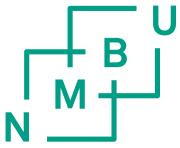
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Christos Dimitriou

Ås, June 2014

Abstract

The experiment was conducted to investigate if Atlantic salmon (Salmo salar) would express satisfactory growth and differences in fatty acid composition in later life stages when fed different EPA and DHA levels in earlier life stages.

In the early stage from 40g to 400g, Atlantic salmon was fed with 14 pre-diets with different inclusion levels of either EPA or DHA alone or a mixture of EPA and DHA (1:1). In the life-stage from 400g to 1 kg, the salmon from all the 14 dietary groups were randomly transferred to 3 new main dietary groups: 0%, 1% inclusion of EPA and DHA and a Control commercial feed with 2.2% EPA+DHA inclusion. Moreover, fish growth and colour from each experimental group and the muscle fatty acid composition were analyzed. EPA and DHA inclusions in diets significantly affect the growth and the colour of the fish. Different fatty acid profiles of the diets were reflected in the fish muscle with significantly higher levels of EPA and DHA acids in dietary groups containing high EPA and DHA inclusions. Few significant differences between pre-diets were found, being the most relevant related to the presence of EPA and DHA in the fillet.

Finally, in our results we showed that the fatty acid composition of the muscle was significantly affected the most at the later life stages by the 3 main diets.

Table of Contents

1. Introduction

The project addresses a main challenge for Norwegian aquaculture industry and for the fish feed producers, the shortage of the long chain omega-3 fatty acids (Vlc Ω-3FA), EPA and DHA. The project's main objective is to increase the knowledge about dietary omega-3 fatty acid requirements in Atlantic salmon that secures growth, health and product quality of the fish in seawater. This master thesis is part of the Norwegian Research Council project "Minimum requirements for omega-3 fatty acids in modern production of Atlantic salmon (MINOMEGA)".

The worldwide production of fish oil (FO) is varied considerably since 1964 till 2010 with a reduction after the large El Nino around 90s' from 1.6 million tons to 1 million tons (Silva et al., 2010).

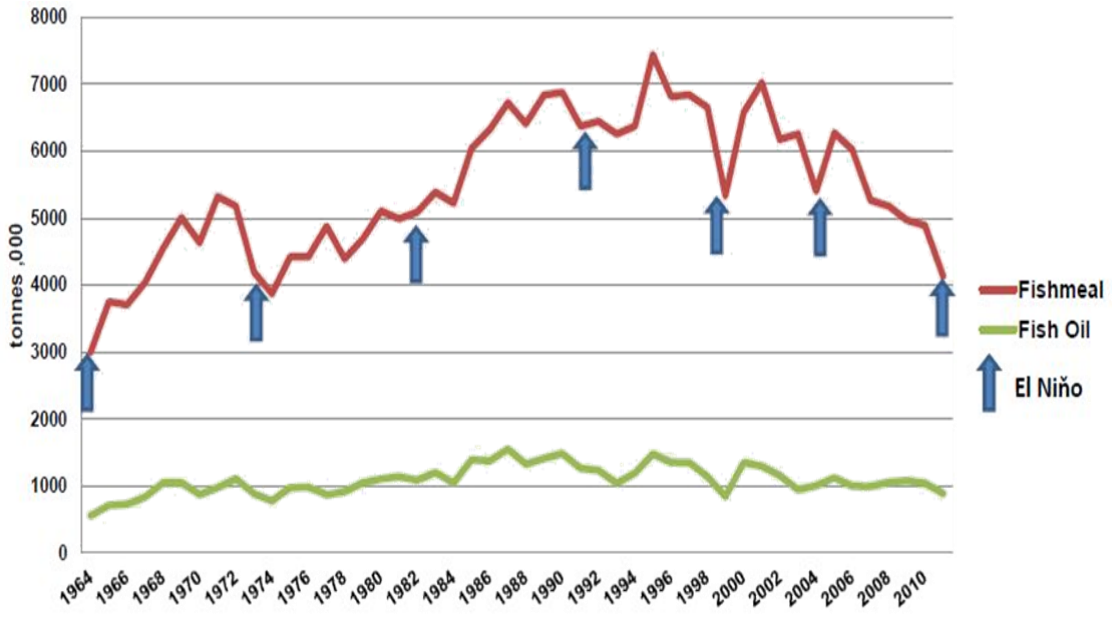

Figure 1. Fish oil and fishmeal production (tonnes) since 1964 till 2010.

Currently FO and fishmeal is used as the only Vlc Ω-3FA source in feed for salmon and trout. Continuously, the demand for FO increases as the world Aquaculture production increases as well as the use of FO directly for human consumption. Already today the usage of FO is dramatically reduced in Atlantic salmon diet (only approximately 37% of the oil today is FO and 63% rapeseed oil), leading to a marked reduction in the EPA and DHA level of salmon filet. Although new alternative sources of Vlc Ω-3FA, such as sea algae, krill and genetic modified oils are under rapidly research, they are not likely to be available at an affordable price for the Aquaculture industry the first five years.

There is a general decrease in Ω-3 FA levels in salmon diets and thereby in the fish itself nowadays, but it is not known what the safe lower levels of EPA and DHA that secure good health and quality of the salmon are. Besides this, salmon require 18:3 n-3 and 18:2 n-6 and certain amounts of DHA and EPA as essential fatty acid (Ruyter et al. 2000a; Ruyter & Thomassen 1999).

The requirements of Atlantic salmon for EPA and DHA are until now only determined in the freshwater stage and not in seawater. The EFA requirement in seawater of EPA and DHA has to be defined in order to avoid reduced growth and increased health risks, in particular under stressful conditions like changing water temperatures, viral and bacterial diseases, handling stress, etc.

The main purpose of this master is to study how different pre-diets with different EPA and DHA levels in early life stages and the 3 main diets at later life stages influence the growth, the colour and fillet composition of Atlantic salmon (Salmo salar).

2. Background & Theory

2.1. Essential fatty acids (EFAs)

There is a need for more knowledge about how a major reduction in the omega-3 fatty acids EPA and DHA influence fish performance, since the aquaculture industry is forced to dramatically reduce these EFAs in fish diets. Early investigation started in the 60s' when people believed that the EFA requirement of fish was the same as in land animals. At the beginning, in aquaculture they therefore used plant oils rich in 18:2 n-6 as dietary lipids (Owen et al., 1972). After sometime they discovered that these oils triggered negative effects on the fish, such as fin erosion, shock syndrome, heart myopathy, swollen livers etc. that were related to EFA deficiency.

A diet with inclusion of FO fed to trout gave faster growth, low mortality and higher feed efficiency (Lee et al., 1967). Studies about feed in Atlantic salmon showed that the 1% levels of dietary Ω-3 FA in feed with EPA and DHA yielded faster growth (Ruyter et al. 2000a). Fish fed with 18:2 n-6 had the lowest growth rate in comparison with fish fed with only 18:3 n-3 Ω-3FAs. From these early studies it was concluded that EPA and DHA were essential in salmon diets and these FAs were therefore classified, as EFAs nutritional lipids (Ruyter et al. 2000b). Not enough studies have been performed in the seawater life stages of Atlantic salmon, in particular the specific requirements for the EFAs DHA and EPA are not known.

The optimization of aquaculture feed has been performed by replacement of FO with substitutes of FO and alternative lipid sources such plant oils (Turchini et al., 2010). The prediction from research areas notices that the marine FO supply is declining and thereby the marine aquafeed industry searches for alternative sources.

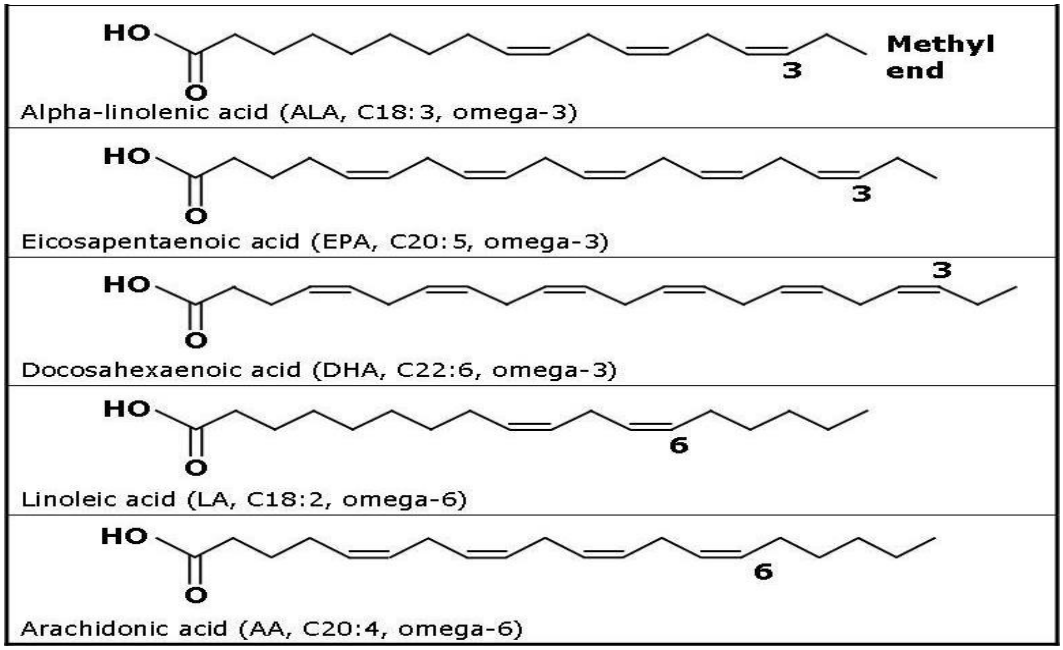

Figure 2. The structures of omega-3 (Alpha-linolenic acid, Eicosapentaenoic acid and Docosahexaenoic acid) and omega-6 fatty acids (Linoleic and Arachidonic acid) with the difference on the double bonds.

2.2. Alternative dietary oils

Several studies have shown that plant oils can replace FO in salmon diets with no negative effects on fish growth (Turchini et al., 2010). Plant oils such as linseed, soybean and rapeseed oil do not negatively affect the welfare of the fish except of the FA composition, with the replacement rate varied from 50 to100% in mix plant oil (Torstensen et al., 2005). Moreover more studies for palm oil, rapeseed and linseed oils showed no changes in growth when are used for FO (Tocher et al., 2003). In Norwegian salmon farming rapeseed oil is used and tend to be highly demanding, since the price on the market is affordable for the industry.

However, some hepatic lipid studies showed increased total lipid content when sunflower and rapeseed oil was used as replacement for FO (Bell et al., 2001). Further, lipid content of liver increased with decreasing temperature, when FO was replaced with soybean oil at 5˚C, while no changes were noticed at 12˚C (Ruyter et al., 2006). Another alternative oil and quite ambitious in aquafeed industry is the poultry oil that is used in aquaculture in South America. The FA composition of this oil largely depends on the chicken diets (Higgs et al., 2006). Higgs et al. (2006) used a combination of poultry oil and canola oil in feed for Atlantic salmon in seawater, and concluded that this mixture was successful and cost efficient lipid source. New oil sources are needed for flexibility in aquafeed and aquaculture industries, therefore poultry oil seems to be quite suitable.

2.3. Health and quality

Fish health and quality is related to the dietary lipid composition, as the organs of the fish are affected by the FA composition from different diets (Hummel, 1993). The high ratio of n-6/n-3 FAs affects salmon and thus the balance between the composition of N-6 and N-3 is quite important. Inclusion of soybean oil into diets of salmon has been shown to affect negatively the macrophages of fish and serum of salmon (Gjoen et al., 2004).

Critical studies performed on rainbow trout suggest that DHA deficiency has negative effects on muscle structure and critical constituents in cell membranes (Castell et al., 1972). Changes in red and white muscle cells can be determined by heart dysfunction. Astaxanthin levels in salmonid fish can be influenced by different diets and FA composition. In addition, astaxanthin can be converted into vitamin A (Schiedt et al., 1985). Moreover, astaxanthin can be converted as an antioxidant and is quite useful for the health of the fish (Guillou et al., 1989). Thus, the levels of EPA and DHA may possibly influence both the metabolism of astaxanthin to vitamin A and the oxidative degradation.

2.4. Approaches and hypothesis

This project aim to give new fundamental understanding and knowledge on how long term feeding with low and high levels of Ω-3 FA influence the growth, colour and FA composition of muscle.

3. Material and Methods

3.1. Fish, experimental diets and sampling

Growth period 40 g to 400g:

Atlantic salmon (Salmo salar) raised in 33 indoor seawater tanks at Sunndalsøra, Norway (Nofima research unit). Tanks of 1 m^2 surface area and 0.6 m depth supplied with 15L seawater of ~3 ppt salinity and ambient temperature. Daily measures and controls about the water temperature which varied between 6.3 and 13.8 ^oC while the oxygen saturation level ensured to ≥ 85%.

The salmon was smoltified by light manipulation and transferred to seawater prior to start of the experiment. There were 70 fish per tank and 14 dietary groups. The start weight of fish was 40g.

1. 0%, 0.5%, 1%, 1.5% and 2% EPA

(0.5%, 1% and 1,5% EPA in duplicate tanks and 0% and 2% EPA in triplicate tanks)

2. 0%, 0.5%, 1%, 1.5% and 2% DHA

(0.5%, 1% and 1,5% DHA in duplicate tanks and 0% and 2% DHA in triplicate tanks)

3. 0%, 0.5%, 1%, 1.5% and 2% EPA+DHA

(0.5%, 1% and 1,5% EPA+DHA in duplicate tanks and 0% and 2% EPA+DHA in triplicate tanks)

4. Control commercial diet (triplicate tanks)

The experimental pre-diets were formulated with different levels of EPA, DHA or both as 0, 0.5, 1, 1.5, and 2 % FA composition of the dry feed weight (Table 2). Though one diet had no EPA and DHA (FO, FM free diet), negative control (0%, NC) and the commercial control diet includes 2.2 % EPA+DHA (CC).

After that point and for 6 months the fish were fed with 13 experimental diets and 1 commercial diet till they reached an average body weight of 400 g.

Growth period 400g to 1 kg:

Further, at 400 g the fish groups were transferred to larger in-door sea water tanks at the same unit at Sunndalsøra, Norway (Nofima research unit). All the 14 dietary groups were randomly redistributed to 9 new tanks, 3 tanks per dietary group (Table 1).

Table 1. Diet distribution per tanks.

14 Pre-diets	3 Diets	9 Tanks
CC, commercial	O %	3(triplicate)
0 %, NC		
0.5, 1, 1.5, 2 % EPA	1%	3(triplicate)
0.5, 1, 1.5, 2 % DHA		
0.5, 1, 1.5, 2% EPA+DHA	Control	3(triplicate)

The FAs composition and total lipid content of the new 3 diets used in the growth period from 400g to 1 Kg (0 % EPA + DHA, 1 % EPA + DHA and Control) are shown on Table 3.

Table 2. FA composition (%) and total lipid content (%) of the experimental pre-diets.

	CC	0%	0.5% EPA	1% EPA	1.5% EPA	2% EPA
14:0	0.7	0.1	0.1	0.1	0.1	0.1
16:0	3.0	3.5	3.4	3.2	3.1	3.0
18:0	0.8	0.9	0.9	0.8	0.8	0.8
20:0	0.1	0.1	0.1	0.1	0.1	0.1
22:0	0.0	0.0	0.0	0.0	0.0	0.0
24:0	0.0	0.0	0.0	0.0	0.0	0.0
Σ Saturated[1]	4.6	4.7	4.6	4.4	4.2	4.1
14:1n–5	0.0	0.0	0.0	0.0	0.0	0.0
16:1n–7	0.8	0.6	0.6	0.6	0.6	0.6
17:1n–7	0.0	0.0	0.0	0.0	0.0	0.0
18:1n–7	0.0	0.0	0.0	0.0	0.0	0.0
18:1n–9	7.5	9.4	9.1	8.7	8.3	7.9
18:1n–11	0.1	n.d.	0.0	0.1	0.1	n.d.
18:1(n–9)+(n–7)+(n–11)	7.6	9.4	9.1	8.7	8.4	7.9
20:1n–7	0.0	0.0	0.0	0.0	0.0	0.0
20:1n–9	0.4	0.1	0.1	0.1	0.1	0.1
20:1n–11	0.2	0.0	0.1	0.1	0.2	0.3
20:1(n–9)+(n–7)+(n–11)	0.7	0.2	0.2	0.3	0.3	0.4
22:1n–7	0.1	n.d.	n.d.	n.d.	n.d.	n.d.
22:1n–9	0.1	0.0	0.0	0.0	0.0	n.d.
22:1n–11	0.4	0.0	0.0	0.0	0.0	0.1
22:1(n–7)+(n–9)+(n–11)	0.5	0.0	0.0	0.0	0.1	0.1
24:1n–9	0.1	0.0	0.0	0.0	0.0	0.0
Σ Monounsaturated[2]	9.7	10.3	10.1	9.7	9.4	8.9
18:2n–6	4.6	4.8	4.7	4.5	4.3	4.2
18:3n–6	0.0	0.0	0.0	0.0	0.0	0.0
20:2n–6	0.0	0.0	0.0	0.0	0.0	0.0
20:3n–6	0.1	0.0	0.0	0.0	0.0	0.0
20:4n–6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ n–6[3]	4.9	4.9	4.8	4.6	4.5	4.3
18:3n–3	1.1	1.0	1.0	0.9	0.9	0.9
18:4n–3	0.0	0.0	0.0	0.0	0.0	0.0
20:4n–3	0.0	0.0	0.0	0.0	0.0	0.0
20:5n–3	1.1	0.0	0.5	1.0	1.5	2.0
22:5n–3	0.2	0.0	0.0	0.0	0.1	0.1
22:6n–3	1.1	0.0	0.1	0.3	0.4	0.5
Σ n–3[4]	3.6	1.1	1.7	2.4	3.0	3.6
EPA + DHA	2.2	0.0	0.6	1.2	1.8	2.5
16:3n–4	0.1	0.0	0.0	0.0	0.1	0.1
Σ Polyunsaturated[5]	8.7	6.0	6.5	7.0	7.5	8.0
Total lipid	25.8	23.4	23.7	23.8	23.7	23.9

[1]Includes 15:0, 17:0.
[2]Includes 14:1n–5, 17:1n–7.
[3]Includes 16:2n–6, 20:4n–6, 22:2n–6.
[4]Includes 20:3n–3.
[5]Includes 16:2n–6, 16:3n–4, 20:3n–3, 20:4n–6, 22:2n–6.

Table 1. (Continued) FA composition (%) and total lipid content (%) of the experimental pre-diets.

	0.5% DHA	1% DHA	1.5% DHA	2% DHA	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA
14:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:0	3.4	3.3	3.2	3.1	3.3	3.2	3.3	3.1
18:0	0.9	0.9	0.9	0.9	0.9	0.8	0.9	0.8
20:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24:0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1
Σ Saturated[1]	4.7	4.5	4.5	4.4	4.5	4.4	4.5	4.3
14:1n–5	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
16:1n–7	0.6	0.6	0.6	0.5	0.6	0.6	0.6	0.6
17:1n–7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:1n–7	0.0	0.0	0.0	0.0	0	0	0	0
18:1n–9	9.2	8.7	8.4	8.1	8.9	8.7	8.7	8.2
18:1n–11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1
18:1(n–9)+(n–7)+(n–11)	9.2	8.7	8.4	8.1	8.9	8.7	8.7	8.3
20:1n–7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:1n–9	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
20:1n–11	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
20:1(n–9)+(n–7)+(n–11)	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
22:1n–7	n.d.	n.d.	0.0	0.0	n.d.	n.d.	0.0	0.0
22:1n–9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:1n–11	0.0	0.0	0.0	0.1	n.d.	0.0	0.0	0.0
22:1(n–7)+(n–9)+(n–11)	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1
24:1n–9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ Monounsaturated[2]	10.1	9.7	9.3	9.0	9.8	9.6	9.8	9.4
18:2n–6	4.7	4.5	4.4	4.2	4.6	4.5	4.5	4.3
18:3n–6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:2n–6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:3n–6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:4n–6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ n–6[3]	4.8	4.6	4.5	4.3	4.7	4.6	4.7	4.4
18:3n–3	1.0	0.9	0.9	0.9	1.0	0.9	0.9	0.9
18:4n–3	0.0	0.0	0.0	n.d.	n.d.	n.d.	n.d.	n.d.
20:4n–3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:5n–3	0.1	0.2	0.2	0.3	0.3	0.6	0.9	1.2
22:5n–3	0.0	0.1	0.1	0.2	0.0	0.1	0.1	0.1
22:6n–3	0.5	0.9	1.4	1.9	0.3	0.5	0.9	1.2
Σ n–3[4]	1.7	2.2	2.7	3.5	1.7	2.2	3.0	3.6
EPA + DHA	0.6	1.1	1.6	2.3	0.6	1.1	1.8	2.4
16:3n–4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ Polyunsaturated[5]	6.5	6.8	7.2	7.8	6.4	6.9	7.6	8.0
Total lipid	23.8	23.6	23.6	23.9	23.3	23.5	24.7	24.4

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

[2]Includes 14:1n–5, 17:1n–7.

[3]Includes 16:2n–6, 20:4n–6, 22:2n–6.

[4]Includes 20:3n–3.

[5]Includes 16:2n–6, 16:3n–4, 20:3n–3, 20:4n–6, 22:2n–6.

Table 3. Fatty acid composition (%) and total lipid content (%) of the experimental main diets.

	Control	1%	Low (0%)
Fat %	28.81	30.66	31.07
14:0	2.2	2	0.9
16:0	9.1	15.2	14.2
18:0	2	4	4
20:0	0.5	0.2	0.3
22:0	0	0.1	0.2
24:0	0.1	0.1	0.1
Σ Saturated[1]	14.3	22.2	19.8
16:1 n-7	2.7	3.8	2.8
18:1 n-9	42.5	31.7	37.7
18:1 n-7	2.9	1.9	1.7
20:1 n-9	1.7	0.9	0.6
22:1 n-11	1	0.7	0.1
24:1 n-9	0.3	0.1	0.1
Σ Monounsaturated[2]	52.5	40.5	43.5
18:2 n-6	16.7	18	21.4
18:3 n-3	7	12.6	13.1
20:2 n-6	0.1	0.2	0.1
20:3 n-6	0.3	0.1	0.1
20:4 n-6	0.2	0.3	0.1
20:5 n-3	3.5	2.2	0.2
22:5 n-3	0.4	0.3	0.1
22:6 n-3	3	1.9	0.2
Σ Polyunsaturated[3]	31.8	36.1	35.7
Σ EPA/DHA	6.6	4.1	0.4
Σ N-3	14.5	17.3	13.7
Σ N-6	17.7	18.9	21.8
Σ N-0	14.2	22.9	20.7
Sum	99.6	99.6	99.6

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

Includes[2]: 14:1 n-5, 15:1, 16:1 n-5, 17:1 n-7, 20:1 n-11, 20:1 n-7, 22:1 n-7, 22:1 n-9.

Includes[3]: 16:2 n-3, 16:2 n-4, 16:2 n-6, 18:3 n-3, 18:3 n-4, 18:4 n-3, 20:3 n-3, 20:4 n-3.

Fish feeds were produced by BioMar AS (Trondheim, Norway) and given by automatic feeders to the fish. A mixture of poultry oil, rapeseed oil and linseseed used as an oil source for the 0% and 1% experimental diets.

When the fish reached the average body weight of 1 kg, aprox. after 9 months, the sampling started. From 9 tanks with 3 different diets 28 fish were collected from each tank. Therefore 2 fish of 28 derived from each 14 pre-diets. The fish were anesthesized in MS222 prior to weighing and killing by cutting off the head and different tissues were collected from the fish (liver, brain, heart, muscle and intestine). However, this master thesis only focus on the fillet, muscle tissue of the fish. The right muscle fillet with the skin-on was packed individually in labeled plastic bags and stored at -40 ^{o}C until analysis of colour and lipid composition.

Prior to this master thesis, fish had been sampled at a body weight of 200 g and 400 g, but analyses of these lifestages are not part of this master thesis which only focus on the influence of the pre-diets and the main diets on growth and fillet composition at 1 kg.

3.2. Colour measurements

Visual colour evaluation:

The colour of fillets of 254(28 fish per tank (2 fish per 14 pre-diets) and 9 tanks) salmon was evaluated visually by the use of Roche SalmoColour Fan™ (DSM Nutritional Products Ltd, Basel, Switzerland). The SalmoFan card based on the colour of salmonid flesh pigmented with astaxanthin and used for colour quality inspection in salmonid fish industry. The colour gradient scale numbered from 20 to 34 (Figure 3). The manual inspections were done in artificial light with the card placed on the same area of the fillet as the Minolta readings. The measurements were done on epaxial muscle anterior to the dorsal fin.

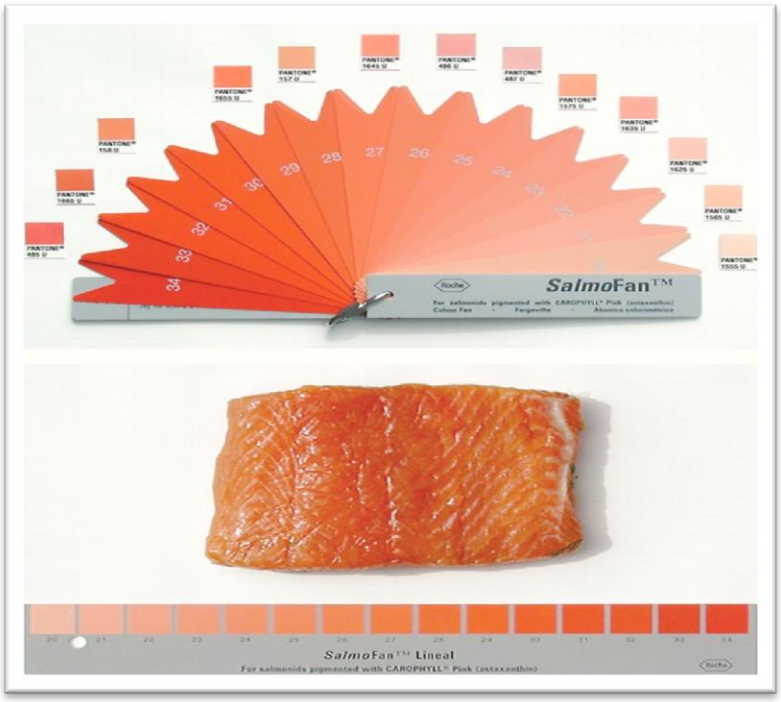

Figure 3. Roche SalmoColour Fan with pigmentation gradient from 20 to 34.

Instrumental colour evaluation:

The colour of the fillets was measured instrumentally using a Minolta Chromameter CR-200/CR231 (Minolta, Osaka, Japan). L^*, a^*, and b^* values (CIE 1976) were measured by reflectance of light from the flesh analogous to a calibration plate. The CIELAB (L^*, a^*, b^*) parameters specified closed to human vision as lightness (L^*), red/green chromaticity (a^*) and yellow/blue chromaticity (b^*) specified by the International Commission on Illumination (CIE). The instrument measures in an area of 8 mm in diameter using a white calibration plate (L^* =97.63, a^*=-0.63, b^*=2.40).

In the measurement of salmon muscle fillets the values L^* matches to lightness, a^* represents to redness and b^* to yellowness (Christiansen et. al., 2000). The quantitative Chroma (C^*_{ab}) measured by LAB values (Hunt, 1977). The expression between a^* and b^* values provide us the intensity of the colour C* and expressed as $C = \sqrt{a^2 + b^2}$. The higher Chroma C* values correspond to higher intensity of colour.

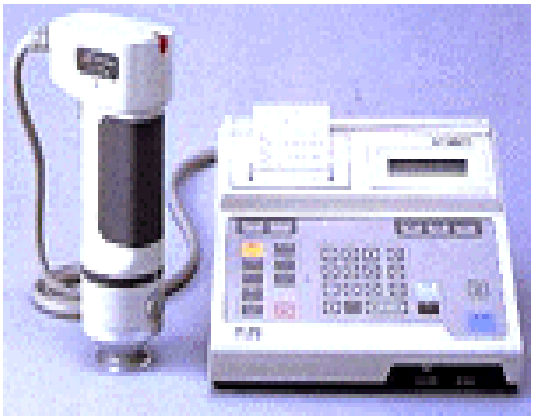

Figure 4. Minolta Chroma Meter CR-200

3.3. Fillet homogenisation

The white muscle tissue is needed from fish fillets for the lipid extraction. Prior to homogenization the fillets were partially thawed for 10 minutes to allow the skin to be peeled off easily. Any adipose tissue directly under the skin was removed, as well as the red muscle tissue along the lateral line. After that, the fillets were sliced into smaller pieces and placed to 1 L kitchen blender for homogenizing. Thereafter, 100 gr of muscle homogenate per individual was taken for used in separately experiment, 2 fish that had been fed the same pre-diet from each tank were pooled to constitute 1 sample, there were 3 tanks per diet which make triplicate samples per pre-diet (n=3). The muscle homogenate was collected in plastic bags and stored at -40 °C prior to analyses.

3.4. Lipid extraction

Lipids were extracted from the homogenized white muscle tissue according to the method described by Folch et al. (1957). The samples were analyzed in duplicates for measuring the total lipid content. For this experiment, 8 pre-diets selected and analyzed with the following methods (CC, 0%, 1%, 2% EPA, 1%, 2% DHA and 1%, 2% EPA+DHA). According to the Folch principle, the final ratio between chloroform:methanol:water needs to be equal to 8:4:3, the method was adapted to the specific tissue volume as follows. Approximately 2 to 2.5 g sample (homogenate tissue) was weighed and transferred into an Erlenmeyer flask. The next steps were performed in the hood. 6 mL 0.9% NaCl and 50 mL chloroform:methanol (2:1) was added to the Erlenmeyer flask with the muscle homogenate. The chloroform:methanol solution contained the antioxidant BHT 0,7 mg/l Butylated hydroxytoluene (2,6-Di-t-butyl-p-cresol), which is a lipophilic antioxidant that protects the lipids against oxidation. The lipids were extracted by homogenization of the muscle fillet with the chloroform:methanol solution for 60 seconds with a homogenizer Heidolph DIAX 900 (Heidolph Elektro, Kelheim, Germany) and then added 6 mL 0.9% NaCl and further mixed for approx. 5 sec more.

Thereafter, the samples were filtered through a cotton filter into a cylinder in order to remove the protein aggregates from the sample.

The samples were left for 1 hour in order to achieve separation of the phases of water soluble components (polar fraction) and almost all lipids (unpolar organic solvent fraction) (with a 3:48:47 and 86:14:1 chloroform:methanol:water ratio respectively). The lipophilic (lower) phase contained the lipid extract, 20 mL of approx. 40 mL were pipetted out and transferred to new vials and further used for analyses of total fat content (**section 3.5**) and total FA composition (**section 3.6**).

3.5. Calculation of total lipid content

20 ml of the total lipid chloroform extracts were transferred to pre-weighed beakers and stayed overnight to evaporate. The next day placed in the oven for 60 min at 102 °C. When all the organic solvent was evaporated after approximately 60 minutes, the beakers were let to cool down at RT. Then the beakers with the extracted fat were weighed. The weight of the lipid in each beaker was found by subtraction of the weight of the empty beaker. The fat percentages of the fillets were calculated as gram lipid per 100 gram fillets (W_{lipid}, g) according to the following formula:

$$Total\ lipid\ content\ = \frac{W_{lipid} * 100}{\frac{(W_S * V_{lipid})}{V_{solvent}}}$$

The W_S is the weight (g) of the sample homogenates, V_{lipid} the volume of the lipid extract used (20 mL) and $V_{solvent}$ the total volume of the lipid extract (37.5 mL).

A mean of the lipid content in each sample was determined in duplicate samples, calculated according to the formula above and presented gram lipid per 100 gram fillet (%). The samples were taken from tank groups with the same experimental diet.

3.6. Total FA composition analysis

The total FA compositions of the different fillets were analyzed by gas-chromatography (GC). The samples were prepared from 1 mL aliquots of the chloroform lipid extracts as follows. First of all, chloroform phases were evaporated at 60 °C under anoxic circumstances (nitrogen overflow; to prevent lipid oxidation). The total lipids were hydrolyzed to free FAs and further methylated by the method described by Mason and Waller (1964). Consequently, FAs were trans-esterified by heating and converted into FA methyl esters (FAMEs). After the FAs were extracted by addition of 2 mL 100 % (v/v) benzene.

Secondly an internal standard 0,02 µL tricosylic acid (C23:0) was added to each sample, in order to calculate the absolute amount of FA per gram of tissue (formulas shown below). 2 mL 3 M methanolic-HCl was added as a catalyst for the methylation reaction, and 0.2 mL 98 % (v/v) 2,2-dimethoxypropane as a water purifier to improve FAME recovery. Thereafter, after mixing well the tubes, leave at room temperature until the next day when the reaction was stopped by adding 2 mL 100 % (v/v) hexane with 3.18 µM BHT and 3 mL 6% $NaHCO_3$ for neutralization. After one hour phase separation in room temperature, the upper unpolar lipid phase was collected and evaporated (likewise above). The FAME samples were dissolved in 100 µL 100 % (v/v) hexane and placed into vials for GC analysis.

The FAMEs were separated using a Hewlett Packard (HP) gas-chromatograph (GC), model 6890 series (HP, Wilmington, US).

From each individual sample, 1 µL was injected by a split injector into a SGE BPX70 capillary column (SGE Analytical Science, Milton Keynes, UK) of 60 m length, with an internal diameter of 0.25 mm and a 0.25 µm thick covering film of BPX 70-0.25 m as the stationary phase.

The GC's mobile phase is helium gas and has additional run a flame ionization detector (FID), which monitored the retention times on the outlet stream.

The temperature program started on an initial temperature for the column of 50 °C for 1.2 min. Then first increased to 170 °C with a rate of 4 °C/min, a further increase to 200 °C with a rate of 0.5 °C/min, and finally to 300 °C with a rate of 10 °C per min. The chromatograph peaks area calculated with the use of HP ChemStation software. The quantification of each FA certified by the respectively muscle was calculated by the following formula:

$$Total\ FA\ content\ = \frac{W_{23:0} * (A_{sum} - A_{23:0})}{A_{23:0}}$$

whereby, $W_{23:0}$ is the amount of the standard (g), A_{sum} the sum of peak areas of all identified FAs (%), also $A_{23:0}$ the peak area report to the standard (%) and A_{FA} is the peak area (%) of the FA of interest (formulas below). The formulas below used for each FA content in mg per g sample and in percentage (%) respectively:

$$FA\ content\ = \frac{\text{total FA content} * A_{FA} * 1000}{A_{sum} - A_{23:0}}$$

$$FA\ \%\ = \frac{A_{FA} * A_{sum}}{A_{sum} - A_{23:0}}$$

3.7. Statistical analysis

All data calculated and diagrams were created using the Microsoft Excel program. Statistics analyzed to two-way analysis of variance (ANOVA) followed by Tukey's multiple range test and the differences are presented as least square means correlation coefficient using JMP Pro 11 (SAS Institute Inc., Cary, NC, USA). The significance level was set at 5% ($P < 0.05$).

4. Results

4.1. Growth

At the final sampling, the average body weight was 1310 kg. Control dietary group gave us the averages of weight 1454 kg, for 0% dietary group an average body weight of 1205 kg, while for 1% dietary group an average value of 1269 kg body weight (Table 4).

Table 4. Body weight (gram) of Atlantic salmon (Salmo salar) in 3 dietary groups (0%, 1%, Control) with pre-diets. (Mean ± SEM, n=6)

Pre-Diets	Control	0%	1%
Control	1410± 61.9	1196± 114.0	1171± 45.6
0	1383± 127.4	1132± 89.2	1204± 67.6
0,5% DHA	1348± 163.5	1087± 91.1	1278± 120.9
0,5% EPA	1390± 86.7	1267± 125.7	1170± 107.9
0,5% EPA+DHA	1546± 65.9	1240± 49.7	1338± 108.9
1% DHA	1258± 86.9	1242± 102.9	1245± 57.8
1% EPA	1505± 94.1	1128± 73.4	1474± 117.5
1% EPA+DHA	1620± 139.3	1160± 117.2	1372± 143.1
1,5% DHA	1358± 109.4	1280± 35.0	1138± 76.3
1,5% EPA	1505± 98.6	1495± 76.7	1216± 122.9
1,5% EPA+DHA	1372± 80.8	1152± 54.6	1254± 100.0
2% DHA	1641± 142.4	1181± 58.2	1440± 78.0
2% EPA	1633± 175.5	1095± 74.7	1243± 86.2
2% EPA+DHA	1390± 112.9	1215± 114.5	1216± 98.4
Mean (kg)	1454± 31.1	1205± 24.3	1269± 26.5

4.2 Muscle colour

The result of the photometer analysis given the values as L.A.B., shows some significance differences between the 3 dietary groups (Figure 5). The statistic analysis gave us a P value < 0.001 in correlation of the 3 dietary groups (0%, 1% and Control) for the Chroma and a P <0.0001 for SalmoFan measurements which showing significant differences in 0% diet with 1% and Control diets. The mean values of Chroma factor are 15.0, 16.3 and 17.5, which present the diets 0%, 1% and Control respectively. As we can see the highest Chroma in salmon fillets correspond with the Control and 1% dietary groups and the lowest in the 0% group.

Visual colour scores by SalmoFan ranged from 21 to 28 with an average of 24.3. The highest colour scores with SalmoFan was found in the fish that had been fed the Control diet while the lowest colour score was found in the 0% dietary group (Figure 6). The mean scores in muscle of SalmoFan per diet are 23.7, 24.4, and 24.8 for 0%, 1% and Control diets respectively.

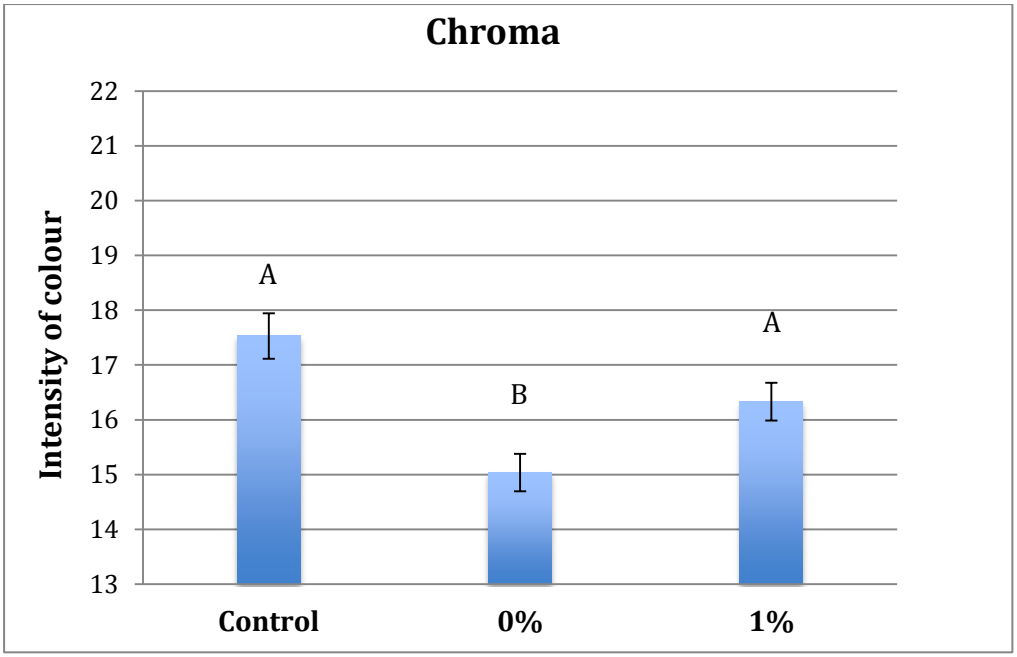

Figure 5. Colour of the 3 dietary groups (Control, 0% and 1%) in muscle of salmon fillets, measured with Chroma (C^*_{ab}). (Mean ± SEM, n=84, levels not connected by same letter are significantly different)

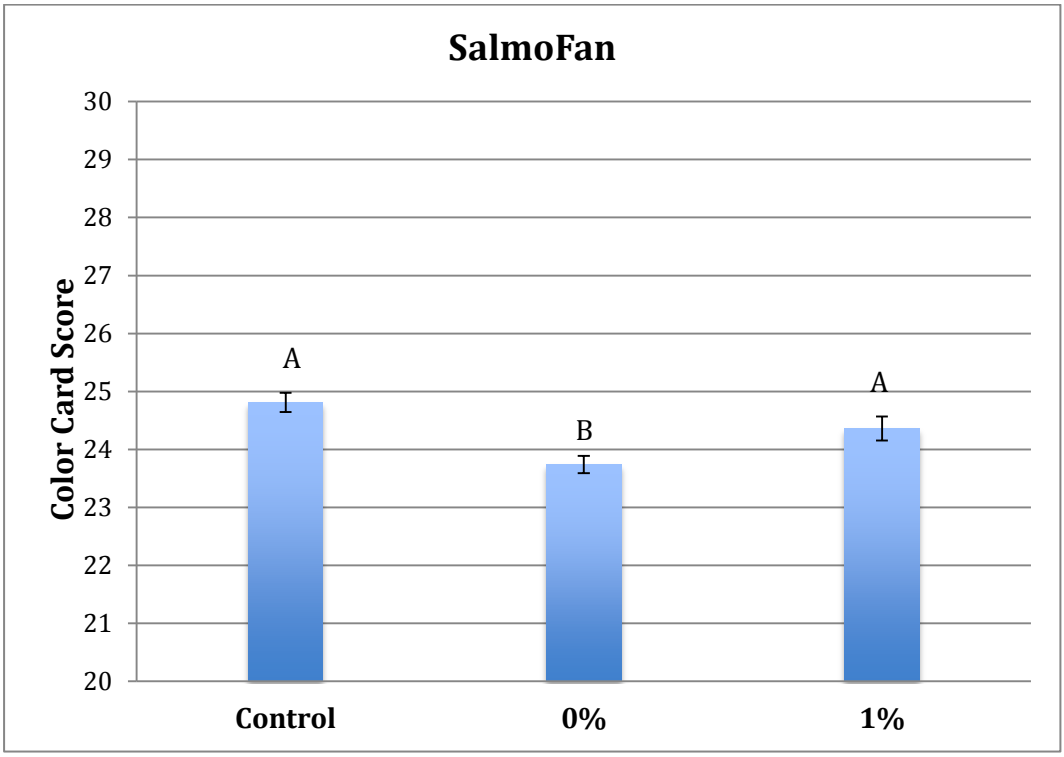

Figure 6. SalmoFan Colour Card Score of the 3 dietary groups (Control, 0% and 1%) in muscle of salmon fillets. (Mean ± SEM, n=84, levels not connected by same letter are significantly different)

It is noticeable that the fish fed the highest dietary EPA and DHA level had also higher colour score with the SalmoFan Card in agreement with the Chroma measurement. It is known that the colour may vary with the size of the fish, therefore any correlation with the growth was also tested (Figure 7, 8, 9).

The correlations between body weight, Chroma and SalmoFan factors for the Control diet, 0% diet and 1% diet are shown in Figure 7, 8 and 9 respectively. Both the average body weight and the colour varied significantly between the different dietary treatments. Figures 7, 8 and 9 show a linear correlation between the Salmofan measurement and the size of the salmon within each dietary groups, showing that the salmon gets more color the larger it is.

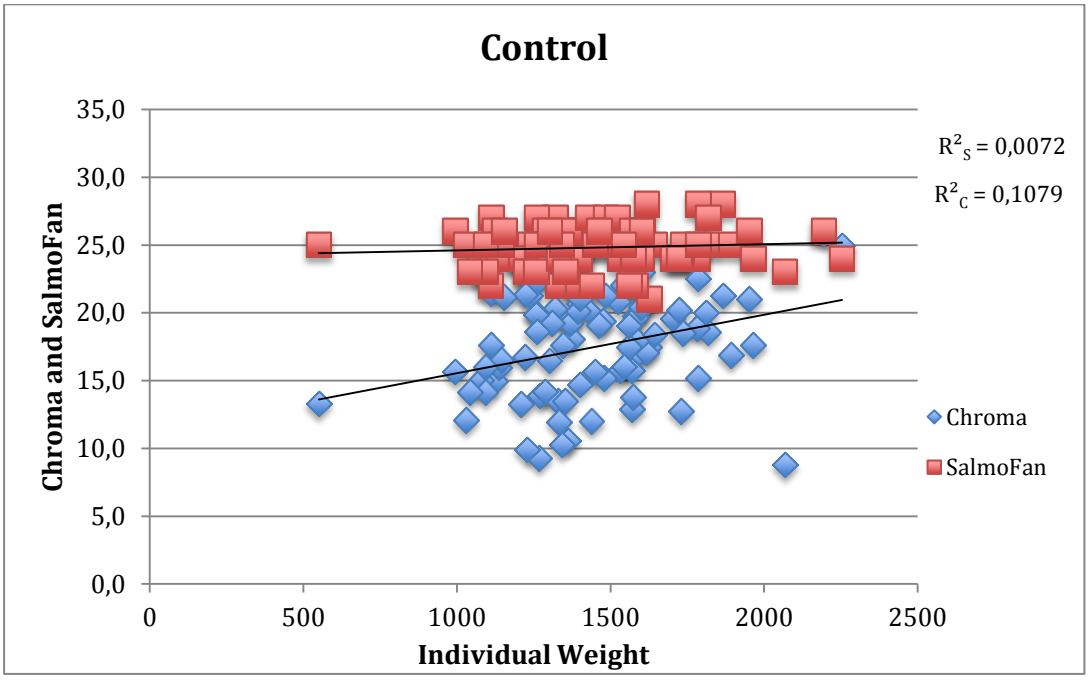

Figure 7. Correlation between Chroma and SalmoFan measured colour in fillet (average values) with the body weight (g) of a salmon fillet in Control Diet. (n=84).

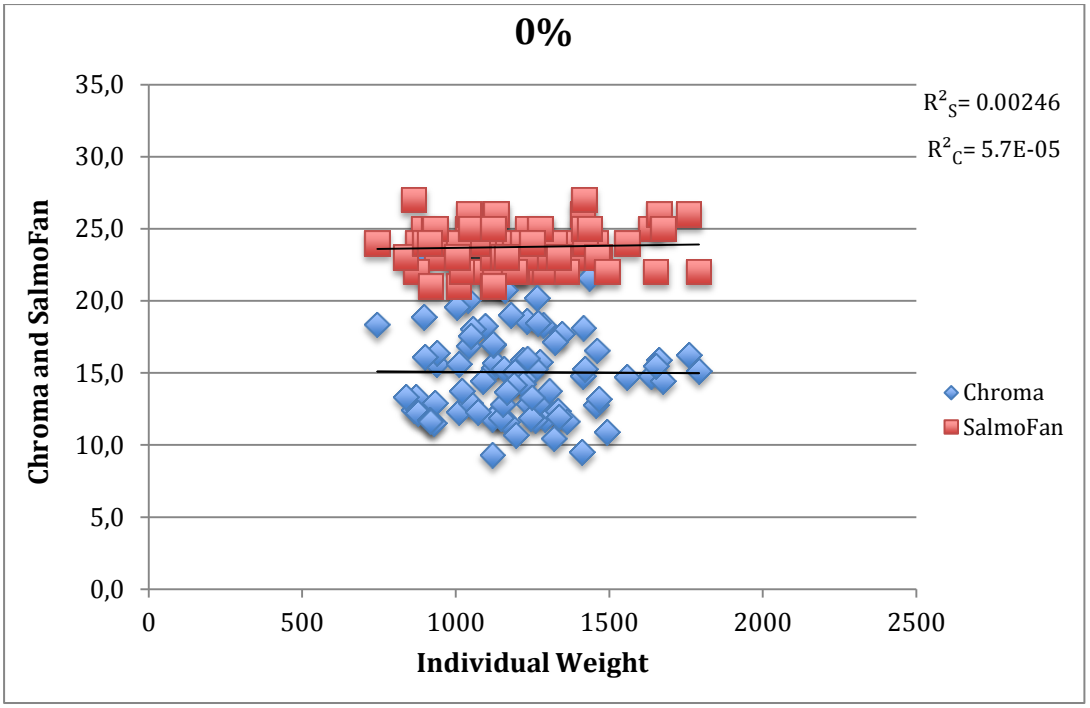

Figure 8. Correlation between Chroma and SalmoFan measured colour in fillet (average values) with the body weight (g) of a salmon fillet in 0% Diet. (n=84).

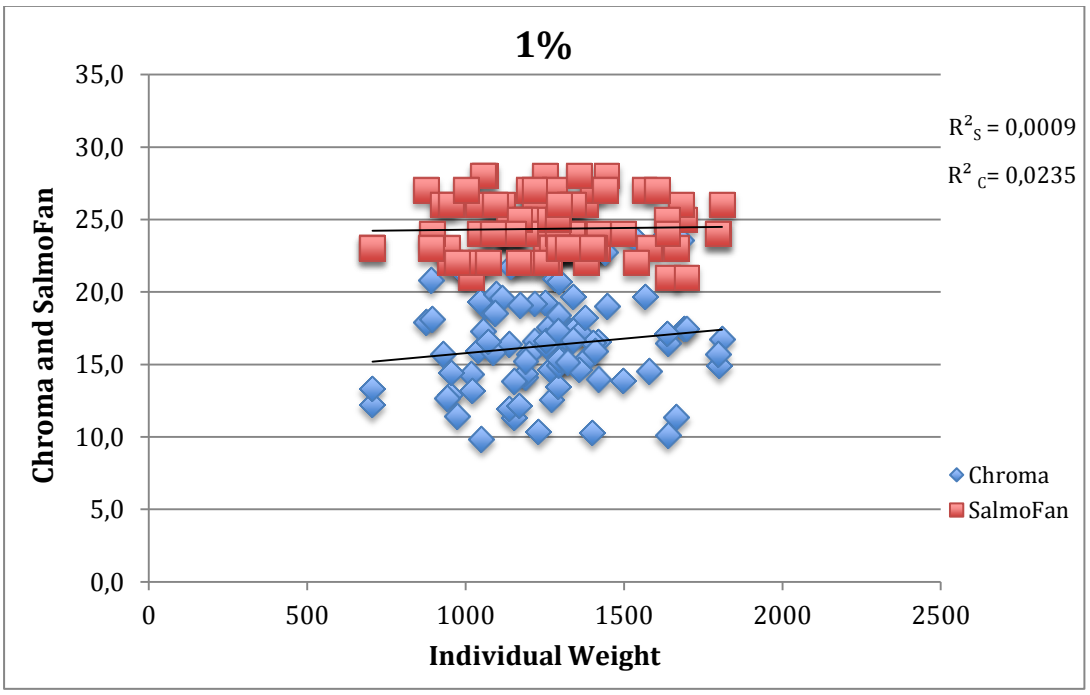

Figure 9. Correlation between Chroma and SalmoFan measured colour in fillet (average values) with the body weight (g) of a salmon fillet in 1% Diet. (n=84).

4.3. Total lipid content

Muscle lipid contents varied from 8.29 % (0%), 9.50 % (1%) to 10.64 % (CC) between the 3 main dietary groups at the final sampling when the fish had reached an average weight of 1,3 kg with P=0.0001. There was a lower fat percentage in the 0% dietary group than in the control and 1% groups. From 14 pre-diets 8 selected for further analysis, however the pre-diet period (from 40g to 400g fish size) did not significantly influence the fat content in the muscle fillet at the final sampling (P= 0.7417).

Table 5. Muscle lipid content (%) of A. salmon in the 3 main dietary groups (CC, 0%, 1%) with 8 pre-diets. (Mean ± SEM, n=2)

Pre-Diets	Control (A)	0 % (C)	1 % (B)
Control	10.3±0.56	8.9±0.74	9.6±0.46
0%	10.4±0.06	7.3±0.42	10.3±1.24
1% EPA	10.3±0.73	7.8±0.27	9.8±0.28
2% EPA	10.0±1.07	7.8±0.51	8.9±0.66
1% DHA	11.6±1.00	8.5±0.65	8.8±0.55
2% DHA	11.0±0.98	8.7±0.22	10.4±0.84
1% EPA+DHA	11.7±0.31	7.7±0.95	9.1±0.36
2% EPA+DHA	9.8±0.60	9.5±0.72	9.1±0.86
Total lipids	10.6±0.40	8.3±0.43	9.5±0.36

4.4. Total FA composition

The total FA composition of the muscle of A. salmon fillets is shown in the tables below (see Table 6, 7, 8) which includes 8 pre-diets from 40 g till 400 g of the fish and 3 main dietary groups of 0%, 1% and Control (CC) from 400 g to 1 kg. The analysis of FAs shows the influence of the pre-diets in the main 3 diets that the fish was fed. There were significant differences related to pre-diet and diets in EPA (20:5 n-3) and DHA (22:6 n-3) with P =0.0001 and P= 0.0062 respectively. Moreover, more significant differences were identified on the Σ EPA+DHA with P=0.0029, on Σ N-3 with a value of P=0.0017. Similarly, there were difference on the Σ N-6 occurred with a significance value of P= 0.0160.

There were relatively few differences in FA composition between the different pre-dietary groups (Table 6). Saturated FAs constituted 15-20% of total FAs, the Monounsaturated approximately 43 of total FAs and the PUFAs 33% of total FAs. ALA (18:3 n-3) and 18:1 n-9 had similar values in related all the pre-diets.

However, Figure 10 shows that the pre-diets in early life stages significantly affected the EPA and DHA level in this 0% group at 1 Kg. N-3 FAs had also significantly affected while N-6 showed not so big differences.

Table 6. Fatty acid compositions (%) of muscle from Atlantic salmon (Salmon salar L.) fed with experimental 8 pre-diets until 400g and then with 0% diet till reach 1 kg. (Mean ± SEM, n=3)

Fatty acids (% of total)	Low (0%)															
	Control		0%		1% DHA		2% DHA		1% EPA		2% EPA		1% EPA+DHA		2% EPA+DHA	
14:0	0.9	±0.07	0.8	±0.01	0.8	±0.01	0.8	±0.004	0.8	±0.01	0.8	±0.003	0.8	±0.02	0.8	±0.01
16:0	8.7	±4.33	13.3	±0.09	13.5	±0.04	8.7	±4.36	13.4	±0.05	13.3	±0.09	8.7	±4.37	13.6	±0.12
18:0	4.5	±0.08	4.5	±0.06	4.4	±0.02	4.4	±0.05	4.4	±0.05	4.3	±0.07	4.3	±0.09	4.5	±0.04
Σ Saturated[1]	15.2	±4.37	19.9	±0.06	19.9	±0.09	15.0	±4.27	19.8	±0.12	19.6	±0.14	15.0	±4.38	20.0	±0.11
16:1 n-7	2.3	±0.03	2.2	±0.01	2.2	±0.03	2.3	±0.02	2.2	±0.01	2.2	±0.01	2.2	±0.03	2.3	±0.02
18:1 n-9	37.5	±0.27	36.9	±0.67	37.5	±0.28	37.4	±0.34	37.2	±0.18	36.9	±0.08	37.4	±0.28	37.5	±0.17
20:1 n-9	1.4	±0.07	1.3	±0.02	1.3	±0.01	1.4	±0.03	1.3	±0.04	1.3	±0.03	1.3	±0.02	1.3	±0.03
20:1 n-11	1.8	±0.03	1.8	±0.05	1.6	±0.06	1.8	±0.05	1.7	±0.11	1.8	±0.04	1.6	±0.05	1.6	±0.06
22:1 n-9	0.2	±0.02	0.2	±0.01	0.2	±0.00	0.2	±0.01	0.2	±0.001	0.2	±0.01	0.2	±0.00	0.2	±0.01
Σ Monounsaturated[2]	43.8	±0.32	43.3	±0.36	43.5	±0.34	43.6	±0.31	43.1	±0.23	42.9	±0.09	43.2	±0.28	43.6	±0.13
18:2 n-6	17.5	±0.18	17.6	±0.17	18.0	±0.07	17.9	±0.11	17.9	±0.04	17.9	±0.02	18.3	±0.20	17.8	±0.09
18:3 n-3	7.7	±0.16	7.6	±0.09	7.7	±0.08	7.7	±0.10	7.5	±0.12	7.7	±0.08	7.9	±0.17	7.7	±0.08
20:4 n-6	1.1	±0.03	1.3	±0.08	1.1	±0.01	1.1	±0.02	1.2	±0.05	1.1	±0.04	1.1	±0.03	1.1	±0.07
20:5 n-3	1.2	±0.03	1.1	±0.03	1.0	±0.04	1.0	±0.03	1.4	±0.07	1.7	±0.09	1.2	±0.10	1.2	±0.01
22:6 n-3	2.5	±0.06	2.0	±0.14	2.7	±0.20	2.8	±0.19	2.6	±0.12	2.6	±0.12	2.7	±0.24	2.6	±0.10
Σ Polyunsaturated[3]	32.5	±0.31	32.0	±0.31	32.8	±0.19	32.9	±0.16	33.0	±0.17	33.4	±0.14	33.5	±0.06	32.6	±0.09
n-3	12.0	±0.14	11.2	±0.24	11.8	±0.25	12.0	±0.14	12.1	±0.21	12.6	±0.17	12.3	±0.21	12.0	±0.07
n-6	20.5	±0.20	20.9	±0.16	21.0	±0.08	20.9	±0.11	20.9	±0.04	20.9	±0.04	21.2	±0.18	20.7	±0.09
20:5 n-3 + 22:6 n-3	3.7	±0.09	3.1	±0.16	3.7	±0.20	3.9	±0.22	4.1	±0.11	4.3	±0.20	3.9	±0.33	3.8	±0.10
Sum %	96.4	±0.05	95.8	±0.35	96.5	±0.13	96.5	±0.06	96.4	±0.12	96.3	±0.16	96.6	±0.12	96.6	±0.19

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

Includes[2]: 17:1 n-7, 18:1 n-9T, 19:1, 22:1 n-7, 24:1 n-9.

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

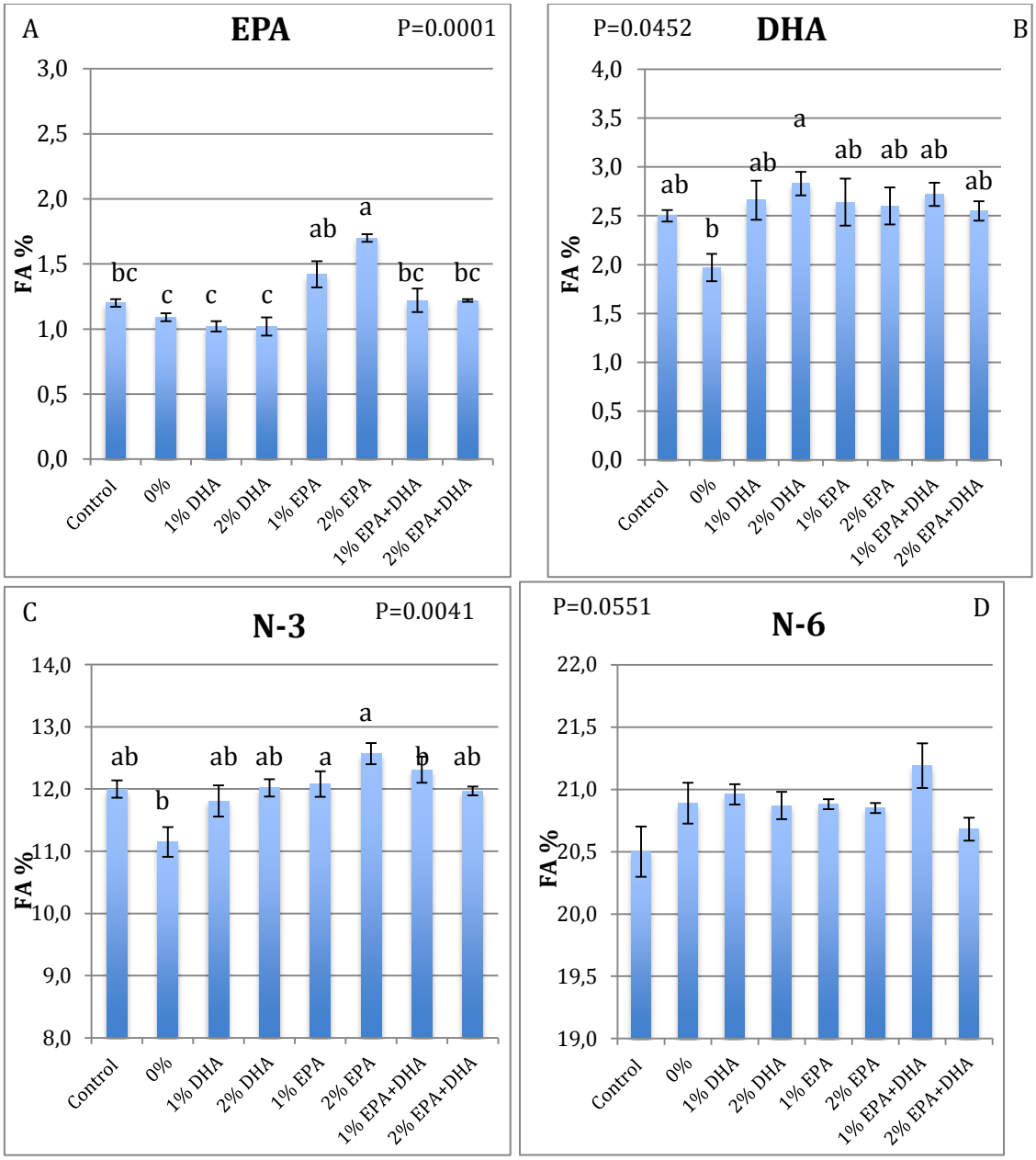

Figure 10. FA compositions of pre-diets (%) of EPA (A), DHA (B), N-3 (C) and N-6 (D) of muscle Atlantic salmon 1kg in the 0% dietary group. (Mean ± SEM, n=3, levels not connected by same letter are significantly different)

The overview of the FA composition in the main dietary group 1%, shown in Table 7, exhibited a slight increase in values of FA in comparison with 0% dietary group except of 18:1 n-9 acid, the Σ N-6 and Σ N-9. There were significance differences of Σ N-9 within the 3 main dietary groups at the P=0.0001 significance level.

There were relatively few differences in FA composition between the different pre-dietary groups (Table 7). Saturated FAs constituted approximately 20% of total FAs, the Monounsaturated approximately 41 of total FAs and the PUFAs 32% of total FAs. ALA (18:3 n-3) and 18:1 n-9 had similar values in related all the pre-diets.

However, Figure 11 shows that the pre-diets in early life stages significantly affected only the N-3 FAs level in 1% dietary group at 1 Kg. EPA, DHA and N-6 didn't affected significally.

Subsequently, the Control dietary group observed another slight increase of EPA, DHA and of the Σ N-9 acids while on the other hand the Σ N-6, Σ N-0 and ALA had a negative influence (Table 8). Saturated FAs constituted approximately 17% of total FAs, the Monounsaturated 50 of total FAs and the PUFAs 24-26% of total FAs. ALA (18:3 n-3) and 18:1 n-9 had similar values in related all the pre-diets. Control pre-diets had high levels due to high FA concentration composition in the diets. DHA pre-diets had lower values in comparison with the EPA pre-diets except only in DHA acid and in some Monounsaturated acids.

This group seem to be less affected by the pre-diets than the 0% group, as example no significant effect on DHA, N-3 and N-6 showed (Figure 12).

Table 7. Fatty acid compositions (%) of muscle from Atlantic salmon (Salmon salar L.) fed with experimental 8 pre-diets until 400g and then with 1% diet till reach 1 kg. (Mean ± SEM, n=3)

Fatty acids (% of total)	1%															
	Control		0%		1% DHA		2% DHA		1% EPA		2% EPA		1% EPA+DHA		2% EPA+DHA	
14:0	2.1	±0.03	1.9	±0.06	1.9	±0.04	1.9	±0.03	1.9	±0.02	1.9	±0.07	1.9	±0.04	1.9	±0.02
16:0	13.1	±0.20	13.2	±0.23	13.4	±0.20	13.0	±0.20	13.3	±0.04	13.4	±0.03	13.4	±0.16	13.1	±0.10
18:0	4.0	±0.04	4.0	±0.03	4.1	±0.09	4.0	±0.12	4.0	±0.03	4.1	±0.03	4.1	±0.06	3.9	±0.03
Σ Saturated[1]	20.2	±0.22	20.3	±0.28	20.4	±0.13	19.8	±0.28	20.2	±0.01	20.3	±0.05	20.4	±0.23	20.0	±0.13
16:1 n-7	3.6	±0.07	3.5	±0.13	3.3	±0.08	3.5	±0.16	3.4	±0.07	3.4	±0.09	3.4	±0.07	3.4	±0.10
18:1 n-9	31.5	±0.97	32.1	±0.78	32.0	±0.86	32.2	±0.76	32.4	±0.87	31.9	±1.13	32.1	±0.89	32.0	±1.10
20:1 n-9	3.8	±0.15	2.4	±1.09	3.4	±0.19	3.6	±0.12	3.4	±0.09	3.2	±0.24	3.5	±0.10	3.4	±0.09
20:1 n-11	1.3	±0.11	1.2	±0.03	1.1	±0.10	1.1	±0.04	1.2	±0.02	1.1	±0.03	1.2	±0.02	1.3	±0.01
22:1 n-9	0.5	±0.02	0.5	±0.02	0.5	±0.03	0.5	±0.02	0.5	±0.02	0.5	±0.05	0.5	±0.02	0.5	±0.03
Σ Monounsaturated[2]	41.5	±0.96	40.5	±1.36	41.1	±0.85	41.6	±1.01	41.6	±0.85	40.9	±0.95	41.5	±0.81	41.3	±1.13
18:2 n-6	14.0	±0.19	14.4	±0.25	14.7	±0.43	14.7	±0.05	14.9	±0.16	14.8	±0.27	14.6	±0.30	14.7	±0.08
18:3 n-3	8.6	±0.12	8.4	±0.001	8.6	±0.28	8.6	±0.02	8.5	±0.10	8.6	±0.11	8.3	±0.18	8.5	±0.01
20:4 n-6	0.6	±0.02	0.7	±0.07	0.6	±0.05	0.6	±0.01	0.6	±0.01	0.6	±0.04	0.7	±0.02	0.7	±0.03
20:5 n-3	1.9	±0.04	1.8	±0.05	1.2	±0.60	1.6	±0.02	1.9	±0.04	2.4	±0.04	1.8	±0.06	1.9	±0.12
22:6 n-3	4.0	±0.19	3.7	±0.32	4.0	±0.07	4.2	±0.12	3.5	±0.03	3.9	±0.13	3.9	±0.05	4.1	±0.34
Σ Polyunsaturated[3]	31.4	±0.49	31.3	±0.53	31.8	±0.51	31.7	±0.07	31.7	±0.23	32.4	±0.38	31.4	±0.30	32.2	±0.36
n-3	15.5	±0.37	14.9	±0.34	14.6	±0.36	14.6	±0.49	14.8	±0.12	15.7	±0.31	13.6	±0.50	15.4	±0.47
n-6	16.0	±0.16	16.4	±0.17	16.6	±0.35	16.6	±0.01	16.9	±0.15	16.7	±0.27	16.6	±0.23	16.8	±0.13
20:5 n-3 + 22:6 n-3	5.9	±0.23	5.5	±0.37	5.2	±0.55	5.3	±0.42	5.4	±0.07	6.2	±0.17	4.5	±0.60	6.0	±0.46
Sum %	93.7	±0.87	92.7	±1.72	93.3	±1.17	93.2	±1.09	94.2	±0.95	94.2	±0.97	92.7	±0.75	94.0	±0.85

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

Includes[2]: 17:1 n-7, 18:1 n-9T, 19:1, 22:1 n-7, 24:1 n-9.

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

Table 8. Fatty acid compositions (%) of muscle from Atlantic salmon (Salmon salar L.) fed with experimental 8 pre-diets until 400g and then with CC, commercial control diet till reach 1 kg. (Mean ± SEM, n=3)

Fatty acids (% of total)	Control (CC)															
	Control		0%		1% DHA		2% DHA		1% EPA		2% EPA		1% EPA+DHA		2% EPA+DHA	
14:0	3.0	±0.01	3.0	±0.20	3.0	±0.02	2.9	±0.10	2.9	±0.13	2.7	±0.17	2.9	±0.04	2.9	±0.14
16:0	10.9	±0.08	11.1	±0.13	11.1	±0.12	11.1	±0.06	10.9	±0.13	11.1	±0.05	11.0	±0.04	11.0	±0.04
18:0	2.6	±0.04	2.7	±0.06	2.6	±0.01	2.8	±0.05	2.7	±0.07	2.8	±0.02	2.7	±0.06	2.7	±0.04
Σ Saturated[1]	17.4	±0.09	17.7	±0.23	17.7	±0.15	17.8	±0.05	17.4	±0.27	17.6	±0.13	17.5	±0.01	17.5	±0.15
16:1 n-7	4.1	±0.09	4.2	±0.28	4.2	±0.12	4.1	±0.06	4.0	±0.22	3.7	±0.20	4.0	±0.09	4.1	±0.06
18:1 n-9	33.1	±0.98	33.2	±1.50	32.3	±1.42	33.3	±1.28	34.0	±1.03	34.4	±1.43	33.9	±1.07	33.3	±1.40
20:1 n-9	7.0	±0.27	7.0	±0.54	7.1	±0.30	7.0	±0.38	6.7	±0.43	6.2	±0.62	6.9	±0.24	6.9	±0.38
20:1 n-11	0.8	±0.02	0.8	±0.05	0.8	±0.01	0.7	±0.01	0.8	±0.02	0.8	±0.02	0.8	±0.04	0.8	±0.01
22:1 n-9	4.1	±1.62	4.4	±1.83	4.2	±1.69	4.2	±1.72	3.9	±1.60	3.7	±1.56	4.0	±1.61	4.3	±1.75
Σ Monounsaturated[2]	50.0	±0.77	50.4	±0.92	49.2	±0.85	50.0	±0.89	50.0	±0.78	49.4	±0.98	50.4	±0.71	50.1	±0.80
18:2 n-6	11.0	±0.13	11.0	±0.39	11.2	±0.05	11.2	±0.24	11.5	±0.35	11.8	±0.37	11.4	±0.08	11.4	±0.18
18:3 n-3	3.8	±0.07	3.6	±0.13	3.7	±0.06	3.7	±0.04	3.8	±0.15	3.9	±0.08	3.8	±0.04	3.7	±0.04
20:4 n-6	0.4	±0.02	0.5	±0.04	0.4	±0.01	0.4	±0.01	0.5	±0.01	0.5	±0.03	0.4	±0.05	0.4	±0.02
20:5 n-3	2.7	±0.05	2.6	±0.09	2.5	±0.07	2.5	±0.06	2.7	±0.05	2.9	±0.07	2.5	±0.05	2.8	±0.03
22:6 n-3	4.3	±0.08	4.1	±0.14	4.7	±0.16	4.7	±0.20	4.2	±0.17	4.7	±0.48	4.1	±0.09	4.6	±0.36
Σ Polyunsaturated[3]	24.6	±0.26	24.1	±0.77	24.8	±0.34	24.7	±0.51	25.1	±0.83	26.2	±1.11	24.6	±0.18	25.1	±0.62
n-3	11.9	±0.13	11.4	±0.27	12.0	±0.36	12.0	±0.32	11.9	±0.43	12.7	±0.73	11.5	±0.10	12.2	±0.42
n-6	12.6	±0.14	12.7	±0.50	12.8	±0.02	12.8	±0.22	13.1	±0.40	13.5	±0.39	13.1	±0.09	12.9	±0.22
20:5 n-3 + 22:6 n-3	6.9	±0.03	6.6	±0.09	7.2	±0.23	7.2	±0.22	6.9	±0.21	7.6	±0.55	6.6	±0.05	7.4	±0.34
Sum %	92.8	±0.64	93.0	±0.70	92.8	±0.63	93.3	±0.45	93.3	±0.63	93.9	±0.27	93.2	±0.56	93.5	±0.32

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

Includes[2]: 17:1 n-7, 18:1 n-9T, 19:1, 22:1 n-7, 24:1 n-9

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

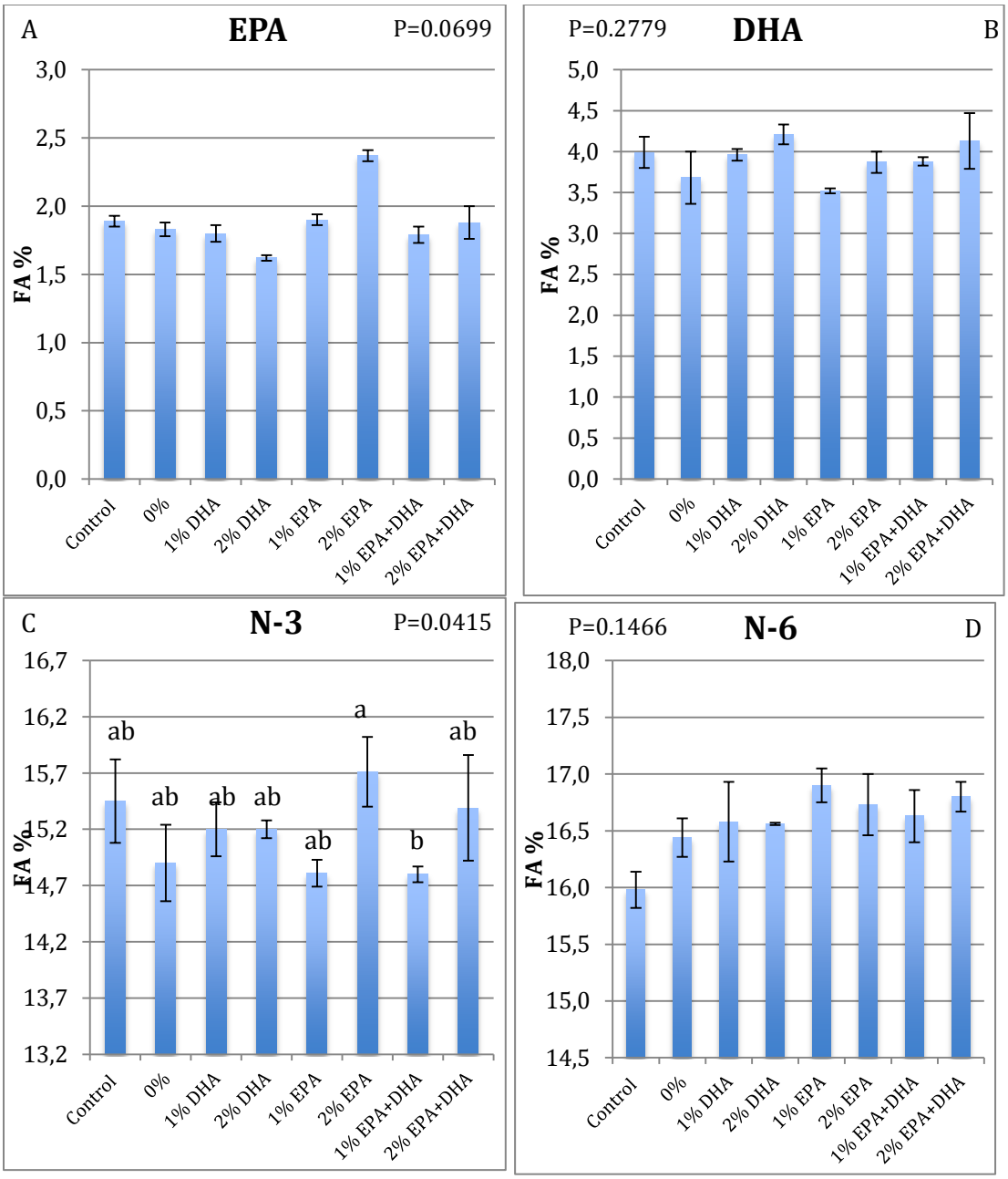

Figure 11. FA compositions of pre-diets (%) of EPA (A), DHA (B), N-3 (C) and N-6 (D) of muscle Atlantic salmon 1kg in the 1% dietary group. (Mean ± SEM, n=3, levels not connected by same letter are significantly different)

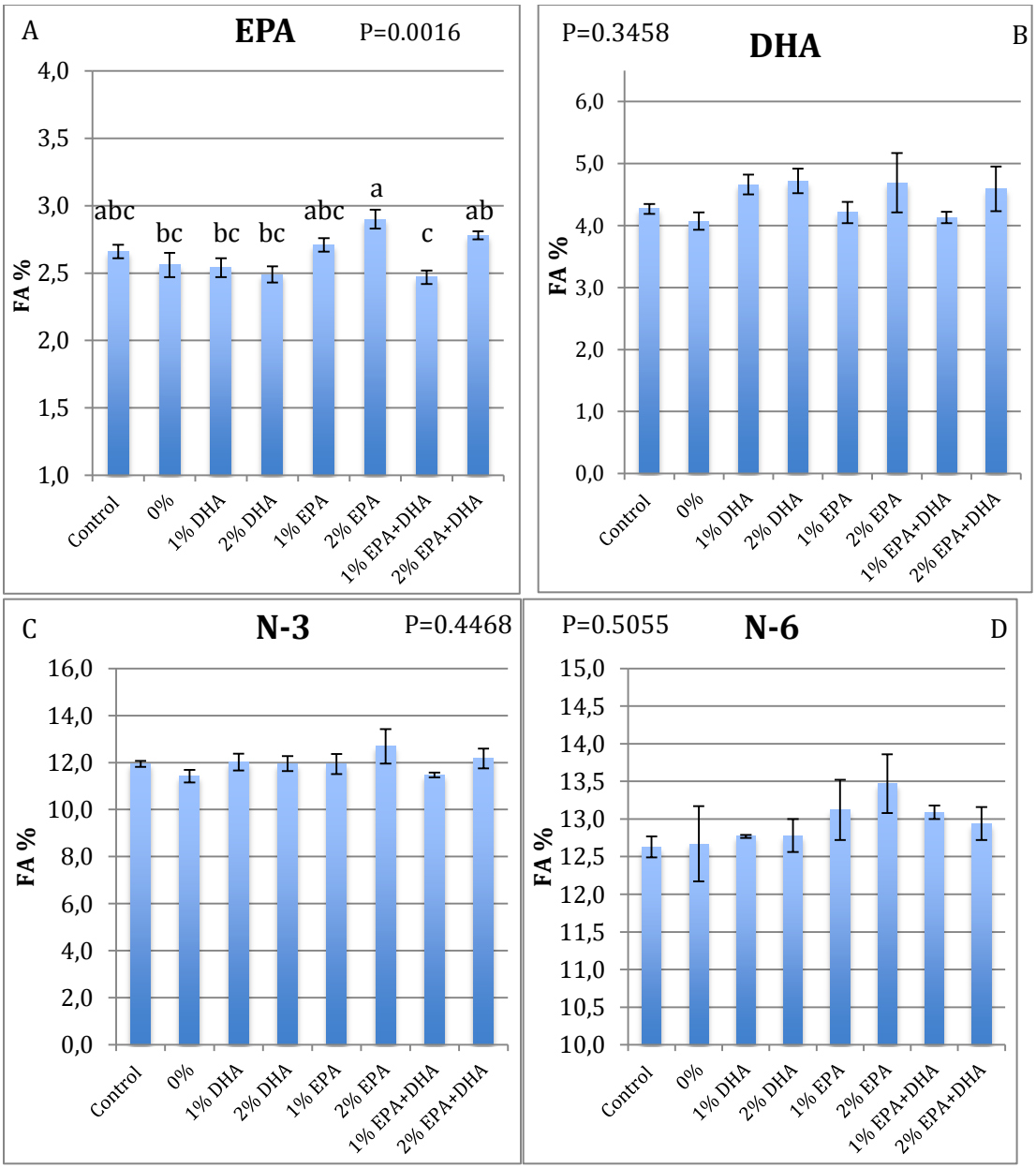

Figure 12. FA compositions of pre-diets (%) of EPA (A), DHA (B), N-3 (C) and N-6 (D) of muscle Atlantic salmon 1kg in the Control dietary group. (Mean ± SEM, n=3, levels not connected by same letter are significantly different)

Table 9. Fatty acids compositions (g per 100g) of muscle from Atlantic salmon (Salmon salar) fed with experimental 8 pre-diets until 400g and then with CC (Commercial Control), Low (0%) and 1% diet till reach 1 kg. (Mean ± SEM, n=3)

Fatty acids (gr per 100 gr)	Control (CC)															
	Control		0%		1% DHA		2% DHA		1% EPA		2% EPA		1% EPA+DHA		2% EPA+DHA	
EPA	2.4	±0.22	2.1	±0.11	2.0	±0.17	2.4	±0.19	2.4	±0.05	2.3	±0.17	2.3	±0.20	2.3	±0.21
DHA	3.9	±0.27	3.4	±0.05	3.7	±0.29	3.7	±0.27	4.0	±0.17	4.3	±0.34	3.7	±0.20	3.7	±0.04
Σ EPA/DHA	6.3	±0.48	5.5	±0.06	5.7	±0.46	6.1	±0.45	6.3	±0.19	6.5	±0.49	6.1	±0.34	5.9	±0.25
Σ N-3	10.8	±0.87	9.5	±0.11	9.5	±0.87	10.5	±0.74	11.0	±0.40	10.9	±0.92	10.2	±0.65	9.8	±0.53
Σ N-6	11.4	±0.87	10.6	±0.23	10.2	±1.17	11.5	±0.79	12.5	±0.45	11.6	±1.16	10.9	±0.95	10.5	±0.79
Σ N-9	40.1	±2.45	37.4	±0.94	34.8	±4.00	39.4	±3.58	43.0	±0.95	40.8	±4.46	36.2	±4.23	36.3	±3.66

Fatty acids (gr per 100 gr)	Low (0%)															
	Control		0%		1% DHA		2% DHA		1% EPA		2% EPA		1% EPA+DHA		2% EPA+DHA	
EPA	0.9	±0.11	0.6	±0.02	0.7	±0.05	0.8	±0.02	0.7	±0.03	0.7	±0.04	1.0	±0.06	0.9	±0.02
DHA	1.8	±0.20	1.2	±0.09	1.7	±0.08	1.6	±0.12	1.6	±0.07	1.9	±0.06	1.6	±0.07	1.9	±0.12
Σ EPA/DHA	2.6	±0.31	1.8	±0.11	2.4	±0.08	2.4	±0.12	2.3	±0.08	2.5	±0.10	2.6	±0.02	2.8	±0.13
Σ N-3	8.5	±0.84	6.6	±0.19	7.8	±0.44	7.1	±0.27	7.3	±0.67	8.0	±0.58	7.7	±0.26	9.0	±0.25
Σ N-6	14.4	±1.52	12.4	±0.30	13.9	±1.09	12.2	±0.44	12.7	±1.45	13.9	±1.17	12.7	±0.37	15.5	±0.30
Σ N-9	27.8	±3.39	23.0	±0.70	26.0	±2.11	22.8	±0.90	23.3	±2.64	26.1	±2.34	23.5	±0.72	29.5	±0.79

Fatty acids (gr per 100 gr)	1%															
	Control		0%		1% DHA		2% DHA		1% EPA		2% EPA		1% EPA+DHA		2% EPA+DHA	
EPA	1.4	±0.18	1.6	±0.15	1.3	±0.06	1.5	±0.06	1.4	±0.09	1.4	±0.13	1.7	±0.15	1.4	±0.04
DHA	2.9	±0.37	3.1	±0.36	2.9	±0.14	2.8	±0.15	2.9	±0.19	3.6	±0.30	2.8	±0.21	3.0	±0.03
Σ EPA/DHA	4.3	±0.55	4.7	±0.49	4.2	±0.19	4.4	±0.21	4.3	±0.28	4.9	±0.43	4.6	±0.36	4.4	±0.08
Σ N-3	11.4	±1.52	12.7	±1.25	11.2	±0.66	11.9	±0.67	11.2	±0.85	12.9	±1.30	11.6	±1.02	11.4	±0.73
Σ N-6	11.8	±1.71	14.0	±1.45	12.2	±0.96	13.6	±0.90	12.6	±1.07	14.1	±1.43	12.4	±1.44	12.6	±1.30
Σ N-9	26.8	±4.45	29.9	±3.51	26.7	±2.31	29.4	±2.47	27.6	±2.65	31.2	±3.87	26.6	±3.38	27.1	±3.38

Differences of FAs between Diet, Pre-Diets and the correlation between them calculated with statistical analysis and the effects of Probability factor shown on the Table 10 with P values.

Table 10. Statistic analysis of fatty acids with the effect of Diet, Pre-Diet and Diet*Pre-Diet indicated significant differences with P value. (Prob.>F)

Fatty acid	Diet	Pre-Diet	Diet *Pre-Diet
14:0	< 0.0001	0.114	0.7178
16:0	< 0.0183	0.5158	0.6567
18:0	< 0.0001	0.821	0.0421
16:1 n-7	< 0.0001	0.3309	0.8381
18:1 n-9	< 0.0001	0.9929	0.9966
20:1 n-9	< 0.0001	0.5534	0.7349
20:1 n-11	< 0.0001	0.0181	0.0191
22:1 n-9	< 0.0001	1.0000	1.0000
18:2 n-6	< 0.0001	0.0058	0.8847
18:3 n-3	< 0.0001	0.0209	0.4498
20:4 n-6	< 0.0001	0.0041	0.4382
20:5 n-3	< 0.0001	0.0001	0.0659
22:6 n-3	< 0.0001	0.0062	0.7459
N-3	< 0.0001	0.0034	0.8115
N-6	< 0.0001	0.0161	0.7656
20:5 n-3 + 22:6 n-3	< 0.0001	0.0013	0.6614

The main 3 dietary groups show us important influences on the salmon fillet in the FAs presented below. In Figure 13 we can see the total FA composition of EPA, DHA and the Σ EPA+DHA in mean values (%) inside of the 3 dietary groups 0%, 1% and Control. DHA have higher values than EPA with both FAs increasingly by 0%, 1% to Control diet.

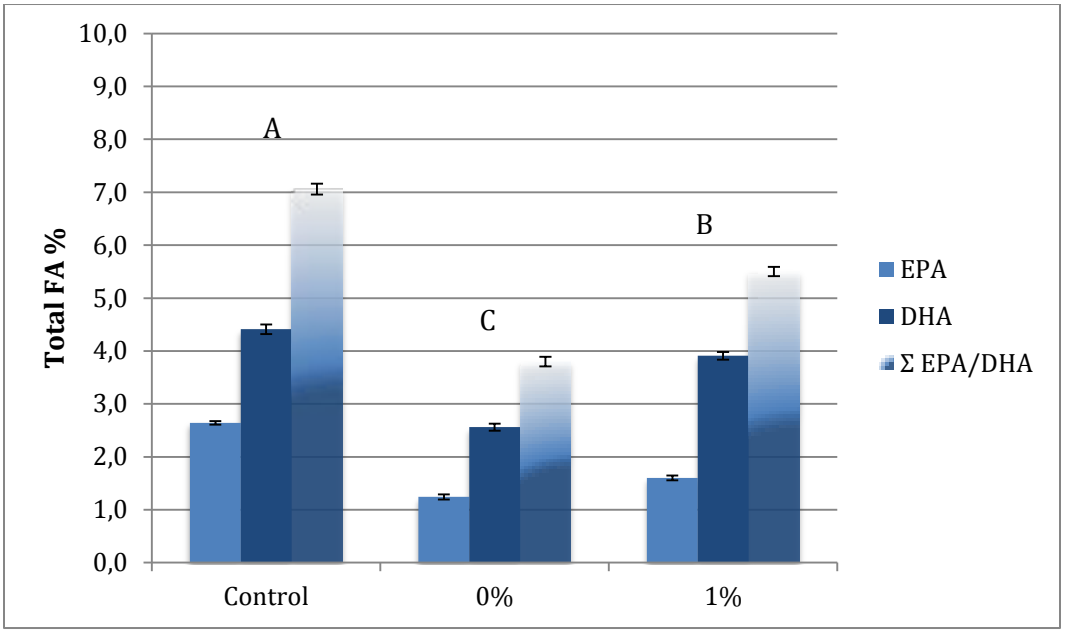

Figure 13. The total FA composition (%) of EPA, DHA and Σ EPA/DHA related to 3 dietary groups (Control, 0% and 1%). (Mean ± SEM, n=3, levels not connected by same letter are significantly different)

In the Σ of N-3 it is noticed some significant differences only at 1% diet with a P value = 0.0001 thus in 0% and Control dietary groups were no essential differences (Figure 14). On the other hand, Σ N-6 showed differences in all diets with P=0.0001 and as it is presented in Figure 10, the highest amounts were found in the 0% diet and then was a decrease of 4% to 1% to 1% and Control diets respectively. Besides, for Σ N-0 noted high differences in 1% diet with P=0.0031 which include the highest levels (%) of Σ N-0 (Figure 15. A). For N-9 acids significance differences were found in the 3 dietary groups with a P=0.0001 with the Control diet showing the highest value and the 1% diet the lowest (Figure 15. B).

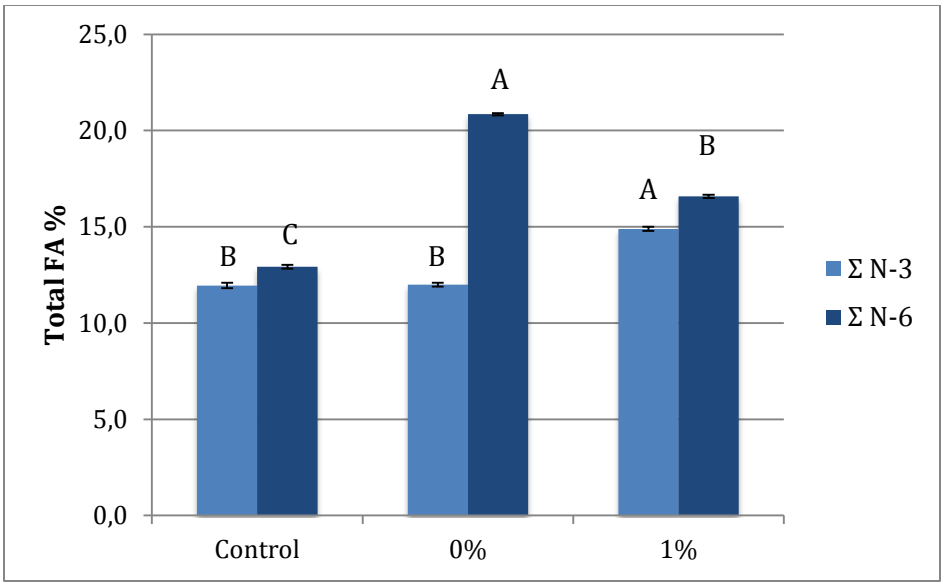

Figure 14. The total FA composition (%) of Σ N-3 and Σ N-6 related to 3 dietary groups (Control, 0% and 1%). (Mean ± SEM, n=3, levels not connected by same letter are significantly different)

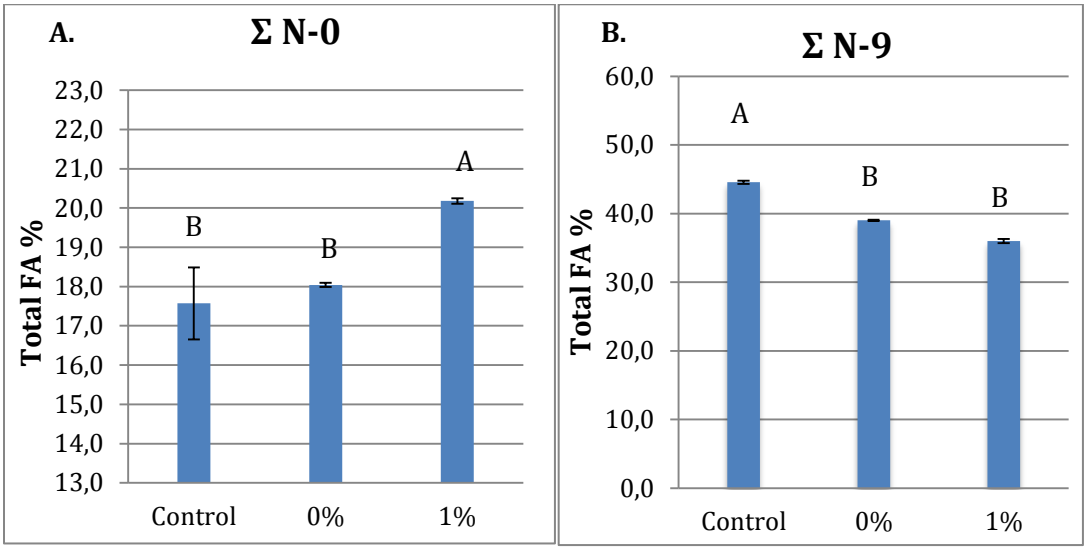

Figure 15. Total fatty acids composition in percentage (%) of Σ N-9 (A.) and of Σ N-0 (B.) in Control, 0% and 1% diets. (Mean ± SEM, n=3, levels not connected by same letter are significantly different)

5. Discussion

5.1. Growth

The growth of the fish seemed to be affected by the 3 main dietary groups. Fish that had been fed with the Commercial Control feed with the highest levels of EPA and DHA had the highest final weight. Moreover, the fish group fed the deficient diet (0%) had lower weight than the Control group. The intermediate group (1%) had a weight in between the 0% and Control groups. As it is mentioned in the study of Bendiksen et. al., (2003) increased levels of omega-3 in the feed provide a positive effect on growth in the fish. Consequently, inclusion of EPA and DHA in the diet of salmon is important to maintain good growth.

5.2. Colour

The colour of salmon fillet is quite important for the aquaculture industry. The salmon industry wants to provide a certain color range of the fillet to the market following the consumer's preference and choice. Significant differences in colour were found between the dietary groups, with the highest colour found in the group with the highest EPA and DHA level inclusion (Control dietary group), and significantly lower colour in the group with the lowest EPA and DHA included (0%) and the intermediate group (1%) in between.

A previous study with salmon fillets has shown that colour, odor, taste, colour shade and colour intensity can be influenced by different diet compositions and lipid sources (Thomassen and Røsjø, 1989).
However it is known that the colour is higher in larger fish than in small (Aksne et al., 1986), therefore the correlation with the weight and the colour provide us a linear regression with the size of the fish.

Based on this, we can say that the weight can influence the difference in colour first and second that the composition of the diet can influence the colour as well.
In order to test this more advanced statistics needs to be run, where is tested how much difference is due to different diets and how much is due to difference in size (however this type of statistics was not performed in this study).

5.3. Fat content

It is known from literature that N-3 fatty acids may reduce the lipid deposition in fish (Leaver et al., 2011). However, it is also known that the fat deposition in salmon increase with the size (Mørkøre and Rørvik, 2001). From our data we show a strong tendency to lower fat level in the deficient group relative to control dietary group. The total fat content of the muscles ranged from 8.29% to 10.64% and showed some significant differences in the 3 main dietary groups. However there were no significant differences in all pre-diets.
The percentage of omega-3 long chain PUFA has a similar increasing relationship with the final weight and the total lipid content of the muscle.
However, quite important variations are remaining on N-3 long chain (PUFA) content throughout the dietary group, according to Leaver et al. (2011).

5.4. Fatty acid composition

There are many studies that show the fatty acid profile of diets for Atlantic salmon and its affect on the muscle tissue of the salmon fillet (Grisdale-Helland et al., 2002; Sargent et al. 2002; Thomassen and Røsjø, 1989;Torstensen et al., 2005).

Our goal was to identify whether pre-diets containing different levels of EPA and DHA in early life stages from 40 gram fish to 400 gram fish would influence the fillet composition at later life stages in Atlantic salmon from 400 gram to 1 Kg when the fish were fed new diets.

In our results, the fatty acid composition of the muscle was significantly affected by the diet. A linear correlation between the FA concentration of the diet and the FA of the tissue has been proven in more studies. (Bell et al., 2001, Torstensen et al., 2005).

We found few significant effects of the pre-diets at 1 kg salmon, however is found that EPA have significant effects in the early life-stages. We also found that DHA pre-diets have high values of DHA in Control and 0% dietary groups with a similar range in the whole main dietary group, corresponding with the dietary levels (Bell et al., 2001).

Further, the main fact from the results is that the diet composition in the growth period from 400 g to 1kg much more influenced the FA composition at 1 Kg than the pre-diets. (Karalazos et al., 2011)

The results of the study showed significant difference in the main dietary groups with the highest inclusion of EPA and DHA increasing by 0% dietary group to 1 % dietary group and the highest at Control dietary group.

Moreover, the result from our study showed that there were significant differences in the percentages of EPA and DHA among the 3 main dietary groups. The levels of EPA influenced more than the DHA levels from the composition of the diet. From this it is evident that dietary lipids interacted to influence tissue fatty acid composition.

According to the results presented in Figure 13 the percentage of DHA ranged from 2.6% to 4.4% and significant differences were seen across diets. The results showed that the muscle percentage of DHA increased related to the diet in agreement with previous studies conducted by Bell et al. (2001) and Kjaer et al. (2008).

Additionally, this can be due to retention of DHA in the body and might occur due to the elongation and desaturation activity in the salmon. On the other hand, some studies show that if you decrease the level of FO in a dietary treatment of salmon the retention of DHA increases in muscle (Caballero et al., 2002; Regost et al., 2003; Torstensen et al., 2004). Another study found that feed including rapeseed oil for salmon converted EPA to DHA by increasing elongase and desaturase activity of the salmon (Thomassen et al., 2012).

Previous studies have highlighted that fatty acid compositions of fish muscle are identified by the type of dietary lipids they consume and furthermore the ability of the fish to modify the fatty acids with different dietary groups through desaturation and elongation reaction (Bell et al., 1993). This effect has been seen in more studies of salmon and can be supported with the studies of Olsen (2011) and Torstensen et al. (2000).

6. Conclusion

In conclusion, the results showed that Atlantic salmon express a satisfactory growth in all diets where EPA and DHA levels formulated. Moreover we found that higher inclusion of EPA and DHA in the diet provide positive growth for the fish. However fillet fat, colour and fatty acids composition varied significantly between the 3 main dietary groups, increasing as the range of EPA and DHA was enhanced. This study shows that dietary groups with different inclusion of EPA and DHA can have a significant impact on the FAs composition of the salmon fillet. Thereby diets with high inclusion of EPA and DHA attribute in more omega-3 FAs composition as EPA and DHA FAs in the muscle of the salmon.

The obtained results for pre-diets indicate an important difference in EPA fatty acid composition of the muscle of Atlantic salmon. Additional, the DHA levels showed some importance for further investigation.

7. References

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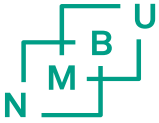

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