivorous species prefer replacements of animal origin rather than plant sources due to palatability. One of the animal protein sources widely used in fish diets is animal by-products, which have several advantages making them more appealing, including being free from antinutritional factors such as phytic acid, phosphorus, or indigestible complex carbohydrates. Furthermore, they contain low amounts of carbohydrates, and are high in crude protein and crude lipids, as well as vitamins such as B_{12}, and trace minerals such as iron, cobalt, and selenium (Francis et al., 2001; NRA, 2003).

In addition to the good nutritional value of poultry by-product meal and hydrolyzed feather meal, they have a very competitive cost advantage over fish meal. Cost per unit protein is typically 25 and 60% lower for poultry by-product meal and feather meal than fish meal (Lim et al., 2008).

1.5 Lipid oxidation

One of the critical problems in oil formulation in diets is lipid oxidation. Lipid oxidation is a process in which free radicals cause unsaturated fatty acids to react with molecular oxygen and form acyl hydroperoxides. High levels of PUFAs in oils make them susceptible to oxidation (Koshio et al., 1994). In contrast, more saturated shorter-chain fatty acids in oils are less affected by oxidation. The presence of catalysts, such as light, heat and enzymes, makes lipids vulnerable to oxidative processes, and causes loss of essential amino acids and fat-soluble vitamins (Shahidi and Zhong, 2005). Therefore, feeds are susceptible to oxidation under different storage conditions. Moreover, feeds and

pellets are usually prepared by steam or extrusion processes which also makes them vulnerable to lipid oxidation (Koshio et al., 1994). It is important to know how fish would be affected by consuming oxidized diets. Hence, some studies have focused on the effects of using oxidized oil in aquafeeds on lipid composition and growth performance of fish.

Zhong et al. (2007) evaluated the effects of oxidized dietary oil and vitamin E supplementation on lipid profiles and oxidation of muscle and liver of juvenile Atlantic cod. Fish were fed four experimental diets including fresh or oxidized fish oil with or without vitamin E (α-tocopherol or mixed tocopherols) for nine weeks. Except for sterols, no significant changes in lipid classes were observed in liver and muscle. Fish fed diets with oxidized oil but without vitamin E, had increasing sterols in their liver and decreasing sterols in their muscle. The fatty acid composition of muscle was affected differently by experimental diets. They observed that fish fed diets containing oxidized oil had a reduction of α-tocopherol deposition in their liver. They also observed that mixed tocopherols had better performance as an antioxidant in comparison to α-tocopherol (Zhong et al., 2007).

The objective of this thesis is to study how using alternative diets with low content of marine resources can affect growth performance, and muscle lipid class and fatty acid composition in farmed Atlantic salmon. This study also evaluates the effects of using these diets on the lipid class, fatty acid, and elemental compositions of liver and head kidney tissues of farmed Atlantic salmon.

1.6 Co-Authorship Statement

I am the first author on all chapters included in this thesis. I assisted in sampling all the tissues used in this study and completed all laboratory analyses (excluding carbon and nitrogen analyses in Chapter 3). I also conducted all statistical analyses of the experimental data.

Dr. Chris Parrish assisted in identifying the research questions in this study, as well as in the experimental design, including sampling protocols and feeding trial planning. He provided expertise and guidance in all aspects of this project and also reviewed this thesis. Jeanette Wells, Richard Taylor, Dr. Matthew Rise, and Dr. Fereidoon Shahidi all helped with both Chapters 2 and 3. Jeanette Wells conducted carbon and nitrogen analysis and assisted in sampling tissues and laboratory analyses. Richard Taylor formulated the diets used in the experiments. Dr. Matthew Rise contributed to the design of the project and research questions in this study and reviewed the thesis. Dr. Fereidoon Shahidi contributed to this project through his guidance in the lipid oxidation part of this study as well as reviewing this thesis.

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2. MINIMIZING MARINE RESOURCE UTILIZATION IN DIETS OF FARMED ATLANTIC SALMON (*SALMO SALAR*): EFFECTS ON GROWTH PERFORMANCE AND MUSCLE LIPID CLASS AND FATTY ACID COMPOSITION

2.1 Abstract

Due to limited fish meal and fish oil resources and their high costs for the aquaculture industry, it is necessary to find alternative sustainable sources of protein and lipids. Therefore, seven different diets were formulated with different protein and lipid sources to feed farmed Atlantic salmon, and their effects on growth performance, muscle lipid class, and fatty acid composition were examined. Growth performance indicated that the diet with the lowest fish meal and fish oil content resulted in the lowest weight gain and final weight, followed by the diet containing the highest level of animal by-products. The lipid class analysis showed no statistical difference in the muscle total lipid content using different diets. However, significant statistical differences were observed between the main lipid classes; triacylglycerols, phospholipids, and sterols. The diet containing 1.4% omega-3 long-chain fatty acids (ω3LC1.41) resulted in the highest content of triacylglycerols and phospholipids. Diets containing medium and low levels of fish oil and fish meal, respectively, led to as high a level of ω3 fatty acids in muscle as when fish were fed diets with high levels of fish meal and fish oil. The results of this study suggest that feeding a diet containing low levels of fish meal and moderate levels of fish oil does not significantly affect ω3 fatty acid composition in muscle. Fish meal

can be reduced to 5% without affecting growth as long as there is a minimum of 5% fish oil, and animal by-products do not exceed 26% of the diet.

2.2 Introduction

Aquafeed production has been growing rapidly, up to 30% annually (Turchini et al., 2010). The major cost in the aquaculture production industry is associated with aquafeeds (50 to 80% of the total cost). Fish meal and fish oil have been the primary ingredients in the aquafeed manufacturing industry. In 2010, 73% of fish meal was consumed in aquaculture (Shepherd, 2012). From 1995 to 2004, global consumption of fish meal and fish oil doubled (Hasan and Halwart, 2009). However, the production of fish meal and fish oil has not changed significantly (Tacon et al., 2006) and even slightly declined in recent years (Tocher, 2015).

While fish meal is known for its high level of essential amino acids, fish oil is the main source of the ω3 polyunsaturated fatty acids (PUFA), 20:5ω3 (eicosapentaenoic acid, EPA) and 22:6ω3 (docosahexaenoic acid, DHA), which are beneficial to the immune system of both animals and humans (Oliveira-Goumas, 2004). EPA and DHA are also important to nervous system, photoreception, and reproductive system (Siriwardhana et al. 2012).

The aquaculture industry is a major contributor to fish food demands, but it consumes about 68% and 89% of the world production of fish meal and fish oil, respectively (Tacon and Metian, 2008). Salmonids dominate the consumption of fish oil at 64%; 49.7% by salmon and 14.8% by trout (Huntington and Hasan, 2009).

Fish meal is the best diet used for carnivorous fish providing the required protein and oil (Hasan and Halwart, 2009). Fish meal contains valuable protein with a superior amino acid profile and with high digestibility, and is an excellent source of 20:5ω3 and 22:6ω3, as well as essential vitamins and minerals (IFOMA, 2001).

One of the most important factors in the sustainability and development of the aquaculture industry is the use and availability of fish oil. Fish oil is a valuable primary resource of lipid providing the required ω3 PUFA (Turchini et al., 2010). Running out of fish oil and fish meal sources from wild fish is one of the aquaculture industry's main concerns. Fish oil and fish meal resources are limited as they are widely used in aquaculture to provide ω3 fatty acids in farmed piscivorous fish (e.g. European seabass, gilthead seabream and Atlantic salmon), as well as to feed omnivorous species (e.g. carp and tilapia) (Naylor et al., 2009).

The increasing need for fish meal and fish oil has also resulted in higher prices of these resources. High costs and limited availability of these resources have led to several evaluations of the effect of replacement of fish meal and fish oil with alternative sustainable sources, such as those derived from terrestrial plants and animals, in the diets of different fish species. While partial replacement generally was successful in terms of growth performance, the full replacement mostly resulted in lower content of ω3 and essential fatty acids, as well as poor growth performance (Jobling et al., 2008; Torstensen et al., 2008; Bell et al., 2010; Turchini et al., 2011).

The objective of this study was to evaluate how using alternative diets with low content of marine resources can affect growth performance, and muscle lipid class and fatty acid composition in farmed Atlantic salmon.

2.3 Materials and Methods

2.3.1 Experimental Diets

Seven different diets were produced in EWOS Innovation AS in Norway. The diets were formulated using different levels of ingredients, including fish meal, fish oil, animal by-products, vegetable oil, vegetable protein, and DHA+EPA. The seven diets were characterized according to the most critical component used and designated as marine, with high levels of fish meal and fish oil; medium marine, containing medium levels of fish meal and fish oil; animal by-product, composed of a high proportion of animal by-products; vegetable protein, including a high level of vegetable protein; ω3LC0, which contained approximately 0% of LC ω3 FAs (long-chain ω3 fatty acids); ω3LC1, with 1% of LC ω3 FAs; and ω3LC1.41, with 1.4% of LC ω3 FAs (Table 2.1).

Table 2.1. Experimental diet ingredients

Ingredients	Marine	Medium marine	Animal by-product	Vegetable protein	ω3LC0	ω3LC1	ω3LC1.41
Fish meal[1] (%)	**35**	**15**	5	5	5	5	5
Animal by-products[2] (%)	15	26	**33**	10	22	21	22
Vegetable protein[3] (%)	9	20	23	**47**	33	34	33
Fish oil (%)	**12**	**6**	5	5	**0**	5	7
Vegetable oil[4] (%)	6	13	14	17	27	22	20
Digestible energy (MJ Kg^{-1})	20	20	20	20	21	21	21
Digestible protein (g Kg^{-1})	375	375	375	375	360	360	360

EPA+DHA (%)	2.91	1.41	1.00	1.00	**0.09**	**1.00**	**1.41**

Boldfaced numbers indicate key components of each diet
[1] Anchovy fish meal
[2] Poultry, feather, and blood meal
[3] Soy protein concentrate, corn and wheat gluten
[4] Rapeseed oil

2.3.2 Experimental fish and feeding

Atlantic salmon (*Salmo salar)* smolts were obtained from Northern Harvest Sea Farms in Stephenville, NL, Canada. Fish were tagged with passive transponder (PIT) and then maintained at 11±1°C in a flow-through seawater system under a 12-h light photoperiod in the Dr. Joe Brown Aquatic Research Building in St. John's, Newfoundland, Canada. Fish (1148 at 139-232 g each) were distributed randomly in 28 tanks (4 tanks per diet), 620 L each. There were 41 fish per tank until day 0, when one fish/tank was sampled. Diets were formulated according to Table 2.1. The fish were hand fed 5 mm experimental pellets to satiation twice a day for 14 weeks. Feed consumption was measured weekly in g and in % body weight per day. Fish weight and fork length were recorded at the beginning and the end of the experiment.

2.3.3 Tissue sampling

Sampling was conducted at week 0 (when no experimental diets were fed), week 7, and week 14. Ten fish were randomly sampled at week 0, followed by 5 fish per tank at week 7 and 14. Fish were euthanized with an overdose of MS-222 (400 mg L^{-1}, Syndel Laboratories, Vancouver, BC, Canada), then fork length and weight were measured. Gut and liver were removed, weighed, sampled, and placed on aluminum weigh boats. Norwegian quality cuts (NQC) (area from directly behind the dorsal fin to the anus) were

removed from the fish body and weighed. With the dorsal fin cut to the right, the skin was cut along the dorsal side, peeled back to expose the skeletal muscle (hereafter referred to as muscle), and a strip of muscle was removed and placed into lipid clean test tubes (rinsed with methanol and chloroform three times each) for lipid analysis. The tubes had been weighed after ashing at 450°C for 8 hrs, and the Teflon lined caps were rinsed three times with methanol and chloroform. Vials were kept on ice during sampling. After sampling, 2 ml of chloroform was poured on the tissues in the test tubes and the remaining space was filled with nitrogen. The tubes were then sealed with Teflon tape and were stored at -20°C. It should be noted that all procedures, including handling, treatment, euthanasia, and dissection were performed according to the guidelines from the Canadian Council of Animal Care (approved Memorial University Institutional Animal Care Protocol 14e71-MR).

2.3.4 Lipid extraction

First, 1 ml of ice-cold methanol was added to the samples, which were ground using a metal rod. The rod was washed into the test tubes with approximately 1 ml of 2:1 chloroform: methanol and then with 1/2 ml of chloroform-extracted water. The ratio of the methanol:chloroform:water was 1:2:1. The tubes were recapped and the mixtures were sonicated for 4 min in an ice bath. They were then centrifuged for 2-3 min at 3000 rpm at room temperature. The organic layer (bottom layer) was removed using the double pipetting technique and transferred into a lipid clean vial (rinsed with methanol and chloroform three times each). Three millilitres of chloroform were added to the extraction test tubes and this procedure was repeated 3 times to extract all the lipids from the

samples. Extractions were evaporated to volume under a gentle stream of nitrogen, sealed with Teflon tape and stored in the freezer at -20°C (Parrish, 1999).

2.3.5 Lipid class determination

The composition of lipid classes was determined through a three-step development method using an Iatroscan (thin-layer chromatography–flame ionization detector) (Parrish 1987). The lipid extracts and standards were spotted on the Chromarods with a Hamilton syringe, and then were focused twice in acetone to produce a narrow band of lipid material near the lower end of the rods. The rods were developed twice (first for 25 min, followed by a 5-min equilibration in the constant humidity chamber, and then for 20 min) in the first development system, which consisted of hexane, diethyl ether, formic acid (98.95:1.0:0.05 ml/ml/ml). The rods were then dried for 5 min and scanned in the Iatroscan. In the second development system, the rods were developed in a mixture of hexane, diethyl ether, formic acid (79:20:1 ml/ml/ml) for 40 min then scanned. The third development system consisted of two steps. First, development in 100% acetone for 15 min twice, and second, twice for 10 min in chloroform, methanol, chloroform-extracted water (50:40:10 ml/ml/ml). As in the previous steps, the rods were scanned, but this time to the end. Before putting the rods in each development system they were dried in a constant humidity chamber and after scanning, the data were collected using PeakSimple software.

2.3.6 Fatty acid methyl ester (FAME) derivatization

Fifty microlitres of lipid extract was transferred into 15 ml lipid clean vials and concentrated to dryness (4-16 mg lipid per 1 ml reagent). Methylene chloride (1.5 ml) and 3 ml of Hilditch reagent (1.5 H_2SO_4: 98.5 anhydrous methanol) were added, then vials were vortexed and sonicated for 4 min. They were filled with nitrogen, capped and heated at 100°C for 1 hr. Saturated sodium bicarbonate solution (0.5 ml) and 1.5 ml hexane were added to the samples which were vortexed, followed by removing the upper layer organic phase. The samples were next dried and refilled with hexane to approximately 0.5 ml. The vials were filled with nitrogen, capped, sealed with Teflon tape, and finally sonicated for another 4 min to re-suspend the fatty acids.

2.3.7 Lipid Oxidation

In order to measure lipid oxidation, one gram of muscle sample (two replicates per fish) was weighed and transferred into centrifuge tubes (plus 1 blank). Then the sample was homogenized with 5 ml of 5 % (w/v) TCA (trichloroacetic acid) by polytron. Samples were centrifuged at 3000 rpm for 10 min and the supernatant (top layer) was filtered through a syringe filter with 0.45 µm pores. Five ml of 0.08 M TBA (thiobarbituric acid) and 2.5 ml of TCA were added and heated in a boiling water bath at 94±1°C for 45 min then cooled to room temperature. Finally, the absorbance was measured at 532 nm using a UV-spectrophotometer. TBARS (thiobarbituric acid reactive substances) values were calculated using a standard curve. Standard curves were prepared using 1,1,3,3-tetramethoxypropane as a precursor of the malondialdehyde (MDA; 0, 1, 2, 3, 4, 5, 6, 7, 8,9 and 10 ppm).

2.3.8 Statistics

In order to ensure representative fish were sampled, only fish with weight gains within the range of twice the standard deviation from the overall tank weight gain means were considered. Correlation and regression analyses were conducted using Minitab version 17 to compare diet ingredients, lipid and fatty acid composition of muscle and diet, and growth characteristics. For statistical analysis of growth data, lipid class, and fatty acid data, nested general linear models were combined with Tukey pairwise comparisons using Minitab to determine the difference between tanks and diets. The normality of residuals was evaluated with the Anderson-Darling normality test. If the test failed ($p<0.05$), a one-way analysis of variance (ANOVA) on ranks was performed in SigmaPlot version 13. Principal components analyses (PCA) were performed using Minitab version 17 to compare diet and muscle fatty acids composition and lipid classes with the seven diets and the graphs were plotted using SigmaPlot version 13. A discriminant analysis was also performed in Minitab to classify the samples according to their major fatty acids and lipid classes.

2.4 Results

2.4.1 Experimental diet composition

Lipid classes of experimental diets were measured as mg g^{-1} wet weight (ww), where moisture in the feeds ranged from 3.88 to 6.39%. Diet lipid composition was mainly triacylglycerol, sterol, and phospholipid, accounted for 54-75, 2.2-5.3, and 3.8-11.5% of the total lipids in the diet, respectively (Table 2.A4). In terms of total lipid, ω3LC0 and ω3LC1.41 diets were statistically higher than the animal by-product diet.

Triacylglycerol content of all diets was similar to the marine diet except ω3LC0 which contained a significantly higher level of triacylglycerol. Sterol and phospholipid content did not differ among diets (Table 2.2).

All diets had the same concentrations of 16:0, ΣSFA, and ΣPUFA as well as the same ratios of P/S and DHA/EPA. The 3 diets formulated to have the highest digestible energy (ω3LC0 to ω3LC1.41) had statistically the same content of 18:1ω9, 18:2ω6, 18:3ω3, and 20:4ω6. The 20:5ω3 and 22:6ω3 contents and proportions (accounted for 0.34-6.24 and 0.41-5.83% of the total fatty acids in the diet, respectively) were significantly lower in the ω3LC0 diet than in the marine, medium marine and ω3LC1.41 diets (Tables 2.3 and 2.A5).

Table 2.2. Lipid classes in experimental diets, as fed (mg g^{-1} wet weight)

Lipid classes (mg g^{-1} wet weight)	Marine n=6	Medium marine n=6	Animal by-products n=9	Vegetable protein n=6	ω3LC0 n=9	ω3LC1 n=9	ω3LC1.41 n=9
Total lipid	161.1±64.5^{ab}	173.4±46.7^{ab}	140.1±39.6^{b}	159.3±27.6^{ab}	229.2±59.2^{a}	203.5±25.6^{ab}	208.7±38.7^{a}
Triacylglycerol	93.3±45.2^{b}	96.1±28.1^{b}	92.1±40.6^{b}	115.4±27.3^{ab}	173.2±55.0^{a}	149.6±27.7^{ab}	147.8±34.9^{ab}
Sterol	6.3±2.6	8.8±1.7	5.7±3.0	4.1±2.6	6.0±3.0	4.4±2.4	6.5±2.8
Phospholipid	9.5±3.1	9.7±2.7	14.9±10.2	14.9±10.5	11.5±9.9	14.0±9.1	8.7±7.4

Data are ± standard deviation for n replicates

Table 2.3. Fatty acids composition of experimental diets, as fed (mg g^{-1} wet weight)

Fatty acid (mg g^{-1} wet weight)	Marine n=6	Medium marine n=6	Animal by-products n=6	Vegetable protein n=6	ω3LC0 n=6	ω3LC1 n=9	ω3LC1.41 n=6
14:0	5.6±2.4^{a}	3.6±1.1^{a}	2.1±0.8^{ab}	2.3±0.5^{ab}	0.5±0.1^{b}	2.5±0.5^{ab}	3.4±0.5^{a}
16:0	14.1±6.1	13.5±4.0	9.9±3.8	10.3±2.5	10.8±1.8	13.0±3.0	13.5±2.1
16:1ω7	5.2±2.2^{a}	3.8±1.1^{a}	2.3±0.9^{ab}	2.3±0.5^{ab}	1.1±0.2^{b}	2.7±0.6^{ab}	3.3±0.5^{a}
18:0	2.9±1.2^{d}	5.7±1.6^{bcd}	6.5±2.9^{abc}	6.4±1.4^{abc}	9.2±1.6^{a}	7.7±1.8^{ab}	4.0±0.6^{cd}
18:1ω7	2.9±1.2^{b}	3.5±1.0^{ab}	1.5±1.0^{b}	3.5±0.8^{ab}	4.5±0.6^{a}	4.4±1.2^{a}	4.1±0.6^{ab}
18:1ω9	30.7 ± 13.2^{e}	49.6 ± 13.8^{cde}	44.6 ± 17.0^{de}	56.6 ± 12.0^{bcd}	88.4 ± 15.4^{a}	77.4 ± 17.0^{ab}	71.0± 11.5^{abc}

18:2ω6	12.3 ± 5.3^{d}	20.1 ± 5.7^{bcd}	17.7± 6.8^{cd}	22.8 ± 5.1^{abc}	32.0 ± 5.8^{a}	29.4 ± 6.7^{ab}	27.1 ± 4.3^{abc}
18:3ω3	4.8 ± 2.1^{e}	7.5 ± 2.1^{cde}	6.6 ± 2.5^{de}	8.8 ± 1.9^{bcd}	12.7 ± 2.5^{a}	11.9 ± 2.7^{ab}	10.9 ± 1.8^{abc}
18:4ω3	3.4±1.5^{a}	2.1±0.6^{a}	1.2±0.5^{ab}	1.4±0.3^{ab}	0.1±0.0^{b}	1.5±0.3^{ab}	2.1±0.3^{a}
20:1ω9	9.6±4.1^{a}	6.8±1.9^{abc}	4.4±1.7^{bc}	5.2±1.1^{bc}	2.8±0.5^{bc}	6.1±1.4^{c}	7.4±1.2^{ab}
20:4ω6	0.5± 0.2^{a}	0.5 ± 0.1^{ab}	0.3 ± 0.1^{abc}	0.2± 0.1^{c}	0.2 ± 0.1^{c}	0.3 ± 0.9^{bc}	0.4 ± 0.1^{abc}
20:5ω3	8.3 ± 3.6^{a}	5.1 ± 1.5^{a}	2.6 ± 1.0^{ab}	3.1± 0.8^{ab}	0.6 ± 0.1^{b}	3.3 ± 0.8^{ab}	4.3 ± 0.7^{a}
22:1ω11(13)	12.1±5.2^{a}	7.8±2.1^{a}	4.6±1.8^{ab}	5.3±1.1^{ab}	0.6±0.1^{b}	5.2±2.1^{ab}	7.9±1.3^{a}
22:5ω3	0.9±0.7^{ab}	0.3±0.1^{a}	0.1±0.0^{ab}	0.1±0.1^{ab}	0.3±0.2^{ab}	0.1±0.0^{b}	0.2±0.0^{ab}
22:6ω3	7.7 ± 3.4^{a}	4.7 ± 1.4^{a}	2.4 ± 0.8^{ab}	3.0± 1.0^{ab}	0.7 ± 0.1^{b}	3.1 ± 0.8^{ab}	3.9 ± 0.7^{a}
Σ Bacterial[1]	1.7 ± 0.7^{a}	1.3 ± 0.4^{ab}	0.8 ± 0.3^{b}	0.9± 0.2^{b}	0.7 ± 0.1^{b}	1.1 ± 0.2^{b}	1.3 ± 0.2^{ab}
Σ SFA[2]	24.1 ± 10.3	24.6 ± 7.2	19.9 ± 7.8	20.7 ± 4.7	22.8 ± 3.8	25.4 ± 5.8	22.9 ± 3.6
Σ MUFA[3]	63.9 ± 27.4^{bc}	74.5 ± 20.6^{abc}	60.7 ± 23.2^{c}	75.3 ± 16.0^{abc}	99.7 ± 17.1^{a}	99.5 ± 21.8^{a}	97.0 ± 15.5^{ab}
Σ PUFA[4]	43.0 ± 18.8	43.6 ± 12.5	32.8 ± 12.4	41.6 ± 9.6	47.5 ± 8.9	52.6 ± 11.9	51.6 ± 8.3
***P/S**[5]	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	2.0 ± 0.0	2.0 ± 0.20	2.0 ± 0.1	2.2 ± 0.0
Σ ω3	26.4 ± 11.6^{a}	20.5 ± 6.0^{ab}	13.4 ± 5.0^{b}	17.0 ± 4.1^{ab}	14.6 ± 3.0^{b}	21.1 ± 5.0^{ab}	22.0 ± 3.6^{ab}
***DHA/EPA**	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	0.9 ± 0.0

* Unitless ratios
[1] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
Data are ± standard deviation for n replicates

2.4.2 Growth performance

Fish mean initial weights ranged from 177-179 g and there was no significant difference among tanks or diets. After 14 weeks of feeding, fish weights increased by 75-94%, and reached final mean weights ranging from 309-342 g with statistical differences among diet treatments (Table 2.4). The marine diet resulted in the highest weight gain/final weights. Final weights were lowest when using the diet with the lowest fish meal and fish oil content (ω3LC0), followed by the diet containing highest level of animal by-products. In terms of length gain, fish size increased by 4.9-5.6 cm, with a similar trend to weight gain results. Mean final lengths were the same for fish fed different diets except there was a significant difference between the ω3LC0 and ω3LC1 treatments. The hepatosomatic index (HSI) and viscerosomatic index (VSI) varied between 1.1-1.5 and 10.6-11.4%, respectively, in week 14. While there was no statistical difference in VSI when feeding different diets, HSI values differed among treatments. Fish fed diets with the highest digestible energy content (ω3LC0, ω3LC1, and ω3LC1.41) had the lowest HSI. The results for the Norwegian quality cut showed no statistical difference among treatments, and varied from 50-60 g at the end of feeding trial. The lowest specific growth rate (SGR) was obtained with the ω3LC0 and animal by-product diets. The use of the seven formulated diets had no significant effect on other factors such as condition factor (CF), apparent feed intake (AFI), and feed conversion ratio (FCR) (Table 2.4).

Table 2.4. Salmon growth data - week 14

Diet	Marine n=134	Medium marine n=134	Animal by-products n=138	Vegetable protein n=137	ω3LC0 n=135	ω3LC1 n=140	ω3LC1.41 n=137
Initial Weight (g)	176.8 ±29.4	179.3 ± 30.3	179.2± 28.8	177.2± 29.8	176.6± 63.7	179.3± 30.1	178.1± 27.5
Final Weight (g)	342.5± 89.5^{a}	325.5± 74.1^{ab}	316.3± 63.6^{b}	332.9± 79.2^{ab}	309.3± 63.7^{b}	339.7± 73.5^{ab}	341.9± 68.7^{ab}
Weight Gain (g)	165.6 ± 77.5^{a}	146.2 ± 54.7^{b}	137.1 ± 47.5^{b}	155.7 ± 64.6^{ab}	132.7 ± 48.6^{b}	160.4 ± 59.7^{ab}	163.8 ± 54.6^{ab}
Initial Length (cm)	24.9± 1.3	25.0± 1.3	25.0± 1.4	25.0±1.4	24.9±1.2	25.0± 1.3	25.0± 1.3
Final Length (cm)	30.4± 2.4^{ab}	30.1± 2.2^{ab}	30.0± 2.1^{ab}	30.2± 2.5^{ab}	26.6± 2.0^{a}	30.6± 2.0^{b}	30.6± 2.0^{ab}
Length Gain (cm)	5.5 ± 1.9^{a}	5.2 ± 1.4^{ab}	5.0 ± 1.3^{b}	5.2 ± 2.0^{ab}	4.9 ± 1.5^{b}	5.6 ± 1.6^{a}	5.6 ± 1.5^{a}
NQC* (g) n= 18-20	59.8± 17.8	57.5± 14.6	49.6± 10.8	58.0± 12.8	54.4± 10.6	58.6± 12.0	58.6± 11.6
SGR^{1} (% day^{-1})	0.65± 0.26^{a}	0.59± 0.18^{bc}	0.57± 0.14^{c}	0.62± 0.23^{ab}	0.56± 0.18^{c}	0.64± 0.21^{ab}	0.66± 0.17^{a}
CF^{2} (g cm^{-1})	1.19± 0.14	1.17± 0.11	1.16± 0.10	1.20± 0.28	1.15± 0.09	1.17± 0.11	1.18± 0.10
VSI^{3} (%) n=29-32	11.4± 1.4	10.6± 1.8	10.7± 1.4	11.1± 1.7	10.9± 1.5	11.3± 1.7	11.1± 1.3
HSI^{4} (%) n=29-32	1.49± 0.40^{a}	1.28± 0.33^{ab}	1.29± 0.38^{ab}	1.19± 0.25^{b}	1.09± 0.22^{b}	1.14± 0.22^{b}	1.10± 0.18^{b}
AFI^{5} (g fish^{-1}) n=4	178.7± 25.2	158.4± 7.0	146.6± 5.5	167.4± 14.4	145.4± 16.7	159.0± 11.2	161.5± 19.7
FCR^{6} n=4	1.09± 0.06	1.09± 0.06	1.07± 0.03	1.08± 0.08	1.10± 0.05	0.99± 0.03	0.99± 0.02

* Norwegian quality cut; the area from directly behind the dorsal fin to the anus
[1] Specific growth rate = 100 × [ln (final body weight) - ln (initial body weight)]/days
[2] Condition factor = body mass/length^{3}
[3] Hepatosomatic index = 100 × (liver mass/body mass)
[4] Viscerosomatic index = 100 × (viscera mass/body mass)
[5] Apparent feed intake = feed consumption/number of fish per tank
[6] Feed conversion ratio = feed consumption/weight gain
Data are ± standard deviation for n replicates

2.4.3 Muscle lipid class composition

There was no significant difference in total lipids among the different treatments. The major lipid classes in muscle tissues were triacylglycerol, phospholipid, and sterol, accounting for 40-56, 1.4-2, and 25-49% of the total lipids in muscle tissues, respectively (Table 2.A6). A comparison between the level of different lipids at week 0 and 14 (Table 2.5) showed that the use of different treatments did not affect the content of triacylglycerol and sterol, but the level of phospholipids dropped after 14 weeks of feeding the marine diet ($p=0.014$). Feeding the marine diet resulted in the lowest content of phospholipid in muscle in comparison to other diets. No significant differences in muscle triacylglycerols were observed when feeding the seven diets, except between the animal by-product and ω3LC1.41 diets, which resulted in the lowest and highest levels of triacylglycerol, respectively. Fish fed the marine and animal by-products diets showed the highest and lowest level of sterol in muscle tissues respectively (Table 2.5).

Table 2.5. Lipid classes of muscle tissue before and after 14 weeks of feeding (mg g^{-1} wet weight)

Lipid classes (mg g^{-1} wet weight)	Initial n=10	Marine n=16-19	Medium marine n=16-19	Animal by-products n=16-19	Vegetable protein n=16-19	ω3LC0 n=16-19	ω3LC1 n=16-19	ω3LC1.41 n=16-19
Total lipid	15.2±7.6	17.8±14.0	9.3±5.2	8.6±4.7	13.7±5.5	10.6±6.0	17.2±12.3	18.1±15.3
Triacylglycerol	7.2±4.8	8.6±7.7^{ab}	5.6±4.5^{ab}	3.4±2.1^{b}	7.9±4.9^{ab}	5.1±3.4^{ab}	8.3±6.4^{ab}	9.2±6.7^{a}
Sterol	0.26±0.17	0.87±0.77^{a}	0.17±0.09^{ab}	0.20±0.33^{b}	0.23±0.04^{a}	0.26±0.16^{a}	0.29±0.27^{ab}	0.28±0.18^{a}
Phospholipid	5.0±2.4	2.8±0.8^{b}	2.9±1.3^{b}	3.9±1.5^{ab}	3.8±0.6^{ab}	4.2±2.8^{ab}	4.4±2.5^{ab}	5.2±3.2^{a}

The underlined data represents the values that are statistically different from week 0
Data are ± standard deviation for n replicates

2.4.4 Muscle fatty acid composition

Muscle tissue fatty acid composition (Table 2.6) showed no significant differences among treatments for 16:0, 16:1ω7, 18:0, 18:4ω3, 22:5ω3, Σ Bacterial, Σ SFA, Σ MUFA, Σ PUFA, and P/S. Table 2.6 shows that in terms of essential long-chain PUFA, the concentration of 20:4ω6 did not change significantly compared to week 0, except when the medium marine diet was used, which resulted in the lowest level of 20:4ω6 in muscle tissues. The content of other long-chain PUFAs (20:5ω3 and 22:6ω3) also decreased significantly when medium the marine diet was fed. Among the treatments, diet ω3LC0 resulted in significantly higher contents of 20:4ω6 than medium marine diet. The total ω3 fatty acids consisted mainly of 20:5ω3 and 22:6ω3, accounted for 2.9-5.8 and 14-20% of the total fatty acids in muscle tissues, respectively (Table 2.A7). The level of 20:5ω3 at the end of feeding trial decreased significantly to about half of its initial value at week 0 when the medium marine, animal by-products, and ω3LC0 diets were fed. 22:6ω3 was affected by different treatments similarly to 20:5ω3. As expected, the level of Σω3 changed in a similar manner to the two dominant ω3 fatty acids, when using the formulated diets. The analysis of DHA/EPA ratio indicated that animal by-products, ω3LC0, and ω3LC1.41 led to the highest ratio, while the marine diet resulted in the lowest ratio of DHA/EPA (Table 2.6). Lipid class and fatty acids proportions (%) in diets and muscles are presented in Tables 2.A4- 2.A7.

Table 2.6. Fatty acid composition of muscle tissue before and after 14 week of feeding trial (mg g^{-1} wet weight)

Fatty acid (mg g^{-1} wet weight)	Initial n=10	Marine n=12-19	Medium marine n=12-19	Animal by-products n=12-19	Vegetable protein n=12-19	ω3LC0 n=12-19	ω3LC1 n=12-19	ω3LC1.41 n=12-19
14:0	0.38±0.24	0.5±0.45^{a}	0.16±0.14^{ab}	0.09±0.06^{ab}	0.18±0.10^{a}	0.07±0.05^{b}	0.21±0.14^{a}	0.25±0.24^{a}
16:0	1.90±0.91	2.18±1.61	1.03±0.58	0.94±0.41	1.36±0.51	0.94±0.65	1.60±0.86	1.59±1.11
16:1ω7	0.65±0.41	0.64±0.63	0.22±0.19	0.13±0.09	0.24±0.15	0.11±0.09	0.30±0.22	1.20±0.39
18:0	0.46±0.24	0.52±0.43	0.25±0.15	0.22±0.11	0.34±0.15	0.27±0.16	0.42±0.27	0.42±0.33
18:1ω7	0.35±0.20	0.40±0.33^{ab}	0.20±0.13^{ab}	0.17±0.08^{b}	0.29±0.14^{ab}	0.23±0.15^{ab}	0.39±0.25^{a}	0.37±0.29^{ab}
18:1ω9	2.29±1.36	3.88±3.72^{ab}	2.13±1.63^{ab}	1.70±1.08^{b}	3.56±2.08^{ab}	2.95±2.09^{ab}	5.02±3.56^{a}	4.55±3.97^{a}
18:2ω6	0.98±0.60	1.25±1.14^{ab}	0.74±0.56^{ab}	0.56±0.37^{b}	1.19±0.66^{ab}	0.89±0.62^{ab}	1.63±1.15^{a}	1.52±1.26^{ab}
18:3ω3	0.12±0.07	0.37±0.32^{ab}	0.20±0.14^{ab}	0.15±0.08^{b}	0.32±0.16^{a}	0.23±0.15^{ab}	0.43±0.32^{a}	0.41±0.29^{a}
18:4ω3	0.11±0.07	0.27±0.25	0.11±0.09	0.09±0.05	0.18±0.11	0.17±0.10	0.22±0.17	0.23±0.21
20:1ω9	0.23±0.15	0.84±0.80^{ab}	0.25±0.20^{ab}	0.16±0.10^{b}	0.33±0.19^{a}	0.12±0.09^{b}	0.39±0.34^{ab}	0.47±0.41^{a}
20:4ω6	0.14±0.06	0.12±0.06^{ab}	0.07±0.03^{b}	0.09±0.04^{ab}	0.11±0.03^{ab}	0.14±0.07^{a}	0.12±0.05^{ab}	0.10±0.05^{ab}
20:5ω3	0.65±0.26	0.67±0.42^{a}	0.29±0.12^{b}	0.28±0.11^{b}	0.34±0.09^{ab}	0.24±0.13^{b}	0.38±0.16^{ab}	0.37±0.20^{ab}
22:1ω11(13)	0.19±0.14	1.97±0.78^{a}	2.09±0.17^{a}	2.18±0.08^{ab}	2.09±0.14^{a}	2.20±0.03^{b}	2.15±0.22^{a}	2.23±0.21^{a}
22:5ω3	0.23±0.11	0.25±0.19	0.09±0.05	0.08±0.04	0.11±0.04	0.08±0.04	0.12±0.06	0.11±0.08
22:6ω3	2.24±0.80	2.48±1.20^{a}	1.26±0.41^{bd}	1.41±0.53^{bd}	1.60±0.38^{abd}	1.25±0.70^{cd}	1.80±0.76^{abd}	1.90±0.77^{ab}
Σ Bacterial[1]	0.13±0.08	0.17±0.15	0.07±0.05	0.05±0.03	0.07±0.04	0.06±0.08	0.09±0.06	0.10±0.09
Σ SFA[2]	2.84±1.45	3.39±2.64	1.54±0.92	1.37±0.62	2.08±0.83	1.47±0.96	2.48±1.40	2.56±2.15

Σ MUFA[3]	3.91±2.36	6.98±6.62	3.16±2.42	2.37±1.49	4.85±2.79	3.58±2.52	6.66±4.74	6.30±5.36
Σ PUFA[4]	4.99±2.24	6.16±4.15	3.06±1.54	2.93±1.17	4.26±1.50	3.34±1.89	5.28±2.91	5.20±3.16
[*]P/S[5]	1.81±0.12	1.97±0.25	2.09±0.23	2.18±0.21	2.09±0.17	2.20±0.10	2.15±0.08	2.23±0.38
Σ ω3	3.47±1.37	4.28±2.54[a]	2.03±0.83[b]	2.07±0.76[b]	2.65±0.73[ab]	2.02±1.10[b]	3.08±1.49[ab]	3.21±1.59[ab]
[*]DHA/EPA	3.54±0.31	4.03±0.77[b]	4.50±0.71[ab]	5.05±0.64[a]	4.77±0.70[ab]	5.22±0.86[a]	4.82±0.57[ab]	4.94±0.90[a]

[*] Unitless ratios
[1] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
The underlined data represents the values that are statistically different from week 0
Data are ± standard deviation for n replicates

2.4.5 Correlation and regression analysis

The correlation and regression analyses were performed among diet ingredients, growth performance, diet lipid classes, diet fatty acid composition, muscle lipid classes, and muscle fatty acid composition (Tables 2.A1, 2.A2, and 2.A3). Growth performance was found to be positively correlated with fish oil content in the diet ($P<0.05$). However, high animal by-products inclusion showed a negative correlation with CF (Table 2.A1). It was also revealed that Σω3 in the diet and muscle had positive correlations with growth performance characteristics ($P<0.05$). This was confirmed through regression analysis (Fig. 2.1), where Σω3 was found to have a linear relationship with growth parameters such as SGR, weight gain, and AFI (R^2 = 73.2, 75.2, 72.1%, respectively). While muscle total lipids and triacylglycerols were positively correlated with growth performance factors ($P<0.01$), they had a highly significant positive correlation ($P<0.001$) with SGR. Regression analysis also validated that growth performance factors (SGR, weight gain, and NQC) increased linearly with the total lipids and triacylglycerols contents in muscle tissue (Figs. 2.2 and 2.3; R^2 = 58.9 – 89.9%). Σ PUFA content in the muscle showed a highly positive correlation with VSI ($P<0.001$). The correlation analysis revealed an inverse relationship between the ratio of DHA/EPA and final length. The results of the regression analysis suggested an inverse linear relationship between HSI and vegetable oil content in the diet ingredients (Fig. 2.4; R^2 = 88.7%). The correlation and regression analysis showed that the relationship between the content of dietary essential fatty acids and muscle fatty acids for 20:5ω3 and 22:6ω3 ($P<0.05$, R^2 = 75.8% and $P<0.05$, 60%, respectively) followed a positive linear trend (Table 2.A3 and Fig. 2.5).

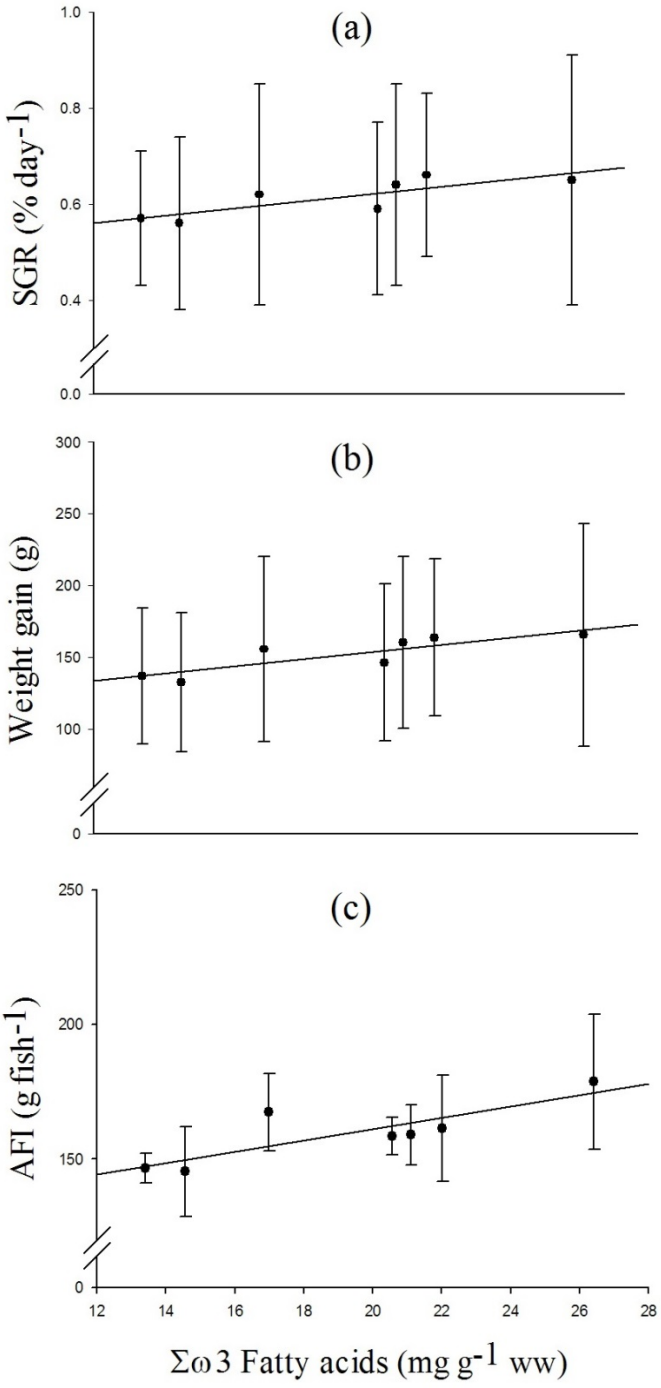

Figure 2.1. Regression analyses between ω3 fatty acids content in the diets and the growth performance factors, including a) specific growth rate, b) weight gain, and c) apparent feed intake.

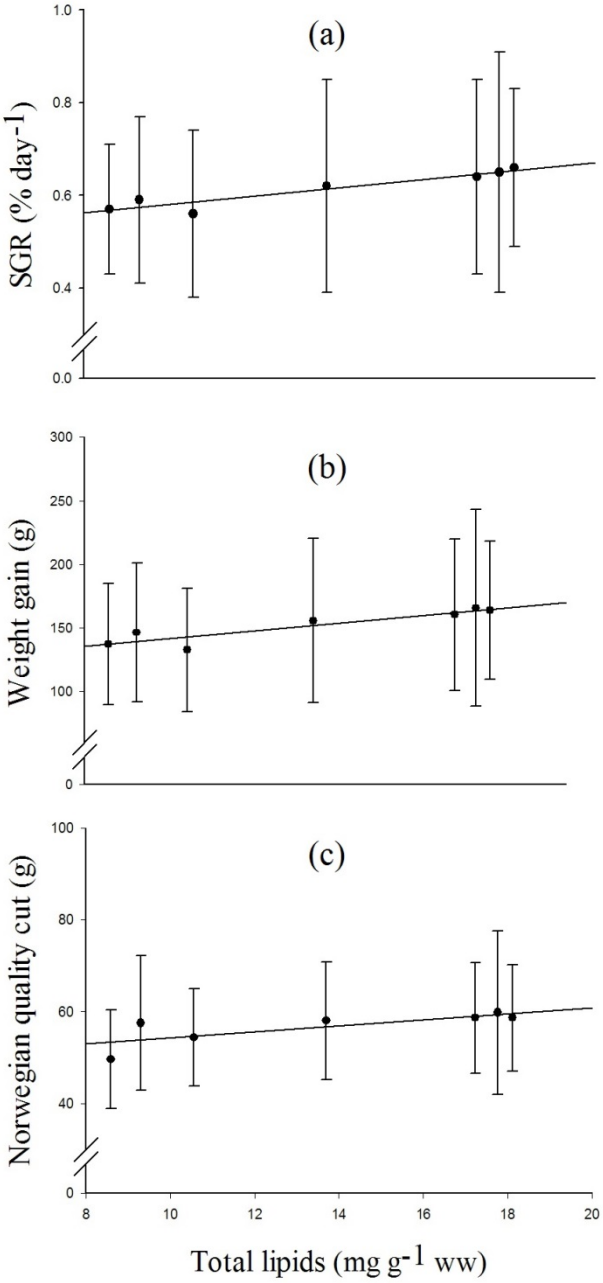

Figure 2.2. Regression analyses between the total lipid content in the muscle tissue and the growth performance factors, including a) specific growth rate, b) weight gain, and c) Norwegian quality cut.

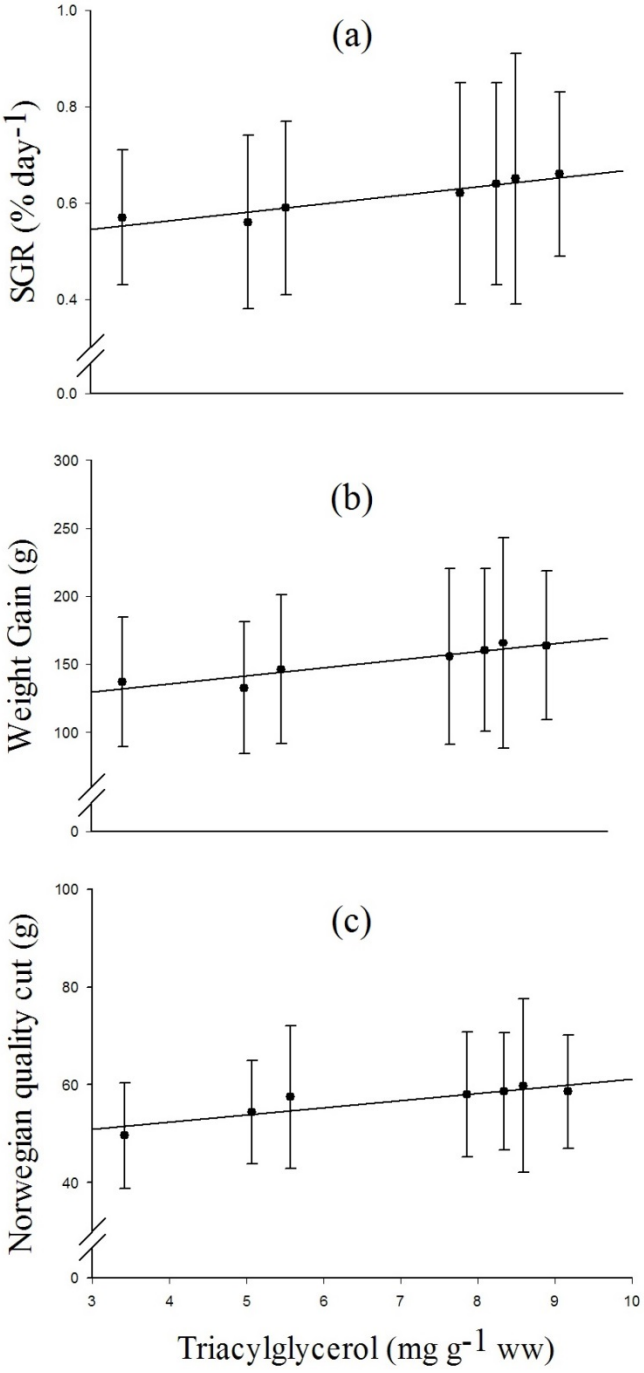

Figure 2.3. Regression analyses between the content of triacylglycerol in the muscle tissue and the growth performance factors, including a) specific growth rate, b) weight gain, and c) Norwegian quality cut.

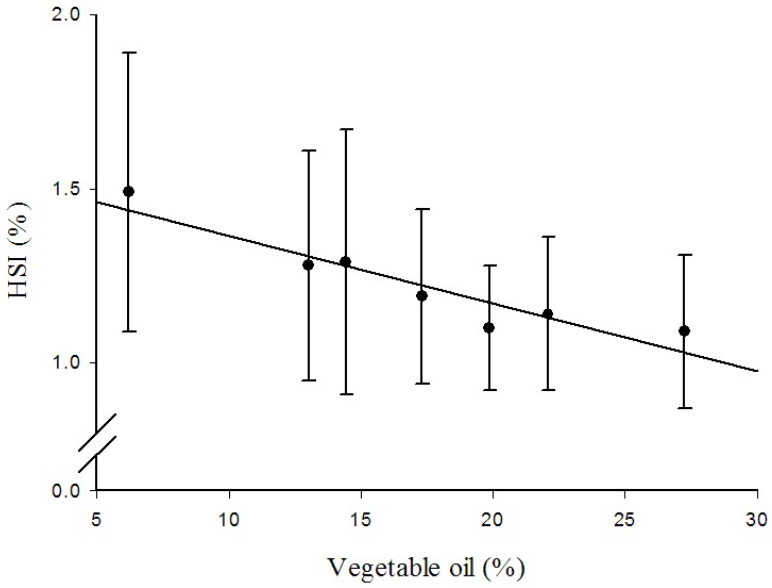

Figure 2.4. Regression analysis between vegetable oil percentage in the diet and hepatosomatic index.

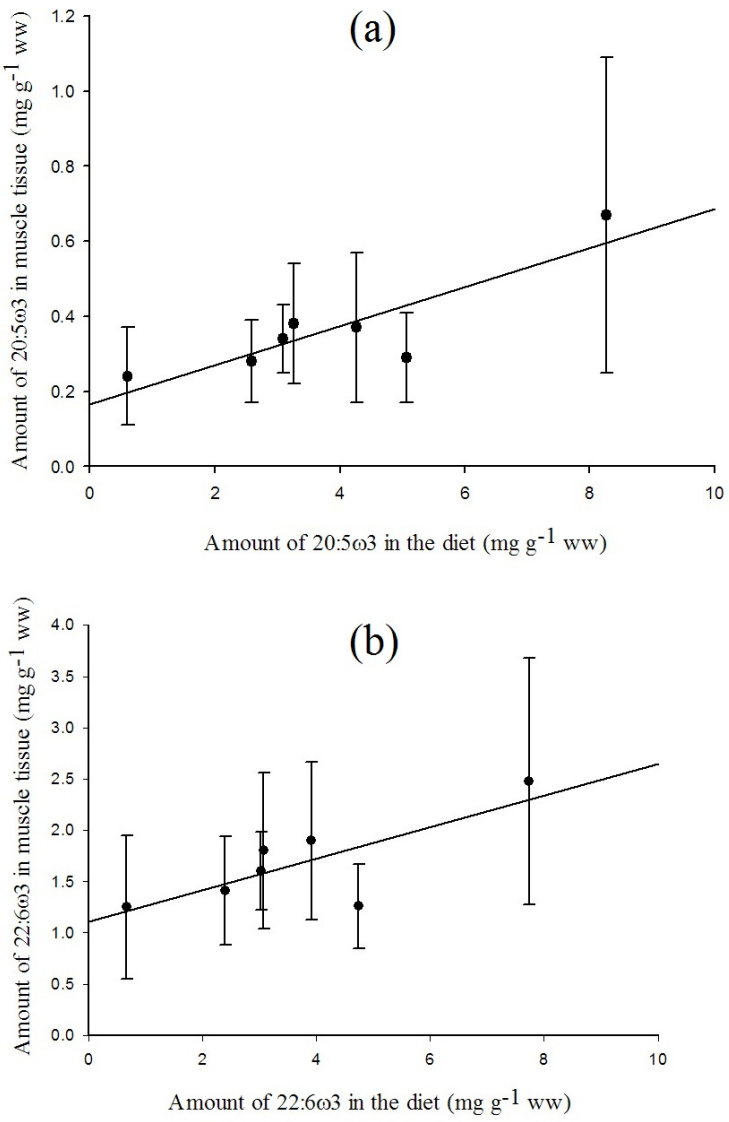

Figure 2.5. Regression analyses between amount of 20:5ω3 (a) and 22:6ω3 (b) in the diet and muscle tissue

2.4.6 Lipid Oxidation

Lipid oxidation was also studied to measure the extent it could affect the lipid in muscle tissues as high content of PUFAs in muscle tissues could make them susceptible to oxidation. Oxidation results were represented by TBARS values for marine, animal by-product, and ω3LC1.41 diets with mean values of 1.15, 1.52, and 1.01 mg MDA kg^{-1} of sample (mg of malondialdehyde equivalents per kg of muscle sample), respectively. While muscle in fish fed the animal by-products diet was expected to be less exposed to lipid oxidation due to the lower content of PUFAs (Table 2.6), the results revealed that these fish muscle samples were oxidized significantly more than the ones fed ω3LC1.41. This may reflect the types of materials used for animal by-products and how they were handled. While the study by Marshall et al. (2006) obtained a TBARS value of about 16.5 mg MDA kg^{-1} after 6 days, the TBARS values in this study did not exceed 3.13 mg MDA kg^{-1} (mean of 1.52 mg MDA kg^{-1}). Therefore, the oxidation results suggested that lipid rancidity was not remarkable for the three diets studied, as the TBARS values were significantly lower than the results of other studies on salmon (e.g. Marshall et al., 2006).

2.5 Discussion

In terms of growth characteristics such as weight gain, length gain, and SGR, the vegetable protein diet gave similar results to when the marine diet was used. Also, previous studies (Bell et al., 2003; Hixson et al., 2014a) showed similar results on growth performance of Atlantic salmon when using vegetable oil. A comparison between the results of different diets on growth performance (Table 2.4) reveals that ω3LC1 and ω3LC1.41 diets were as efficient as the marine diet probably because the required

minimum level of essential fatty acids were provided in these two diets. This result is in agreement with the results of the study by Hixson et al. (2014a), in which the authors stated that to support fish growth, a minimum level of marine lipid must be provided in the diet of Atlantic salmon. Unlike the ω3LC1, ω3LC1.41, and vegetable protein diets, the animal by-products and ω3LC0 diets significantly reduced weight gain, length gain, and SGR compared to when the marine diet was fed. However, previous studies showed that feeding diets containing up to 15% (Hatlen et al., 2013) and 40% (Deslauriers and Rideout, 2009) animal by-products had no significant effect on growth rate in comparison with the control (fish oil) diet. The difference between the growth results of this research and previous studies is due to the inclusion level of fish meal and fish oil in the diet, which was considerably lower in this study. For example, Bell et al. (2010) reduced the content of fish meal in the diet of Atlantic salmon to 25% with no significant change in growth and feed conversion. However, the present study further reduced the fish meal content to as low as 5% and still led to the same growth performance as when diets with 15 and 35% fish meal were used. This is reinforced by the significant correlations ($P<0.05$) between fish oil and weight gain and final weight, as final weight and weight gain increase with the inclusion level of fish oil (Table 2.A1). Fish fed the marine diet had the highest HSI level (Table 2.4) and there were significant positive correlations between HSI and fish meal, fish oil, and EPA+DHA ($P<0.01$, $P<0.05$, and $P<0.05$, respectively: Table 2.A1). The high content of fish meal, fish oil, and EPA+DHA might have caused the highest HSI level, but in a study by Hixson et al. (2013), HSI (8.5-8.8%) of Atlantic cod did not significantly differ between fish fed three different treatments,

including a fish oil control diet and two other diets in which 40 and 80% of the fish oil were replaced with camelina oil. In another study on Atlantic salmon (Espe et al., 2006), HSI did not differ among fish fed the four different experimental diets (a control fish meal diet (49%), and three other diets with no fish meal content), and unlike our results here, fish fed a control fish meal diet resulted in the lowest HSI.

The concentration of sterol and triacylglycerol in muscle samples did not change from week 0 to week 14 for all diets. However, phospholipid concentration decreased at the end of the feeding trial only when the marine diet was fed. Comparing the marine diet at the end of the feeding trial with the other diets, triacylglycerol concentration in muscle tissues did not significantly differ using different treatments. Compared to the marine diet, sterol concentration decreased using the animal by-product diet while phospholipid content increased when the ω3LC1.41 diet was fed (Table 2.5). It should be noted that these significant differences were not observed when sterol and phospholipid proportions (% total lipids) were compared (Table 2.A6), suggesting differences in class concentrations were driven by changes in total lipids.

As seen previously (Bell et al., 2003, Torstensen et al., 2005, Hixson et al., 2014b), the fatty acid composition of tissues in this study were readily influenced by the fatty acid composition of the diet, as evident from the correlation between ω3 fatty acids (20:5ω3 and 22:6ω3) of diets and muscle tissues (Table 2.A3). The slope of the regression line for dietary content of 20:5ω3 and 22:6ω3 against their contents in muscle tissue indicates the extent of this dependency. The higher slope for 22:6ω3 (0.30 mg g^{-1}

wet weight) compared to 20:5ω3 (0.08 mg g^{-1} wet weight) showed that 22:6ω3 was deposited four times more than 20:5ω3 in muscle tissue. A similar result was observed when fish oil was replaced with crude palm oil in Atlantic salmon diets (Bell et al., 2002), where 22:6ω3 (slope = 0.81) was more deposited than 20:5ω3 (slope = 0.58) in muscle tissues when proportions (% total fatty acids) were compared.

The fatty acid compositions of muscle tissues (Table 2.6) show that the sum of EPA and DHA levels ranges from 1.49 (0.24+1.25) to 3.15 (0.67+2.48) mg g^{-1} wet weight. According to the Canada's Food Guide (Government of Canada, 2007), one serving of cooked fish is 75 g. Therefore, a serving of cooked Atlantic salmon fed the seven dietary treatments includes EPA+DHA levels ranging from 112 (1.49×75) to 236 (3.15×75) mg which would be less than the daily requirement (250 mg) recommended by the World Health Organization (WHO, 2008).

Although animal fats generally have high levels of saturated fatty acids (Turchini et al, 2009), the animal by-product diet was characterized by a significantly lower concentration of MUFA (Table 2.3). The muscle of animals fed this diet had lower concentrations of a single MUFA (20:1ω9), and two PUFA (18:2ω6 and 18:3ω3).

Table 2.6 shows the fatty acid composition of fish fed vegetable protein, ω3LC1, and ω3LC1.41 diets was the same as fish fed the marine diet. Comparing the fatty acid composition in muscle tissues of fish fed the marine diet with the ones fed animal by-products, vegetable protein, ω3LC0, ω3LC1, and ω3LC1.41 diets reveals that they did not affect the muscle fatty acid composition of 18:1ω9, 18:2ω6, 18:3ω3, and 20:4ω6.

Although the diet containing 0% of LC ω3 fatty acids had the lowest level of 20:4ω6, fish fed this diet resulted in the highest proportion and concentration of 20:4ω6 in muscle tissues (Table 2.A7 and Table 2.6). This diet (ω3LC0) contains a high inclusion of rapeseed oil which has a high level of 18:2ω6. Therefore, it is assumed that 18:2ω6 was converted to 20:4ω6 resulting in the high percentage of 20:4ω6 in muscle tissues (Isseroff et al., 1987). The correlation analysis shows that growth performance characteristics are positively correlated with total concentrations of ω3 fatty acids in the diets. It is important to note there was no significant correlation with individual ω3 fatty acids in the diets (Table 2.A1) suggesting that an improvement in growth performance may be obtained irrespective of ω3 fatty acid chain-length or degree of unsaturation. In turn this indicates an interchangeability among these fatty acids in terms of function or biochemically through modification of chemical structures. This result was also confirmed through the regression analysis, which showed improved growth performance when higher inclusion levels of ω3 was used in the diet (Fig. 2.1). Therefore, marine, vegetable protein, ω3LC1, and ω3LC1.41 diets which contained high level of ω3 led to the best growth performance. In another study using the same dietary treatments, the vegetable protein diet was found to increase the immune response of Atlantic salmon (Caballero-Solares et al., 2017). Also, in past studies replacing fish oil and fish meal with camelina oil in the diet of Atlantic salmon, fish fed the highest level of ω3 fatty acids had the best performance in terms of weight gain, length gain, and SGR (Hixson et al. 2014a; Hixson et al. 2014b). The poor performance of fish fed ω3LC0 diet is undoubtedly associated with the complete lack of fish oil in this diet. This is in agreement with the

results of study by Bendiksen et al. (2011) in which the limitation of fish oil was identified as a more important factor than fish meal deficiency. Generally, a relatively high content of fish meal in the diet reduces the possibility of having low levels of essential fatty acids (Turchini et al., 2009). This study also confirms that a diet with relatively high content of fish meal (marine diet with 35% fish meal) resulted in the highest muscle levels of essential fatty acids per wet weight (Table 2.6).

The regression analysis showed that growth performance was directly correlated with the total lipid content in muscle as there was a correlation between total lipid and growth performance factors such as SGR, NQC, and weight gain (Fig. 2.2). The muscle total lipid was found to be a function of triacylglycerol as it was the main lipid class. Therefore, a positive relationship between the growth parameters and triacylglycerol was also observed (Fig. 2.3). The inverse correlation between HSI and vegetable oil content in the diet (Fig. 2.4) is why the diet with the highest inclusion of vegetable oil (ω3LC0) resulted in the lowest HSI.

2.5.1 Multivariate statistics

Diet and muscle fatty acid and lipid class composition were compared among the seven dietary treatments. The diet compositions, as well as muscle compositions, were very different as discriminant analysis correctly classified 44 of 45 (for diet) and 76 of 92 (for muscle) samples on the basis of major fatty acids and lipid classes.

The PCA for the diet showed that the fatty acids and lipid classes were categorized into four clusters (given on a dendrogram) according to their similarity

through cluster analysis of variables. The larger cluster in the top right quadrant of Fig. 2.6 shows the importance of 20:5ω3, 22:6ω3, and total sterols to the lipid composition of the marine and medium marine diets. The cluster in the top left quadrant shows the importance of triacylglycerols for energy and 18:2ω6 and 18:3ω3 as precursors of long-chain PUFA in diets ω3LC1 and ω3LC0. The cluster in the lower left of Fig. 2.6 shows the importance of phospholipids in the animal by-product and vegetable protein diets and the high DHA/EPA ratio in the vegetable protein and ω3LC0 diets.

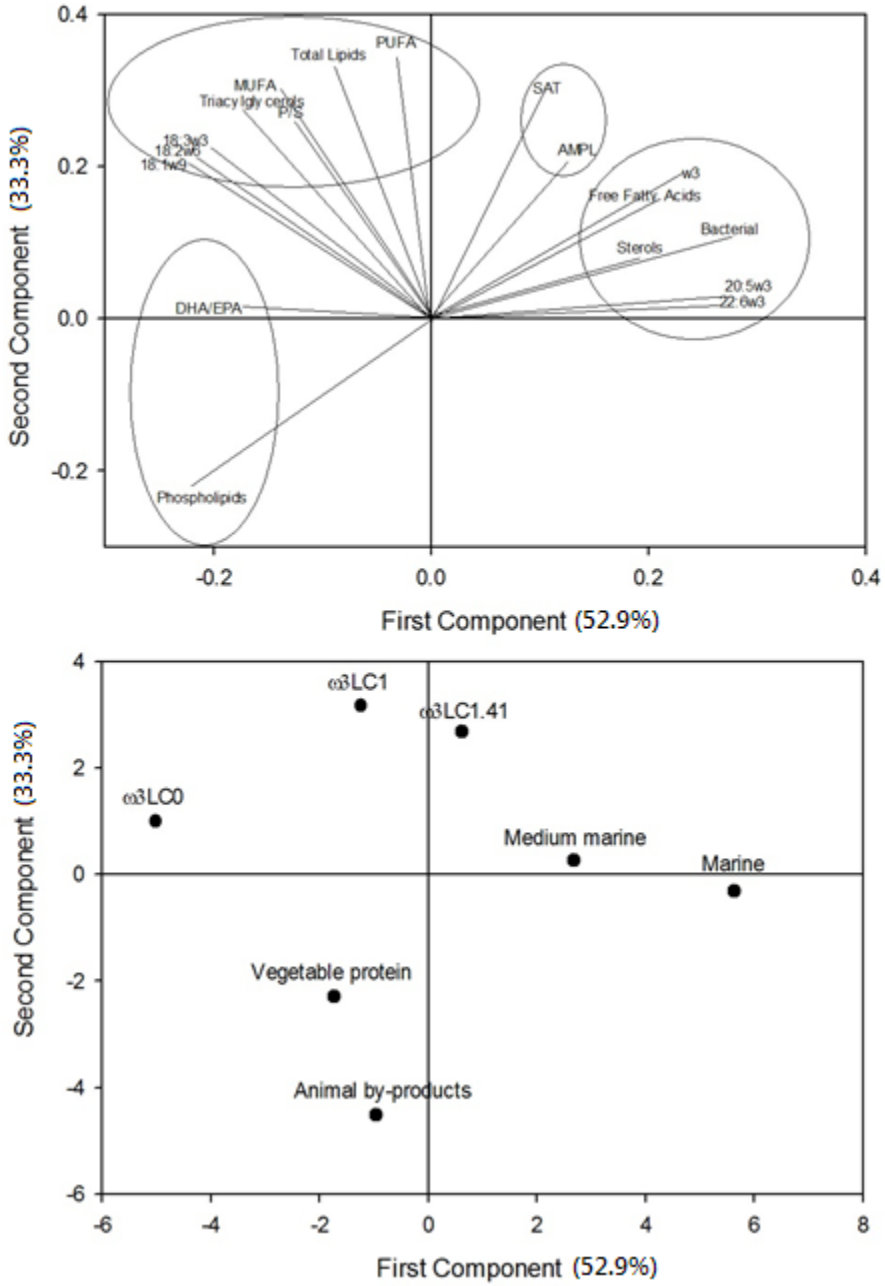

Figure 2.6. Principal components analysis of diet lipid and fatty acid contents (mg g^{-1} wet weight) with the seven diets

The PCA for the muscle tissue showed similar clusters of lipids as in the PCA for the diets. The cluster in the top right quadrant of Fig. 2.7 shows the role of triacyglycerols as energy storage with the ω3LC1 and ω3LC1.41 dietary treatments. The cluster in the top left quadrant shows the importance of phospholipids, and the P/S and DHA/EPA ratios with the ω3LC0 diet suggesting effects on membrane structure. The cluster in the lower right of Fig. 2.7 is associated with the marine dietary treatment. This correlation is the result of high inclusion of DHA+EPA in marine diet and correlation analysis confirmed that fatty acid composition of muscle tissue is directly influenced by the level of EPA+DHA in the diet. This cluster includes components that are important to both energy storage and membrane structure. While saturated fatty acids could contribute to energy storage, 20:5ω3, 22:6ω3, and sterols are important to the structure of cell membranes. The PCA for muscle tissue also showed a separation between 20:4ω6, with a more central location, and other lipids. The central location of the vegetable protein dietary treatment in the PCA results from the generally intermediate lipid composition of the muscle tissue.

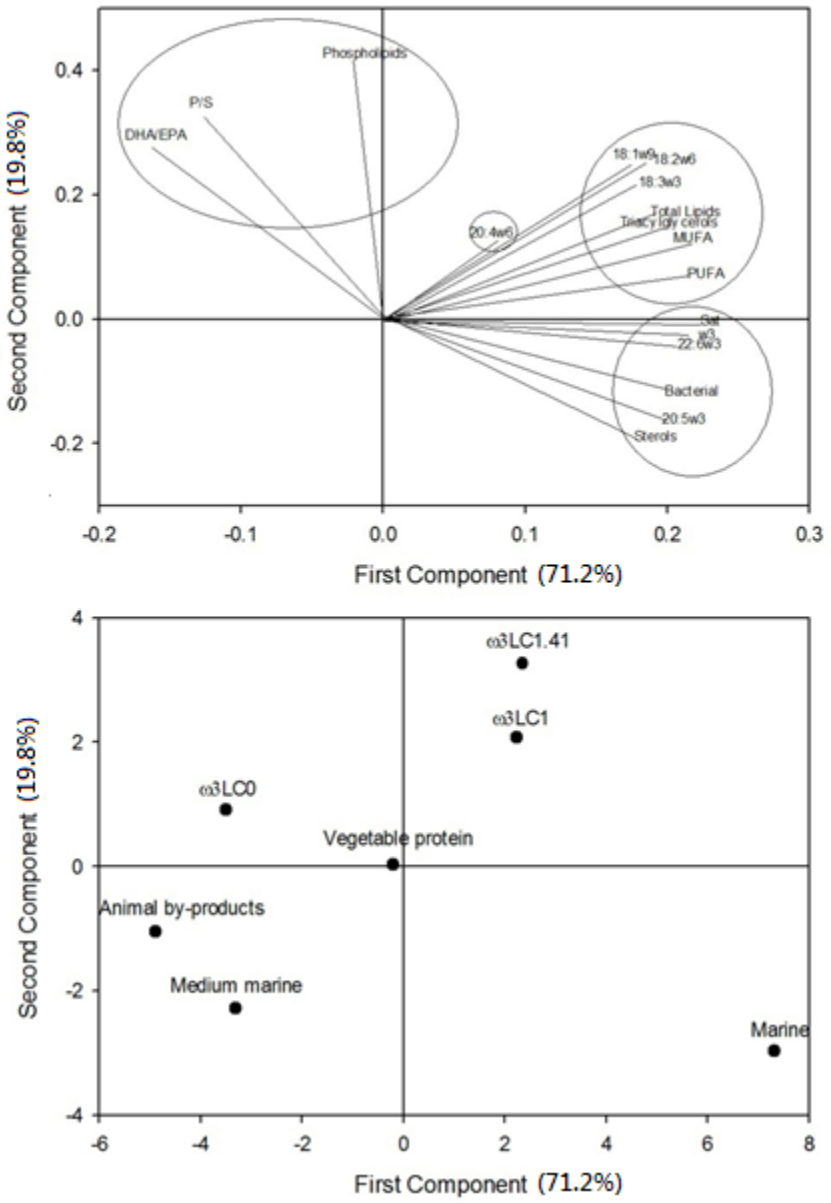

Figure 2.7. Principal components analysis of lipid and fatty acid contents (mg g^{-1} wet weight) in muscle tissue with the seven diets

2.6 Conclusions

This study evaluated the effects of minimizing marine resource utilization in diets of farmed Atlantic salmon on growth and muscle lipid composition. It was concluded that reducing marine resource utilization to less than 10% of diets in farmed Atlantic salmon affects growth, lipid classes and fatty acid composition of the muscle tissues. Also, replacement of fish meal and fish oil in aquafeeds with animal by-products and rapeseed oil at levels used in this study (33 and 27%, respectively) affects growth performance. However, lower level replacement might not affect growth. This study suggests that fish meal can be reduced to as low as 5% without affecting growth, as long as there is a minimum of 5% fish oil and animal by-products do not exceed 26% of diet.

2.7 References

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2.8 Appendix

Table 2.A1. Correlation analysis r values among diet ingredients, growth performance, diet (D) lipid classes, diet fatty acid composition, muscle (M) lipid classes, and muscle fatty acid composition (data with*, **, and *** represent P≤0.05, P ≤0.01, and P≤0.001, respectively)

Fish performance and diet and fish composition	Final weight (g)	Weight gain (g)	Length gain (cm)	HSI[1] (%)	VSI[2] (%)	NQC[3] (g)	Final length (cm)	SGR[4] (% day^{-1})	CF[5] (g cm^{-1})	AFI[6] (g fish^{-1})
VSI (%)		0.780*								
NQC (g)	0.807*	0.829*	0.760*							
Fish meal (%)				0.881**						
Animal by-products (%)									-0.770*	
Vegetable protein (%)				-0.805*						
Fish oil (%)	0.760*	0.759*		0.767*						0.842*
Vegetable oil (%)				-0.942**						
Digestible protein (g Kg^{-1})				0.766*						
EPA+DHA (%)				0.831*						0.843*
Total Lipid (M)*	0.891**	0.912**	0.901**		0.911**	0.768*		0.945***		
Triacylglycerol (M)	0.914**	0.937**	0.878**		0.827*	0.900**		0.948***		0.771*
Phospholipid (M)				-0.819*						

18:1ω9 (M)	0.772*	0.788*	0.828*	0.863*		0.833*	
18:2ω6 (M)	0.817*	0.828*	0.864*	0.840*	0.761*	0.869*	
18:3ω3 (M)	0.866*	0.880**	0.893**	0.883**	0.797*	0.913**	
20:5ω3 (M)				0.756*			0.869*
22:6ω3 (M)	0.814*	0.841*	0.755*	0.853*		0.830*	0.830*
Σ Bacterial[7] (M)		0.761*		0.756*			0.845*
Σ SFA[8] (M)	0.875**	0.903**	0.826*	0.897**		0.891**	0.866*
Σ MUFA[9] (M)	0.886**	0.909**	0.892**	0.937**	0.816*	0.923**	
Σ PUFA[10] (M)	0.880**	0.906**	0.861*	0.945***	0.756*	0.912**	0.791*
P/S[11] (M)			-0.851*				-0.783*
Σ ω3 (M)	0.838*	0.867*	0.785*	0.881**		0.855*	0.849*
DHA/EPA (M)			-0.835*				-0.861*
Triacylglycerol (D)**			-0.836*				
18:1ω9 (D)			-0.930**				
18:3ω3 (D)			-0.938**				
20:4ω6 (D)			0.772*				
20:5ω3 (D)			0.799*				0.850*
22:6ω3 (D)			0.804*				0.859*
Σ Bacterial (D)	0.768*	0.769*					0.824*
Σ MUFA (D)			-0.843*				

Σ ω3 (D)	0.847*	0.853*	0.837*	0.840*	0.822*	0.834*
DHA/EPA (D)				-0.980***		

[1] Hepatosomatic index
[2] Viscerosomatic index
[3] Norwegian quality cut
[4] Specific growth rate
[5] Condition factor
[6] Apparent feed intake
[7] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[8] Saturated fatty acids
[9] Monounsaturated fatty acids
[10] Polyunsaturated fatty acids
[11] Polyunsaturated/saturated fatty acids
* The unit for all lipids and fatty acids compositions is mg g^{-1} ww

Table 2.A2. Correlation analysis r values among diet ingredients, growth performance, diet lipid classes, diet (D) fatty acid composition, muscle (M) lipid classes, and muscle fatty acid composition (data with*, **, and *** represent P≤0.05, P ≤0.01, and P≤0.001, respectively)

Fish performance and diet and fish composition	Fish meal (%)	Vegetable protein (%)	Fish oil (%)	Vegetable oil (%)	EPA+DHA (%)
Sterol (M)	0.887**				0.817*
20:5ω3 (M)	0.855*		0.881**		0.911**
22:6ω3 (M)			0.840*		0.832*
Σ Bacterial (M)	0.843*		0.847*		0.881**
Σ SFA[1] (M)			0.792*		0.784*

P/S[2] (M)	-0.867*			0.793*	-0.767*
Σ ω3 (M)			0.816*		0.811*
DHA/EPA (M)	-0.916**		-0.871*	0.852*	-0.919**
Total Lipid (D)				0.785*	
Triacylglycerol (D)				0.914**	
18:1ω9 (D)			-0.770*	0.98***	
18:2ω6 (D)			-0.770*	0.976***	-0.797*
18:3ω3 (D)				0.968***	-0.757*
20:4ω6 (D)	0.817*	-0.824*	0.861*	-0.841*	0.885**
20:5ω3 (D)	0.884**		0.968***	-0.867*	0.986***
22:6ω3 (D)	0.890**		0.964***	-0.868*	0.985***
Σ Bacterial[3] (D)	0.826*		0.922**		0.934**
Σ MUFA[4] (D)				0.855*	
Σ ω3 (D)			0.833*		0.838*
DHA/EPA (D)			-0.766*		

[1] Saturated fatty acids
[2] Polyunsaturated/saturated fatty acids
[3] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[4] Monounsaturated fatty acids
* The unit for all lipids and fatty acids compositions is mg g^{-1} ww

Table 2.A3. Correlation analysis r values among diet ingredients, growth performance, diet lipid classes, diet (D) fatty acid composition, muscle (M) lipid classes, and muscle fatty acid composition (data with*, **, and *** represent P≤0.05, P ≤0.01, and P≤0.001, respectively)

Fish performance and diet and fish composition	Total Lipid (M)	Triacylglycerol (M)	Sterol (M)	Phospholipid (M)	18:1ω9 (M)	18:2ω6 (M)	18:3ω3 (M)	20:5ω3 (M)	22:6ω3 (M)	Σ Bacterial (M)	Σ SFA (M)	Σ MUFA (M)	P/S (M)	DHA/EPA (M)
Triacylglycerol (M)	0.949***													
18:1ω9 (M)	0.935**	0.904**												
18:2ω6 (M)	0.940**	0.926**			0.995***									
18:3ω3 (M)	0.974***	0.955***			0.985***	0.991***								
20:5ω3 (M)			0.953***											
22:6ω3 (M)	0.847*		0.876**					0.955***						
Σ Bacterial[1] (M)			0.941**					0.974***	0.945***					
Σ SFA[2] (M)	0.914**	0.855*	0.829*				0.826*	0.925**	0.977***	0.943***				
Σ MUFA[3] (M)	0.987***	0.943***			0.928**	0.928**	0.968***		0.860*	0.796*	0.936**			
Σ PUFA[4] (M)	0.967***	0.897**			0.850*	0.848*	0.909**	0.847*	0.941**	0.876**	0.982***	0.981***		
P/S [5] (M)				0.844*				-0.755*						
Σ ω3 (M)	0.881**	0.798*	0.868*				0.773*	0.949***	0.994***	0.955***	0.994***	0.901**		
DHA/EPA (M)			-0.758*					-0.840*		-0.808*			0.925**	
20:4ω6 (D)														-0.864*

20:5ω3 (D)			0.766*						0.871*	0.775*	0.861*	0.754*		-0.781*	-0.951***	
22:6ω3 (D)			0.772*						0.875**	0.775*	0.863*	0.756*		-0.797*	-0.958***	
Σ Bacterial (D)									0.850*	0.791*	0.889**	0.808*			-0.894**	
Σ PUFA (D)					0.806*	0.792*										
P/S (D)				0.791*					0.834*	0.818*	0.901**					
Σ ω3 (D)	0.761*	0.775*										0.878**	0.806*		-0.817*	

[1] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
* The unit for all lipids and fatty acids compositions is mg g^{-1} ww

Table 2.A4. Lipid class proportions in experimental diets (% total lipid classes)

Lipid classes (% total)	Marine n=6	Medium marine n=6	Animal by-products n=9	Vegetable protein n=6	ω3LC0 n=9	ω3LC1 n=9	ω3LC1.41 n=9
Triacylglycerol	54.4±15.1^{ab}	55.3±5.0^{b}	62.3±18.8^{ab}	72.2±10.7^{ab}	75.0±8.4^{a}	72.4±5.0^{ab}	71.3±13.8^{ab}
Sterol	4.2±1.4^{ab}	5.3±1.0^{a}	4.6±4.0^{ab}	2.7±1.9^{ab}	2.5±1.2^{ab}	2.2±1.3^{b}	3.2±1.9^{ab}
Phospholipid	6.3±1.4^{ab}	5.8±1.5^{ab}	11.5±8.8^{a}	8.6±4.9^{ab}	5.1±3.5^{ab}	6.7±4.2^{ab}	3.8±1.9^{b}

Data are ± standard deviation for n replicates

Table 2.A5. Fatty acid proportions of experimental diets (% identified fatty acids)

Fatty acid (% identified fatty acids)	Marine n=6	Medium marine n=6	Animal by-products n=6	Vegetable protein n=6	ω3LC0 n=6	ω3LC1 n=9	ω3LC1.41 n=6
14:0	4.30±0.30^{a}	2.51±0.20^{ab}	1.84±0.03^{abd}	1.67±0.03^{bd}	0.28±0.03^{d}	1.43±0.03^{cd}	1.96±0.03^{abc}
16:0	10.7±0.3^{a}	9.37±0.39^{ab}	8.65±0.24^{abc}	7.47±0.18^{bcd}	6.34±0.16^{d}	7.35±0.09^{cd}	7.83±0.08^{abcd}
16:1ω7	3.93±0.10^{a}	2.59±0.07^{ab}	1.99±0.05^{abc}	1.65±0.02^{bcd}	0.62±0.05^{d}	1.52±0.02^{cd}	1.92±0.02^{abcd}
18:0	2.17±0.04^{c}	3.96±0.05^{bc}	5.64±1.08^{a}	4.62±0.20^{ab}	5.51±0.38^{a}	4.38±0.18^{abc}	2.30±0.05^{bc}
18:1ω7	2.20±0.03^{b}	2.44±0.03^{ab}	2.47±0.05^{ab}	2.54±0.02^{a}	2.87±0.38^{ab}	2.57±0.15^{a}	2.40±0.15^{ab}
18:1ω9	23.3±0.3^{c}	34.7±0.2^{c}	39.2±0.6^{bc}	41.1±0.6^{ac}	51.7±1.1^{a}	43.4±0.4^{ab}	41.2±0.2^{ac}

18:2ω6	9.30±0.05^{b}	14.0±0.2^{bd}	15.5±0.3^{bc}	16.5±0.2^{acd}	18.4±1.1^{a}	16.6±0.1^{ac}	15.7±0.1^{abc}
18:3ω3	3.67±0.08^{b}	5.23±0.09^{bd}	5.84±0.08^{bc}	6.42±0.10^{acd}	7.27±0.74^{a}	6.70±0.05^{ac}	6.34±0.06^{abc}
18:4ω3	2.59±0.07^{a}	1.50±0.04^{ab}	1.07±0.02^{abd}	1.00±0.01^{bd}	0.07±0.02^{d}	0.86±0.01^{cd}	1.20±0.02^{abc}
20:1ω9	7.27±0.34^{a}	4.79±0.18^{ab}	3.90±0.07^{abd}	3.81±0.09^{abd}	1.67±0.13^{d}	3.41±0.06^{cd}	4.29±0.04^{abc}
20:4ω6	0.40±0.02^{a}	0.33±0.02^{b}	0.28±0.02^{c}	0.17±0.03^{dh}	0.10±0.03^{f}	0.18±0.01^{eh}	0.21±0.01^{g}
20:5ω3	6.24±0.17^{a}	3.52±0.11^{a}	2.29±0.09^{ab}	2.22±0.15^{ab}	0.34±0.04^{b}	1.82±0.04^{bc}	2.47±0.03^{ac}
22:1ω11(13)	9.20±0.27^{a}	5.49±0.29^{ab}	4.05±0.17^{abd}	3.84±0.04^{abd}	0.34±0.04^{d}	2.88±0.97^{cd}	4.57±0.05^{abc}
22:5ω3	0.63±0.29^{a}	0.23±0.01^{a}	0.13±0.02^{ab}	0.11±0.02^{ab}	0.13±0.08^{ab}	0.09±0.03^{b}	0.09±0.01^{b}
22:6ω3	5.83±0.24^{a}	3.29±0.13^{a}	2.15±0.18^{ab}	2.16±0.33^{ab}	0.41±0.05^{b}	1.75±0.11^{bc}	2.27±0.04^{ac}
Σ Bacterial[1]	1.28±0.07^{a}	0.90±0.02^{ac}	0.73±0.02^{ab}	0.67±0.01^{bc}	0.41±0.02^{b}	0.63±0.02^{bd}	0.74±0.03^{acd}
Σ SFA[2]	18.3±0.8^{a}	17.1±0.5^{ac}	17.4±1.1^{ac}	15.0±0.3^{ad}	13.6±0.5^{bd}	14.4±0.2^{bcd}	13.3±0.1^{bd}
Σ MUFA[3]	48.5±0.7^{b}	52.1±0.6^{bd}	53.4±0.8^{bcd}	54.6±0.6^{ab}	59.1±1.5^{a}	56.0±0.5^{ad}	56.4±0.1^{ac}
Σ PUFA[4]	32.5±0.7^{a}	30.4±0.3^{ac}	28.9±0.4^{bc}	30.1±0.5^{ac}	27.3±1.9^{b}	29.4±0.6^{bc}	30.0±0.1^{ad}
***P/S[5]**	1.78±0.11^{bc}	1.78±0.06^{bc}	1.67±0.12^{b}	2.00±0.03^{ab}	2.02±0.20^{ac}	2.04±0.07^{ab}	2.25±0.03^{a}
Σ ω3	19.9±0.6^{a}	14.3±0.3^{ac}	11.9±0.3^{bce}	12.3±0.4^{ae}	8.3±0.9^{be}	11.7±0.6^{bde}	12.8±0.1^{acd}
***DHA/EPA**	0.93±0.02^{ab}	0.94±0.01^{ab}	0.94±0.04^{a}	0.97±0.08^{ab}	1.21±0.12^{b}	0.96±0.05^{ab}	0.92±0.01^{a}

* Unitless ratios
[1] Σ Bacterial is a sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
Data are ± standard deviation for n replicates

Table 2.A6. Lipid class proportions of muscle tissue before and after 14 week of feeding trial (% total lipid classes)

Lipid classes (% total)	Initial n=10	Marine n=9-19	Medium marine n=9-19	Animal by-products n=9-19	Vegetable protein n=9-19	ω3LC0 n=9-19	ω3LC1 n=9-19	ω3LC1.41 n=9-19
Triacylglycerol	47.4±14.3	56.0±23.8	55.9±19.5	40.2±10.1	53.0±15.0	45.2±15.2	50.9±19.5	51.5.±11.6
Sterol	1.5±0.5	1.9±1.3	1.8±0.9	1.7±0.6	1.9±0.7	2.0±0.6	1.4±0.6	1.9±0.9
Phospholipid	36.0±18.6	26.2±20.2^{b}	35.9±19.3^{ab}	49.1±12.0^{a}	32.7±13.7^{b}	44.2±18.4^{a}	35.9±19.8^{ab}	34.1±12.8^{ab}

Data are ± standard deviation for n replicates

Table 2.A7. Fatty acid proportions of muscle tissue before and after 14 weeks of feeding (% identified fatty acids)

Fatty acid (% identified fatty acids)	Initial n=10	Marine n=12-19	Medium marine n=12-19	Animal by-products n=12-19	Vegetable protein n=12-19	ω3LC0 n=12-19	ω3LC1 n=12-19	ω3LC1.41 n=12-19
14:0	3.02±0.45	2.69±0.62^{a}	1.89±0.46^{ab}	1.34±0.22^{c}	1.51±0.24^{bc}	0.73±0.24^{d}	1.42±0.18^{bc}	1.59±0.36^{abc}
16:0	16.3±0.6	14.0±1.6^{ab}	13.8±1.3^{ab}	14.2±0.8^{a}	12.5±1.2^{bc}	10.5±4.4^{c}	11.6±1.3^{c}	11.8±1.8^{c}
16:1ω7	5.14±0.76	3.28±0.98^{a}	2.55±0.67^{ab}	1.82±0.47^{bc}	2.00±0.44^{ab}	1.19±0.50^{c}	1.92±0.33^{abc}	2.12±0.70^{ab}
18:0	3.97±0.15	3.17±0.27^{ab}	3.28±0.26^{ab}	3.38±0.30^{a}	3.10±0.28^{ab}	3.23±0.26^{ab}	2.96±0.26^{b}	2.99±0.20^{b}
18:1ω7	2.86±0.18	2.38±0.16^{d}	2.48±0.17^{bd}	2.47±0.12^{bd}	2.55±0.16^{bc}	2.73±0.16^{a}	2.66±0.12^{ac}	2.57±0.10^{bc}
18:1ω9	18.8±1.6	20.5±4.3^{b}	25.1±4.5^{b}	24.4±3.6^{b}	30.1±4.8^{a}	32.8±6.4^{a}	33.1±4.1^{a}	29.9±4.5^{a}
18:2ω6	7.92±0.87	6.69±1.45^{c}	8.81±1.53^{bc}	7.91±2.17^{bc}	10.2±1.3^{ab}	10.0±1.5^{ab}	10.8±1.1^{a}	10.1±1.4^{ab}
18:3ω3	0.99±0.08	2.03±0.36^{c}	2.54±0.60^{ac}	2.27±0.25^{bc}	2.78±0.29^{a}	2.66±0.33^{ab}	2.93±0.45^{a}	2.91±0.25^{a}

18:4ω3	0.92±0.13	1.43±0.34^{bc}	1.27±0.30^{cd}	1.27±0.19^{cd}	1.50±0.29^{bc}	1.95±0.26^{a}	1.65±0.32^{ab}	1.49±0.28^{bc}
20:1ω9	1.87±0.28	4.41±1.13^{a}	2.94±0.65^{abc}	2.26±0.37^{cd}	2.78±0.46^{bc}	1.31±0.37^{d}	2.53±0.87^{bc}	3.09±0.50^{ab}
20:4ω6	1.26±0.20	0.91±0.33^{b}	1.10±0.32^{bc}	1.45±0.26^{ac}	1.07±0.32^{bc}	1.89±0.60^{a}	0.95±0.30^{b}	0.92±0.30^{b}
20:5ω3	5.77±0.65	4.77±1.20^{a}	4.24±1.03^{ab}	4.41±0.74^{a}	3.37±1.00^{bc}	3.26±1.01^{bc}	2.94±0.88^{c}	3.26±1.06^{bc}
22:1ω11(13)	1.51±0.29	4.21±1.22^{a}	2.28±0.60^{abc}	1.61±0.37^{cd}	1.89±0.36^{bc}	0.39±0.18^{d}	1.81±0.37^{bc}	2.23±0.62^{abc}
22:5ω3	1.93±0.14	1.63±0.19^{a}	1.29±0.18^{ab}	1.24±0.33^{ab}	0.99±0.24^{bc}	0.99±0.25^{bc}	0.92±0.23^{c}	0.97±0.31^{bc}
22:6ω3	20.5±3.5	19.9±8.0^{ab}	19.4±6.7^{ab}	22.3±4.6^{a}	16.1±5.4^{ab}	17.3±6.9^{ab}	14.1±4.3^{b}	16.8±6.1^{ab}
Σ Bacterial[1]	1.03±0.12	0.92±0.24^{a}	0.78±0.28^{a}	0.71±0.28^{ab}	0.64±0.10^{a}	0.49±0.08^{b}	0.63±0.07^{ab}	0.65±0.13^{a}
Σ SFA[2]	24.1±0.4	21.1±1.3^{a}	20.2±1.4^{acd}	20.6±0.9^{ac}	18.9±1.3^{bc}	16.9±4.3^{b}	17.7±1.4^{b}	18.0±1.8^{bd}
Σ MUFA[3]	31.8±3.1	37.0±8.1^{ab}	37.2±6.7^{ab}	34.2±4.7^{b}	41.1±6.1^{a}	39.9±7.3^{ab}	44.0±5.3^{a}	41.7±5.9^{a}
Σ PUFA[4]	43.6±3.0	41.6±7.1^{ab}	42.32±5.8^{ab}	44.9±4.3^{a}	39.7±5.0^{ab}	42.1±6.7^{ab}	38.1±3.9^{b}	40.0±5.5^{ab}
***P/S**[5]	1.81±0.12	1.97±0.25	2.09±0.23	2.18±0.21	2.09±0.17	2.20±0.10	2.15±0.08	2.23±0.38
Σ ω3	31.2±3.9	31.1±8.6^{ab}	29.8±7.2^{ab}	32.4±5.1^{a}	25.7±6.1^{ab}	26.8±7.7^{ab}	23.3±5.1^{b}	26.5±6.7^{ab}
***DHA/EPA**	3.54±0.31	4.03±0.77^{b}	4.50±0.71^{ab}	5.05±0.64^{a}	4.77±0.70^{ab}	5.22±0.86^{a}	4.82±0.57^{ab}	4.94±0.90^{a}

* Unitless ratios
[1] Σ Bacterial is a sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, ai16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
Data are ± standard deviation for n replicates

3. MINIMIZING MARINE RESOURCE UTILIZATION IN DIETS OF FARMED ATLANTIC SALMON (*SALMO SALAR*): EFFECTS ON LIVER AND HEAD KIDNEY LIPID CLASS, FATTY ACID AND ELEMENTAL COMPOSITION

3.1 Abstract

The limited supply of fish meal and fish oil for aquafeed has necessitated research on the use of alternative sources of protein and lipids that can provide the nutritional requirements for finfish. Seven dietary treatments with different protein and lipid sources were formulated to feed farmed Atlantic salmon, and their effects on liver and head kidney lipid class, fatty acid and elemental composition were studied. The elemental analysis showed that the ratio of carbon to nitrogen (C/N) was higher in head kidney tissues than it was in liver tissues, which is consistent with higher content of total lipids in head kidney tissues in comparison to liver tissues. The results also showed that the livers from fish fed the low fish meal diet containing 1.4% ω3 long chain fatty acids (ω3LC1.4) had a higher nitrogen concentration and a lower C/N ratio than livers from all other dietary treatments. Also, livers and head kidneys from fish fed the lowest amount of fish meal and fish oil had the least EPA (20:5ω3) and DHA (22:6ω3) and the highest ARA (20:4ω6) levels. This study suggests that fish meal and fish oil reduction levels to less than 10% in diets of farmed Atlantic salmon affects elemental and chemical compositions of the liver and head kidney tissues. This implies that at least 10% of the diet should contain fish meal and fish oil to achieve comparable lipid contents to fish fed marine-based diets in order to reduce susceptibility to inflammation.

3.2 Introduction

The major cost in the finfish aquaculture production industry is associated with the production of aquafeeds (50-80% of the total cost), which has been growing significantly, up to 30% annually (Turchini et al., 2010). The finfish aquaculture industry is a major contributor to fish food demands, but it is heavily dependent on marine resources, consuming about 68 and 89% of the world production of fish meal and fish oil, respectively (Tacon and Metian, 2008, Shepherd, 2012). While global fish meal and fish oil consumption almost doubled from 1995 to 2004 (Hasan and Halwart, 2009), the production of fish meal and fish oil has not increased significantly (Tacon et al. 2006).

One of the most important factors in the sustainability and development of the aquaculture industry is the use and availability of fish oil. Fish oil is beneficial to the immune system of animals and humans (Oliveira-Goumas, 2004) and is known as a valuable primary resource of lipid providing the required ω3 PUFAs (polyunsaturated fatty acids, PUFA) (Turchini et al., 2010). Fish oil has high levels of 20:5ω3 (eicosapentaenoic acid, EPA) and 22:6ω3 (docosahexaenoic acid, DHA). The consumption of fish oil is dominated by salmonids at 64%; 49.7% by salmon and 14.8% by trout (Huntington and Hasan, 2009).

Fish meal is an excellent diet for carnivorous fish as it provides the required protein and oil (Hasan and Halwart, 2009). Fish meal is highly digestible and has a valuable protein content with a superior amino acid profile and contains high levels of essential amino acids. It is also a good source of 20:5ω3, 22:6ω3, and essential vitamins and minerals (IFOMA, 2001).

The limited availability of wild fish as the main source of fish meal and fish oil is one of the main concerns of aquaculture industry. Fish meal and fish oil resources are depleting as they are widely used in aquaculture to provide the required ω3 fatty acids in farmed piscivorous (e.g. European seabass, gilthead seabream and Atlantic salmon) and omnivorous fish (e.g. carp and tilapia) (Naylor et al., 2009).

The increasing demand for the consumption of fish meal and fish oil has also increased the price of these resources. Due to the high cost and the limited availability of fish meal and fish oil resources, and in order to reduce the consumption of marine resources in aquaculture, it is important to replace fish meal and fish oil with alternative sustainable sources of lipid and protein (e.g. terrestrial plants and animals). This study evaluates the effects of using alternative diets containing low levels of marine resources on the lipid class, fatty acid, and elemental compositions of liver and head kidney tissues of farmed Atlantic salmon. These two tissues were selected for this study as they were found to be responsive to dietary treatments (Eslamloo et al., 2017). While liver tissues are representative of fish metabolism and growth, head kidney tissues are important in studying the fish immune system (Caballero-Solares et al., 2017).

3.3 Materials and Methods

3.3.1 Experimental Diets

Seven different diets which are described in Chapter 2 (Section 2.3.1) were provided by EWOS Innovation AS in Norway (Table 2.1).

3.3.2 Experimental fish and feeding

Atlantic salmon (*Salmo salar)* smolts obtained from Northern Harvest Sea Farms in Stephenville, NL, Canada were tagged and fed as described in Chapter 2 (Section 2.3.2).

3.3.3 Tissue sampling

Sampling was conducted at week 0 (when no experimental diets were fed), week 7, and week 14. Ten fish were randomly sampled at week 0, followed by 5 fish per tank at week 7 and 14. Fish were euthanized with an overdose of MS-222 (400 mg L^{-1}, Syndel Laboratories, Vancouver, BC, Canada), then fork length and weight were measured. Gut and liver tissues were removed, weighed, and sampled. The membrane covering the head kidney was removed (head kidney was defined as the anterior one-quarter of the kidney located directly behind the head). The head kidney was removed using the scoop end of a spatula. Liver and head kidney samples were separated into three subsamples. The largest subsample was placed into lipid clean test tubes (rinsed with methanol and chloroform three times each), the second piece was placed into an ashed scintillation vial for carbon (C) and nitrogen (N) analysis and the third subsample was left on a weigh boat for dry/ash analysis. The tubes had been weighed after ashing at 450°C for 8 hrs, and the Teflon lined caps were rinsed three times with methanol and chloroform. Vials were kept on ice during sampling. After sampling, 2 ml of chloroform was poured on the tissues in the test tubes and the remaining space was filled with nitrogen. The tubes were then sealed with Teflon tape and were stored at -20°C. It should be noted that all procedures, including handling, treatment, euthanasia, and dissection were performed according to

the guidelines from the Canadian Council of Animal Care (approved Memorial University Institutional Animal Care Protocol 14e71-MR).

3.3.4 Lipid extraction

Lipid were extracted in methanol:chloroform:water as described in Chapter 2 (Section 2.3.4).

3.3.5 Lipid class determination

Lipid classes were separated by thin-layer chromatography and measured by flame ionization detection as described in Chapter 2 (Section 2.3.5).

3.3.6 Fatty acid methyl ester derivatization

Fatty acid methyl esters were derivatized using the Hilditch reagent as explained in Chapter 2 (Section 2.3.6).

3.3.7 C and N analysis

Samples were collected in 20 ml vials that had been placed in a muffle furnace at 450°C for 8 hrs. The vials were covered with aluminum foil that had also been heated for 8 hrs at 450°C. The plastic cap was screwed on over the aluminum foil. The samples were stored at -20°C until processing.

The vials had the cover and the aluminum foils removed and were placed in a 60°C oven for at least 24 hrs. After this drying period, the samples were ground to a fine powder using a mortar and pestle. The samples were then moved to a descicator containing an open beaker of hydrochloric acid for a second 24-hr period. This helps

break down the sample and removes the inorganic carbon. After this 24-hr period, the samples were placed back in the oven at 60°C for a further 24 hrs.

Samples were then weighed using a Mettler Toledo UMT 2 balance into tin capsules (Isomass), folded to ensure no loss of sample and placed into a 96 well plate. The plate was stored in a desiccator until the samples were run on a Perkin Elmer Series II analyzer. The C and N were calibrated before the run using acetanilide and a standard was run after every 10 samples to ensure the results were no more than 10% off expected values.

3.3.8 Statistics

In order to ensure representative fish were sampled, only fish with weight gains within the range of twice the standard deviation from the overall tank weight gain means were considered. Correlation and regression analyses were conducted using Minitab version 17 to compare diet ingredients, lipid and fatty acid composition of tissue and diet, and growth characteristics. For statistical analysis of lipid class, fatty acid and elemental composition data, nested general linear models were combined with Tukey pairwise comparisons using Minitab to determine the difference between tanks and diets. The normality of residuals was evaluated with the Anderson-Darling normality test. If the test failed ($p<0.05$), a one-way ANOVA on ranks was performed in SigmaPlot version 13. Principal components analyses (PCA) were performed using Minitab version 17 to compare diet, liver and head kidney fatty acid composition and lipid classes with the seven diets and the graphs were plotted using SigmaPlot version 13. A discriminant

analysis was also performed in Minitab to classify the samples according to their major fatty acids and lipid classes.

3.4 Results

3.4.1 Experimental diet composition

Diets consisted mainly of triacylglycerol and had the same concentrations of 16:0, ΣSFA, and ΣPUFA as well as the same ratios of P/S and DHA/EPA as detailed in Chapter 2 (Section 2.4.1).

3.4.2 Elemental composition

Elemental compositions were measured at week 7 and 14 in head kidney, liver and muscle tissue (Norwegian quality cut: Chapter 2) for comparison. Although there was no significant difference in the carbon (C) and nitrogen (N) contents of liver and head kidney tissues among the seven diets after week 7, the C/N ratio and N % in muscle tissues differ significantly. The animal by-products diet resulted in the lowest C/N ratio and highest N % in muscle. However, no significant differences in liver C/N ratio and N % were observed when feeding the seven diets, except between the marine and ω3LC1.41 diets, which resulted in the highest and lowest levels of C/N ratio and N %, respectively. While the ω3 LC 0 diet resulted the lowest C % in head kidney, fish fed the marine and medium marine diets had the highest C % in their head kidney tissues (Table 3.1).

Table 3.1. Elemental composition of muscle (M), liver (L), and head kidney (HK) tissues after week 14 (% dry weight)

Elemental Composition (% dry weight)	Marine n=20	Medium marine n=20	Animal by-products n=20	Vegetable protein n=20	ω3 LC 0 n=20	ω3 LC 1 n=20	ω3 LC 1.41 n=20
C/N (M)	3.7±0.4	3.5±0.2	3.4±0.3	3.7±0.5	3.5±0.2	3.6±0.2	3.7±0.4
C[1] (M)	49.3±3.3	47.9±3.4	49.6±3.8	49.3±1.8	47.8±5.4	49.3±1.6	48.9±3.8
N[2] (M)	13.4±0.7^{b}	13.6±0.7^{ab}	14.9±2.7^{a}	13.5±1.3^{ab}	13.6±1.5^{ab}	13.9±0.6^{ab}	13.4±1.1^{ab}
C/N (L)	5.8 ± 1.1^{a}	5.7 ± 1.2^{ab}	5.6 ± 0.9^{ab}	5.3 ± 0.8^{ab}	5.1 ± 0.8^{ab}	5.2 ± 0.7^{ab}	4.8 ± 0.3^{b}
C (L)	48.3 ± 1.2	48.6 ± 1.3	49.1 ± 1.5	48.9 ± 1.0	49.5 ± 1.9	48.6 ± 2.0	48.9 ± 0.8
N (L)	8.5 ± 1.4^{b}	8.8 ± 1.5^{ab}	9.0 ± 1.4^{ab}	9.4 ± 1.2^{ab}	9.9 ± 1.4^{ab}	9.5 ± 0.9^{ab}	10.2 ± 0.5^{a}
C/N (HK)	6.5 ± 1.5	6.3 ± 1.1	5.3 ± 1.1	5.4 ± 1.1	5.2 ± 1.0	5.4 ± 1.2	5.7 ± 1.6
C (HK)	55.3 ± 3.0^{a}	54.5 ± 5.5^{a}	50.6 ± 4.6^{ab}	51.3 ± 3.1^{ab}	49.4 ± 4.4^{b}	51.5 ± 4.0^{ab}	51.9 ± 3.7^{ab}
N (HK)	8.8 ± 1.5	8.8 ± 1.1	9.8 ± 1.2	9.8 ± 1.2	9.6 ± 1.2	9.7 ± 1.4	9.5 ± 1.6

[1] Carbon
[2] Nitrogen
Data are ± standard deviation for n replicates

3.4.3 Liver lipid class composition

There was no significant difference in total lipids and major lipid classes among the different treatments. The major lipid classes in liver tissues were phospholipid, triacylglycerol, and sterol, accounting for 52-64, 17-28, and 10-12% of the total lipids in liver tissues, respectively (Table A.6). A comparison between the level of different lipids at week 0 and 14 (Table 3.2) showed that the use of different treatments did not affect the total lipids, but triacylglycerol, and sterol increased after 14 weeks except for marine and ω3 LC 1.4 diets which did not change. Phospholipid content dropped significantly after 14 weeks except when medium marine and ω3 LC 1.4 were fed (Table 3.2).

Table 3.2. Lipid classes of liver tissue before and after 14 week of feeding trial (mg g^{-1} wet weight)

Lipid classes (mg g^{-1} wet weight)	Initial n=10	Marine n=12	Medium marine n=12	Animal by-product n=12	Vegetable protein n=12	ω3 LC 0 n=12	ω3 LC 1 n=12	ω3 LC 1.4 n=11-12
Total lipid	30.6±3.3	34.7 ± 7.7	39.2 ± 13.9	33.9 ± 11.7	36.4 ± 12.2	35.3 ± 16.6	41.2 ± 19.4	34.3 ± 6.8
Triacylglycerol	1.69±0.96	8.9 ± 5.7	10.4 ± 10.4	9.5 ± 6.0	10.1 ± 10.2	10.7 ± 11.5	14.2 ± 16.9	6.2 ± 3.0
Sterol	1.19±0.08	3.2 ± 0.8	3.8 ± 0.9	3.4 ± 0.9	3.7 ± 0.9	3.7 ± 1.4	3.7 ± 0.7	3.7 ± 1.0
Phospholipid	27.0±3.2	19.2 ± 4.1	20.3 ± 4.1	18.3 ± 7.0	19.0 ± 4.3	17.2 ± 5.6	19.0 ± 2.4	21.8 ± 4.3

The underlined data represents the values that are statistically different from week 0
Data are ± standard deviation for n replicates

3.4.4 Liver fatty acid composition

Liver tissue fatty acid composition (Table 3.3) showed no significant differences among treatments for 16:0, 18:0, 18:1ω7, 18:1ω9, 18:3ω3, 20:1ω9, Σ SFA, Σ MUFA, and Σ PUFA. Table 3.3 shows that in terms of essential long-chain PUFA, the concentration of 20:4ω6 did not change significantly compared to week 0, except when the marine and ω3 LC 1.4 diets were used, which resulted in the lowest level of 20:4ω6 in liver tissues. The content of other long-chain PUFAs (20:5ω3 and 22:6ω3) also decreased significantly when using different treatments except when marine and medium marine diets were fed. Among the treatments, diet ω3LC0 resulted in the highest contents of both 18:2ω6 and 20:4ω6 in liver. The total ω3 fatty acids consisted mainly of 22:6ω3 and 20:5ω3, accounted for 13-25.8 and 2.5-5% of the total fatty acids in liver tissues, respectively (Table 3.A5). Feeding the marine diet resulted in the highest content of 20:5ω3, while both marine and medium marine diets resulted the highest content of 22:6ω3 in liver. As expected, the level of Σω3 was the lowest when ω3LC0 was fed. The analysis of the DHA/EPA ratio indicated that vegetable protein, ω3LC1, and ω3LC1.41 led to the highest ratio, while the marine diet resulted in the lowest ratio of DHA/EPA (Table 3.3). It should be noted that lipid class and fatty acids proportions (%) in diets and in liver and head kidney are presented in Tables 2.A4 and 2.A5 and in 3.A3-3.A7.

Table 3.3. Fatty acid composition of liver tissue before and after 14 week of feeding trial (mg g^{-1} wet weight)

Fatty acid (mg g^{-1} wet weight)	Initial n=10	Marine n=12	Medium marine n=12	Animal by-product n=12	Vegetable protein n=12	ω3 LC 0 n=12	ω3 LC 1 n=12	ω3 LC 1.4 n=12
14:0	0.34±0.03	0.49±0.22^{a}	0.38±0.16^{a}	0.32±0.17^{a}	0.28±0.14^{ab}	0.14±0.07^{b}	0.31±0.20^{ab}	0.26±0.08^{ab}
16:0	3.87±0.36	2.98±0.85	3.20±1.05	2.75±0.89	2.63±0.80	2.33±0.79	2.72±0.85	2.52±0.57
16:1ω7	0.53±0.10	0.67±0.34^{a}	0.65±0.54^{ab}	0.49±0.27^{ab}	0.38±0.27^{ab}	0.25±0.14^{b}	0.45±0.35^{ab}	0.34±0.13^{ab}
18:0	1.32±0.17	1.05±0.25	1.35±0.76	1.07±0.37	1.14±0.44	1.10±0.52	1.17±0.49	0.97±0.24
18:1ω7	0.53±0.09	0.67±0.24	0.84±0.45	0.64±0.36	0.66±0.40	0.75±0.51	0.91±0.49	0.84±0.29
18:1ω9	2.64±0.55	5.53±2.34	8.18±6.02	7.49±3.93	8.36±5.89	9.73±7.96	10.88±9.93	6.09±2.09
18:2ω6	0.98±0.18	1.24±0.53^{b}	1.91±0.84^{ab}	1.97±0.98^{ab}	2.10±1.19^{ab}	2.41±1.52^{a}	2.89±2.19^{ab}	1.89±0.51^{ab}
18:3ω3	0.08±0.02	0.25±0.13	0.33±0.17	0.30±0.17	0.34±0.21	0.33±0.22	0.52±0.48	0.34±0.11
20:1ω9	0.18±0.05	1.07±0.47	1.01±0.60	0.82±0.52	1.05±0.86	0.35±0.18	0.87±0.47	0.77±0.34
20:4ω6	0.93±0.12	0.6 ± 0.1^{b}	0.7 ± 0.1^{ab}	0.8 ± 0.3^{ab}	0.8 ± 0.2^{ab}	1.3 ± 0.5^{a}	0.8 ± 0.1^{ab}	0.6 ± 0.2^{ab}
20:5ω3	1.53±0.24	1.2 ± 0.3^{a}	1.1 ± 0.3^{ab}	0.8 ± 0.3^{b}	0.8 ± 0.2^{b}	0.6 ± 0.2^{b}	0.8 ± 0.2^{b}	0.9 ± 0.2^{b}
22:1ω11(13)	0.04±0.01	0.38±0.25^{a}	0.23±0.19^{ab}	0.19±0.15^{ab}	0.16±0.10^{ab}	0.05±0.01^{b}	0.16±0.12^{ab}	0.13±0.7^{ab}
22:5ω3	0.41±0.05	0.35±0.09^{a}	0.30±0.11^{ab}	0.25±0.12^{ab}	0.21±0.06^{b}	0.21±0.06^{ab}	0.21±0.05^{b}	0.21±0.07^{b}
22:6ω3	6.68±0.69	5.8 ±1.1^{a}	5.7 ± 1.2^{a}	4.5 ±1.3^{b}	4.7 ± 7.8^{ab}	3.1 ± 1.0^{c}	4.9 ± 0.9^{ab}	5.1 ± 1.0^{ab}
Σ Bacterial[1]	0.20±0.02	0.2 ± 0.08^{a}	0.2 ± 0.08^{ab}	0.2 ± 0.06^{ab}	0.2 ± 0.06^{ab}	0.1 ± 0.08^{b}	0.2 ± 0.1^{ab}	0.2 ± 0.04^{ab}
Σ SFA[2]	5.73±0.51	4.7 ± 1.3	5.1 ± 2.0	4.2 ± 1.4	4.2 ± 1.4	3.7 ± 1.4	4.4 ± 1.5	3.9 ± 0.9
Σ MUFA[3]	4.21±0.80	9.0 ± 3.8	11.6 ± 8.3	10.3 ± 5.3	11.2 ± 7.7	11.9 ± 9.7	14.5 ± 12.7	8.8 ± 3.0

Σ PUFA[4]	11.46±1.25	10.7 ± 2.4	11.6 ± 3.0	10.3 ± 3.5	10.8 ± 2.7	10.3 ± 4.2	12.3 ± 4.6	10.5 ± 2.3
[*]P/S[5]	2.00±0.14	2.3 ± 0.2 [b]	2.4 ± 0.3 [b]	2.4 ± 0.1 [b]	2.6 ± 0.2 [b]	2.8 ± 0.2 [a]	2.8 ± 0.1 [a]	2.7 ± 0.1 [a]
Σ ω3	8.93±0.94	8.1 ± 1.7 [a]	7.8 ± 1.8 [a]	6.2 ± 2.0 [ab]	6.5 ± 1.1[ab]	4.7 ± 1.5[b]	7.0 ±1.7[a]	6.9 ± 1.5 [a]
[*]DHA/EPA	4.42±0.40	4.9 ± 0.6 [b]	5.5 ± 0.7 [ab]	5.6 ± 0.8 [ab]	6.1 ± 1.0 [a]	5.4 ± 0.9 [ab]	6.2 ± 1.1 [a]	6.0 ± 0.6 [a]

[*] Unitless ratios
[1] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
The underlined data represents the values that are statistically different from week 0
Data are ± standard deviation for n replicates

3.4.5 Head kidney lipid class composition

There was no significant difference in total lipids, triacylglycerol, and phospholipid among the different treatment. However, feeding the medium marine diet resulted the highest content of sterol in head kidney tissues. The major lipid classes in head kidney tissues were triacylglycerol, phospholipid, and sterol, accounting for 44-73, 27-41, and 2.5-6% of the total lipids in muscle tissues, respectively (Table 3.A6). A comparison between the level of different lipids at week 0 and 14 (Table 3.4) showed that the use of different treatments did not affect the content of total lipids, nor the major lipid classes after 14 weeks.

Table 3.4. Lipid classes of head kidney tissue before and after 14 week of feeding trial (mg g^{-1} wet weight)

Lipid classes (mg g^{-1} wet weight)	Initial n=10	Marine n=12	Medium marine n=10-12	Animal by-product n=11-12	Vegetable protein n=11-12	ω3 LC 0 n=12	ω3 LC 1 n=11-12	ω3 LC 1.4 n=10-12
Total lipid	63.4±35.1	58.5±20.4	54.1±13.7	45.7±22.0	59.3±28.1	46.2±17.2	46.6±21.0	58.9±26.2
Triacylglycerol	28.1±19.4	31.8±17.3	33.8±21.8	20.0±13.0	36.7±18.8	29.7±18.5	31.7±24.5	42.4±22.9
Sterol	2.46±1.60	2.25±1.26^{ab}	3.58±1.01^{a}	2.36±1.36^{ab}	1.41±0.08^{b}	1.70±1.03^{b}	1.60±1.21^{b}	1.53±0.72^{b}
Phospholipid	16.8±6.1	17.0±7.2	16.7±5.8	15.8±6.1	13.0±4.0	13.7±3.3	14.0±5.4	12.4±4.2

Data are ± standard deviation for n replicates

3.4.6 Head kidney fatty acid composition

Head kidney tissue fatty acid composition (Table 3.5) showed no significant differences among treatments for 16:0, 18:0, 18:1ω7, 18:1ω9, 18:2ω6,18:3ω3, 18:4ω3, 20:4ω6, 20:5ω3, 22:5ω3, 22:6ω3 Σ SFA, Σ MUFA, Σ PUFA, P/S, and Σ ω3. A comparison between the level of different fatty acids at week 0 and 14 (Table 3.5) showed that 14:0, 16:1ω7, Σ Bacterial, and Σ SFA dropped significantly from week 0 to week 14 except when the marine diet was used. 16:0, 18:0, Σ PUFA, and Σ ω3 also decreased significantly from week 0 to week 14 when using all the seven treatments. Although, use of different treatments did not affect essential long-chain PUFAs such as 20:4ω6, 20:5ω3, and 22:6ω3 at week 14, the level of 20:4ω6 dropped in the head kidney of fish fed all the diets except ω3 LC 1. In week 14 compared with week 0, the level of 20:5ω3 and 22:6ω3 also decreased when fish were fed the animal by-product, the ω3 LC 0, and the ω3 LC 1 diets. Feeding the ω3 LC 0 diet resulted in the highest ratio of DHA/EPA in head kidney tissues (Table 3.5).

Table 3.5. Fatty acid composition of head kidney tissue before and after 14 week of feeding trial (mg g^{-1} wet weight)

Fatty acid (mg g^{-1} wet weight)	Initial n=10	Marine n=10-12	Medium marine n=10-12	Animal by-product n=12	Vegetable protein n=11-12	ω3 LC 0 n=12	ω3 LC 1 n=11-12	ω3 LC 1.4 n=10-12
14:0	1.90±0.97	1.70±0.64[a]	1.14±0.32[ab]	0.96±0.46[ab]	1.31±0.64[ab]	0.89±0.42[b]	0.88±0.52[b]	1.31±0.69[ab]
16:0	8.00±4.02	6.16±2.04	6.00±2.79	4.62±1.99	6.05±2.73	4.44±1.65	5.09±2.62	6.52±3.38
16:1ω7	3.48±2.22	2.42±0.92[a]	1.78±0.55[ab]	1.56±0.75[ab]	2.00±1.03[ab]	1.31±0.63[ab]	1.53±0.95[b]	1.99±1.06[ab]
18:0	2.22±1.17	1.58±0.51	1.74±0.80	1.35±0.59	1.75±0.82	1.33±0.47	1.62±0.91	1.88±0.98
18:1ω7	1.76±1.07	1.34±0.46	1.42±0.72	1.08±0.49	1.49±0.70	1.13±0.47	1.37±0.79	1.64±0.85
18:1ω9	11.8±7.9	11.6±4.7	12.6±3.9	11.1±5.1	14.9±6.1	12.8±6.5	13.8±6.5	16.9±8.5
18:2ω6	4.51±3.37	3.74±1.77	4.93±2.91	3.28±2.07	5.55±3.15	4.32±2.22	4.78±3.55	6.28±3.50
18:3ω3	0.51±0.43	0.83±0.50	1.21±0.91	0.56±0.44	1.14±0.70	0.95±0.52	0.89±0.74	1.52±0.94
18:4ω3	0.57±0.50	0.66±0.40	0.81±0.66	0.40±0.36	0.80±0.50	0.62±0.34	0.79±0.68	1.06±0.72
20:1ω9	1.21±0.92	2.21±1.00[a]	1.74±1.00[ab]	1.12±0.56[b]	1.66±0.87[ab]	1.16±0.58[b]	0.95±0.63[ab]	2.03±1.08[ab]
20:4ω6	0.74±0.42	0.44±0.19	0.60±0.28	0.43±0.37	0.56±0.29	0.53±0.13	0.78±0.47	0.56±0.34
20:5ω3	2.44±1.94	1.75±0.95	1.59±0.81	0.86±0.80	1.42±0.82	1.09±0.43	1.02±0.71	1.56±0.95
22:1ω11(13)	1.04±0.76	1.65±0.49[a]	1.15±0.32[ab]	0.83±0.42[ac]	1.18±0.62[ab]	0.78±0.41[c]	0.46±0.27[bc]	1.56±0.84[ab]
22:5ω3	0.89±0.73	0.64±0.37	0.48±0.20	0.31±0.29	0.52±0.34	0.37±0.17	0.40±0.30	0.49±0.26
22:6ω3	5.89±4.19	4.75±2.42	4.61±2.19	2.64±2.39	4.14±2.22	3.58±0.89	2.87±1.92	4.41±2.37
Σ Bacterial[1]	0.67±0.42	0.58±0.19[a]	0.44±0.12[ab]	0.39±0.18[ab]	0.49±0.25[ab]	0.35±0.15[ab]	0.38±0.22[b]	0.52±0.26[ab]
Σ SFA[2]	12.6±6.4	9.89±3.34	8.28±1.89	7.28±3.16	9.58±4.42	7.00±2.68	8.00±4.25	9.26±4.11

Σ MUFA[3]	30.4±13.5	20.9±8.4	22.4±12.2	16.6±7.7	24.6±13.0	18.1±8.9	21.9±14.7	28.0±15.6
Σ PUFA[4]	18.0±13.4	14.9±7.5	16.3±8.8	10.0±7.7	16.7±9.4	13.4±5.4	14.1±9.9	18.4±10.1
[*]P/S[5]	1.29±0.47	1.50±0.47	1.67±0.35	1.23±0.63	1.64±0.48	1.90±0.11	1.58±0.62	1.72±0.48
Σ ω3	10.9±8.2	9.21±4.91	8.99±4.71	5.05±4.46	8.51±4.83	6.98±2.46	6.32±4.45	9.44±5.27
[*]DHA/EPA	2.70±0.66	2.72±0.24[b]	2.87±0.27[b]	3.05±0.55[ab]	3.05±0.33[ab]	3.33±0.43[a]	2.89±0.30[ab]	2.87±0.38[b]

[*] Unitless ratios
[1] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
The underlined data represents the values that are statistically different from week 0
Data are ± standard deviation for n replicates

3.4.7 Correlation and regression analyses

The correlation and regression analyses were performed among diet ingredients, elemental composition, diet lipid classes, diet fatty acid composition, liver lipid classes, liver fatty acid composition, head kidney lipid classes, and head kidney fatty acid composition (Tables 3.A1, 3.A2, and 3.A3). The proportion of nitrogen in the muscle at week 14 had negative correlations with growth performance factors (Chapter 2) such as final weight, weight gain, and NQC ($P<0.05$, $P<0.05$, and $P=0.001$, respectively). This was confirmed with regression analysis (Fig. 3.1), where the muscle nitrogen percentage was found to have significant negative linear relationships with the growth performance factors (R^2 = 82.0, 79.4, and 95.2%, respectively). The correlation and regression analysis showed that the relationship between the content of 20:5ω3 in the diet and its deposition in liver and head kidney ($P<0.001$, R^2 = 92.6 % and $P<0.05$, R^2 = 60.6 %, respectively) followed positive linear trends (Table 3.A3 and Fig. 3.2). As expected, the essential fatty acids (20:5ω3 and 22:6ω3) contents in the diet were found to be positively correlated with the amount of Σ ω3 in the liver tissues ($P<0.01$), which was confirmed through both correlation and regression analyses (R^2 = 83.3 % and 82.7 %, respectively) (Table 3.A3 and Figs. 3.3 and 3.4). This positive correlation resulted in a positive relationship between the total amount of ω3 in the diet and liver ($P<0.05$, R^2 = 67.4 %) (Table 3.A3 and Fig. 3.5), meaning that 20:5ω3 and 22:6ω3 contribute the most (2.5-5.1% and 13.4-24.5% of the total fatty acids, respectively) to the total ω3 fatty acids content in liver tissues.

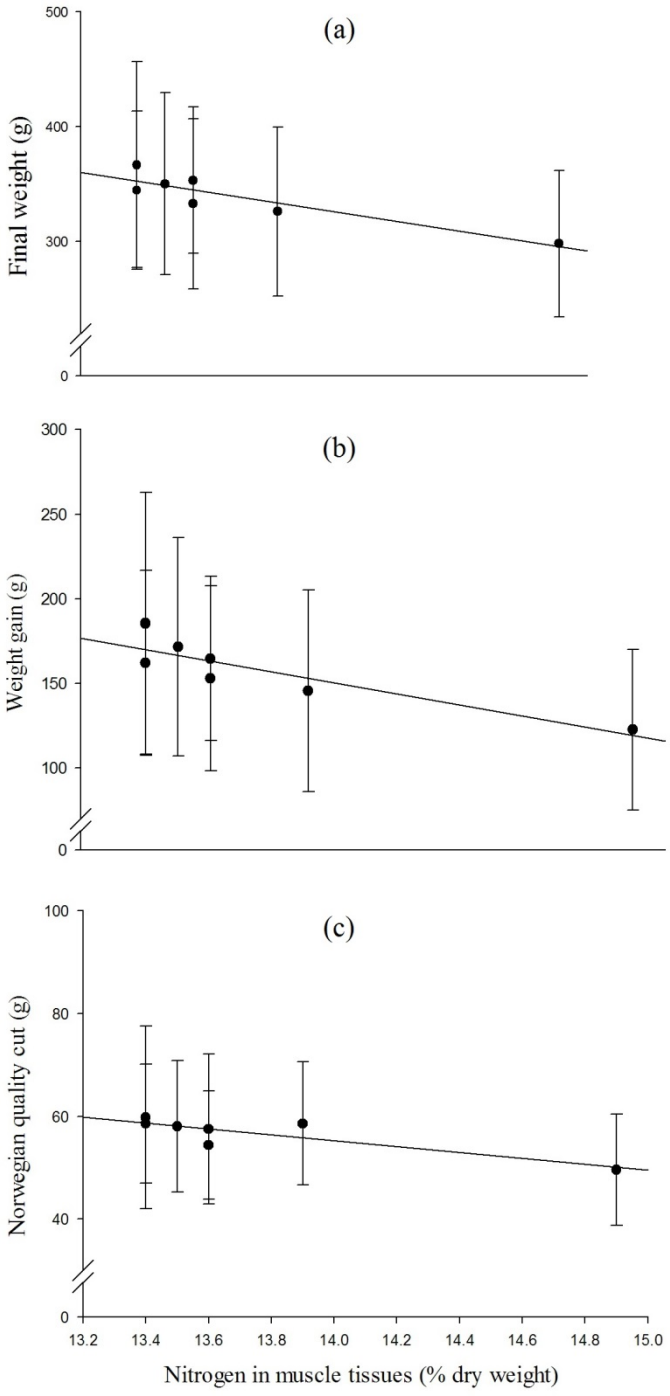

Figure 3.1. Regression analysis between nitrogen concentration in muscle tissue and growth performance factors, including a) final weight, b) weight gain, and c) Norwegian quality cut.

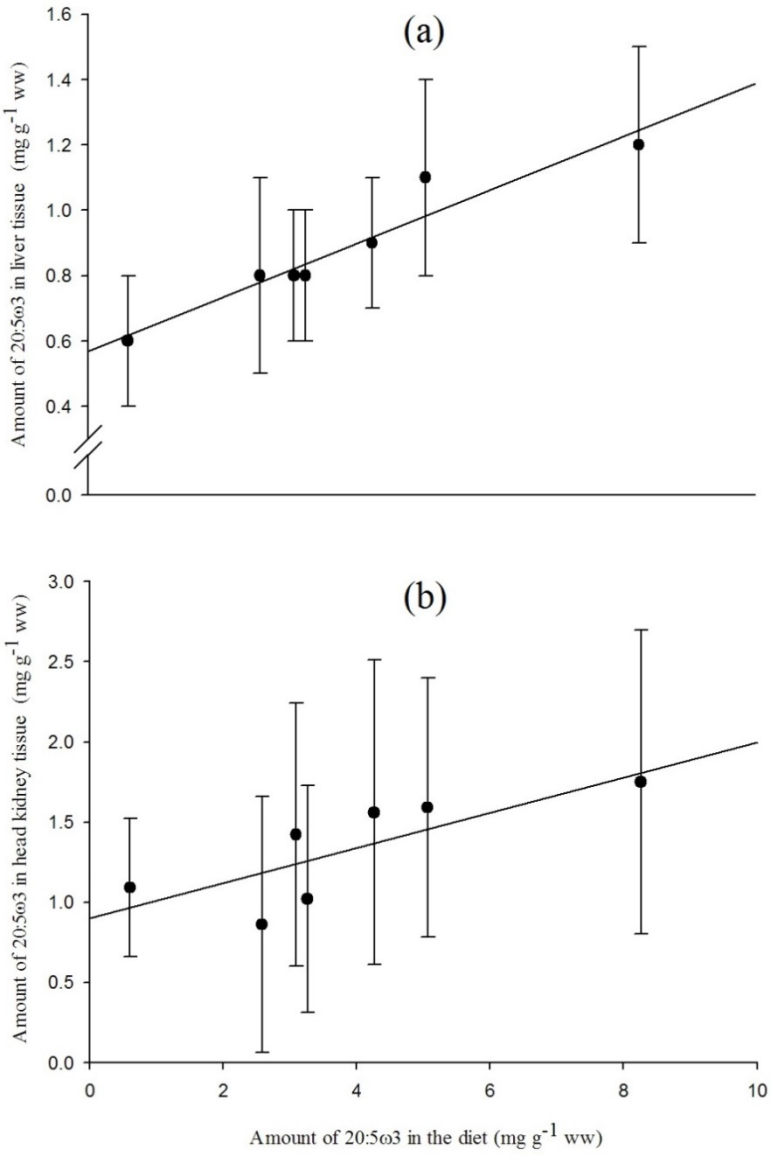

Figure 3.2. Regression analysis between the amounts of 20:5ω3 in the diet and liver (a) or head kidney (b).

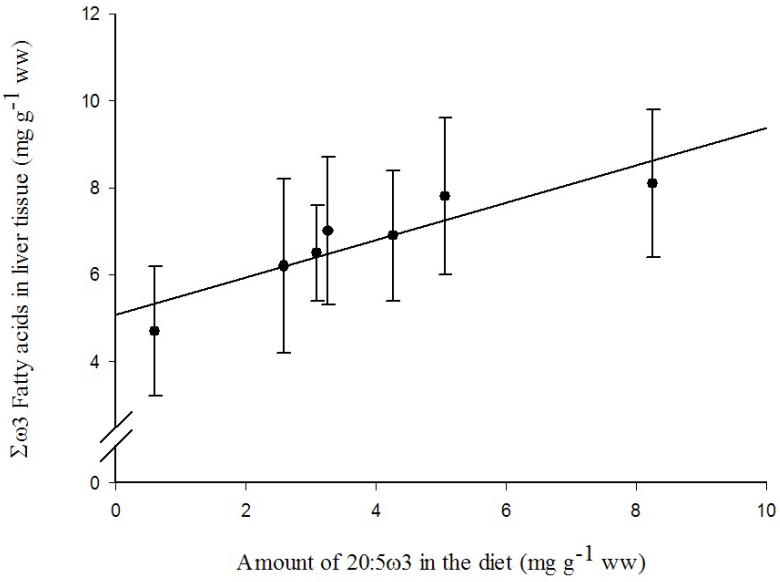

Figure 3.3. Regression analysis between the amounts of 20:5ω3 in the diet and the content of Σ ω3 fatty acids in liver tissue.

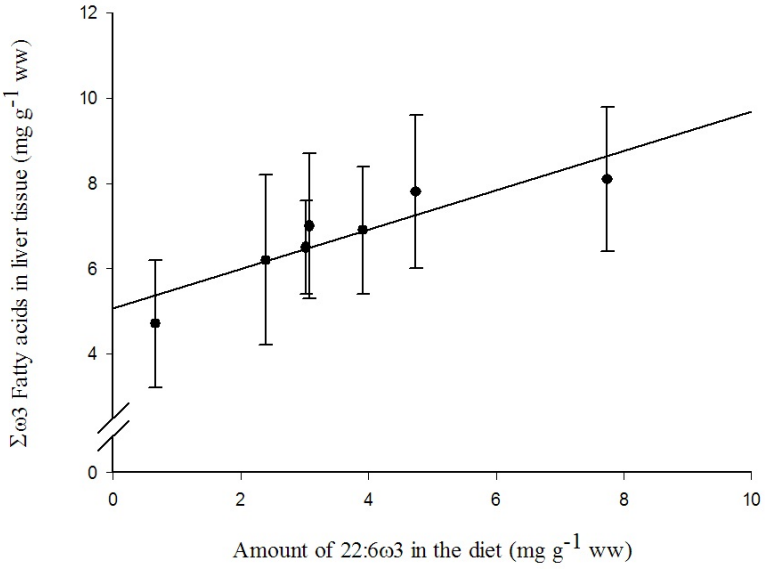

Figure 3.4. Regression analysis between the amount of 22:6ω3 in the diet and the content of Σ ω3 fatty acids in liver tissue.

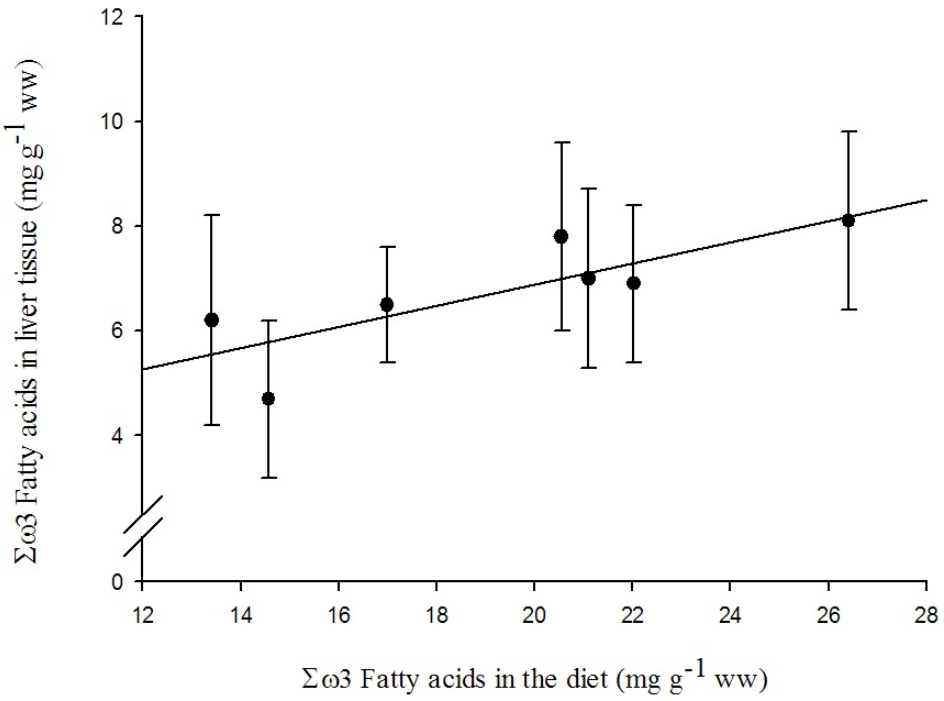

Figure 3.5. Regression analysis between the amounts of Σ ω3 fatty acids in the diet and in liver tissue.

3.5 Discussion

According to previous studies (Bodin et al., 2007; Post et al., 2007), the lipid content in aquatic animals has a positive correlation with C/N ratio as most lipids contain high levels of carbon and little or no nitrogen. However, changes in protein content may be driving the C/N ratios rather than lipid. This is in agreement with the elemental composition results of this study since whenever nitrogen had the lowest content with a dietary treatment, the C/N ratio with the same dietary treatment was at its highest level (Table 3.1). Therefore, the diet which had the lowest lipid content (animal by-products) (Table 2.2) gave the highest nitrogen (% dry weight) in muscle and head kidney tissues.

Also, lipid class composition of the three tissues showed that the animal by-products diet had the lowest lipid content (Tables 2.5, 3.5 and 3.7). The elemental compositions of the three tissues show that nitrogen was preferentially deposited in muscle (13.4-14.9 % dry weight) and relatively equal in liver (8.5-10.2 % dry weight) and head kidney (8.8-9.8 % dry weight) tissues.

A comparison among the contents of total lipids and lipid classes in different tissues revealed that the total lipid content of head kidney (46-59 mg g^{-1} wet weight) was relatively higher than that in liver (34-41 mg g^{-1} wet weight) or muscle (9-18 mg g^{-1} wet weight) tissues (Tables 2.5, 3.5, and 3.7). This variation was also seen in the content and proportions of triacylglycerol which were present at higher values in head kidney (20-42 mg g^{-1} wet weight and 44-73%, respectively) compared to muscle (6-14 mg g^{-1} wet weight and 40-56%, respectively) and liver (3-9 mg g^{-1} wet weight and 17-28%, respectively). Lipid metabolism and fatty acid synthesis (e.g. membrane fatty acids) have been reported to play key roles in function and differentiation of mammalian stem cells (Folmes et al., 2013). Head kidney is the main haematopoietic tissue (i.e. differentiation site of leukocytes and erythrocytes) in teleosts (Tort et al., 2003), and it contains a large population of stem cells. Therefore, a high level of lipid content observed in the head kidney of samples herein may be caused by fatty acid synthesis and membrane lipid accumulation required for the function and differentiation of haematopoietic stem cells. Furthermore, leukocytes form lipid bodies for activation of different immune functions (e.g. inflammation) and signaling pathways (Bozza et al., 2009). Hence, the high level of triacylglycerol recorded in the head kidney samples of the present study may have

occurred as a result of the storage of lipids by differentiated leukocytes in head kidney of the fish. However, the contents of sterol (3.2-3.7, 1.4-3.6, and 0.2-0.9 mg g^{-1} wet weight in the liver, head kidney, and muscle, respectively) and phospholipid (17-22, 12-17, and 3-5 mg g^{-1} wet weight in the liver, head kidney, and muscle, respectively) in the liver tissues were generally highest among the three examined tissues (Tables 2.5, 3.5, and 3.7). As phospholipid and sterol contribute to membrane structure, this suggests that there was more membrane material in liver than head kidney and muscle. The level of sterol decreased while phospholipid increased in head kidney leukocytes of salmon fed ω3LC1.4 diet compared to those fed ω3LC1 diet (Eslamloo et al. 2017), suggesting the influence of dietary EPA+DHA on composition of cell membranes. Here neither sterols nor phospholipids showed significant differences in head kidney samples but sterol concentrations and proportions followed the same trend.

In terms of ω3 essential fatty acids (20:5ω3, 22:6ω3), a higher range of 20:5ω3 was found in the head kidney (0.9-1.7 mg g^{-1} wet weight) samples than the liver and muscle tissues (0.6-1.2 and 0.2-0.7 mg g^{-1} wet weight, respectively), while the content of 22:6ω3 in the liver tissues was generally higher (3.1-5.8 mg g^{-1} wet weight), compared to head kidney and muscle tissues (2.6-4.7 and 1.2-2.5 mg g^{-1} wet weight, respectively) (Tables 2.6, 3.6, and 3.8). This result is in agreement with the study by Torstensen et al. (2004), in which dietary fish oil was replaced with increasing levels of rapeseed oil in diets of Atlantic salmon, and the tissue levels of the essential fatty acids 20:5ω3 and 22:6ω3 showed a higher accumulation in liver compared to muscle. For the ω6 essential fatty acid (20:4ω6) the greatest range was in the head kidney where there was a 3 fold

difference in concentration. Caballero-Solares et al. (2017) suggest the high ARA/EPA ratio of the animal by-product diet favored increased expression of transcripts involved in the synthesis of pro-inflammatory eicosanoids. They compared immune response in salmon fed the marine, vegetable protein and animal by-product diets used here. The ARA/EPA ratio is up to 2 fold higher in head kidneys from fish fed the animal by-product diet compared to the marine or vegetable protein diets which exactly reflects the differences in dietary ratios.

As in previous studies (Bell et al., 2001, Torstensen et al., 2004) the fatty acid composition of tissues in this study are readily influenced by the fatty acid composition of the diet, as evident from the correlations between the amounts of Σ ω3 fatty acids in the diet and in the liver tissues, as well as the amounts of 20:5ω3 and 22:6ω3 in the diet and liver (Table A3.3). The slope of the regression line for dietary content of 20:5ω3 and 22:6ω3 against their contents in liver tissues indicates the extent of this dependency. The higher slope for 22:6ω3 (0.36 mg g^{-1} wet weight) compared to 20:5ω3 (0.08 mg g^{-1} wet weight) showed that 22:6ω3 was deposited about four times more than 20:5ω3 in liver tissue. A similar result was observed for muscle tissues when the same experimental diets were used, in which 22:6ω3 was deposited four times more than 20:5ω3 in muscle tissues (Chapter 2). Also, in another study, when fish oil was replaced with increasing rapeseed oil and 50 % olive oil in the diet of Atlantic salmon (Torstensen et al., 2004), where 22:6ω3 (slope = 3.25) was more deposited than 20:5ω3 (slope = 0.95) in liver tissues when proportions (% total fatty acids) were compared. Eslamloo et al. (2017) reported that lower levels of dietary EPA+DHA (i.e. ω3LC1 *vs* ω3LC1.4) decrease and increase,

respectively, the levels of EPA and linoleic acid in head kidney leukocytes of salmon; moreover, it modulates the expression of genes associated with fatty acid metabolism and inflammatory responses in salmon macrophage-like cells. Here neither EPA nor linoleic acid showed significant differences in head kidney samples but EPA concentrations followed the same trend. As for the essential fatty acids in head kidney, there was only a correlation and regression between the amounts of 20:5ω3 in the diet and head kidney (Fig. 3.2), but no correlation was found between the content of 22:6ω3 in the diet and head kidney. The close slopes in Figures 3.3 and 3.4 (0.43 and 0.46, respectively) showed that 22:6ω3 and 20:5ω3 had fairly identical effects on the total ω3 fatty acids content in liver.

Fatty acid compositions of head kidney showed that using the seven dietary treatments did not affect the contents (mg g^{-1} wet weight) of most major fatty acids such as ω3, ω6, and ω9 fatty acids (Table 3.5). However, all proportions of major fatty acids were affected by these different diets (Table 3.A7).

Although the diet containing 0 % of LC ω3 fatty acids had the lowest level of 20:4ω6, fish fed this diet resulted in the highest proportion and concentration of 20:4ω6 in liver tissues (Table 3.A5 and Table 3.3). This diet (ω3LC0) contains a high inclusion of rapeseed oil which is rich in 18:2ω6. Therefore, it is assumed that 18:2ω6 was converted to 20:4ω6, therefore resulting in the high percentage of 20:4ω6 in liver tissues (Isseroff et al., 1987). This result is similar to the muscle fatty acid compositions of the fish fed the same dietary treatments (Chapter 2).

This study showed that diet with the highest content of rapeseed oil (ω3LC0) resulted in the lowest level of essential fatty acids, and in contrast, diet with the lowest level of rapeseed oil (marine) resulted in the highest content of 20:5ω3 and 22:6ω3 in the liver tissues (Table 3.3). This is in agreement with other studies (e.g. Torstensen et al., 2004; Tocher et al., 2003; Torstensen et al., 2011) where the replacement of fish oil with increasing levels of rapeseed oil or other vegetable oils in the diet of Atlantic salmon resulted in lower levels of 20:5ω3 and 22:6ω3 in the liver tissues. However, the DHA/EPA ratio is the lowest for liver with the marine diet, as a result of DHA content changing less than EPA content with dietary replacement. This suggests conservation of DHA content in phospholipids of liver membranes, as evident from PCA of liver tissues in which EPA, DHA, and phospholipids were co-located as contributors to the structure of cell membranes especially in the liver of fish fed the marine diet (Fig. 3.6). As stated by Torstensen et al. (2004), this study also showed that while ω3LC0 diet, which contained the highest level of rapeseed oil, resulted in the highest level of ω6 fatty acids (e.g. 18:2ω6 and 20:4ω6) in the liver tissues, the diet with the lowest content of rapeseed oil (marine) led to the lowest 18:2ω6 and 20:4ω6 levels in the liver tissues (Table 3.3). In terms of fatty acid proportions, liver and head kidney from the marine treatment usually showed the highest or lowest values, and this treatment had the greatest number of significant differences (Table 3.A5 and 3.A7). This indicates that the fatty acids proportions in this treatment were more different than in other treatments. A similar result was obtained by Bell et al., (1996), where fish oil diet resulted in the highest proportion of almost all of the studied fatty acids in kidney tissues of Atlantic salmon.

A comparison between the deposition rates (contents) of fatty acids in the three tissues reveals that SFA and MUFA were deposited about four times and two times more in head kidney (7-9.9 and 16.6-28.0 mg g^{-1} wet weight, respectively) and liver (3.9-5.1 and 8.8-14.5 mg g^{-1} wet weight, respectively) than they were deposited in muscle (1.4-3.4 and 2.4-7.0 mg g^{-1} wet weight, respectively) tissues (Tables 2.6, 3.6, and 3.8). Also, 18:2ω6 and 18:3ω3 fatty acids, which are the precursors of the essential fatty acids 20:4ω6 and 20:5ω3 and 22:6ω3, were preferentially deposited in head kidney (3.3-6.3 and 0.6-1.5 mg g^{-1} wet weight, respectively), followed by liver (1.2-2.9 and 0.2-0.5 mg g^{-1} wet weight, respectively) and muscle (0.6-1.6 and 0.1-0.4 mg g^{-1} wet weight, respectively) tissues. 20:4ω6, however, had the highest deposition in the liver (0.6-1.3 mg g^{-1} wet weight), followed by head kidney (0.4-0.8 mg g^{-1} wet weight) and muscle (0.1-0.2 mg g^{-1} wet weight) tissues (Tables 2.6, 3.6, and 3.8).

Generally, a relatively high content of fish meal in the diet reduces the possibility of having low levels of essential fatty acids (Turchini et al, 2009). This study also confirms that a diet with relatively high content of fish meal (marine diet with 35% fish meal) resulted in the highest levels of essential fatty acids per wet weight in both liver and head kidney tissues (Tables 3.6 and 3.8).

3.5.1 Multivariate statistics

Diet, liver, and head kidney fatty acid and lipid class composition were compared among the seven dietary treatments. The diet, liver, and head kidney compositions were very different as discriminant analysis correctly classified 44 of 45 (for diet), 62 of 65

(for liver) and 67 of 72 (for head kidney) samples on the basis of major fatty acids and lipid classes.

The PCA for the diet showed that the fatty acids and lipid classes were categorized into four clusters (given on a dendrogram) according to their similarity through cluster analysis of variables. The larger cluster in the top right quadrant of Fig. 2.6 shows the importance of 20:5ω3, 22:6ω3, and total sterols to the lipid composition of the marine and medium marine diets. The cluster in the top left quadrant shows the importance of triacylglycerols for energy and 18:2ω3 and 18:3ω3 as precursors of long-chain PUFA in diets ω3LC1 and ω3LC0. The cluster in the lower left of Fig. 2.6 shows the importance of phospholipids in the animal by-product and vegetable protein diets and the high DHA/EPA ratio in the vegetable protein and ω3LC0 diets.

The results of PCA for the liver tissues categorized the fatty acids and lipid classes into three clusters. The cluster in the top right quadrant of Fig. 3.6 shows the importance of triacylglycerols for energy and 18:2ω3 and 18:3ω3 as precursors of long-chain PUFA in ω3LC1 and vegetable protein diets. The cluster in the top left of Fig. 3.6 is associated with the marine and medium marine dietary treatments. This correlation is the result of high inclusion of DHA+EPA in the marine diet and correlation analysis confirmed that fatty acid composition of liver tissue is directly influenced by the level of EPA+DHA in the diet. This cluster includes components that are important to both energy storage and membrane structure. While saturated fatty acids could contribute to energy storage, 20:5ω3, 22:6ω3, and phospholipids are important to the structure of cell

membranes. The PCA for liver tissue also showed a separation between 20:4ω6 and other lipids and fatty acids. According to Figs. 3.6, this cluster is associated with ω3LC0 diet, which is expected as this diet was found to have the highest level of 20:4ω6 (Table 3.3).

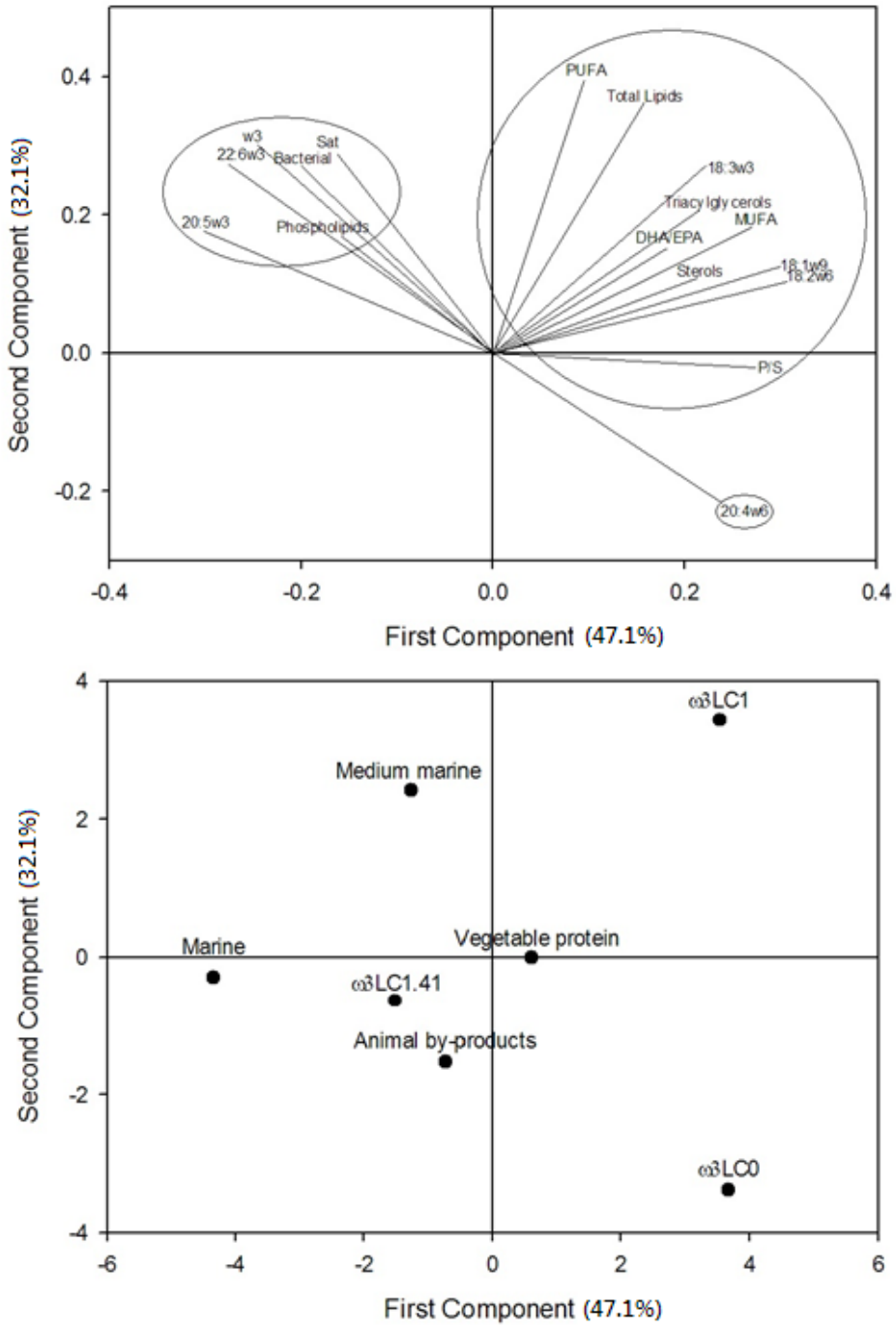

Figure 3.6. Principal components analysis of lipid and fatty acid contents (mg g^{-1} wet weight) in liver tissue

As for the PCA for head kidney, the fatty acids and lipid classes were grouped into six clusters. The cluster in the top right quadrant shows the importance of triacylglycerols for energy and 18:2ω3 and 18:3ω3 as precursors of long-chain PUFA in ω3LC1.4 and vegetable protein diets. The cluster in the lower right of Fig. 3.7 is associated with the marine and medium marine dietary treatments. As stated previously, this correlation is resulted from the direct deposition of DHA+EPA content in the diet into head kidney tissues. This cluster also has components (e.g. saturated fatty acids, 20:5ω3, and 22:6ω3) that could contribute to energy storage and cell membrane structure. The cluster in the lower left quadrant shows the importance of phospholipids and sterols with the animal by-product diet suggesting effects on membrane structure. It should be noted that while the PCA for liver showed a considerable distance between ω3LC0 and ω3LC1 diets (Fig. 3.6), these diets were close in the PCA for head kidney (Fig. 3.7), as the lipid and fatty acid compositions in both tissues were similar using the two diets (Tables 3.4 and 3.5).

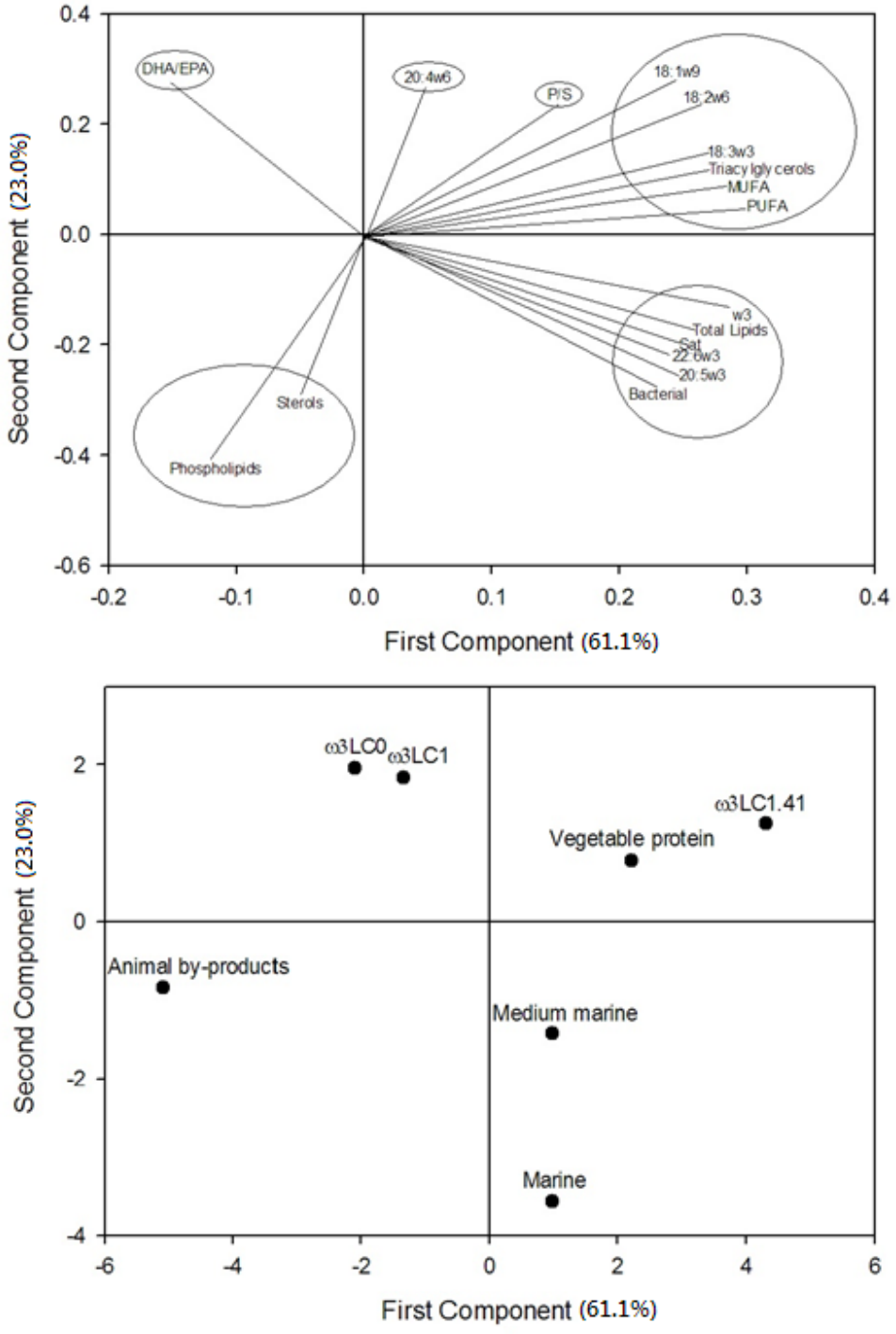

Figure 3.7. Principal components analysis of lipid and fatty acid contents (mg g^{-1} wet weight) in head kidney tissue

3.6 Conclusions

This study evaluated the effects of minimizing marine resource utilization in diets of farmed Atlantic salmon on liver and head kidney elemental and lipid compositions. The study showed that C/N ratio in head kidney was the highest among the three tissues. Consequently, feeding fish with the formulated diets resulted in higher total lipids content in head kidney than liver and muscle tissues. It was also revealed that SFA, MUFA, 18:2ω6, and 18:3ω3 were deposited more in head kidney, than liver and muscle tissues. In terms of essential fatty acids, while head kidney was found as the preferred tissue for the deposition of 20:5ω3, 22:6ω3 was more accumulated in the liver tissue. The replacement of fish meal and fish oil in aquafeeds with animal by-products and rapeseed oil at levels used in this study (33 and 27%, respectively) affects liver and head kidney elemental compositions. It was concluded that reducing marine resource utilization to less than 10% in diets of farmed Atlantic salmon affects lipid classes and fatty acid compositions of the liver and head kidney tissues with negative effects on essential fatty acids.

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3.8 Appendix

Table 3.A1. Correlation analysis r values among diet ingredients, elemental composition at week 14, diet (D) lipid classes, diet fatty acid composition, lipid classes of liver (L) and head kidney (HK), and fatty acid composition of liver and head kidney (data with*, **, and *** represent P≤0.05, P ≤0.01, and P≤0.001, respectively.

Elemental composition, diet and fish composition	Fish meal	Fish oil	Vegetable oil	Vegetable protein	EPA+ DHA	Carbon (L)	Nitrogen (L)	Carbon (HK)	Nitrogen (HK)
Vegetable oil					-0.884**				
EPA+DHA	0.881**	0.987***							
C/N ratio[1] (L)							-0.989***		
C/N ratio (HK)		0.874**						0.980***	-0.957***
Carbon (HK)					0.887**	-0.890**			-0.898**
Σ MUFA[2] (D)			0.855*						
18:2ω6 (L)			0.821*						
20:4ω6 (L)		-0.871**							
P/S (L)			0.944***						
DHA/EPA (L)				0.824*					
Phospholipid (HK)			-0.754*	-0.887**					
Σ SFA[4] (HK)		0.777*							
DHA/EPA (HK)		-0.922**							

[1] Carbon to nitrogen ratio
[2] Monounsaturated fatty acids
[3] Polyunsaturated/saturated fatty acids
[4] Saturated fatty acids
[*] The unit for all lipids and fatty acids compositions is mg g^{-1} wet weight, and for elemental composition is % dry weight.

Table 3.A2. Correlation analysis r values among diet ingredients, elemental composition at week 14, diet (D) lipid classes, diet fatty acid composition, lipid classes of liver (L) and head kidney (HK), and fatty acid composition of liver and head kidney (data with*, **, and *** represent P≤0.05, P ≤0.01, and P≤0.001, respectively).

Elemental composition, diet and fish composition	TL[1] (L)	TAG[2] (L)	18:2ω6 (L)	18:3ω3 (L)	20:4ω6 (L)	20:5ω3 (L)	22:6ω3 (L)	Σ SFA (L)	ΣMUFA (L)	ΣPUFA (L)	P/S (L)	Σ ω3 (L)
18:1ω9 (L)		0.873**										
18:3ω3 (L)	0.792*											
P/S[4] (L)			0.818*			-0.767*						
Σ MUFA[5] (L)	0.845*	0.949***	0.879**	0.838*								
Σ PUFA[6] (L)	0.968***			0.774*								
Σ SFA[7] (L)						0.814*	0.805*					
PL (HK)								0.772*			-0.834*	
Sterol (HK)								0.789*				
20:4ω6 (HK)	0.894**		0.775*	0.944***					0.803*	0.867*		
20:5ω3 (HK)						0.775*						
DHA/EPA (HK)					0.927**		-0.960***					-0.967***

[1] Total lipid
[2] Triacylglycerol

[3] Phospholipid
[4] Polyunsaturated/saturated fatty acids
[5] Monounsaturated fatty acids
[6] Polyunsaturated fatty acids
[7] Saturated fatty acids
[*] The unit for all lipids and fatty acids compositions is mg g^{-1} wet weight.

Table 3.A2. Continued. Correlation analysis r values among diet ingredients, elemental composition at week 14, diet (D) lipid classes, diet fatty acid composition, lipid classes of liver (L) and head kidney (HK), and fatty acid composition of liver and head kidney (data with*, **, and *** represent P≤0.05, P ≤0.01, and P≤0.001, respectively).

Elemental composition, diet and fish composition	TL (HK)	TAG (HK)	Sterol (HK)	PL[3] (HK)	18:1ω9 (HK)	18:2ω6 (HK)	18:3ω3 (HK)	20:5ω3 (HK)	22:6ω3 (HK)	Σ SFA (HK)	Σ MUFA (HK)	Σ PUFA (HK)
PL (HK)			0.814*									
18:1ω9 (HK)		0.861*		-0.844*								
18:2ω6 (HK)		0.919**										
18:3ω3 (HK)		0.937**										
20:5ω3 (HK)	0.895**											
Σ ω3 (HK)	0.901**	0.832*								0.789*		0.897**
Σ MUFA (HK)	0.762*	0.949***			0.892**	0.927**	0.896**					
Σ PUFA (HK)	0.812*	0.984***			0.782*	0.882**	0.933**	0.776*	0.777*		0.935**	
Σ SFA (HK)	0.940***							0.824*				

Table 3.A3. Correlation analysis r values among diet ingredients, elemental composition at week 14, diet (D) lipid classes, diet fatty acid composition, lipid classes of liver (L) and head kidney (HK), and fatty acid composition of liver and head kidney (data with *, **, and *** represent P≤0.05, P ≤0.01, and P≤0.001, respectively).

Elemental composition, diet and fish composition	TL[1] (D)	TAG[2] (D)	PL[3] (D)	18:1ω9 (D)	18:2ω6 (D)	18:3ω3 (D)	20:4ω6 (D)	20:5ω3 (D)	22:6ω3 (D)	ΣMUFA (D)	Σω3 (D)	P/S (D)
18:1ω9 (D)		0.960***										
18:2ω6 (D)		0.939**										
18:3ω3 (D)		0.957***										
Σ MUFA[4] (D)		0.952***										
P/S [5] (D)		0.776*										
18:2ω6 (L)						0.815*						
20:4ω6 (L)								-0.795*	-0.785*			
20:5ω3 (L)					-0.775*	-0.760*	0.975***	0.962***	0.960***		0.796*	
22:6ω3 (L)							0.893**	0.892**	0.887**		0.779*	
Σ ω3 (L)							0.908**	0.913**	0.910**		0.821*	
P/S (L)	0.828*	0.950***		0.969***	0.973***	0.984***				0.938**		
Σ SFA[6] (L)							0.806*					
Total lipid (HK)												0.784*

TAG (HK)									0.784*
PL (HK)			-0.768*	-0.780*					-0.894
18:1ω9 (HK)									0.928**
18:2ω6 (HK)									0.842*
20:5ω3 (HK)						0.779*		0.767*	
22:6ω3 (HK)	-0.760*								
DHA/EPA (HK)					-0.875**	-0.907**	-0.899**	-0.869	

[1] Total lipid
[2] Triacylglycerol
[3] Phospholipid
[4] Monounsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
[6] Saturated fatty acids
[*] The unit for all lipids and fatty acids compositions is mg g^{-1} wet weight.

Table 3.A4. Lipid classes of liver tissue before and after 14 week of feeding trial (% total lipid classes)

Lipid classes (% total)	Initial n=10	Marine n=12	Medium marine n=12	Animal by-product n=12	Vegetable protein n=12	ω3 LC 0 n=12	ω3 LC 1 n=12	ω3 LC 1.4 n=11-12
Triacylglycerol	5.6±3.4	24.4 ± 10.9	23.0 ± 13.2	26.4 ± 11.5	23.4 ± 15.3	24.8 ± 13.7	27.7 ± 17.4	17.5 ± 6.4
Sterol	3.92±0.49	9.6 ± 3.0	10.6 ± 3.8	11.3 ± 6.4	11.2 ± 4.4	11.9 ± 6.1	10.3 ± 3.5	10.9 ± 3.5
Phospholipid	88.1±4.7	56.2 ± 11.2	55.2 ± 14.2	53.4 ± 16.4	55.6 ± 14.5	51.8 ± 11.1	52.2 ± 16.5	64.2 ± 7.0

Data are ± standard deviation for n replicates

Table 3.A5. Fatty acid composition of liver tissue before and after 14 week of feeding trial (% identified fatty acids)

Fatty acid (% identified fatty acids)	Initial n=10	Marine n=12	Medium marine n=12	Animal by-product n=12	Vegetable protein n=12	ω3 LC 0 n=12	ω3 LC 1 n=12	ω3 LC 1.4 n=12
14:0	1.57±0.15	1.94±0.39^{a}	1.35±0.18^{ab}	1.27±0.26^{ab}	1.06±0.10^{b}	0.53±0.10^{c}	0.96±0.18^{bc}	1.11±0.19^{b}
16:0	18.1±1.0	12.2±1.9^{a}	11.7±2.0^{ab}	11.5±1.7^{ab}	10.6±2.0^{ab}	9.8±2.3^{ab}	9.7±2.2^{b}	11.0 ±1.5^{ab}
16:1ω7	2.47±0.34	2.58 ±0.69^{a}	2.05±0.60^{ab}	1.91±0.44^{ac}	1.35±0.33^{cd}	0.95±0.14^{d}	1.34±0.33^{bcd}	1.44±0.31^{bcd}
18:0	6.15±0.60	4.38±0.67^{sb}	4.59±0.50^{a}	4.43±0.56^{sb}	4.41±0.42^{sb}	4.38±0.5^{ab}	3.95±0.62^{b}	4.22±0.51^{ab}
18:1ω7	2.45±0.22	2.68±0.27^{b}	2.92±0.45^{ab}	2.54 ±0.85^{b}	2.38±0.45^{b}	2.84 ±0.38^{ab}	2.94±0.51^{ab}	3.67±0.77^{a}
18:1ω9	12.2±1.5	22.0±4.5^{b}	26.6±6.9^{ab}	28.8±6.1^{ab}	29.0 ±7.1^{ab}	34.9±7.3^{a}	31.3±7.6^{a}	26.1 ±4.3^{ab}
18:2ω6	4.55±1.21	4.94 ±0.85^{d}	6.72±0.72^{c}	7.62±1.06^{bc}	7.70±0.91^{bc}	9.27±1.03^{a}	8.79 ±1.11^{ab}	8.18±0.70^{ab}
18:3ω3	0.39±0.09	1.00±0.28^{b}	1.14±0.22^{b}	1.13±0.29^{b}	1.23±0.24^{ab}	1.24±0.20^{ab}	1.51±0.40^{a}	1.48±0.23^{a}

20:1ω9	0.83±0.18	4.36±1.47^{a}	3.45±1.12^{ab}	3.09±1.10^{ab}	3.51±1.45^{ab}	2.10±1.00^{b}	3.39±1.22^{ab}	3.21±0.81^{ab}
20:4ω6	4.33±0.34	2.4 ± 0.60^{b}	2.8 ± 0.8^{b}	3.5 ± 1.0^{b}	3.2 ± 1.0^{b}	5.2 ± 1.4^{a}	2.9 ± 0.9^{b}	2.7 ± 0.5^{b}
20:5ω3	7.08±0.39	5.1 ± 1.0^{a}	4.1 ± 1.1^{ab}	3.5 ± 0.9^{bc}	3.3 ± 0.8^{bc}	2.5 ± 0.8^{c}	3.0 ± 1.0^{bc}	3.7 ± 0.7^{b}
22:5ω3	1.90±0.14	1.45±0.22^{a}	1.11±0.27^{b}	1.04 ±0.29^{bc}	0.89±0.23^{bc}	0.94 ±0.31^{bc}	0.79±0.27^{c}	0.92±0.20^{bc}
22:6ω3	31.2±2.2	24.6 ± 4.7^{a}	22.1 ± 5.7^{a}	19.3 ± 4.4^{ab}	20.4 ± 6.1^{a}	13.4 ± 4.2^{b}	18.4 ± 5.6^{ab}	22.2 ± 3.8^{a}
Σ Bacterial[1]	0.92±0.06	1.0 ± 0.1^{a}	0.8 ± 0.1^{b}	0.7 ± 0.1^{bc}	0.6 ± 0.04^{c}	0.5 ± 0.1^{d}	0.6 ± 0.1^{c}	0.7 ± 0.1^{bc}
Σ SFA[2]	26.9±5.2	19.3 ± 2.2^{a}	18.3 ± 2.1^{a}	17.8 ± 2.1^{ab}	16.6 ± 2.4^{ab}	15.2 ± 2.7^{b}	15.2 ± 2.6^{b}	16.9 ± 1.9^{ab}
Σ MUFA[3]	19.5±2.0	35.9 ± 6.9	38.1 ± 9.1	39.5 ± 7.3	39.3 ± 9.1	42.6 ± 8.5	42.0 ± 9.3	37.4 ± 5.8
Σ PUFA[4]	53.4±2.2	44.4 ± 5.1	43.4 ± 7.3	42.5 ± 5.4	43.9 ± 7.0	42.0 ± 5.9	42.6 ± 6.8	45.5 ± 4.0
***P/S**[5]	2.00±0.14	2.3 ± 0.2^{a}	2.4 ± 0.3^{a}	2.4 ± 0.1^{a}	2.6 ± 0.2^{a}	2.8 ± 0.2^{b}	2.8 ± 0.1^{b}	2.7 ± 0.1^{b}
Σ ω3	41.6±2.0	33.8 ± 5.3^{a}	29.8 ± 6.8^{ab}	26.3 ± 5.3^{bc}	27.2 ± 6.7^{ab}	19.8 ± 5.1^{c}	25.4 ± 6.4^{bc}	30.0 ± 4.1^{ab}
***DHA/EPA**	4.42±0.40	4.9 ± 0.6^{b}	5.5 ± 0.7^{ab}	5.6 ± 0.8^{ab}	6.1 ± 1.0^{a}	5.4 ± 0.9^{ab}	6.2 ± 1.1^{a}	6.0 ± 0.6^{a}

* Unitless ratios
[1] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
Data are ± standard deviation for n replicates

Table 3.A6. Lipid classes of head kidney tissue before and after 14 week of feeding trial (% total lipid classes)

Lipid classes	Initial	Marine	Medium marine	Animal by-product	Vegetable protein	ω3 LC 0	ω3 LC 1	ω3 LC 1.4
(% total)	**n=10**	**n=12**	**n=10-12**	**n=11-12**	**n=11-12**	**n=12**	**n=11-12**	**n=10-12**

Triacylglycerol	50.0±19.3	53.1±18.8^{bc}	53.4±11.3^{bc}	43.7±9.0^{c}	71.6±13.0^{a}	57.2±14.9^{ac}	66.0±15.9^{ab}	73.1±13.8^{a}
Sterol	4.40±2.54	4.05±2.30^{ab}	6.34±3.16^{a}	6.16±3.83^{ab}	2.81±1.26^{ab}	3.22±1.64^{ab}	4.27±2.77^{ab}	2.50±1.20^{b}
Phospholipid	31.4±14.6	29.9±8.3^{ab}	28.2±8.6^{ab}	41.5±8.8^{a}	27.3±11.0^{ab}	37.8±18.3^{ab}	37.0±17.4^{ab}	27.0±17.9^{b}

Data are ± standard deviation for n replicates

Table 3.A7. Fatty acid composition of head kidney tissue before and after 14 week of feeding trial (% identified fatty acids)

Fatty acid (% identified fatty acids)	**Initial n=10**	**Marine n=10-12**	**Medium marine n=10-12**	**Animal by-product n=12**	**Vegetable protein n=11-12**	**ω3 LC 0 n=12**	**ω3 LC 1 n=11-12**	**ω3 LC 1.4 n=10-12**
14:0	3.97±0.64	3.64±0.39^{a}	2.81±0.30^{ab}	2.99±0.61^{ab}	2.66±0.43^{bc}	2.09±0.48^{c}	2.29±0.25^{bc}	2.65±0.41^{bc}
16:0	17.1±3.6	13.7±1.8^{a}	12.7±1.4^{ab}	14.4±2.1^{a}	12.4±1.8^{ab}	12.6±2.4^{ab}	11.8±0.9^{b}	12.1±2.3^{b}
16:1ω7	6.92±0.73	5.09±0.62^{a}	4.41±0.61^{ab}	4.88±1.05^{a}	4.01±0.65^{ac}	3.59±0.78^{bc}	3.39±0.49^{c}	4.21±0.86^{ac}
18:0	4.72±0.99	3.54±0.48^{b}	3.68±0.43^{ab}	4.17±0.52^{a}	3.57±0.46^{b}	3.92±0.57^{ab}	3.56±0.40^{ab}	3.45±0.51^{b}
18:1ω7	3.56±0.34	2.96±0.35^{ab}	2.90±0.28^{b}	3.32±0.43^{ab}	3.02±0.37^{ab}	3.28±0.43^{a}	2.95±0.10^{ab}	2.95±0.33^{ab}
18:1ω9	23.5±3.1	25.3±3.5^{c}	30.2±3.2^{b}	34.0±4.9^{ab}	33.9±3.3^{ab}	37.3±5.0^{a}	32.5±2.5^{ab}	32.9±3.1^{ab}
18:2ω6	8.30±1.21	7.96±0.95^{c}	9.95±0.80^{abc}	9.09±1.49^{bc}	10.5±1.3^{ab}	10.1±1.9^{ab}	10.9±0.8^{a}	10.6±1.3^{ab}
18:3ω3	0.92±0.25	1.71±0.45^{bc}	2.02±0.39^{ac}	1.45±0.57^{c}	2.11±0.53^{ab}	1.77±0.65^{ac}	2.37±0.32^{a}	2.22±0.52^{ab}
18:4ω3	0.94±0.39	1.37±0.45^{ab}	1.31±0.32^{ab}	0.98±0.54^{b}	1.44±0.48^{ab}	1.52±0.69^{a}	1.56±0.22^{ab}	1.47±0.43^{ab}
20:1ω9	2.29±0.27	4.77±0.63^{a}	3.57±0.42^{ab}	3.35±0.37^{bc}	3.32±0.35^{bc}	2.20±0.34^{d}	2.94±0.20^{cd}	3.51±0.35^{abc}
20:4ω6	1.46±0.39	0.97±0.36^{bc}	1.16±0.42^{abc}	1.10±0.75^{abc}	1.07±0.39^{abc}	1.56±0.74^{a}	1.47±0.30^{ab}	0.90±0.36^{c}
20:5ω3	4.25±1.58	3.73±1.38^{a}	3.29±0.96^{a}	2.08±1.38^{b}	2.57±0.98^{ab}	2.07±1.08^{b}	2.89±0.35^{ab}	2.71±0.91^{ab}

22:1ω11(13)	1.97±0.18	4.31±0.65^{a}	2.83±0.46^{ab}	2.50±0.35^{bc}	2.35±0.26^{bc}	1.12±0.30^{d}	1.97±0.21^{cd}	2.69±0.30^{ab}
22:5ω3	1.52±0.61	1.33±0.47^{a}	1.15±0.29^{ab}	0.76±0.45^{b}	0.92±0.33^{b}	0.80±0.41^{b}	0.96±0.13^{b}	1.00±0.34^{ab}
22:6ω3	10.7±3.8	10.3±4.0^{a}	9.55±2.95^{ab}	6.54±4.78^{ab}	7.67±2.76^{ab}	6.04±3.30^{b}	9.91±1.96^{ab}	7.95±3.17^{ab}
Σ Bacterial[1]	1.32±0.07	1.26±0.11^{a}	1.06±0.10^{ab}	1.11±0.15^{ab}	1.00±0.17^{b}	0.92±0.16^{b}	0.91±0.07^{b}	1.03±0.12^{ab}
Σ SFA[2]	26.9±5.2	22.0±2.9^{a}	20.2±2.1^{ab}	22.6±3.4^{a}	19.6±2.7^{b}	19.6±3.4^{ab}	18.5±1.3^{b}	19.1±3.1^{b}
Σ MUFA[3]	40.2±4.2	45.6±5.8	46.1±4.4	50.9±7.4	49.0±5.1	50.9±7.2	46.0±2.5	48.9±4.5
Σ PUFA[4]	32.4±8.9	31.9±8.5	33.2±5.7	26.0±10.7	31.0±7.6	29.2±9.7	35.1±1.5	31.6±6.8
***P/S**[5]	1.29±0.47	1.50±0.47^{b}	1.67±0.35^{ab}	1.23±0.63^{b}	1.64±0.48^{ab}	1.58±0.62^{ab}	1.90±0.11^{a}	1.72±0.48^{ab}
Σ ω3	19.4±6.7	19.6±6.8^{a}	18.4±4.6^{ab}	12.5±7.8^{b}	15.6±5.2^{b}	12.9±6.0^{b}	18.6±1.8^{ab}	16.4±5.1^{ab}
***DHA/EPA**	2.70±0.66	2.72±0.24^{b}	2.87±0.27^{b}	3.05±0.55^{ab}	3.05±0.33^{ab}	2.89±0.30^{ab}	3.33±0.43^{a}	2.87±0.38^{b}

[*] Unitless ratios
[1] Σ Bacterial is the sum of all the bacterial fatty acids, including *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 17:1, and 18:1ω6
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4] Polyunsaturated fatty acids
[5] Polyunsaturated/saturated fatty acids
Data are ± standard deviation for n replicates

4. Summary

Seven experimental diets containing different protein and lipid sources were formulated to evaluate the effects of minimizing marine resource utilization in diets of farmed Atlantic salmon on growth, as well as on elemental, lipid, and fatty acid composition of muscle, liver, and head kidney tissues.

The growth performance results revealed that the diet with the lowest fish meal and fish oil content led to the lowest weight gain, followed by the diet with the highest content of animal by-products. The analysis of lipid classes for all the tissues showed that while the seven different treatments did not significantly influence the total lipid content of tissues, significant statistical differences were observed in the major lipid classes. This study also showed that feeding fish with diets containing medium and low levels of fish oil and fish meal, respectively, generally led to as high a level of ω3 fatty acids as when fish were fed diets containing high levels of fish meal and fish oil. Tissue fatty acid profiles showed reductions in the contents of DHA and EPA when fish meal and fish oil were minimized in the diets. Therefore, Σω3 fatty acids which consisted mainly of DHA and EPA was also reduced using the diets with minimized marine resources. The elemental composition analysis showed that the C/N ratio was the highest in head kidney, followed by liver and muscle tissues. Relatedly, feeding fish with the formulated diets resulted in the highest total lipids content in head kidney among the three tissues. It was also observed that fatty acids such as SFA, MUFA, 18:2ω6, and 18:3ω3 were preferentially deposited in head kidney, followed by liver and muscle tissues. While 20:5ω3 had higher deposition in head kidney, 22:6ω3 accumulated more in the liver

tissue. However, a comparison of significant differences in 22 individual fatty acids, groups and ratios in the three tissues revealed that muscle and liver tissues were more responsive to the dietary treatments.

Growth performance and elemental compositions of the examined tissues in this study were affected when fish meal and fish oil were replaced with animal by-products and rapeseed oil at levels of 33 and 27%, respectively. However, replacement at lower levels might not affect growth. This study suggests that fish meal can be reduced to as low as 5% without affecting growth, if a minimum of 5% fish oil and a maximum of 26 % animal by-products is provided in the diet. The study also concluded that reducing marine resource utilization to less than 10% in diets of farmed Atlantic salmon affected lipid classes and fatty acid compositions of the muscle, liver, and head kidney tissues. This result highlights the importance of inclusion of at least 10% fish meal and fish oil in the diet to achieve comparable lipid content and growth performance to fish fed marine-based diets in order to reduce susceptibility to inflammation.