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Effects of replacing fish oil with microalgae biomass (*Schizochytrium spp)* as a source of n-3 LC-PUFA to Atlantic salmon *(Salmo salar)* on growth performance, fillet quality and fatty acid composition.

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Master of Science in Feed Manufacturing Technology

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List of Abbreviations

AA	Arachidonic Acid
AA	Microalgae diet
ALA	Alpha-linolenic acid
DHA	Docosahexaenoic acid
DM	Dry matter
EFAs	Essential Fatty Acids
EPA	Eicosapentanoic acid
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FO	Fish Oil
GLA	Gamma Linolenic acid
GC	Gas Chromatography
HSI%	Hepatosomatic index
HCl	Hydrochloric acid
K	Condition Factor
LA	Linoleic acid
LC-PUFA	Long chain Polyunsaturated Fatty Acids
MUFAs	Monounsaturated Fatty Acids
NaCl	Sodium Chloride
$NaHCO_3$	Sodium bicarbonate
PUFAs	Polyunsaturated Fatty Acids
SFAs	Saturated Fatty Acids
SGR	Specific Growth Rate
SEM	Standard Error Mean
SFan	Salmon Fan
TCA	Tricarboxylic Acid
TFA	Total Fatty Acid
VSI%	Visceral somatic Index

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Abstract

Low levels of EFA in fish feed as a result of changes in diet composition brings a need of finding a novel ingredient that will supplement Essential Fatty Acids (EFA) in fish feed. Microalgae have ability of producing omega-3 polyunsaturated fatty acids (PUFA) and for that are predicted to be a reliable feed ingredients in replacing fish oil in the near future. Main objective of present study was to evaluate effects of replacing fish oil (FO) with microalgae biomass (*Schizochytrium spp*.) (AA) as a source of n-3 LC-PUFA to Atlantic salmon (*Salmo salar*) on growth performance, fillet quality and fatty acid composition. Atlantic salmon were fed two diets: FO control diet and AA enriched diet for 400 days. Fish that were fed AA diet showed higher final weight, specific growth rate (SGR) and condition factor (K) than fish fed the FO control diet. Fillet quality was good and analysis showed no significant difference between fish in the two dietary group on gaping score, fillet yield and redness. Although a significant difference was noted on fillet yellowness in fish from AA dietary group.

Furthermore, Folch method was used to analyse total fat content in liver and fillet. Statistical analysis showed no significant difference in fat content between the two dietary groups. Fatty acid composition was also analysed by using gas chromatography (GC) method. Liver and muscle fatty acid compositions varied between the two dietary groups. Percentage of eicosapentaenoic acid (EPA) was lower and the percentage of docosahexaenoic acid (DHA) was higher in AA dietary group fish than in fish from FO dietary group. Generally, results from our study shows that inclusion of (*Schizochytrium spp)* biomass (6.25% of the diet) as source of DHA is able to improve final weight, SGR, K, deposition of DHA in tissues (fillet and liver) without reducing the quality of fillet in terms of red color and gaping score.

Keywords: Atlantic salmon, fish oil, *Schizochytrium spp,* fillet quality, fatty acid composition, DHA, EPA

1. Introduction

1.1 Aquaculture and Salmon production

Aquaculture production has been able to achieve tremendous changes over the course of years, from being a less important sector to a reliable one in feeding world's population. Currently there are about 600 aquatic species that are farmed under different intensities and technological sophistication using freshwater, brackish and marine water. Although, aquaculture sector has grown tremendously, there is still imbalance on distribution of aquaculture production among countries, with China being the leading producer. (http://worldatlas.com). Records provided by FAO showed that about 181 countries and territories are involved in aquaculture production with an estimate of a total value of US 119 billion. In Europe aquaculture production has grown for about 30% within 20 years (1990 to 2010) mainly due to the increase in Atlantic salmon farming (FAO 2012).

Norway is the world's leading producer of Atlantic salmon and in 2012 Atlantic salmon production was approximately 1.3 million tons. Salmon belongs to anadromous family, meaning salmon spend its life both in freshwater and seawater. Juvenile wild salmon spend their early life in freshwater (rivers and streams) and at later stages migrate to the sea. Normally wild salmon juveniles spend the first 1-4 years in freshwater before migrating to the sea where they feed and mature for 1-3 winters. Farmed Atlantic salmon spend 10-16 months in freshwater before transferred to the sea where they grow until harvest for about 14-22 months (http://seafish.org: Liu et al., 2010).

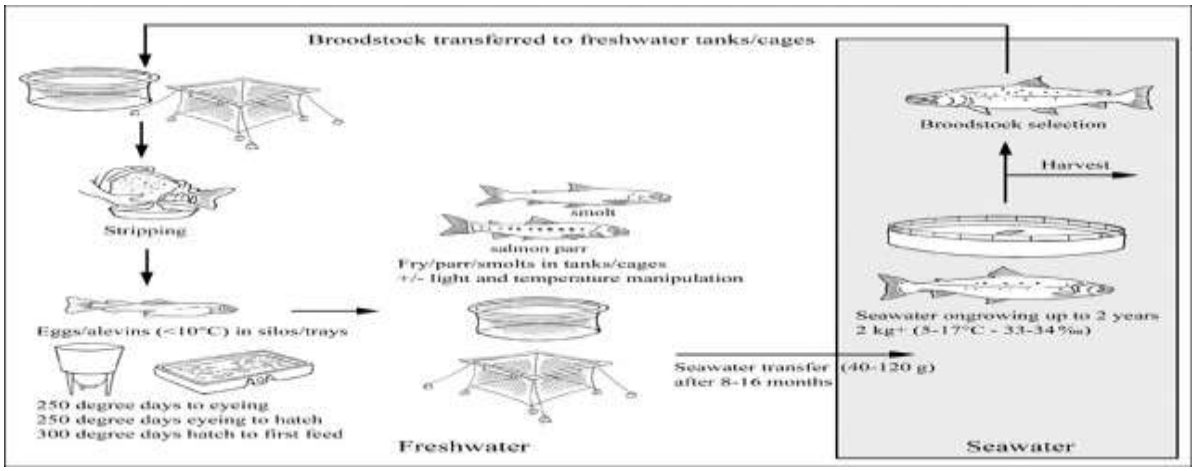


Figure 1 Production cycle of Atlantic salmon (source; Google)

1.2 Salmon diet Composition

Similar to other fish diets, salmon diets are composed of carbohydrates, proteins, lipids, vitamins and minerals. However, marine fish especially carnivorous like Atlantic salmon have low ability of utilizing carbohydrates as energy source and thus depend mainly on lipids and proteins as energy sources. But lipid can also minimize the amount of dietary protein which is used and this effect is called protein-sparing effect (Tocher, 2003). Fish meal and fish oil have been and still are important ingredients in Atlantic salmon diets. A report by FAO, showed that salmonids are main consumers of fish meal followed by marine fish (FAO, 2012). In 2012, salmonids consumed about 21% of fish meal and 53% of fish oil of the world production (Ytrestøyl T et al., 2014).

Required changes in fish diet composition over time, are well explained by Ytrestøyl et al., (2014). In 1990, 90% of fish diets was composed mainly of marine ingredients, whereas in 2013 marine ingredients contributed only by 30%. Decrease in the content of marine ingredients was accompanied by an increase in plant oil and plant protein in fish diet. The need of finding alternatives to fish meal and fish oil is important because most of plant ingredients have low levels of essential fatty acids (EFA) and as result, fish performance and health is affected.

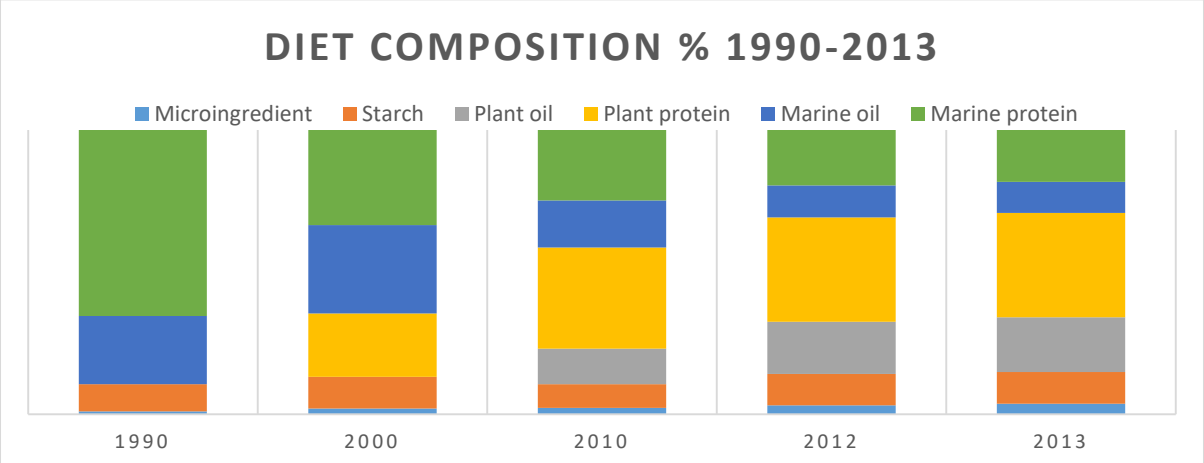


Figure 2 shows percentage on how fish diet composition changed between years 1990 to 2013 ingredients presented in the figure are micro ingredients, starch, plant oil, plant protein, marine oil and marine protein (Ytrestøyl et al., 2014).

1.3 Lipid and fatty acids

Lipids are made up of a) Triacylglycerols b) Wax c) Phospholipids d) Sphingolipids and e) Sterols and lipids have two main properties: water insolubility and their solubility in non-polar solvents such as acetone or chloroform. Triacylglycerols contain three molecules of fatty acids esterified to the sn-1, sn-2 and sn-3 positions of L-glycerol and these are main storage form of metabolic energy in fish. Phospholipids are made two molecules of fatty acids esterified to L-glycerol 3-phosphate at position sn-1 and sn-2 on the phosphatidic acid backbone and main role of phospholipid is to build cell membrane bilayers (Sargent et al., 2002).

All classes of lipids except sterol are made up of fatty acids. Fatty acids are carbon chains with a methyl group on one end and carboxyl group on another. Fatty acids can be classified depending on a number of characteristics. According to degree of unsaturation, fatty acids are divided into three groups, saturated (with no double bond), monounsaturated (with one double bond) and polyunsaturated (with two or more double bond) (Sargent et al., 2002). Also position of the first double bond is used to describe fatty acid for example 18:3n-3 indicates, a fatty acid with 18 carbons, three double bonds and the first double bond is on third carbon atom from the methyl group. Furthermore, in Polyunsaturated fatty acids (PUFAs) position of double bond is used to describe fatty acids even broader for instance omega-3 refers to group of fatty acids with first double bond between third and fourth carbons while omega-6 refers to a group of fatty acids with first double bond between carbon six and seven (Akoh, 2008). Omega-3 and Omega-6 are also known as EFAs because mammals and fish cannot synthesize these and thus must be supplied through diet (FAO 1980).

1.3.1 Synthesis of essential fatty acids

18:2n-6 and 18:3n-3 fatty acids are main precursors in synthesizing longer chain omega-3 and omega-6 fatty acids from diets. 18:2n-6 is used to synthesize Arachidonic acid (AA) while Eicosapentanoic acid (EPA) and Docosahexaenoic acid (DHA) are synthesized from 18:3n-3 (Monroig, et al., 2013).

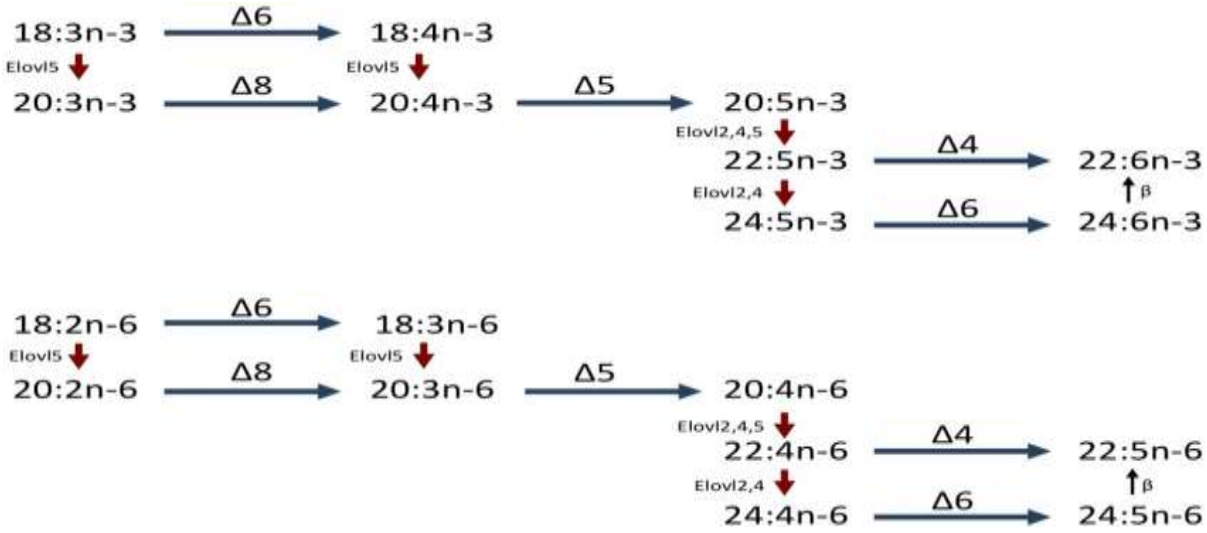


Figure 3. Biosynthesis of EFA (AA, EPA and DHA) from two precursor linoleic acid (LA) and alpha-linolenic acid (ALA). Indicating Δ6 and Δ5 desaturase: Elovl 2 and 5 elongase and β-oxidation (Monroig, et al., 2013).

1.3.2 Requirements and deficiency of EFAs

As mentioned above that fish do not have ability of synthesizing EFAs de novo and thus EFAs must be supplied in diets. Adequate levels of EPA and DHA in diets are important in maintaining fish health in order to influence ontogenesis, growth, survival, pigmentation and resistance to stress and disease as well as in the development and functionality of the brain, vision and nervous system (Sargent et al., 2002). Common symptoms of EFA deficiencies are associated with slow grow rate, fin erosion, reduced feed efficiency, shock syndrome and increased mortality (Lall 2000: Ruyter 2000a). Determination of sufficient levels of EFA to fish is limited by several factors such as species, natural habitats and fish diets (Ruyter et al., 2000a). Thus it is still unclear on how much is required in terms of long-chain PUFA (LC-PUFA) EPA and DHA to meet a salmon's requirements. A study in Atlantic salmon, fed diets with different levels of EPA + DHA (2g/kg and 10g/kg) and reared for 13 months to a final weight of 3kgs suggested that minimum of 1% (10g/Kg) of EFA in fish diet is sufficient for normal growth but this amount is not enough to prevent long term effects on fish health (Bou et al., 2017).

To humans EPA and DHA are essential as well. Benefits of EPA and DHA to humans relate to increasing body ability on resisting diseases and conditions like hypertension, diabetes, arthritis, atherosclerosis, skin diseases and other inflammatory and autoimmune disorders (Zivkovic et al., 2011). Apart from EPA and DHA, another important EFA is AA. AA is the main

precursor of eicosanoids in biosynthesis of prostanoids, leukotrienes and thromboxanes. AA also has important physiological functions in reproduction, cardiovascular, blood clotting and immune response (Otohinoyi et al., 2016)

1.4 Microalgae

Microalgae are likely to be reliable replacement of fish oil in the near future, due to their ability of producing omega-3 LC-PUFA specifically DHA (Taelman et al., 2013: Kousoulaki et al., 2015) which are crucial fatty acid for growth and health of both fish and humans. In addition to LC-PUFA other nutrients like pigments and vitamins are also available in microalgae as shown in Table 1.

Table 1 essential nutrients in microalgae (Priyadarshani and Rath 2012).

Pigments/Carotenoids	B-carotene, astaxanthin, lutein, zeaxanthin, canthaxanthin, chlorophyll, phycocyanin, phycoerythrin, fucoxanthin
PUFA	DHA(C22:6), EPA(C20:5), ARA(C20:4), GAL(C18:3)
Vitamins	A, B1, B6, B12, C, E, biotin, riboflavin, nicotinic acid, pantothenate, folic acid
Other	Antimicrobial, antifungal, antiviral agents, toxins, amino acids, proteins, sterols, MAAs for light protection.

Microalgae are applied in a number of industries. In aquafeed, microalgae are used as source of lipid, pigments, vitamins and more. In humans microalgae are used as food supplement and in cosmetics. In energy industries, microalgae are used as a source of biofuel (Priyadarshani and Rath 2012). Microalgae can either be heterotrophic or autotrophic. Heterotrophic refers to kind of microalgae that are not able to synthesize their own food and thus depend on external organic substances (Rameshprabu et al., 2009) such as glucose (Jiang et al., 2004) and acetate (Kiseleva and Kotlova 2007). Main characteristic of heterotrophic microalgae is their ability of metabolizing without light. Whereas, autotrophic microalgae are the opposite of heterotrophic because these are capable of producing organic compounds by using light or through chemical reactions like oxidation. Chemical compositions of different

microalgae differ according to their species, nutrient availability (nitrogen and phosphorus), light, temperature, pH and salinity (Ren, 2014). Microalgae lipid content ranges between 1% and 70% (Mettings, 1996), depending on species and culture conditions and there are even huge variation in their fatty acid compositions (Tonon et al., 2002). Another benefit of using algal biomass comparing to other sources of oil was mentioned by Ratledge (2005) that because algal is fermented generally it becomes free from contaminants such as heavy metals.

1.4.1 Schizochytrium spp

Comparing to other species in thraustochytrid family, *Schizochytrium spp*. has the fastest growth rate with a relatively simple culture process compared to other single-cell microalgae (Ganuza et al., 2008; Lewis et al. 1999). *Schizochytrium spp* are heterotrophic algae and found in marine and other salty environments. Thraustochytrid show higher potential than other species of becoming a relevant replacement to fish oil as a source of DHA for aquaculture, (Lewis et al., 1999: Barclay and Zeller 1996; Nichols et al., 1996;) mainly because of high percentage of lipid content 55-75% in DM (Miranda et al., 2015: Ren et al., 2010: Christi 2007). High levels of DHA is a main reason of using microalgae (*Schizochytrium spp)* to substitute fish oil. DHA in *Schizochytrium spp* is approximately to be 43.2 % of total fatty acid (Sarker et al., 2016: Ludevese-Pascual et al., 2016) while DHA level in fish oil is around 13% of total fatty acid.

Schizochytrium spp has been incorporated in fish diets at different inclusion levels to different fish species. For example Sarker et al., (2016) used *Schizochytrium spp* as whole cell in juvenile Nile tilapia at 4%, 8%, 12.5% and 16.1% inclusion level of the diet. Sprague et al., (2015) fed algal meal from *Schizochytrium spp* to Atlantic salmon at inclusion level of 11% and 5.5% of diet and Sea Bream were fed dried microalgae at inclusion levels of 11% and 10.7% of the diet (Eryalçın and Yıldız (2015). Novel feed ingredients like yeast, microalgae and likes need to meet desired characteristics such as having required levels of EFA and without causing negative effects to fish performance in terms of growth, health, digestibility and product quality. Several studies on marine algae focused their experiments on using microalgae at relatively low inclusion levels, aiming to determine microalgae potentiality in aquafeed. For example Norambuena et al., (2015) used derived products (dry algae meal), Verdemin (derived from *Ulva ohnoi*) and Rosamin (derived from diatom *Entomoneis spp*.) at 2.5% and

5% levels to feed juvenile Atlantic salmon and Valente et al., (2006) fed *Gracilaria bursa-pastoris, Ulva rigida* and *Gracilaria cornea* to European sea bass at levels up to 10% of diet. Kousoulaki et al., (2015) used different inclusion levels 0.1%, 0.6% and 1.5% of *Schizochytrium spp* (whole biomass) to Atlantic salmon and results showed there was no effect on fish survival, feed intake, feed conversion and protein efficiency rates despite diet difference. These studied analysed the possibility of incorporating microalgae in aquafeed up to a level of 10% without causing negative effects. Recent studies aim on determining the possibility of using microalgae to even higher levels and not only at salmon early life stage but throughout growth period. Sprague et al., fed Atlantic salmon post-smolts algal meal for 19 weeks at levels 11% and 5.5% of the diet without negative effects. Studies observed no effect on fillet lipid percentage although a noted a difference was in DHA levels with fish that fed algal treatment having higher levels of DHA (Miller et al., 2007: Norambuena et al., 2015: Sarker et al 2016). Despite promising results by microalgae inclusion in diet still it has some limitations most of which are associated with high production costs and high technological development requirements (Jiang et al 2004: Norsker et al., 2011).

Table 2 shows fatty acids composition from two sources of lipids: *Schizochytrium spp* (whole cell dried) and fish oil (menhaden) (Sarker et al., 2016).

	Fish oil (menhaden)	*Schizochytrium spp*
Fatty acids	% of TFA	% of TFA
C14:0	8.1	9.3
C16:0	17.9	24.4
C18:0	0.6	0.5
C16:1n-7	13.9	0.2
C18:1n-9	5.2	0.1
C18:1n-7	3.3	ND
C18:2n-6	1.5	ND
C18:3n-6	0.3	0.2
C18:3n-3 *ALA*	1.5	ND
C20:4n-3	1.4	0.8
C20:5n-3 *EPA*	14.9	0.8
C22:5n-3	2.6	0.4
C22:6n-3 *DHA*	13	43.2

1.5 Main Objective

The overall aim of this study was to evaluate effects of using microalgae (biomass) *Schizochytrium spp* as source of DHA in the diet of Atlantic salmon in a long term production.

1.5.1 Specific Objectives

i. To determine effects of microalgae (biomass) on fish performance parameters like final weight, specific growth rate and condition factor.

ii. To study effects of microalgae (biomass) on visceral-somatic and hepatosomatic index and fillet quality traits.

iii. To determine effects of replacing fish oil with microalgae (biomass) on lipid composition of liver and muscle fillets.

2. Materials and methods

2.1 Materials

2.1.1 Chemicals and equipment

Chemicals and equipment	**Producer**
Chloroform	VWR International, PA, USA
Methanol	VWR International, PA, USA
Sodium Chloride	VWR International, PA, USA
Benzene	VWR International, PA, USA
Hexane	VWR International, PA, USA
Methanolic HCl	Supelco Inc, Bellefonte, PA, USA
2,2-dimethoxypropane	Sigma Chemical Co., St Louis. MO, USA
Butylated hydroxytoluen (2,6-Di-t-butyl-p-cresol, BHT)	Sigma Chemical Co., St Louis. MO, USA
Hewlett Packard 6890 gas chromatograph	Avondale, PA, USA

2.2 Methods

2.2.2 Fish trial, experimental diets and sampling

The fish feeding experiment took place at the previous NOFIMA research station, now known as Marine Harvest in Averøya, Norway. Atlantic salmon were reared from a start weight of 865 g for 400days to a slaughter weight of approximately 3 Kg from 11th December, 2015 to 17th January, 2017.

Figure 2.1 Map of Møre and Romsdal Count showing Averøya Kommune where the experimental trial took place.

The fish were fed two different diets, one diet supplemented with fish oil (FO) and another was supplemented with microalgae (AA). There were triplicate sea cages per dietary group, 6 cages in total. Composition of the two diets that were used as a source of DHA during this feed trial is shown in table 1.

Diet Name	Fish Oil (FO)	Microalgae (AA)
	%	%
Soy protein concentrate (SPC)	25	25
Fish Meal	10	10
Krill meal hydrolysate (wet)	2.5	2.5
Aquate	0.4	0.4
Choline chloride	0.5	0.5
Cholesterol	0.5	0.5
vitamin mix	3	3
Alltech Mineral Plex	0.64	0.64
Yttrium oxide	0.01	0.01
Soya lecithin	0.5	0.5
Lysine (99 %, 19.41 % Cl)	0.5	0.5
Carophyll Pink (10%)	0.05	0.05
Mono Calcium Phosphate	2	2
Methionine 99%	0.25	0.25
Horse bean meal 6/15	16.56	13.35
Wheat gluten 4/15	5.15	5.45
Rapeseed oil	14.34	17.5
Linseed oil	9.25	10.6
Palm oil	4.55	1
Fish oil O7/14	4.3	
Alltech AlG Rich (microalgae)		6.25
Sum	100	100

Table 2. The fatty acid compositions of diets (%, g/Kg) in feed DM)

Fatty acids	**FO**	**AA**
14:0	0.4	0.3
16:0	4.1	4.4
18:0	0.9	0.8
20:0	0.1	0.1
22:0	0.1	0.1
∑ SFAs	5.5	5.7
16:1 n-7	0.4	0.1
18:1 (n-9)+(n-7)+(n-5)	12.2	12.7
20:1 (n-9)+(n-7)	0.3	0.3
22:1 (n-11)+(n-9)+(n-7)	0.2	0.2
24:1 n-9	-	-
∑MUFAs	13.1	13.3
18:2 n-6	5.0	5.5
18:3 n-6	-	-
20:2 n-6	-	-
20:3 n-6	-	-
20:4 n-6	-	-
22:4 n-6	-	-
22:5 n-6	-	-
∑ PUFA (n-6)	5.1	5.5
18:3 n-3	5.9	7.1
18:4 n-3	0.1	-
20:3 n-3	-	-
20:4 n-3	-	-
20:5 n-3	0.7	0.1
21:5 n-3	-	-
22:5 n-3	0.1	-
22:6 n-3	0.5	1.1
∑PUFA (n-3)	7.3	8.3
EPA+DHA	1.1	1.2

FO: fish oil; AA: Microalgae

SFAs; saturated fatty acids:

MUFAs; monounsaturated fatty acids:

PUFAs; polyunsaturated fatty acids:

Fish sample collection took place from January 17th to 19th, 2017 at Averøya. A total of 412 fish were harvested. The number of Atlantic salmon fed fish oil (FO) and microalgae (AA) diets was n=213 and 199, respectively from six sea pens. All fish were used for the analyses of weight and growth rate, other parameters final length, final weight, gutted weight, liver weight, and were recorded during harvesting process. In addition, liver, blood, intestine and fillet samples were taken. Only liver and fillet samples were analysed in this thesis.

Not all harvested fish were selected for further analysis in the laboratory instead two groups were made. The first group of fish were selected for quality analyses and this involved sample size of 9 individual fish from each sea pen making a total of 54 fish and the second group selected for fatty acid analysis comprised 12 individual fish from each sea pen making a total of 72 fish. For sample collection, fish were killed in two ways depending on belonging group. Fish for fatty acid analysis were killed by using anaesthetizes and this was done by placing them in water that was mixed with Tricaine methanesulfonate (MS222). This step was followed by weighing final weight and length, gutting and lastly samples collection (muscle, blood, liver and intestines). Fish selected for muscle quality analyses were killed by being hit on the head (stunning) then gills cut out and placed in ice water for 10 mins in order to let them bleed out prior to fillet sample collection. Fillet samples were collected as whole Liver samples were collected by removing an entire liver from digestive system, then weighed and placed in a sampling bag and blood samples were taken. Fillet samples were frozen in -20^{0}C and liver samples in -40^{0}C for storage until further analysis in the laboratory.

2.2.3 Laboratory work

Laboratory experiment took place at NOFIMA research station in Ås. Fillet quality analyses took place on 27th of January, 2017 and fatty acid analysis began from 1st of March to 17th of March, 2017. A total of 54 fish were used for quality analyses from which half were fed the AA and the other half were fed the FO diet. Another 72 fish were used for fatty acid analyses of which 36 were fed the AA diet and the other 36 were fed the FO diet.

2.2.4 Gaping score

Gaping in fish fillet occurs as a result of interaction between forces pulling the muscle apart and the strength of the tissue. Gaping score was measured according to Andersen et al., (1994) by observing amount and size of slits/holes on the fillet. Gaping scale ranged 0-5 that:

0 (no gaping), 1 (less than 5 slits), 2 (less than 10), 3 (less than 10), 4 (more than 10) and 5 (large holes)

2.2.5 Fillet color

Global way of measuring fillet color involves a method of visually comparing fillets against the *Salmo*Colour Fan. The salmoColour fan has a scale ranging from 20-34 in which 20 indicate palest fillet colour and 34 is the most intense. Color assessment was performed in two different locations on the ventral fillet part between the posterior part of the dorsal fin and the gut (NQC) and under the anterior part of the dorsal fin.

Figure 2.3. Illustrating SalmonFan

Furthermore fillets colour were measured using a hand-held Minolta. A software program was developed to capture images and to obtain colour results based on L* (lightness), a*(redness) and b*(yellowness) values. After color assessment fillets were cut into pieces and stored in -20^{0}C freezer until further analysis.

2.2.6 Fat content and Fatty acid composition analysis

Homogenization and pooling

Homogenization of the 72 individual fish samples was conducted by first letting fillet and liver samples to thaw for about an hour before placed in a mixer until thoroughly blended and thereafter 12 individual homogenized fish samples were pooled to three pooled samples per cage (4 fish per pooled sample and a total of 3 pooled samples per cage). For each pen three samples were made and each pooled sample consisted four individual fish. A total of 9 pooled samples were made from each diet group (36 fish in total). 18 samples analysed for both

dietary groups (72 fish). Pooled liver homogenates were stored in plastic tubes at -40^{O}C while pooled fillet homogenates were stored in plastic bags at -40^{O}C until analysis.

Figure 2.4. Illustrating some of muscles pooled samples

Figure 2.5. Illustrating some of livers pooled samples

2.2.7 Fat content analysis

Analysis of tissue fat percentage was done using Folch method. Folch extraction (Folch et al., 1957) is a method for lipid extraction that is based on lipids' solubility in organics solvent. Folch extraction solution is a mixture of two parts polar (water and methanol) and non-polar solvents (organic chloroform). Fat is easily dissolved in non-polar solvent following the

principle that the polarity of fat is similar to non-polar solvent. In Folch method, fat is extracted by organic solvent and kept in organic phase, after evaporation of this organic phase, the mass difference before and after evaporation was the amount of fat.

2g of homogenized sample was transferred into a 100ml Erlenmeyer flask where 6ml of 0.9% NaCl and 50ml chloroform: methanol (2:1), with antioxidant BHT 0.7mg/l was added. Sample was homogenized with a homogenizator (IKA T25 digital ultra turrax) at 17,000 rpm for 60 seconds, followed by addition of 6ml 0.9% NaCl and thereafter sample was homogenized again for 5 seconds more. The mix was separated into two phases, the lower phase contained chloroform: methanol: water in the ratio of 86:14.This phase contained almost all lipids and the upper phase was chloroform: methanol: water in the ratio of 3:48:47 that contained mostly water soluble components

The homogenate was filtered through cotton filter inside a cylinder and once all homogenate was filtered, the cylinder was capped and kept in freezer until the next day. Empty beakers were weighed in order to be used in the following day.

On the second day, upper phase of the liquid that contained water/methanol was removed by a water-vacuum pump-pipette. The lower phase with lipids was pipetted (20ml) into beakers in order to be used for fat content analysis and the rest was transferred to a test tube so as to be used for fatty acid composition in gas chromatograph (GC).

For fat content determination beakers with liquid (20ml) were placed on heating plate in order for the solvent to completely evaporate. Then, beakers were transferred into a dryer at 102^0C for 20min in order to dry any remaining water. After 20 mins beakers were removed from the dryer and weighed again. Formula used for calculating fat content was:

$$\% \text{ fat} = \frac{g\ fat\ *\ 100}{I\ *\ U/37.5}$$

g fat = evaporated sample in beaker

100 = %

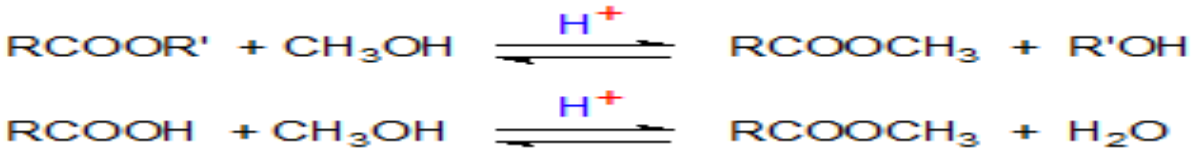
I = weight of the sample in g

U = Pipetted chloroform extract (20ml*) in mL beaker

37.5 = Total volume of solvent (33.3 mL *100/89) = 37.5 mL (Chloroform in extract solution = 50*2/3 = 33.3 mL)

2.2.8 Fatty acid composition analysis

Analysis of fatty acid composition started with methylation process. This is the analysis of fatty acid in GC as fatty acid methyl esters. Fatty acid tendency of forming hydrogen bonds in polar state is a reason why fatty acids are often analysed by using methylation process. Reducing their polarity make them more amenable for analysis.



The first step involved transferring 0.5ml of each sample to a new test tube and evaporated at 60^{O}C with nitrogen flow. Aim of nitrogen flow was for preventing oxidation and pushing chloroform out. Next step was addition of 10µL and 20µL of C23: 0.61769/50ml to liver's and fillet's samples respectively. C23: 0.61769/50ml is a known concentration internal standard that mainly used to quantify amount of fatty acid found in samples.

Thereafter Benzene, metanolic-HCl and dimetoxypropan solution were added in 2mL, 2Ml and 0.2mL respectively (Mason and Waller 1964). Afterward, samples were heated at 80^{0}C for 20mins. After samples were heated, hexane and 6% $NaHCO_3$ by 2mL and 2mL respectively. $NaHCO_3$ is added for the purpose of neutralization. The mix that was obtained contained two phases, upper phase contained hexane and benzene together with fatty acids while the lower phase contained salt solution and water. The upper was transferred to a new test tube and evaporated with nitrogen flow until dry. Thereafter 5 drops of hexane were added into a tube and transferred to GC sample bottle. Addition of hexane was repeated until desired volume was attained for GC analysis.

From the GC individual fatty acids was determined based on two factors: retention time that indicated specific fatty acid and area (%) that indicated its amount. Formula used to calculate fatty acid composition based on concentration fatty acid from the GC, sum of fatty acid from the GC and concentration of internal standard from the GC.

% FA= $(con.of\ FA\ *SUM\ FA\ )/(SUM\ FA - con.of\ int.std)$

2.2.9 Biometric Traits

i. Condition factor (K) indicates the relationship between salmonid length and weight. The value of K is influenced by age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. Formula used to calculate K was:

$$K = \frac{final\ weight}{L^3}$$

ii. Specific growth rate shows the percentage increase in size per day.

Formula used to calculate SGR was:

$$SGR = \frac{ln(^{final\ weight}/_{initial\ weight})}{number\ of\ days} * 100$$

iii. Visceral somatic index (VSI %) determines how much fat is deposited in visceral rather than in muscle. Hepatosomatic Index (HSI %) is basically the ratio of liver weight to total body weight and it is used to measure the fish energy reserves.

Formula used to calculate visceral somatic index was

$$VSI\ \% = \frac{visceral\ weight}{final\ weight} * 100$$

Formula used to calculate hepatosomatic index was

$$HSI\ \% = \frac{liver\ weight}{final\ weight} * 10$$

2.2.10 Statistical Analysis

All the data were processed and figures were created using the Microsoft Excel Program. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test at a significance level of $P<0.05$. Data are expressed in Mean and Standard error mean (SEM).

3. Results

We conducted a 400 days feed experiment with microalgae (biomass) diet and results found are discussed into three main sections in this chapter. The first section is concerning diet composition and performance parameters of experimental fish. The second section is about fillet quality assessment and the last section presents fat content and fatty acid analysis results from liver and muscle tissues.

3. 1. Experimental fish

3.1.1. Initial and final weights

The mean initial weight of fish fed FO was 867.5g and for fish fed AA was 862.8g and mean final weight of fish fed FO was 2803.2g and for fish fed AA was 3298g. Statistical analysis showed no significant difference ($p>0.05$) in initial weight between two dietary groups while final weight varied significantly ($p<0.05$) between the two dietary groups (Figure 3.1).

3.1.2. Condition factor (K) and Specific Growth Rate (SGR)

The mean K of fish fed FO was 1.19 and for fish fed AA was 1.23. Statistical analysis presented significant difference ($p<0.05$) in K between two dietary groups. The mean SGR of fish fed FO was 0.23 and of fish fed AA was 0.27. Statistical analysis revealed there is a significant difference ($p<0.05$) in specific growth rate between two dietary groups (Figure 3.2).

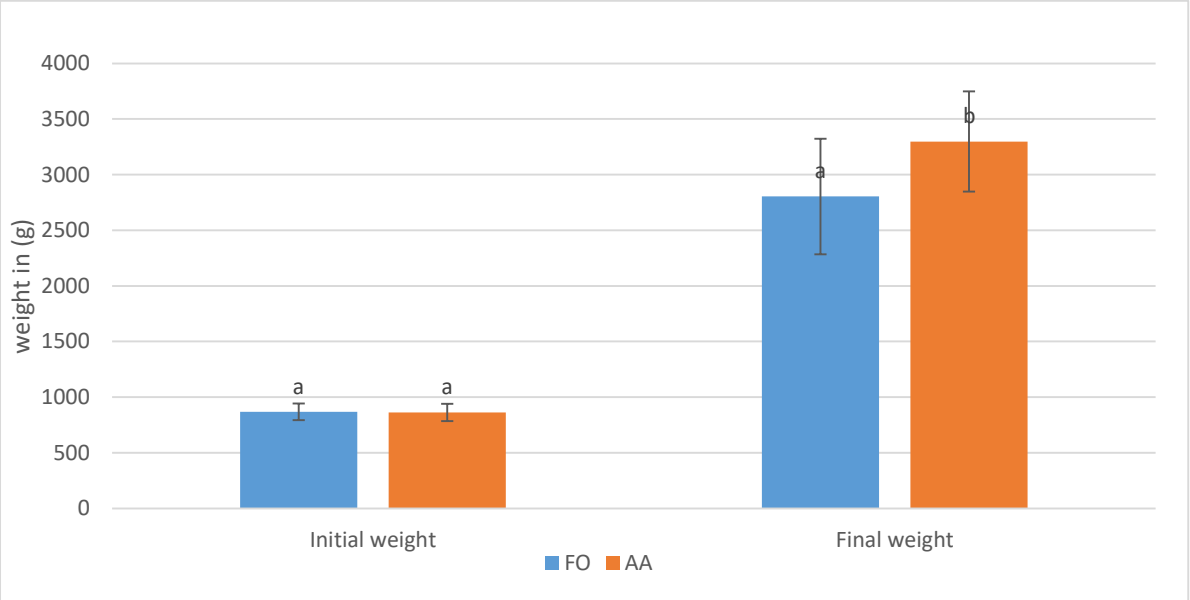


Figure 3.1: Initial weight and final weight Atlantic salmon fed two diets FO and AA. N= 3 (triplicate sea pens per diets). Data are expressed in Mean ± SEM. Differing letters indicate significant differences ($p<0.05$) between two dietary groups.

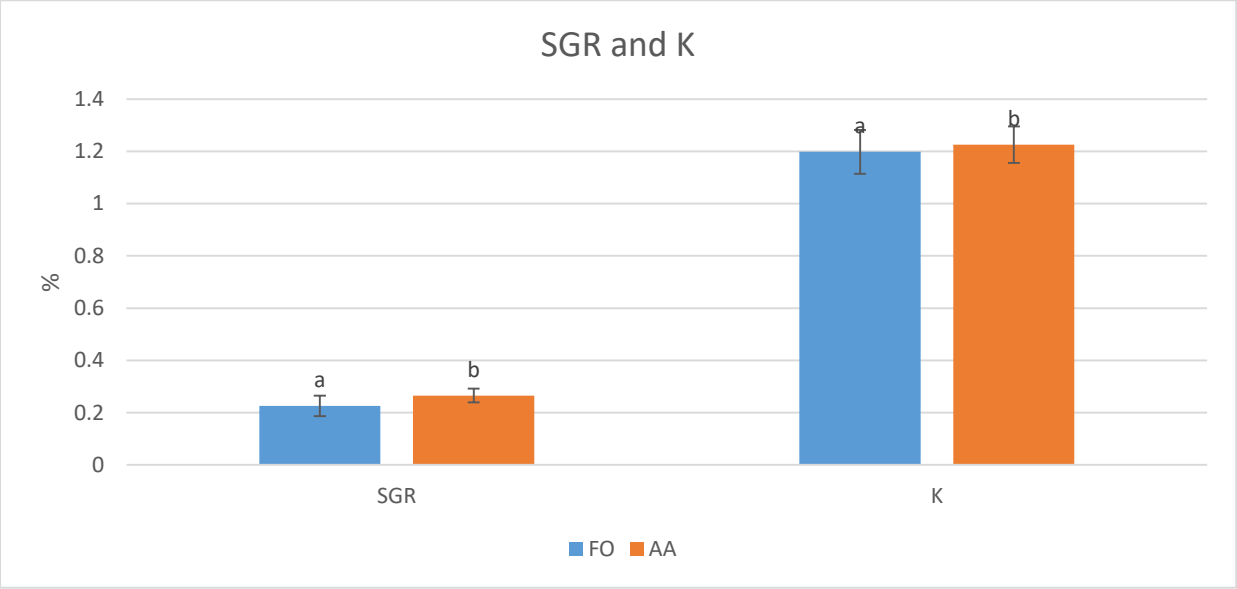


Figure 3.2 SGR and K from Atlantic salmon fed two diets FO and AA. N= 3 (triplicate sea cages per diets). Data are expressed in Mean ± SEM. Differing letters indicate significant differences ($p<0.05$) between two dietary groups.

3.2. Quality assessment

3.2.1. Fillet Yield

The mean fillet yield of fish fed FO was 58% and of fish fed AA was 58%. Statistical analysis showed no significant difference ($p>0.05$) in fillet yield between two dietary groups (Figure 3.3).

3.2.2. VSI % and HSI%

The mean VSI% of fish fed FO was 13.1 whereof the mean VSI% of fish fed AA was 11.6. Statistical analysis showed significant difference ($p<0.05$) in Visceral index between two dietary groups (Figure 3.4).

The mean HSI% of fish fed FO was 1.1 whereof the mean HSI% of fish fed AA was 1.2. Single factor Anova by Excel, 2013 indicated no significant difference ($p<0.05$) in HSI% between two dietary groups (Figure 3.4).

3.2.3. Gaping

The mean gaping score of fish fed FO was 0.2 whereas of fish fed AA was 0.4. Statistical analysis showed no significant difference ($p>0.05$) in gaping between two dietary groups (Table 3.2).

3.2.4. Liver weight

Liver weights of Atlantic salmon fed two diets (FO) and (AA) were 33.45g and 38.42g respectively, statistical analysis showed significant difference between two dietary groups (Table 3.1).

3.2.5. Fillet color assessment

Fillet color was observed using SFan and Minolta. SFan results showed that there is no significant difference in red colour between the two dietary groups (Table 3.2). Minolta chroma meter measurements showed no significant difference on red chromaticity (Figure 3.5) and the lightness (Figure 3.7) between the two dietary groups. However, yellow chromaticity data showed that there is a significant difference on three fillet positions (dorsal posterior, ventral anterior and ventral posterior) where measurements were taken (Figure 3.6).

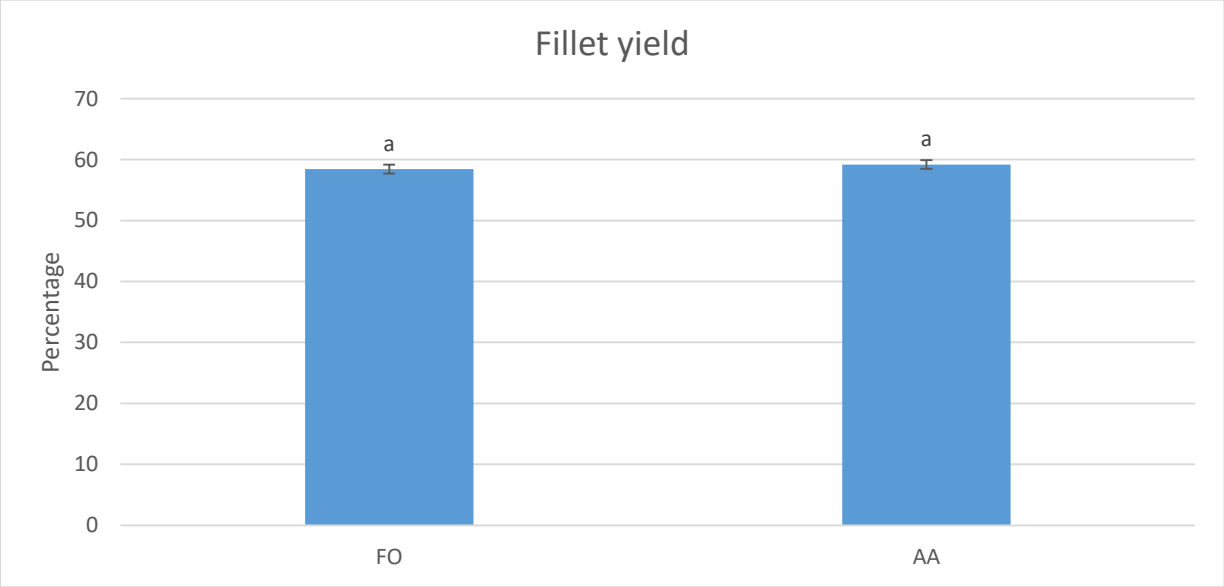


Figure 3.3. The mean fillet yield of fish fed FO was 58.4% while for fish fed AA was 59.2%. N=3 (triplicate sea cages per diets). Differing letters indicate significant difference ($p<0.05$) in fillet yield between two dietary groups.

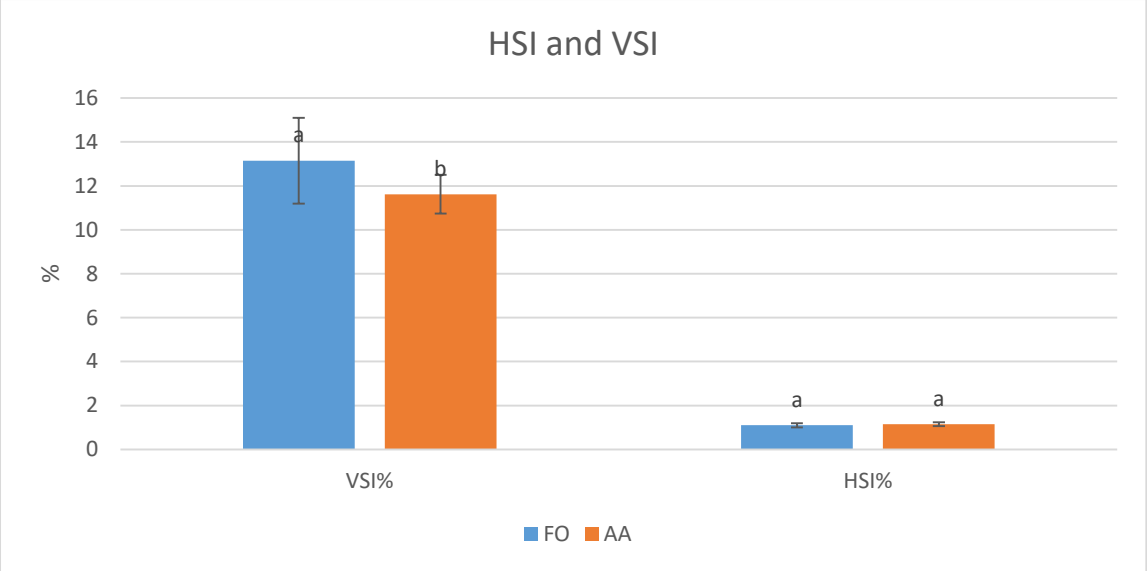


Figure 3.4. HSI% and VSI% from Atlantic salmon fed two diets FO and AA. N= 3 (triplicate sea cages per diets). Data are expressed in Mean ± SEM. Differing letters indicate significant difference ($p< 0.05$) in qualities between two dietary groups.

Table 3.1 Liver weight from Atlantic salmon fed two diets FO and AA. Sample size for liver weight was 72 livers (12 liver samples from each sea pen) N= 3 (triplicate sea pens per diets). Data are expressed in Mean ± SEM. Differing letters indicate significant difference ($p< 0.05$) in qualities between two dietary groups.

	FO	**AA**
Liver weight (g)	33.45 ± 1.05^{a}	38.42 ± 0.93^{b}

Table 3.2. SFan and gaping score results from Atlantic salmon fed two diets FO and AA. N= 3 (triplicate sea pens per diets). Data are expressed in Mean ± SEM. Differing letters indicate significant difference ($p< 0.05$) between two dietary groups.

	FO	**AA**
Sfan_X	25.87 ± 0.69^{a}	26.56 ± 0.81^{a}
Gaping score	0.24 ± 0.12^{a}	0.43 ± 0.13^{a}

Figure 3.5 Red chromaticity (a*). N=3, (triplicate sea pens per diets) and data are expressed in Mean of the three replicate sea pens per diet. Letters indicate significant differences ($p<0.05$) between two dietary groups.

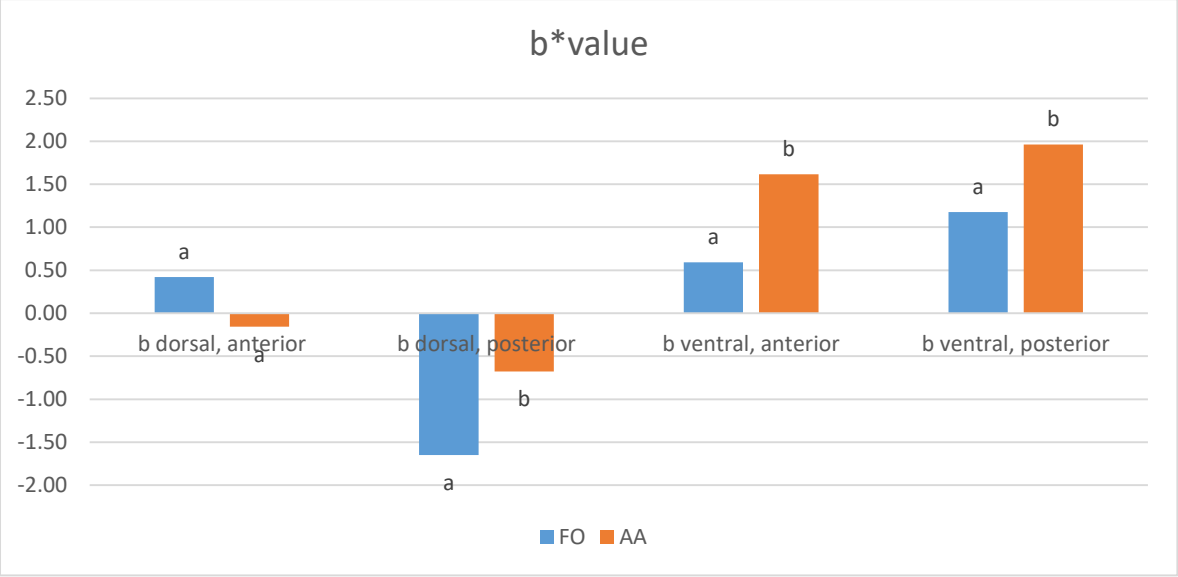


Figure 3.6. Yellow chromaticity (b*value).N=3(triplicate sea pens per diets) and data are expressed in Mean. Differing letters indicate significant differences ($p<0.05$) between two dietary groups.

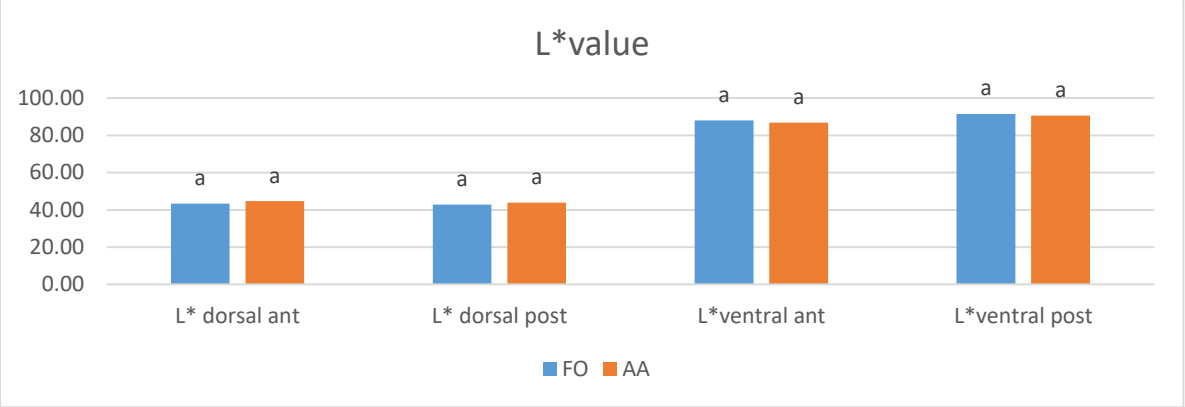


Figure 3.7. Lightness (L*value). N=3 (triplicate sea pens per diets) and data are expressed in Mean. Differing letters indicate significant differences ($p<0.05$) between dietary groups.

3.3. Fatty Acid Analysis

3.3.1. Liver fat content and fatty acid composition

The overall amount of lipid in liver tissues of the Atlantic salmon did not differ between the two dietary groups (Figure 3.8).

Liver Fatty acid composition (% of total fatty acids) was influenced by dietary groups. With the exception of 22:0 all other SFA showed significant diet effects. When saturated fatty acids were summed results showed that fish from FO group had higher values comparing to AA group with 16:0 having higher percentage in liver than all other SFAs.

Regarding MUFAs, 18:1n-9 varied significantly between two dietary groups with fish from AA dietary group having higher values than from FO dietary group. Total MUFA also showed significant difference with fish from AA dietary group having higher percentage than FO group.

Omega-3 fatty acid, with exception of 20:4n-3 and 20:3n-3, the rest varied greatly due to effects of microalgae although the total omega-3 fatty acids did not differ significantly. Percentage of 20:5n-3 was lower in AA dietary group fish comparing to fish from FO dietary group while percentage of 22:6n-3 was higher in fish from AA dietary group.

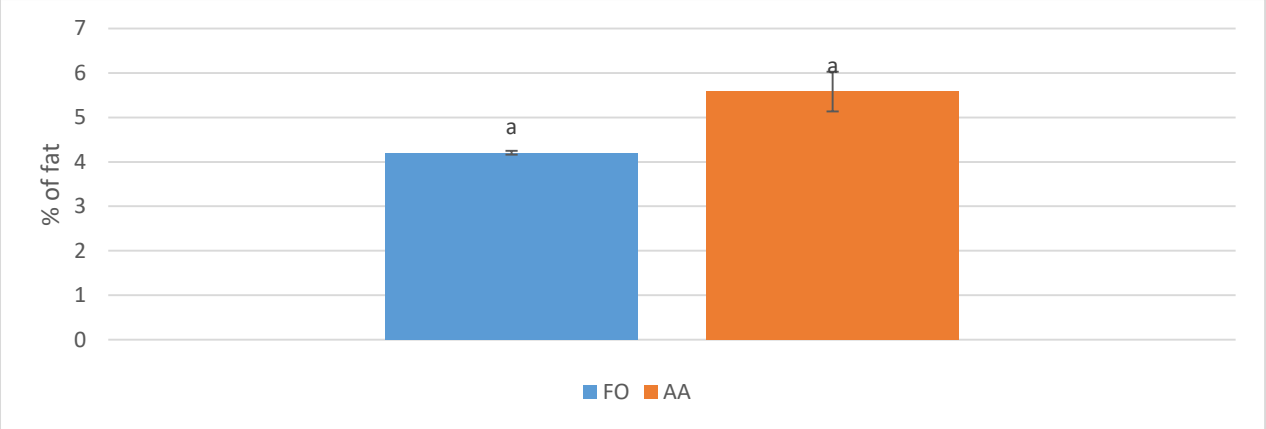


Figure 3.8. Fat percentage of liver tissue of Atlantic salmon fed two diets FO and AA. Data are expressed as mean ± SEM, N=3 (triplicate sea pens per diets). Letters indicate significant difference ($p<0.05$) between two dietary groups.

Table 3.3 Liver fatty acid composition (percentage of total fatty acids) of the two dietary groups. Data are expressed as mean ± SEM. Letters indicate significant difference ($p<0.05$) between two dietary group.

Fatty acids	**FO**	**AA**
16:0	13.42±0.30^{a}	10.91±0.59^{b}
18:0	4.53±0.07^{a}	2.90±0.39^{b}
SUM N-O	18.97±0.46^{a}	14.36±0.96^{b}
18:1 n-9	22.16±0.20^{a}	27.61±0.80^{b}
18:1 n-7	1.76±0.20^{a}	1.83±0.02^{a}
20:1 n-9	1.45±0.10^{a}	2.04±0.18^{b}
22:1 n-7	1.89±0.11^{a}	1.75±0.10^{a}
SUM MUFA	28.73±0.34^{a}	34.26±1.04^{b}
18:2 n-6	10.07±0.11^{a}	12.07±0.27^{b}
20:2 n-6	1.24±0.04^{a}	1.49±0.07^{b}
20:4 n-6	1.46±0.04^{a}	0.69±0.03^{b}

SUM N-6	13.72±0.17[a]	14.91±0.30[b]
18:3 n-3	8.17±0.24[a]	10.64±0.21[b]
20:3 n-3	1.08±0.00[a]	1.48±0.16[a]
20:5 n-3	7.88±0.10[a]	2.16±0.42[b]
22:5 n-3	2.24±0.05[a]	0.54±0.05[b]
22:6 n-3	11.98±0.12[a]	14.13±0.77[b]
SUM N-3	31.59±0.23[a]	29.13±1.19[a]
SUM EPA+DHA	19.86±0.15[a]	16.30±1.18[b]

Fatty acids lower than 1% are not included in this table. Sum N-O (saturated fatty acids) also include 22:0 and 14:0; Sum MUFA (monounsaturated fatty acids) also include 16:1 n-7, 16:1 n-5, 18:1 n-11 and 22:1 n-11; Sum N-6 (omega-6 series polyunsaturated fatty acids) include 18:3 n-6 and 20:3 n-6; Sum N-3 (omega-3 series polyunsaturated fatty acids) include C20:4 n-3. Data are expressed as mean ± SEM, N=3 (triplicate sea pens per diets).Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

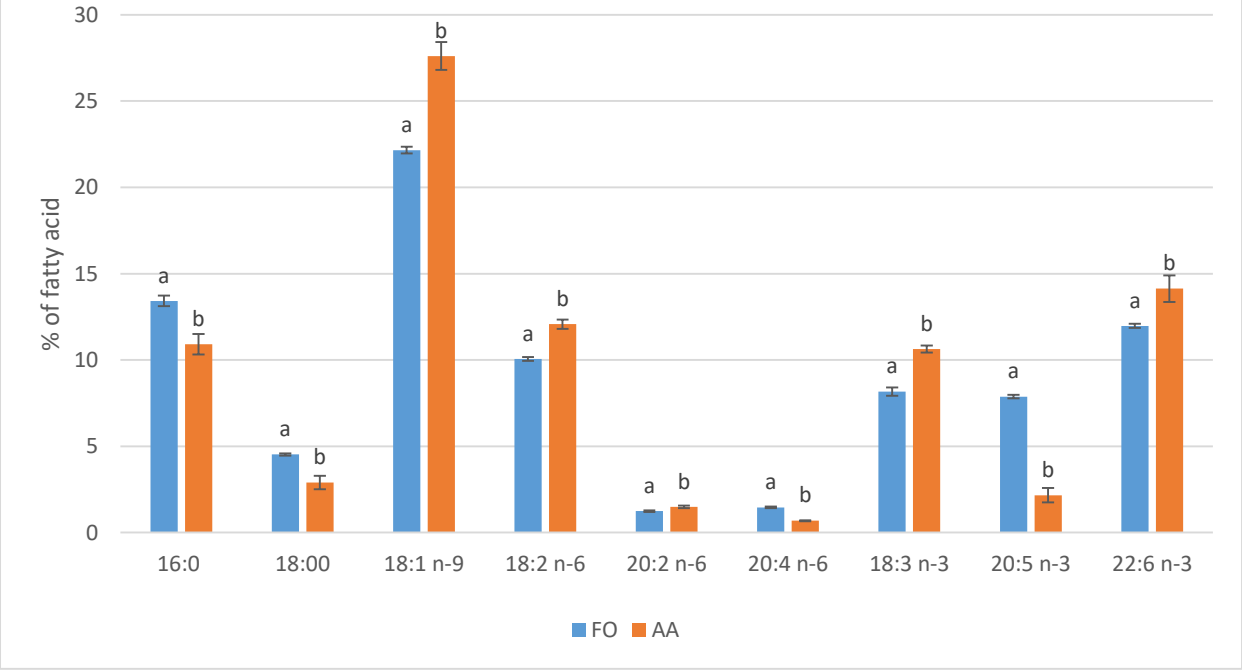


Figure 3.9. Effect of diets FO and AA on selected percentage of fatty acids in liver lipids. Data are expressed as mean ± SEM, N=3 (triplicate sea pens per diets). Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

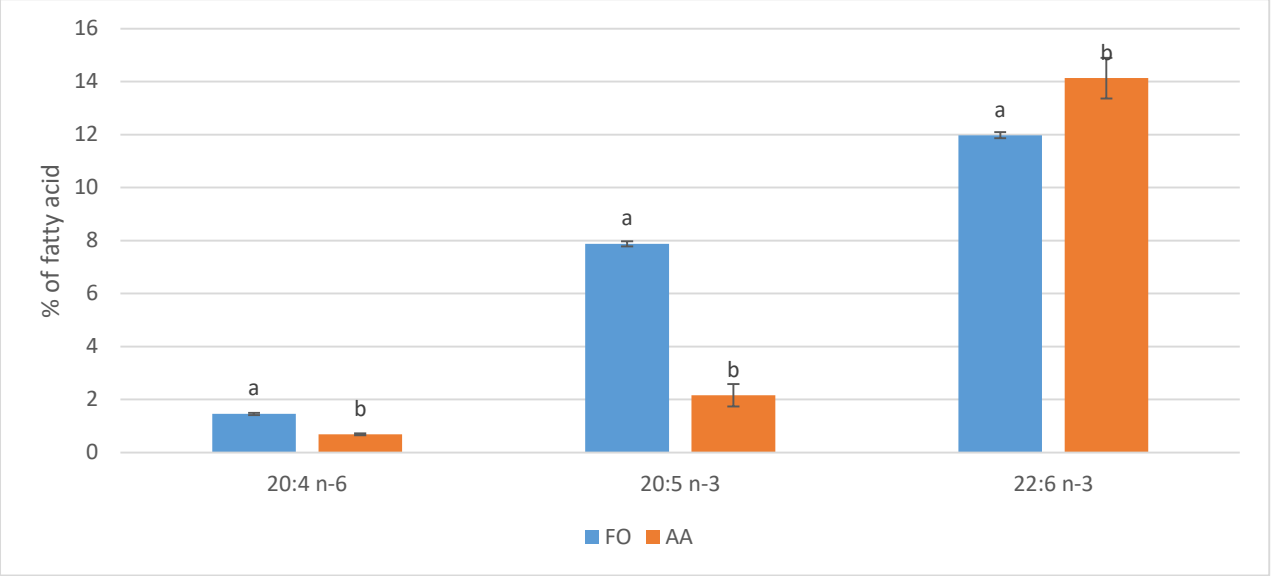


Figure 3.10. Effect of diets FO and AA on three fatty acids 20:4 n-6, 20:5 n-3and 22:6 n-3. Data are expressed as mean ± SEM, N=3 (triplicate sea pens per diets). Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

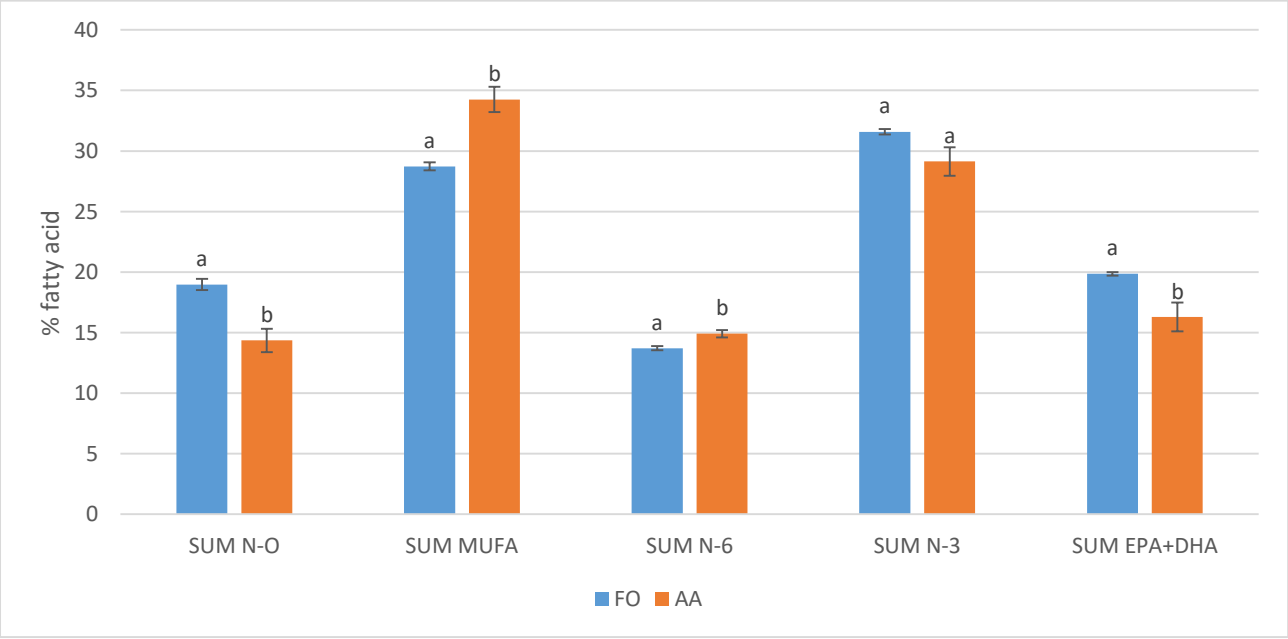


Figure 3.11. Effect of diets FO and AA on the percentage of total fatty acids. SUM N-O, SUM-MUFA, SUM N-6, SUM N-3 and SUM EPA +DHA. Data are expressed as mean ± standard error

SEM, N=3(triplicate sea pens per diets). Differing letters indicate significant difference ($p<0.05$) between two dietary group.

3.3.2. Fillet fatty acid composition

Lipid percentage in fillet was not affected by dietary effects and though AA diet shows a higher mean of fat percentage, there was no significant difference between two dietary groups (Figure 3.12).

Fatty acid composition in fillets (% of fatty acids) was also influenced by dietary groups. All SFA showed significant effects due to diets with 16:0 having higher percentage in fillet than all other SFAs. When SFAs were summed it was noted that fish from FO group had higher values of SUM-SFA comparing to AA group (Table 3.4).

Regarding MUFAs, with exception of 18:1n-9 and 22:1n-7 all other MUFAs varied significantly between two dietary groups. Total MUFA also showed significant difference with fish from FO dietary group having high percentage than from AA group (Table 3.4).

Omega-3 fatty acids varied greatly due to effects of microalgae also when summed it was noticed that fish from AA dietary group have higher percentage than fish from FO group. Percentage of 20:5n-3 was lower in AA dietary group fish comparing to fish from FO dietary group while percentage of 22:6n-3 was high in fish from AA dietary group (Table 3.4).

Figure 3.12. Fat percentage (Folch %) from muscle tissue of Atlantic salmon fed two diets FO and AA. Data are expressed as mean ± SEM, N=3, (triplicate sea pens per diets). Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

Table 3.4 Fillet fatty acid composition of two diets. Data are expressed as mean ± standard error mean. Letters indicate significant difference ($p<0.05$) between two dietary group.

Fatty acids	FO	AA
14:0	1.14±0.00[a]	0.64±0.01[b]
16:0	10.71±0.05[a]	9.82±0.04[b]
18:0	2.84±0.03[a]	2.57±0.01[b]
SUM N-O	14.87±0.08[a]	13.25±0.06[b]
16:1 n-7	1.20±0.01[a]	0.48±0.00[b]
18:1 n-9	36.67±0.05[a]	36.79±0.01[a]
18:1 n-7	2.19±0.02[a]	2.09±0.01[b]
20:1 n-9	1.83±0.01[a]	1.89±0.00[b]
SUM MUFA	43.46±0.05[a]	42.59±0.01[b]
18:2 n-6	15.43±0.01[a]	15.91±0.04[b]
20:2 n-6	1.03±0.01[a]	1.21±0.01[b]
SUM N-6	17.03±0.01[a]	17.57±0.04[b]
18:3 n-3	15.80±0.01[a]	17.47±0.03[b]
20:3 n-3	1.18±0.01[a]	1.53±0.03[b]
20:5 n-3	1.65±0.02[a]	0.60±0.01[b]
22:5 n-3	0.67±0.00[a]	0.27±0.00[b]
22:6 n-3	2.31±0.01[a]	3.69±0.02[b]
SUM N-3	21.65±0.01[a]	23.61±0.04[b]
EPA+DHA	3.95±0.01[a]	4.29±0.03[b]

Fatty acids lower than 1% except 22:5 n-3 are not included in this table. Sum N-O (saturated fatty acids) also include C22:0; Sum MUFA also include 22:1 n-7 and 22:1 n-11; Sum N-6 include 18:3 n-6, 20:3 n-6 and 20:4 n-6. Sum N-3 include 20:4 n-3. Data are expressed as mean ± SEM, N=3 (triplicate sea pens per diets).Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

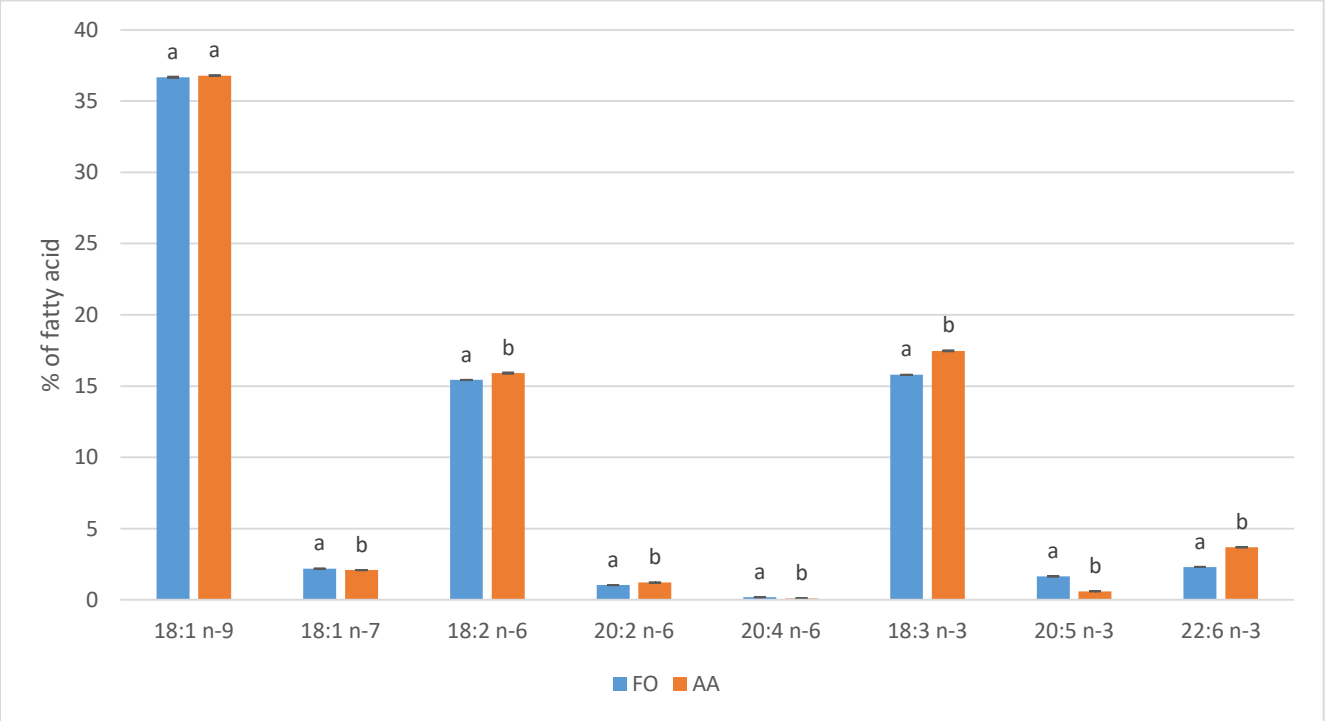


Figure 3.13. Effect of diets FO and AA on selected percentage of fatty acids. Data are expressed as mean ± SEM, N=3, (triplicate sea pens per diets).Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

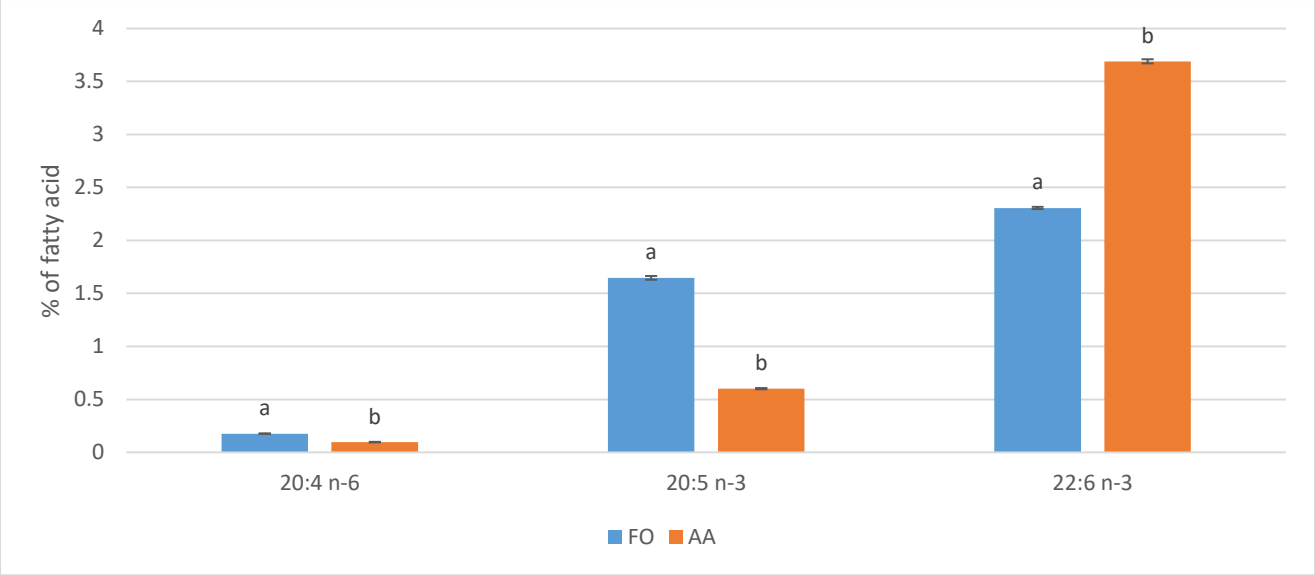


Figure 3.14. Effect of diets FO and AA on percentage of three fatty acids 20:4n-6, 20:50n-3 and 22:6n-3. Data are expressed as mean ± SEM. N=3, (triplicate sea pens per diets). Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

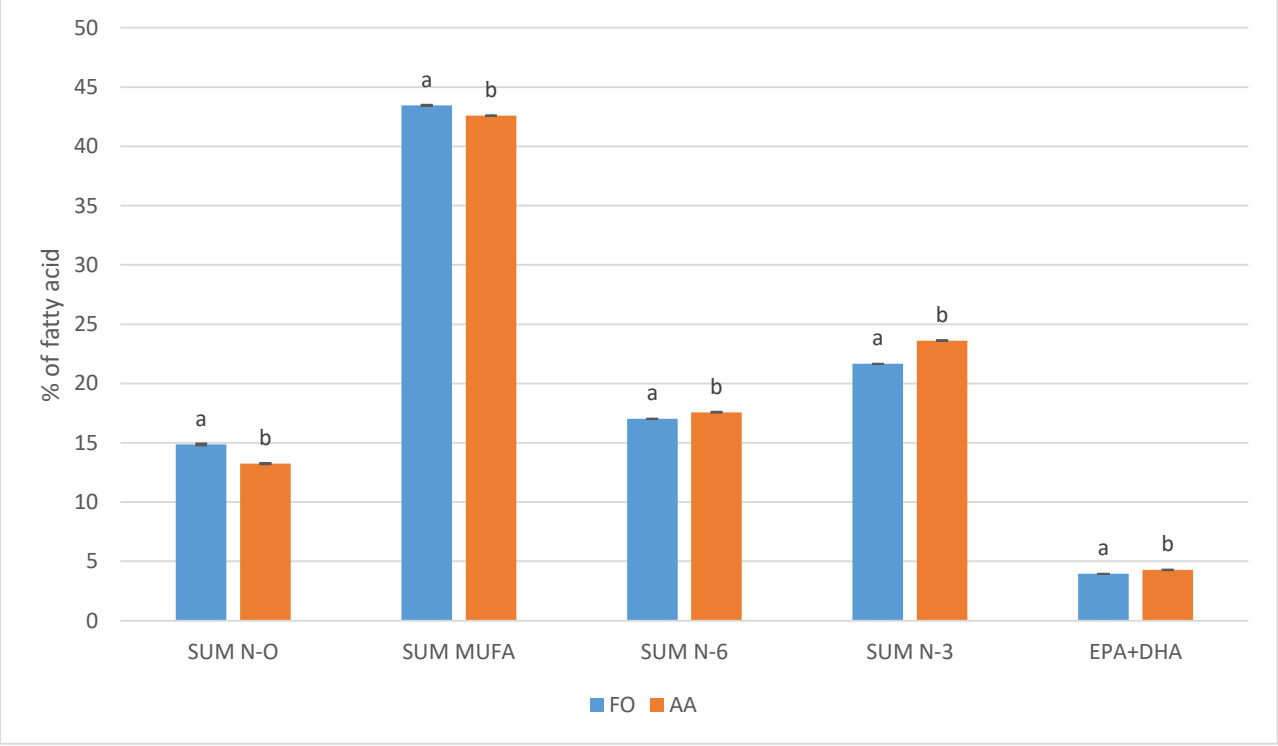


Figure 3.15. Effect of diets FO and AA on percentage of total fatty acid of SUM N-O, SUM-MUFA, SUM N-6, SUM N-3 and SUM EPA +DHA. Data are expressed as mean ± SEM, N=3(triplicate sea pens per diets). Differing letters indicate significant difference ($p<0.05$) between two dietary groups.

4. Discussion

4.1 Effect of Microalgae to fish performance parameters

Fish final weight and length are often used to determine whether a particular fish has reached a market demand or not and final weight is influenced by growth rate. In the present study, results showed a significant difference on final weight between fish in FO dietary group (control diet) and AA dietary group (treatment group), with fish in AA dietary group weighing about 0.5 kg more than fish in the control diet. Although in our study we did not measure feed conversion ratio (FCR), but higher final weight in fish from AA dietary group may indicate that fish in this group had better FCR and hence improved weight: results agree with interpretation from Kousoulaki et al., (2015) that although there was no significant difference in feed intake between treatments used in their study, *Schizochytrium spp* diet stimulated muscle growth that contributed to final weight. On the other hand our results are in contrast with a study by Sprague et al (2015) who noted that despite replacing fish oil with algae meal at (11% and 5.5% of the diet) to Atlantic salmon for trial period of 19 weeks to a size of 3kg, final weight was not affected by dietary groups. Possible reasons for this variation are differences existing in diet compositions, microalgae inclusion levels and length of the trial experiment between studies, since our study was conducted for 57 weeks with microalgae (biomass) inclusion of 6.25% of the diet.

Similar to final weight, specific growth rate (SGR) was also affected by dietary effects. Fish in AA dietary group had higher SGR than control group. SGR results from our study were 0.27 for AA dietary group at inclusion level of 6.25% of the diet and 0.23 for control dietary group. Good digestibility in fish that were fed AA diet might be a possible explanation for these results. Reitan et al., (2012) replaced fish oil with different microalgae species (*Nannochloropsis* sp., *Phaeodactylum tricornutum* and *Isochrysis galbana*) and results presented that while 6% microalgae inclusion had good digestibility increase in algal inclusion to 12% lowered fish digestibility. Furthermore higher levels of micronutrients such as carotenoid astaxanthin found in *Schizochytrium spp* whole cell of about 7.7mgL-1 (Yamasaki et al 2006) also contribute to high growth rate in fish of AA dietary group (Sarker et al., 2016). Improved palatability in microalgae diet is another reason on why fish in AA dietary group had higher SGR than in control group. A study in sea urchin that were fed different species of

microalgae Ulva (Chlorophyta) as an additive showed that inclusion of 200gkg-1 dried Ulva improved feed palatability significantly (Cyrus et al., 2015).

Results for condition factor (K) presented a significant difference with fish in AA dietary group having higher K (1.23) than fish fed FO diet (1.19). Furthermore, condition factor results are related to final weight as it was also observed that fish in AA dietary group had both higher final weight and condition factor and for that reason it can be suggested that by feeding fish with microalgae diet, fish status was not negatively affected.

4.2 Effect of Microalgae to quality traits

Fillets are significant product from fish, because of their market value (Gjedrem 2008: Kousoulaki et al 2016). Fillet yield results from our study did not show significant difference between the two dietary groups (FO and AA) as both means were 58%. This low fillet yield percentage is complemented with the fact that fish were trimmed during fillet collection, since fillets were to be used for other analyses such as sensory analyses. Nonetheless fillet yield from our trial are slightly low but they still lie within normal range of 40% to 70% (Rørå et al. 2001).

Slits and holes appear on fillet as a result of broken connective tissues (Love, 1970), gaping on fillet reduce its value especially in the processing industry (Michie, 2001). In this study gaping score was at low level with an average of 0.3 and did not show significant difference between dietary groups. A number of studies mention absence of organic minerals in diet as an influence on gaping score (Kousoulaki et al., 2016: Mørkøre and Austreng 2004: Mahmoud and Edens, 2003). However this part was not discussed in present study thus cannot be used to make a conclusion. However, results agree with previous study using similar microalgae specie at different levels (0.1%, 0.6% and 1.5%) in which microalgae diet had no effect on flesh firmness and it was established that less gaped flesh are less prone to problems likely to occur during filleting and processing (Kousoulaki et al 2015).

Studies show that levels of DHA in the diet influence fillet color (redness) (Ytrestøyl (n.d.). Thomassen and Røsjo (1989) in their study that substituted capelin oil (CO) with vegetable oils: soybean oil (SO), low- and high-erucic acid rapeseed oil (LERO and HERO) for 18 weeks. Noted that color strength (redness) was high in fish fed CO mainly because of high DHA level in diet of 4.8% FA comparing to other diets SO, LERO and HERO that was 2.7%, 2.5% and 2.1% respectively. In another study that fed Atlantic salmon three different diets: 100% fish oil (FO), 75% vegetable oil (VO) and 100% VO to harvest weight. Results showed that flesh color was intensified in the fish fed FO indicating the influence of DHA to fillet color as fillet fatty composition showed higher DHA level from fish fed FO diet (Torstensen et al., 2005). In a more related study by Betiku et al., (2016), similar results were recorded that there was a significant difference in fillet quality when fish oil was 100% replaced with plant oil (supplemented with 30 mg g^{-1}: 60 mg g^{-1}: 90 mg g^{-1} DHA-Gold algal oil) showing that inclusion of microalgae in diet even to a small quantity influence flesh color. Those studies agree to a fact that redness in fillet is due to higher levels of DHA in the diet. However that was not observed in our study because inclusion of microalgae in the diet did not have effects on the red color of the fillet instead dietary effect was noted in b* value (yellowness) at three locations: dorsal posterior, ventral anterior and ventral posterior with fish from AA dietary group showing higher values than control diet. Reason for this is not known but it might due to carotenoids other than astaxanthin available in the microalgae.

Schmitt and Dethloff (2000), stated that welfare of the fish is determined by analysing both condition factor and organo-somatic indices (VSI% and HSI %). On the case of HSI% our results agrees with other previous studies who also noted no dietary effects to organo-somatic indices due to microalgae diet. For instance, when Thraustochyrid *Schizochytrium spp* oil was added at 100% level in Atlantic salmon parr, no effects were noted on HSI% (Miller et al 2007). Also similar results were obtained when Atlantic salmon were fed microalgae diets at different levels (0.1%, 0.6% and 1.5%) (Kousoulaki et al., 2015). In the present study results on VSI% showed significant difference between two dietary groups with fish in FO dietary group having higher VSI% but lower final weight than AA dietary group. Higher accumulation of visceral fat has been related to low levels of n-3 LC-PUFA in diet. For instance, in a study that involved full substitution of fish oil with *Camelina sativa* oil (CO) with partial substitution of fish meal with camelina meal in for farmed Atlantic salmon fed for 16 weeks to final weight

of around 600g. Results showed higher VSI% of 12% in fish that fed 100% CO while VSI% in fish fed FO diet was 10% indicating influence of DHA to VSI% (Hixson et al 2014). Thus, although in present study total level of EPA + DHA was the same in both diets, higher levels of DHA in AA diet can be a factor led to lower VSI% in fish fed AA diet.

4.3 Effect of Microalgae diets to Fat content and Fatty Acid composition of liver and muscle

4.3.1 Liver total fat content and fatty acid composition

Although livers from fish that were fed 6.25% AA diet had a 1% higher fat percentage than fish in control group. There were no significant differences in the fat levels in livers of fish between FO and AA dietary. Marine sources like microalgae because they contain high levels DHA have effect of decreasing fatty liver risk, thus fatty livers were not expected potential risk in the present study.

Results from liver fatty acid composition showed significant differences in several fatty acids but most important were 18:1n-9, 18:3n-3, 18:2n-6, 20:5n-3 and 22:6n-3. Deposition of two essential FAs, EPA and DHA differed significantly between two dietary groups. While EPA content was higher to fish in control group, DHA percentage was higher in treatment diet group. Obtained results are related to presence of fish oil in control diet. The percentage of 22:6n-3 was significantly higher in liver of fish from AA dietary group than in control group due to higher DHA level in the microalgae. Another notably result was the percentage of the monounsaturated FAs 18:1n – 9, indicating that Δ^9 desaturase activity was higher in fish from AA dietary group than in control group. Results agree with Sprague et al., (2015) who showed that microalgae diets increased deposition of 18:1n – 9.

4.3.2 Muscle total fat content and fatty acid composition

Results from our trial showed no significant difference on amount of muscle fat deposited in fish between the two dietary groups with fat percentage in fish from AA dietary group was 18.7%. Results agree with other studies for instance a study by Sprague et al (2015) who reported no significant difference on lipid percentage among four dietary treatments (northern fish oil, southern fish oil, 11% microalgae diet and 5.5% microalgae diet) that was fed to Atlantic salmon (post-smolts) for 19 weeks. Also Norambuena et al., (2015) found similar results despite using different microalgae species (*Ulva ohnoi* at 2.5% and *Entomoneis spp* at 5%) to feed Juvenile Atlantic Salmon for 84 days. Nevertheless a reason for no variation

from our study between two dietary groups is still unclear although it might relate the observation that fish in this study had lower VSI% (leaner fish) and thus energy in diet it was mostly used for growth rather than fat deposition in the stomach area.

Deposition of total ω-3, from our study differed significantly in muscle tissues between the two dietary groups. Higher levels of total ω-3 in muscle tissues of fish in AA dietary group supports a theory that fillet composition adapts composition of the diet used. Results disagree with Kousoulaki et al (2015) study in which fillet total ω-3 was not affected by different inclusion levels of microalgae in diets. It should be noted that both studies agree with the fact that, fillet composition is influenced by the DHA content from the diet. However, variation observed between our study and Kousoulaki et al (2015) in deposition of total ω-3 can be related to differences in trial period length, microalgae inclusion levels and inclusion of plant oils such as rape seed oil, palm oil and linseed oil that also contain 18:3n-3 that is a DHA precursor. In our study trial period was 400 days at 6.25% inclusion level while Kousoulaki et al., (2015) study was conducted for 84 days at 0.1%, 0.6% and 1.5% inclusion levels, suggesting that length of the experiment and inclusion level influence deposited ω-3. In general, n-3 LC-PUFA are important for the somatic growth of marine fish with DHA the most highly retained PUFA in a variety of species (Sargent et al., 2002).

EPA and DHA from diets need to be deposited in fillet due to their significance to both fish and human health. Accumulation of two EFAs, EPA and DHA differed significantly with fish in FO dietary group having higher levels of EPA, while amount of DHA was noted to be in higher levels on fish in AA dietary group. Lower levels of EPA in muscle tissues in fish from AA dietary are not odd as same results have been obtained from earlier studies that fed microalgae diet in replacement of fish oil (Ganuza et al., 2008 ; Miller et al., 2007: Eryalçin et al., 2013). Also EPA requirements that is supposed to be half of DHA levels (Rodríguez et al., 1998) were not met (DHA: EPA 3:0.6) in this study. Lower levels of EPA in fish from AA group indicate that retro-conversion of DHA to EPA or synthesis through Δ^6 and Δ^5 desaturation of 18:3n-3 to produce EPA in AA fed fish was not sufficient to maintain body levels of EPA relative to a FO fed fish (Tocher and Ghioni, 1999).

Higher levels of DHA on fillets from fish in AA dietary group are related with higher levels of DHA in the diet. Results agree with previous studies, who observed higher flesh DHA levels in salmon parr-smolts when *Schizochytrium* sp. replaced fish oil as oil source at 100% inclusion

level for over 9 weeks (Miller et al. 2007) and as dried product at 100% inclusion level for 51 days (Carter et al. 2003). However, caution should be applied when comparing results since the former authors utilised the richer oil extract as the sole oil source. In a more related study by Sprague et al., (2015) it was noted no significant difference on DHA levels between fish that were fed FO and the one were fed AA diet however DHA levels in diet were not balanced at the same level instead microalga diet had higher levels of DHA than in FO diet. Obvious difference from their study was between two algal treatments (11% and 5.5%) showing that higher levels of DHA in the diet led to higher deposition levels in fillets. In our study that was not the case because fish in the AA group were fed more DHA and less EPA than in fish from FO group with the sum of EPA+DHA balanced to the same level in both dietary group. Despite most studies focusing on DHA levels it should be noted that EPA is as important as DHA because its deficiency can also lead to slow growth, reduce survival rate and stress (Liu et al., 2002: Bou et al., 2017) and also because EPA plays a role as precursor of eicosanoids, its deficiency can be associated with cell immune and the stress responses (Ganga et al., 2005, 2006). Inclusion of algal to levels higher than 11% might be able to improve amount of DHA deposited in flesh to resemble or even be above to the amount that is deposited by fish oil diets (Sprague et al. 2015).

Conclusion

Present study described effects of using microalgae *Schizochytrium spp* as a supplement to Atlantic salmon diets from 860 grams fish size to approximately 3 kg. Studied parameters included the ones assessed fish performance, fillet quality and fatty acid composition between fish in two dietary groups: AA and FO. In general, fish performance parameters showed fish from AA dietary group performed better than in FO group. Fish final weight varied significantly, with fish from AA dietary group having higher mean final weight of 3.3kg compared to mean final weight of 2.8kg of fish from FO dietary group. K value was 1.23 for fish from AA dietary and 1.19 for FO dietary group, SGR was also high in fish from AA dietary group (0.27). Other qualities like gaping score, fillet yield, HSI%, a*value and L*value showed no significant difference between the two dietary groups. Regarding fatty acid composition it was interesting to see higher levels of DHA in fillets from fish in AA dietary group and since this is the first study that involved feeding *Schizochytrium spp* to Atlantic salmon as n3-LC PUFA source for a long period of time (400 days) those were encouraging results. This study shows that it is possible for *Schizochytrium spp* to replace fish oil at a 6.25% as source of n-3 LC PUFA source in Atlantic salmon fish until they reach market size without causing negative effect to their performance, fillet quality and fatty acid composition. On the other hand main nutritional concern of using algal diet alone is low levels of EPA in fillets and therefore more studies are needed to counteract existing challenges.

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