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The influence of dietary n-3 fatty acid deficiency on fatty acid composition of total lipid fraction and individual phospholipid classes in erythrocytes of Atlantic salmon (*Salmo salar* L.)

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Ivana Vukoje

Abbreviations

AA	Arachidonic acid (C20:4 n-6)
ALA	alpha-linolenic acid (C18:3 n-3)
DHA	Docosahexaenoic acid (C22:6 n-3)
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid (C20:5 n-3)
FAME	Fatty acid methyl esters
GC	Gas chromatography
LA	linoleic acid (C18:2 n-6)
LC-PUFA	Long-chain polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
n-3	Omega-3
n-6	Omega-6
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PL	Phospholipids
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
S.E.M.	Standard error of mean
TLC	Thin-layer chromatography

Summary

While global population and its demand for seafood are constantly on the rise, aquaculture is facing a significant challenge how to provide safe and nutritious fish when fish oil supplies are decreasing. More emphasis has been placed on farmed Atlantic salmon since it is an important source of health promoting long-chain polyunsaturated fatty acids in human diet. To obtain good growth, health and ultimately high nutritional quality, Atlantic salmon requires essential fatty acids, mainly EPA (Eicosapentaenoic acid, C20:5 n-3) and DHA (Docosahexaenoic acid, C22:6 n-3). However, sustainable alternatives for fish oil in modern fish industry are lacking those fatty acids and therefore question imposes itself: what is the minimum inclusion of EPA and DHA in the feed that can secure good health and growth of Atlantic salmon and keep the production sustainable. Previous studies have shown that freshwater salmon has a minimum requirement of about 1 % EPA and DHA combined in the feed to maintain good growth. However, the requirements in seawater Atlantic salmon are yet to be established. Therefore, of great importance is to research and fully comprehend how low levels of EPA and DHA in the salmon diet influence fish health in different phases of the life cycle. This study is based on long-term feeding of Atlantic salmon from 40 g to 3,5 kg with low dietary EPA and DHA and focuses on changes, induced by dietary EPA and DHA inclusions of 0,2 % and 1, 7 %, on fatty acid composition of total lipid fraction and individual phospholipid classes (PC, PS, PI, and PE) in Salmon erythrocytes, during last phase of life cycle prior to slaughtering. Results show that Atlantic salmon fed with n-3 deficient diet, containing 0,2 % EPA and DHA combined, as a compensation for those fatty acids tends to accumulate AA (Arachidonic acid, C20:4 n-6) in erythrocytes and phospholipid fractions, especially in phosphatidylinositol (PI). Higher level of 20-carbon (n-6) fatty acids observed in fish fed n-3 deficient diet lead to low ratio between n-3 and n-6 fatty acids. In the end, those changes may disturb inflammatory status in the fish. Therefore, 0, 2 % inclusion of EPA and DHA combined in feed is not sufficient to maintain DHA status in erythrocytes of Atlantic salmon and this may have severe negative effects on fish health.

Key words: Atlantic salmon, erythrocytes, EPA, DHA, PC, PS, PI, PE

1. Introduction

As the global population and its demand for seafood increases more emphasis has been placed on Atlantic salmon production. Salmon is a global product shown to be a rich source of the health promoting PUFAs, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. To maintain sustainable production of Atlantic salmon implementing substitutes for fish oil has become of a great importance. However, replacing the traditional marine ingredients, fishmeal (FM) and fish oil (FO), in farmed salmon feeds with sustainable substitutes of plant origin, lacking EPA and DHA, presents a significant challenge for the aquaculture industry. When marine ingredients are replaced with substitutes of terrestrial origin to large extent, the amount of dietary EPA and DHA, as well omega-6 and monounsaturated fats is also modified (Torstensen et al., 2013). It is to be expected that changes in dietary fatty acid composition will also have impact on fish growth, development, and health due to the well-established and proven role of essential fatty acids in diverse biological functions, such as regulation of cell growth, maintenance of proper structure and function of biomembranes, development of neural tissues and modulation of expression in variety of genes, including those involved in fatty acid metabolism and inflammation. To which extent changes in fatty acid composition of the feed have impact on the health and welfare somewhat depends on the minimum requirements for essential fatty acids, particularly EPA and DHA, of the fish. The principles for determining the nutritional requirements are yet to be established and more clearly defined. Previous studies have shown that freshwater salmon has a minimum requirement of 1 % EPA and DHA combined in the feed to maintain good growth (Ruyter et al., 2000). Hitherto, nutritional requirements of salmon mainly have been defined by groundwork including growth and survival in the short-term experiments (Torstensen et al., 2013). It is suggested that a new definition for the nutritional requirements should also include information about the optimum level of EPA and DHA that assures optimum performance due to a variety of criteria related to growth, absence of symptoms of deficiency and optimal fish health including resistance to disease. It is of great importance to define early symptoms of omega-3 fatty acid deficiency, since sub-optimal levels of EFAs during early life stages undoubtedly can increase health risks in later stages of life, even when obvious symptoms such as reduced growth and increased mortality do not manifest the deficit. It is yet to be researched and fully comprehended how low levels of EPA and DHA in the salmon diet influence fish health in different parts of the life cycle with emphasis on later phases of life prior to slaughtering. This study observed changes in fatty acid composition in total lipid fraction and individual phospholipid classes (PC, PS, PI, and PE) in erythrocytes during a seawater production cycle in Atlantic salmon reared from 1.2 - 3,5 kg fed with diets including 0,2 % and 1,7 %. of EPA and DHA combined.

2. Background knowledge

2.1. Basic Lipid chemistry

2.1.1. Lipids and Fatty acids

Lipids are a diverse group of compounds which is characterized by the property of insolubility in water (hydrophobicity), and the solubility in non-polar solvents such as acetone, ether, or chloroform. Lipids can be divided into several groups: Fatty acids; triacylglycerols; waxes; phospholipids; Sphingophospholipids and isoprenoids. Together with phospholipids and sphingolipids, which are extremely important for organism as they build membranes, triacylglycerols are also very important group of lipids as they represent a major storage form of metabolic energy in the body. Fatty acids are carbon chains with a methyl group at one end of the molecule and a carboxyl group at the other end. By the degree of saturation fatty acids can be divided into saturated, monounsaturated, and polyunsaturated. Saturated fatty acids lack double bond while mono- have one and polyunsaturated two or more. The letter n is used to indicate the position of the double bond closest to the methyl end. In polyunsaturated fatty acids (PUFAs) the first double bond can be found between the third and the fourth carbon atom from the n carbon therefore these are termed as Omega-3 (n-3 fatty) acids. In case the first double bond is between the sixth and seventh carbon atom they are termed as Omega -6 (n-6) fatty acids (Akoh, 2008).

Omega-3 and Omega- 6 fatty acids are also known as essential fatty acids (EFAs) because mammals and fish are unable to synthesize them and must obtain them via diet (FAO, 1980; Simopoulos, 1991).

2.1.2. Synthesis of essential fatty acids

There are two essential fatty acids, both polyunsaturated fatty acids, linoleic acid (LA) which is a precursor of omega-6 fatty acids and alpha-linolenic (ALA), which is a precursor of omega-3 fatty acids. Arachidonic acid (AA) is synthesized from linoleic, while from alpha-linolenic acid are being synthesized eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid (FAO, 1980; Simopoulos, 1991).

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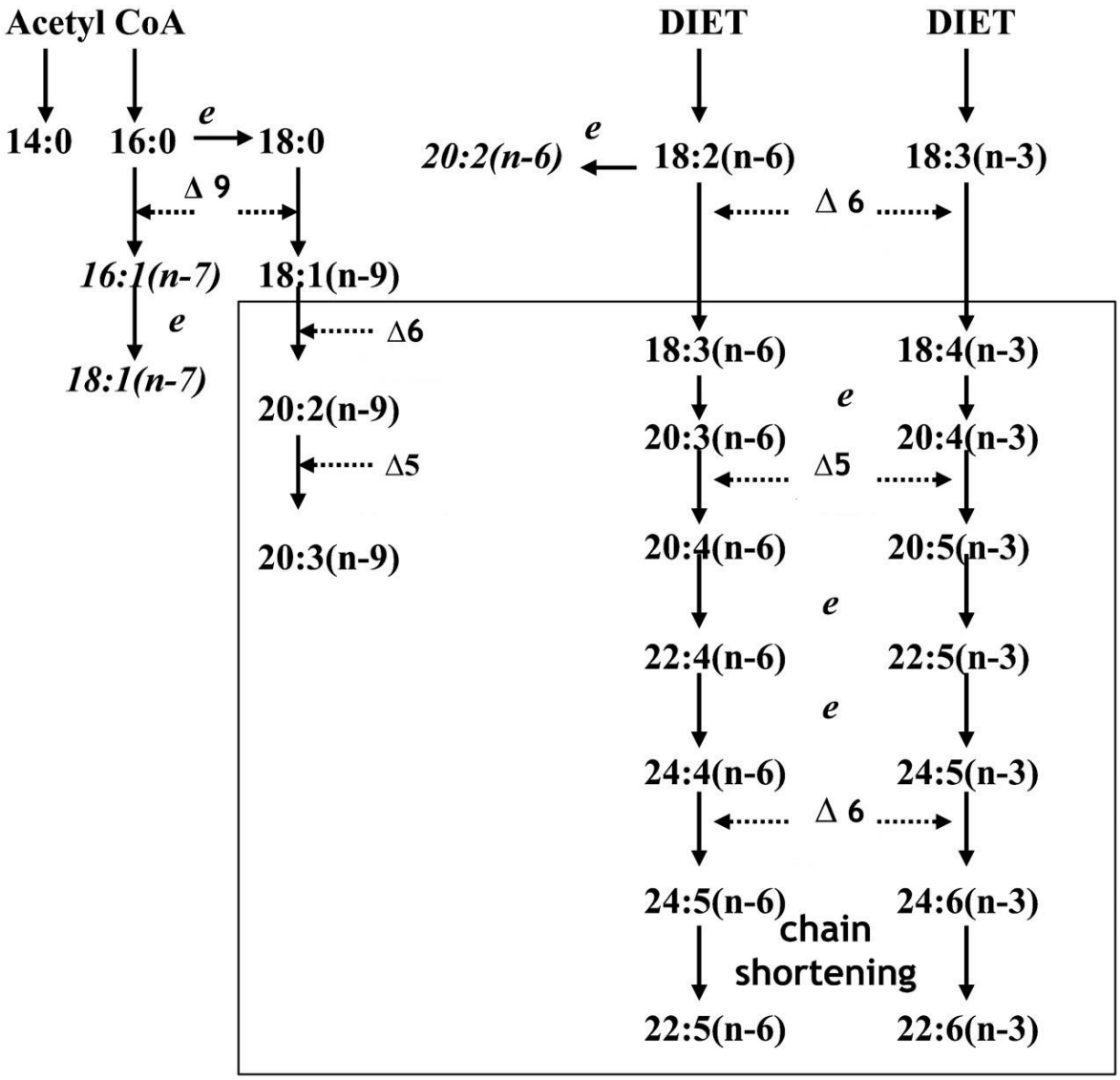

Figure. 2.1.2. Modified scheme of major steps in fatty acid desaturation and elongation (Xie and Innis, 2008).

Long-chain omega-3 and omega-6 fatty acids compete for the same enzymes Δ6- and Δ 5-desaturase, therefore for the outcome of these metabolic pathways a key factor is the ratio of omega-3 and omega-6 fatty acids in the diet (FAO, 1980; Simopoulos, 1991) (Figure. 2.1.2.).

Importance of this is going to be further discussed in the next chapter.

2.1.3. Phospholipids

Phospholipid are the main constituents of all biological membranes and most common among them are phosphoglycerides. They are complex lipids which consist of glycerol, two fatty acids, phosphate and second molecule of alcohol attached to the phosphate (Berg et al, 2002).

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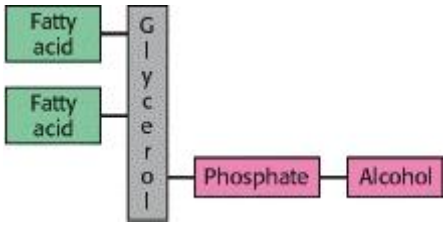


Figure 2.1.3.a. Phospholipid basic structure (Adopted from Berg et al., 2002).

The common alcohol moieties of phosphoglycerides are the amino acid serine, ethanolamine, choline, and the inositol. The structural formulas of phosphatidylcholine and the other principal phosphoglycerides are given in the figure (Berg et al, 2002).

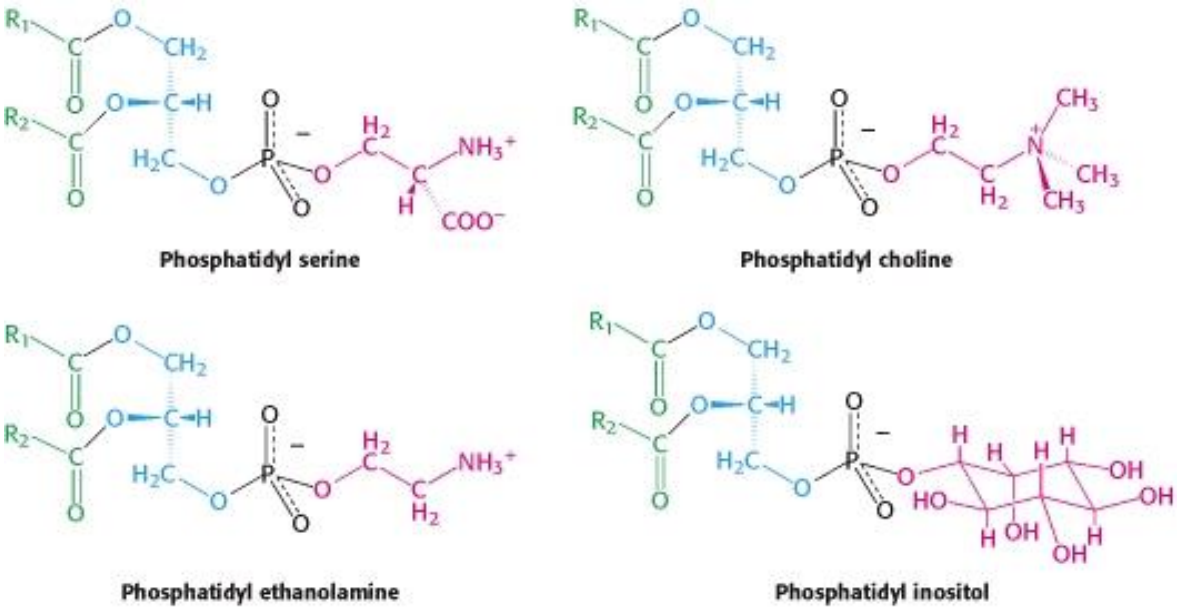


Figure 2.1.3.b. Structural formulas of phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) (adopted from Berg et al., 2002).

2.1.4. Synthesis of relevant phospholipid classes

2.1.4.1. Phosphatidylcholine (PC)

Phosphatidilcholines is a class of phospholipids also called the lechitins. PC primarily contains C16:0 or C18:0 acid at first carbon and C18:1(n-9), C18:2(n-6), or C18:3(n-3) at second carbon. Firstly, choline is activated by phosphorylation and then by bounding to Cytidine 5` di phosphate (CDP) prior to attachment to phosphatidic acid. As well it can be synthetized by the addition of choline to CDP - activated 1,2 – diacylglycerol. The third pathway to synthesis of PC involves the conversion of either phosphatidylserine (PS) or phosphatidylethanolamine (PE) to PC requiring decarboxylation of PS to get PE which then undergoes a series of three methylation reactions utilizing S – adenosylmethionine (SAM) as methyl group.

2.1.4.2. Phosphatidylethanolamine (PE)

PE primarily contains C16:0 or C18:0 acid on first carbon and LC – PUFA for example C18:2, 20:4 and 22:6 on second carbon. Synthesis of PE can occur by two pathways. First ethanolamine can be activated by phosphorylation and then bounded to CDP. Ethanolamine is then transferred from CDP – ethanolamine to phosphatidic acid to get PE. The second pathway includes the decarboxylation of PS.

2.1.4.3. Phosphatidylserine (PS)

PS is composed of fatty acids similar to PE. PS synthesis involves an exchange reaction of serine for ethanolamine in PE. Mentioned exchange occurs when PE is in the lipid bilayer of the membrane.

2.1.4.4. Phosphatidylinositol (PI)

PI contains almost solely C18:0 at first carbon and C20:4 (n-6) at a second. Synthesis of PI involves CDP – activated 1,2 – diacylglycerol condensation with myo – inositol. PI is critically important membrane phospholipid involved in transmission of signals for cell growth and differentiation of the cell (Berg et al, 2002).

2.2. Lipid nutrition in Atlantic salmon

2.2.1. Dietary lipid in aquaculture industry

Global consumption of fish is constantly on the rise, while the amount of captured fish is declining, hence it is predicted that aquaculture will provide the most reliable supply of seafood in the future. As the world's population is rapidly growing, it is of great importance to rear safe and nutritious fish. Nevertheless, there are many issues related to fish nutrition that need to be considered with the aim of aquaculture sustainability which is an environmental, economic, and social concern. Certain matters are associated with modern fish nutrition such as feed and nutrient efficiency, overfeeding and waste, biotechnology, sustainable feed ingredients, fish health and welfare, and furthermore human health and final product acceptance. Those topics have become controversial since they impact the environment and/or alter the final product for consumption. In due course, each of these concerns may affect the final product for human consumption, either nutritionally, environmentally, or economically. Realizing a balanced relation between quality and safe food production and environmental sustainability will be a challenge for the modern industry (Hixson, 2014; FAO, 2014; Strobel et al., 2012; Abedi and Sahari, 2014; Turchini and Tocher, 2010; Bell and Tocher, 2009).

Terrestrial plant oils are the most used alternative to FO (Fish oil) in aquaculture diets despite the fact that they are relatively poor sources of n -3 PUFA compared with marine FO. In contrary, they are rich sources of n -6 and n- 9 fatty acids, mainly 18:2 (n-6) and 18:1 (n-9), with the exclusion of certain oilseeds. Even though they are an adequate energy source, using terrestrial plant oils in feed certainly results in lower levels of DHA and EPA in tissues of fish fed plant oils (Sprague et al, 2016; Kitessa et al., 2014; Hixson, 2014; Turchini and Tocher, 2010).

Substitute for FO in fish feeds should avoid excessive deposition of 18:2 (n-6) and enhance conversion of 18:3 (n-3) to 20:5 (n-3) and 22:6 (n-3), and in order to increase n-3/n-6 ratio contain higher amounts of 18:3 (n-3) and lower of 18:2 (n-6) (Bell et al., 2001; Pettersson et al., 2009 a, b; Ramezani-Fard and Kamarudin, 2013).

Opposing to above mentioned criteria a study based on evaluation of four different alternative oils, used in rainbow trout feed, on deposition efficiency of n-3 LC-PUFAs is showing that n- 6 rich diet could spare catabolism of n-3 LC-PUFAs and as such was suggested to be adequate substitute for FO (Francis et al., 2014). Other recent studies have described that diets with high inclusion of 18:3 (n-3) are not adequate as a solitary replacement for FO, since fish did not properly utilize the 18:3 (n-3) in favour of n- 3 PUFA biosynthesis, but rather for

catabolization and use as source of energy (Barlow et al, 2003; Palmquist, 2009). Hence, the fatty acid composition of the dietary lipid has a significant influence on the tissue fatty acid composition of the fish (Kim et al. 2012; Jobling and Bendiksen, 2003; Webster and Lim, 2002; Sargent et al., 1989).

What is the minimum inclusion of EPA and DHA that should be in the salmons feed in order to obtain good health and growth is yet to be established (Monroig et al, 2011; Gracey 2014).

2.2.2. Biological Activities of Essential fatty acids

When Omega- 3 and Omega-6 are consumed, they are incorporated into cell membranes in all tissues of the body. Due to this fact, dietary changes in the composition of PUFAs can have a profound impact on a cells function because the membrane lipids serve as source of precursors for the synthesis of important signalling molecules involved in cell growth and development as well as modulation of inflammation. The key to maintain fluidity and elasticity of the membranes is the structure of phospholipid molecules, respectively, the fact is that the sn-2 position of the glycerol molecule in phospholipids is bounded to unsaturated fatty acid. As well fish individual phospholipids show characteristic differences in fatty acid composition. For example, 16:0, 18:1 n-9 and 22:6 n-3 are shown to be major fatty acids in PE of trout livers while PS is containing similar content but shown to be more abundant in 18:0 fatty acid (Hazel, 1979). In general, phospholipids of marine fish resemble the fatty acids composition of that of the trout excepting n-3 PUFAs which seem to be more dominant (Ackman, 1980; Tocher 1988). To function properly, membranes need to be in a fluid state regardless of fluctuations in temperature. Acyl chains of PUFAs molecules have trans configuration, therefore, they are weakly bounding with the neighbouring saturated chains that are straight and long. Therefore, membrane fluidity will be greater with a higher number of double covalent bonds, i.e., a greater degree of unsaturation of the fatty acid chains. PUFAs are more present inside, cytosol part, of bilayer therefore this part of the fluid bilayer is more fluid than inner part. Their presence as well contributes to asymmetry of the distribution of cholesterol in the membrane, because cholesterol is better associated with saturated acyl chain fatty acids at positions sn-1 of glycerol, and extends away from polyunsaturated acyl chains. Thus, the content of PUFA, especially DHA, in the cytosol layer contributes to the lateral organization of cholesterol in the membrane and supports its moving in the opposite part of the bilayer. By removing cholesterol from its immediate surroundings, DHA increases fluidity, which is a characteristic of membranes parts which are easily connected with other membranes, or else release vesicles and modulates the thickness of the membrane (due to the exceptional flexibility it is able for terminal methyl group to approach to the cell surface). Based on the

described it can be concluded that balanced lipid composition of biological membranes provides asymmetry, fluidity, and elasticity which are necessary for normal functioning of all membrane functions (Van Meer et al., 2008; Simons, 2004; Jacobson et al, 2007; Singer and Nicolson, 1972). Since fish is poikilothermic organism, meaning that cannot control its body temperature, its ability to survive at lower temperatures depends on the ability to change the entire metabolism, especially maintain cell membrane fluidity since it decreases with temperature. The organism adjusts to it via quantitative and qualitative changes in the lipid composition of the membrane by increase in the degree of unsaturation of fatty acids. Particularly the proportion of phosphatidylethanolamine is increasing relative to phosphatidylcholine, cholesterol content is reduced in comparison to the phospholipids, the composition of membrane in general is changing in favour of structures having an unsaturated fatty acid in the lipid anchor and structures that have polar hydrophilic moiety (Hazel, 1984; Bell et al., 1986; Waagbø, 1994; Ernst et al, 2016). The role of dietary n-3 PUFA during homeoviscous adaptation in fish is explained in more details by Hazel (1984). Keeping that information on mind, one may conclude that ratio between phosphatidylcholine and phosphatidylethanolamine or ratio between PUFAs and saturated fatty acids can be used as an index of fish adaptation to fluctuations in environmental temperatures (Logue, 2000).

The most important omega-6 PUFA which can be found in phospholipids is arachidonic acid (AA). AA acts as a powerful second messenger which can be metabolised by a series of cytosolic enzymes (cyclooxygenase, lipoxygenase, epoxygenase, cytochrome P450 enzyme family) into various eicosanoids (compounds composed of 20 carbons: prostaglandins, leukotrienes, thromboxanes, lipoxins, Hydroxyeicosatetraenoic acid). These compounds are potent mediators of inflammatory processes in the cell, initiate downstream cellular processes of signalling and cell proliferation (Bell et al, 2003; Berge et al, 2009; Smith and Murphy, 2002.)

Probably the most important biological role of the omega-3 PUFAs, EPA and DHA is that they serve as precursors for potent anti- inflammatory lipids. Enzyme cytosolic phospholipase A independent of calcium catalyses the release of DHA from sn-2 position of the phospholipid molecules, predominantly phosphatidylethanolamines. Released, DHA can be reincorporated into the phospholipid molecule, or be enzymatically metabolized in different docosanoids (compounds built of 22 carbon atoms: protectins and resolvins). Docosanoids participate in intracell signalling, but their effects are contrary to those of eicosanoids. Unlike most eicosanoids that stimulate inflammatory processes in the cell, activity of docosanoids is anti-inflammatory, neuroprotective and antiapoptotic (Ernst et al, 2016; Smith and Murphy, 2002.)

2.2.3. Essential fatty acid requirement and deficiency

As mentioned above, fish cannot synthesize either 18:2 (n-6) or 18:3 (n-3) de novo. Hence one or both fatty acids must be obtained via diet, depending on the EFA requirements. In addition, fish vary considerably in their ability to convert 18-carbon unsaturated fatty acids to longer-chain, more highly unsaturated fatty acids of the same series. However, it is known that marine fish require dietary lipid in form of EPA and DHA comparing with freshwater fish requiring either LA or ALA or both (Tocher, 2010).

Although Atlantic salmon is one of the most successfully reared fish from the family of salmonids, relatively little information is existing on the EFA requirements for this species, especially for later life stages (Tocher, 2010). The estimated EFA requirement in Atlantic salmon fry, based on total body and tissue fatty acid composition data, is established to be 1 % of EPA and DHA combined in the diet (Ruyter et al., 2000).

The most common indicators of EFA deficiency in various fish species include a shock syndrome, fin rotening, myocarditis, reduced growth rate and feed efficiency, skeletal abnormalities, and increased mortality (Lall, 1758; Leray et al., 1985; Lall, 2000; NRC, 2011).

Essential fatty acid deficiency has also been shown to reduce the reproductive performance of rainbow trout (Leray et al., 1985) and Mediterranean fish species (Palacios et al., 1995; Abrehouch et al., 2007).

In fish species that are able for further desaturation and elongation of 18:2 (n-6) or 18:3 (n-6), an avoid of either of these fatty acids in the diet leads to accumulation of 20:3(n-9) in the phospholipids, formed via desaturation and elongation of 18:1(n-9), which is an indicator of EFA deficiency in many terrestrial animals (Roberts, 2012; Ichi et al., 2014; Bond et al., 2015).

It has been suggested that the ratio of 20:3(n-9)/20:5(n-3) in polar lipids from the liver of rainbow trout might be an indicator of EFA deficiency if it is greater than 0.4 (Sargent et al., 1989). Regarding to Mediterranean fish species is considered that the diet with sufficient EFA could be termed if this ratio in phospholipids is less than 0.10 (Webster, 1995).

Knowing that omega-3, omega-6, and omega-9 fatty acids compete for the same desaturase enzymes it can be concluded that synthesis of the 20:3n-9 increases only when dietary intakes of omega-3 and omega-6 fatty acids are very low. Also, an important ratio that should be considered is between n-3 and n-6 PUFAs. In fish phospholipids, this ratio is shown to be 10-15:1 whereas the n-6/n-3 ratio should not extent 5:1 in modern human diet; this ratio is yet to be established for fish species (Ackman 1980; Rizzo et al, 2010).

2.3. Fish Erythrocytes as a biomarker

Fish erythrocytes are blood circulating nucleated cells of different size and half-life of 80–500 days. Erythrocyte count depends on fish activity, water temperature and dissolved oxygen concentration as well as on other environmental factors showing significant seasonal changeability. As well it depends on age, sex, reproductive, and nutritional status, and may vary among different populations of the same species. It usually ranges from 0.5–1.5 × 10^6/mm^3 in less active species to 3.0–4.2 × 10^6/mm^3 in more active ones. Fishes have a weak barrier between hematopoietic tissue and circulating blood in which numerous undeveloped cells are present, often containing over 10 % of all erythrocytes. As in other vertebrates, fish erythrocytes contain tetrameric haemoglobins of different oxygen affinity. Oxygen affinity is lower in species living in well-oxygenated water than in those that experience hypoxia (Fischer et al.,1998). Fish erythrocytes are moreover sensitive to environmental pollution, and their morphological evaluation can be used as a bioindicator of toxicity (Wlasow, 1984; Witeska, 2013; Ferencz, 2015). In addition, dietary lipid has an impact on fish erythrocytes like in mammals (Cowey and Sargent, 1977). As mentioned previously, major phospholipids (PE, PS, PC, and PI) have a structure role in red blood cell (RBC) membrane (Smith, 1987; Cooper, 2000). Dietary fatty acids as major constituents of phospholipids modulate blood concentrations of fatty acids and biological processes in plasma membrane thus they serve as a great bioindicator of PUFAs status in mammals and following the same analogy in the fish as well. EPA plus DHA expressed as a percentage of total fatty acids (TFA), in red blood cell (RBC) membrane of mammals, also called Omega-3 index, present a validated bioindicator of tissue membrane n-3 PUFA status (Von Schacky et al.,1985; Harris et al., 2004; Block et al., 2008; Arnold et al., 2010; Harris and Thomas, 2010; Harris et al., 2013; William et al., 2013; Gurzell et al, 2014). Several observational studies in human report that red blood cells in comparison with plasma provide a more reliable estimate of Omega-3 index and suggest that it should be used as a biomarker for cardiovascular disease risk with proposed range identifying following: high risk, below 4%; intermediate risk, from 4 to 8 %; and low risk, above 8 % (Harris, 2008; Von Schacky, 2014).

3. Materials and methods

3.1. Materials

Table 3.1. Chemicals and equipment

Chemicals and equipment	**Producer**
2,2-dimethoxypropane	Sigma Chemical Co., St Louis. MO, USA
2',7'-dichlorofluorescein	Merck, Darmstadt, Germany
Acetic acid	Merck, Darmstadt, Germany
Benzene	VWR International, PA, USA
Butylatedhydroxytoluen (BHT)	Sigma Chemical Co., St Louis. MO, USA
Chloroform	VWR International, PA, USA
Diethyl ether	Sigma Chemical Co., St Louis. MO, USA
Ethanol	VWR International, PA, USA
Hewlett Packard 6890 gas chromatograph	Avondale, PA, USA
Hexane	VWR International, PA, USA
Methanol	VWR International, PA, USA
Methanolic HCl	Supelco Inc., Bellefonte, PA, USA
Petroleum ether	Sigma Chemical Co., St Louis. MO, USA
*TLC silica gel 60g 0,25 mm Glass plates 20*20 cm*	Merck, Darmstadt, Germany

3.2. Methods

3.2.1 Experimental fish and diets

The experiment of concern for this Master thesis is based on a project carried out at NOFIMA's research station at Sunndalsøra and Helgeland Aquaculture Research Station Dønna, Norway. The salmon was followed from start weight of 40 grams up to 1 kg in indoor tanks at Nofima Sunndalsøra. At the start of the experiment after a smoltification (40 grams) fish was ready to be transferred into seawater. Fish of 40 grams was reared to an average body weight (BW) of 400 grams in Phase 1 and of 400 grams to approximately 1 Kg in Phase 2. In Phase 3 fish was reared until reaching a slaughter size of approximately 3,5 kg. In the Phase 1 fish were fed with different concentrations (0%, 0.5%, 1%, 1.5% and 2%) of EPA alone, DHA alone and a mixture of EPA and DHA which in total comprised 14 different diets. Basic feed was a fish oil

(FO) - and fishmeal (FM)– free, on the other hand commercial control (CC) with approximately of 2,2 % EPA and DHA contained FO and FM.

In Phase 2 fish from the 14 experimental groups was transferred to 9 tanks (5m * 5m) comprising 3 different diets (3 replicates per diet). All fish were individually marked. Fish from the various pre-diet feeding groups were equally represented in the 9 tanks and fed with different concentrations of a mixture of EPA and DHA as follows: 1.7 % EPA and DHA (6.6 % of TFA) (approximately commercial control); 1 % EPA and DHA (4.1 % of TFA) and 0.2 % EPA and DHA (0.4 % of TFA). For the ease of understanding and comprehension, further in the thesis diet with inclusion of 1.7 % EPA and DHA is going to be called Commercial control (CC) and others as follow: 1 % EPA and DHA, 1 % Main Diet (MD) and 0.2 % EPA and DHA, Low Diet (LD).

After the first two phases concerning 2 early life stages, fish was transferred to the sea and fed with the same diet as mentioned above until reaching a slaughter size of approximately 3,5 kg. The fish was individually weighed before transportation from Sunndalsøra, and average weight in the 9 groups ranged from 1172 to 1430 grams. The fish were transferred from the 9 tanks in Sunndalsøra to 9 sea cages at Helgeland Aquaculture Station Dønna by truck. Shortly after transportation wounds were observed on a portion of fish, especially around the caudal fin section, which indicated damage caused by handling. Over the next 4 weeks relatively high mortality in all the 9 cages was recorded.

Because of different incidence of mortality in various cages, odd number of fish was left in cages. Increasing levels of DHA in the diet in the earliest stages of life led to lower mortality. From the transfer from Sunndalsøra to Helgeland Aquaculture Research Station until April 20 had about 60 % of fish died in the LD group compared with 40% in the CC. Taking this into concern, in next 10 days it was decided to reduce the number of cages from 9 to 3, one cage per diet with approximately 200 fish per cage. Various pre-diet groups were equally represented in each cage.

3.2.2. Temperature and feeding regime

From stocking in cages in February 2014 until mid-April, the temperature was around 5 ° C. The temperature rose gradually until the end of July reaching maximum of 17.5 ° C. In mid-November 2014 at the end of the experiment temperature was 9 ° C (Havforsk, 2016).

Feeding strategies were similar for all diet groups in the experiment and followed the procedures developed by Helgeland Aquaculture Research Station Dønna. Feeding was performed to appetite level and lead to an overfeeding of about 10 %. Two meal per day are used at temperatures above 8 ° C, and one meal per day at temperatures below 8 ° C. Feed

intake per cage was recorded weekly based on amount fed, and correction for residual feed removed using Akva Lift-UP system. Fatty acid composition of the feed is shown in **Table 4.2.2.**

Table 4.2.2. Fatty acid composition of feed (% of total fatty acids)

Fatty acid (% of TFA)	*Low Diet* ***0,2% EPA + DHA***	*Main Diet* ***1% EPA + DHA***	*Commercial control* ***1,7% EPA+DHA***
C 12:0	0,8	0,4	0,1
C 14:0	1,0	3,3	4,5
C 16:0	14,2	11,9	8,3
C 18:0	4,2	3,2	1,5
C 20:0	0,3	0,3	0,4
C 24:0	0,1	0,1	0,2
Sum SAT	20,6	19,1	14,8
C 16:1 n-9	0,2	0,2	0,1
C 16:1 n-7	2,8	4,5	5,4
C 18:1 n-9	38,1	26,2	26,8
C 18:1 n-7	1,2	1,6	2,2
C 20:1 n-7	0,0	0,3	0,5
C 22:1 n-11	0,4	8,1	12,5
C 22:1 n-9	0,1	0,8	1,3
C 24:1 n-9	0,1	0,3	0,6
Sum MUFA	43,2	42,8	50,3
C 18:2 n-6	21,1	13,1	8,1
C 18:3 n-6	0,1	0,1	0,1
C 18:3 n-4	0,1	0,1	0,0
C 18:3 n-3	11,4	11,4	4,2
C 20:4 n-3	0,2	0,8	1,2
C 20:1 n-9	0,8	7,0	10,5
C 20:2 n-6	0,1	0,2	0,2
C 20:3 n-6	0,1	0,1	0,0
C 20:4 n-6	0,3	0,2	0,2
C 20:5 n-3	0,4	2,4	3,9
C 22:5 n-3	0,1	0,2	0,4
C 22:6 n-3	0,3	1,1	2,1
Sum PUFA	35,0	36,5	30,6
Sum EPA+DHA	0,7	3,5	6,0
Sum N-3	12,4	15,8	11,6
Sum N-6	21,8	13,7	8,6

SUM SAT includes fatty acids 15: 0, 17: 0 and SUM MUFA include 14: 1 n-5, 16: 1n-5, 17: 1n-7, 22: 1n-7 (≤ 1 % of total fatty acids)

LD was based on a FM-free and a FO-free basic feed, where oil source was canola oil, poultry oil and flaxseed oil. The main protein source was poultry meal. MD was also FM- free but with

inclusion of FO to the level of EPA and DHA of 1 % in the feed. CC contained both FM and FO. Nofima vitamin and mineral mixture was used in feed of LD and MD. In all tree diets 3.0 % krill meal was used as an appetite enhancer.

All feed used in the experiment was produced by BioMar AS.

3.2.3. Experimental sampling

The data consists of slaughter records on individual basis, a total of 225 fish. Of fish that was harvested at the end of the experiment at Helgeland Aquaculture Research Station Dønna, had 65 fish received LD, 69 fish had received MD, and 91 fish had CC. Individual registrations of all fish in the current pre-diets are used as the basis for statistical calculations of growth and harvest parameters. Five pre-diets (extreme diets) were selected for further analysis, and in each group, were 6 individual samples used. Two and two samples were combined so that the data for statistical analysis of the pre-diet effects includes 3 values (n = 3). For the effect of the main diets (the three diets used in cages Dønna), there are 15 analysis values (n = 15). Before slaughtering fish was anesthetized and blood samples were taken using Venoject® vacuum tubes. To separate plasma and erythrocytes centrifuge (3000 rpm, 10 min, 5-8°C) was performed. Erythrocytes samples were transferred to vacutainer tubes containing EDTA as an anticoagulant, frozen in liquid nitrogen and stored at -70 °C until analysed.

3.2.4. Total Lipid extraction

Total lipids were extracted from erythrocytes using Folch extraction method (Folch et al., 1957). Erythrocytes samples were taken from freezer (-70 °C) and placed on a dry ice, those which are immediately used for weighting are placed into the cold water to defrost. 25 µl of the sample was transferred into small plastic vials and stored at -70 °C for additional RNA analysis not covered by this thesis. Remained aliquot was transferred to glass tubes and weighted. In each sample was added 1,25 ml of NaCl. Sample was homogenized by using vortex. Further 7 ml of methanol (containing 0,5 µl of BHT) and 14 ml of chloroform were added homogenizing the sample with vortex in between. To make sure sample is being homogenised properly, knife was used for 60 seconds. Samples were centrifuged (2000 rpm, 4 °C, 10 min).

After centrifuge, upper phase was carefully removed using a disposable glass pipette with vacuum suction and properly discarded while lower (chlorophorm) phase was transferred to small glass tubes. Further 1 ml of aliquot was transferred to another glass tube by means of syringe which was washed with chlorophorm between taking different samples. The remaining

chlorophorm phase was stored at – 40 °C until further separation of lipid groups by TLC. Former was evaporated at 60°C with nitrogen overflow.

Methylation was performed by adding, in order as follows, 2 ml of benzene, 2 ml of methanolic HCl and 0,2 ml of dimetoxypropan. Tubes were caped, mixed well and incubated at room temperature until the next day. The reaction was stopped by adding 2 ml of hexane and for neutralization 3 ml of 6 % $NaHCO_3$ was used. After the visible separation of the two layers, upper phase was removed and sample was evaporated at 60°C with nitrogen overflow according to Mason and Waller (1964). Prior to gas-liquid chromatography (GC) analysis dried lipid sample was dissolved in 1 ml hexane.

3.2.5. Chromatography

By using chromatographic methods, it is possible to separate the two or more similar ingredients of the mixture which with other analytical processes is rather difficult to achieve. Term chromatography comprises methods of separation based on different components of samples between two phases, which move one compared to another. Stationary phase can be solid or liquid, a mobile liquid or gas. The components of the sample need to be soluble in the mobile phase, but also must somehow work with the stationary phase: resolve, adsorb, or chemically react. The result of components being differently distributed between two phases is the basis for their chromatographic separation. Gas chromatography is used for separation, isolation, and quantitative analysis of components in mixtures, to determine the purity of the substance and help with the identification. It is based on separation of components of the mixture due to differences in adsorption or partition on the stationary phase with gas as a mobile phase. Due to the stationary phase this method is divided into Gas-solid Chromatography and Gas-liquid chromatography. In Gas-solid chromatography stationary phase is an adsorbent (silica gel, aluminium oxide, diatomaceous earth), which specifically, bounds components of the mixture. Gas-liquid chromatography stationary phase is liquid (silicone oils, liquid hydrocarbons of high molecular weight, esters, and alcohols with high boiling points). Due to differences in solubility of components of the mixture in the stationary phase, comes to their separation. By Gas chromatography (GC), qualitative and quantitative analysis of mixtures can be achieved with better resolution and in a shorter time than in most today established analytical procedures. Mobile phase - carrier gas – has low viscosity therefore much longer, and thus the more effective columns can be used. The detection of small quantities of gases and vapours is much simpler and more accurate than the determination of small amounts of a substance in the liquid state. However, this method is suitable only for the analysis of volatile (Molecular mass < 500), not too polar (long stick to the column) and thermally stable compounds (column operating temperatures are from -70 to 400

° C). These conditions are characteristically for relatively few compounds (about 15 %). Compounds that do not meet the above conditions can be derivatized and then analysed by gas chromatography. Thus, fatty acids were trans-esterified and converted into fatty acid methyl esters (FAMEs) by methylation as already described above.

Thin layer chromatography is based on the distribution of the substance between the solid adsorbent, and liquid mobile phase. Due to the capillary action solvent flows up the solid adsorbent. As the stationary phase for thin layer chromatography, commonly is used silica and alumina applied in a thin layer on glass, metal, or plastic plate. The mixture to be separated is applied on a thin layer near one end of the plate to the point of a smaller diameter. The eluent is poured into a glass container with a lid (developing chamber) so to cover the bottom. The plate is immersed into the solvent with side of the plate, on which sample was applied (starting line), facing bottom. Due to the capillary action eluent flows up the adsorbent and a separation of components of the mixture occurs. When the solvent is closer to the top edge of the plate, the plate is removed from the chamber and occurred distance of solvent is marked (solvent front). If the components are not coloured, they can be visualized in several ways. The most common way is using ultraviolet light (UV), reversible addition of iodine, spraying with reagent, or with concentrated sulfuric acid (compounds become visible after heating).

The use of thin layer chromatography is various. TLC is used to identify substances, control the purity of a compound, and to monitor the course of the reaction (monitor the formation of the product, or disappearance of reactants). Finally, before each chromatography on a column, TLC should be used to find solvent with optimum polarity and ability to separate components of the mixture. In addition to the analytical, TLC may be used in preparative purposes (Snyder Kirkland, 2010; Seppänen-Laakso et al., 2002; Bele and Khale, 2011).

3.2.5.1. Gas chromatography (GC)

As mentioned above, the samples were evaporated at 60 °C with nitrogen overflow and dissolved in hexane. FAMEs were separated and quantified by GLC using a 60 m*0.25 mm ID capillary column (SGE, Pty Ltd. Victoria, Australia). Hydrogen was used as carrier gas and temperature programming was from 50 °C to 170 °C at 4°C/min, next to 200 °C at 0.5 °C/min and then to 300 °C at 10 °C/ min. In comparison with retention times of fatty acid methyl esters known standards, obtained from Sigma, individual fatty acids were identified.

3.2.5.2. Separation of lipid classes with Thin-layer chromatography (TLC)

TLC plates were preconditioned in methanol allowing migration to the top. According to the direction of migration top side was marked. After preconditioning they were dried at 120 °C in 20 min and stored in exicator until used. As a mobile phase solvent of Petroleum eter, Dietyleter and Acetic acid, in ratios 113:20:1 by volume, was used. One centimetre from the bottom of the plate, samples were applied and plate was immersed into the developing chamber containing the mobile phase. Sufficient time for the migration of lipid samples was allowed. Migration of the mobile phase was approximately 18 cm. The plate was removed from developing chamber and left until dried up. All the mentioned steps were performed in the fume hood. The plate was sprayed with 2,7 – diclorfluorescin (2 % in 96 % ethanol). After plate was dried in the fume hood, detection of lipid classes was performed under the UV light on 366 nm (lipid classes appeared as yellow spots). Spots corresponding to phospholipids were marked and the area was scraped and transferred into a glass tube while fractions corresponding to tryacilglycerols and cholesterols were scraped and properly stored at -40 °C until further analysis, not included in this thesis. The phospholipid fraction of each sample was eluated from the silica gel in a polar solvent, prepared with 500 ml of chloroform, 390 ml of methanol, 10 ml of acetic acid and 100 ml of water (chloroform-methanol-acetic acid-water 50:39:1:10) also known as Arvidson's solvent (Arvidson, 1968). After added 4 ml of Arvidson's solvent, phospholipids samples were centrifuged (2000 rpm, 4 °C, 60 sec), 1,4 ml of 0.9% NaCl was added and centrifugation was repeated to separate the phases. Upper phase was carefully removed using a disposable glass pipette with vacuum suction and properly discarded while lower (chlorophorm) phase was transferred to small glass tubes and stored at -40 °C until phospholipids analysis.

3.2.5.3. Separation of phospholipids classes with TLC

The samples containing total phospholipid fraction, previously stored at -40 °C were left under the fume hood until reaching the room temperature and then evaporated at 60 °C with nitrogen overflow. Four to six droplets of pure chloroform were added into the samples and then applied to the TLC plate in a manner described above. Solvent containing chloroform, methanol, acetic acid, and water, in the ratios of 100:75:6:2 by volume, was used as the mobile phase. Sufficient time for the migration of lipid samples was allowed. Migration of the mobile phase was approximately 18 cm. The plate was removed from developing chamber and left until dried up. All the mentioned steps were performed in the fume hood. The plate was sprayed with 2,7 – diclorfluorescin (2 % in 96 % ethanol). After plate was dried in the fume hood, detection of phospholipid classes was performed under the UV light on 366 nm by comparison with known standards (Sigma Chemical Co., St Louis. MO, USA). The spots corresponding to PC, PE, PI, and PS were scraped off into glass tubes, 10 ul chloroform and C23:0 (0.6176g/50 ml) as an internal standard was added and used for further quantification of the fatty acids, described later in the text. Using the same method described above, fatty acid composition of phospholipid classes was determined.

3.2.6. Data analysis

The total FA content (g) of the lipid extract was calculated by following formula: Total FA content (extract) = $W_{c23}*(A_{sum}-A_{c23}) / A_{c23}$ where W_{c23} is the amount of added standard (g), A_{sum} the sum peak area of all detected FAs (%) and A_{c23} the peak area corresponding to the standard (%). Further, the total FA content per g sample was calculated: Total FA content (sample) =total FA content (extract) $* V_{solvent} / (V_{extract} * W_s)$ where $V_{solvent}$ represents volume of the solvent, $V_{extract}$ volume of sample taken for GC analysis and W_s weight of the sample (g). Thus, quantity of each fatty acid can be calculated by following formula: FA content (mg/g) = total FA content (sample) $* A_{FA} / (A_{sum}-A_{c23})$ where A_{FA} is the area of peak (%) of the related fatty acid. At the end, percentage of each fatty acid can be calculated by means of this formula: FA content (%) = $A_{FA} * A_{sum} / (A_{sum}-A_{c23})$. Everything mentioned above was calculated by using Microsoft Excel (2016).

3.2.7. Statistical analysis

All the data in this study was subjected to one-way ANOVA followed by Duncan's multiple range test. Software package UNISTAT (London, England) was used and level of significance was set to $P \leq 0.05$.

4. Results

There was no effect of five pre - diets (extreme diets) on fatty acid profile (% of TFA) in slaughtered fish (refer to the tables in **Appendix**). As a base for statistical analysis three major diet groups are chosen. The influence, on total fatty acid profile and individual phospholipid classes (PC, PS, PI, and PE) in Salmon erythrocytes, of diet with inclusion of 1 % EPA and DHA - 1 % Main Diet (MD), 0.2 % EPA and DHA - Low Diet (LD) and 1.7 % EPA and DHA - Commercial control (CC) has been observed. However, the most significant differences are detected in fatty acid composition between 0.2 % EPA and DHA - Low Diet (LD) and 1.7 % EPA and DHA - Commercial control (CC) and those results are presented and discussed.

4.1. Fatty Acid Composition in Salmon erythrocytes

The results show that dietary EPA and DHA are effecting the total fatty acid composition in erythrocytes, particularly the levels of certain fatty acids (**Figure 4.1.a**). As the dietary level of EPA and DHA increases consequently the level of those fatty acids is increasing in the erythrocytes, on the other hand with lower inclusion of EPA and DHA in the diet the levels of C18, C18:1(n-9), C18:2(n-6), as well as C20:4(n-6) as expected, are increasing, with significant difference between both dietary groups (**Table 4.1.**).

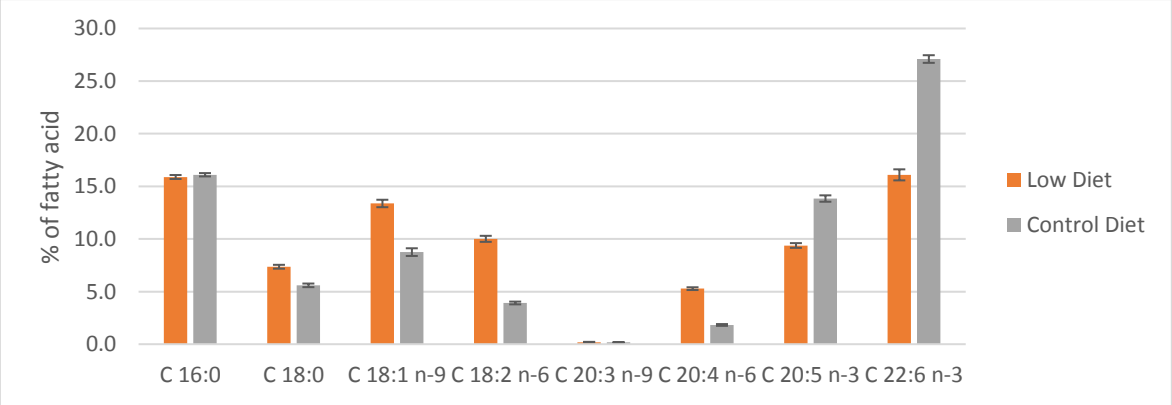
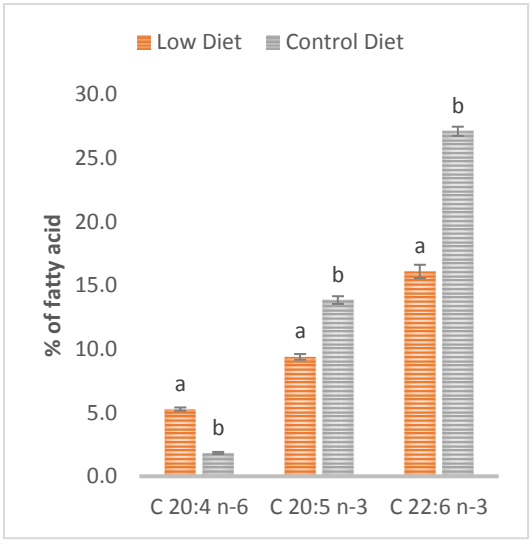


Figure 4.1.a Effect of diet on the level of selected fatty acids in Erythrocytes (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.)

Table 4.1. Effect of diet on the level of selected fatty acids in Erythrocytes (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences ($P \leq 0.05$) between the different dietary groups.

FATTY ACID (% OF TFA)	*LOW DIET* *0,2% EPA + DHA*			*CONTROL DIET* *1,7% EPA+DHA*		
C 16:0	15.89	±	0.19	16.10	±	0.16
C18:1 N-7	1.37	±	0.02[a]	1.83	±	0.04[b]
C 18:0	7.36	±	0.18[a]	5.59	±	0.17[b]
C 18:1 N-9	13.37	±	0.35[a]	8.75	±	0.36[b]
C 20:3 N-9	0.19	±	0.03	0.17	±	0.02
C 18:2 N-6	10.01	±	0.29[a]	3.92	±	0.13[b]
C 20:3 N-6	3.37	±	0.08[a]	0.57	±	0.02[b]
C 20:4 N-6	5.28	±	0.13[a]	1.83	±	0.08[b]
C 20:5 N-3	9.38	±	0.22[a]	13.84	±	0.30[b]
C 22:6 N-3	16.09	±	0.52[a]	27.10	±	0.36[b]



The ratio between C20:4(n-6) and C20:5(n-3) in LD group is 0,6 while in CC group is 0,1.

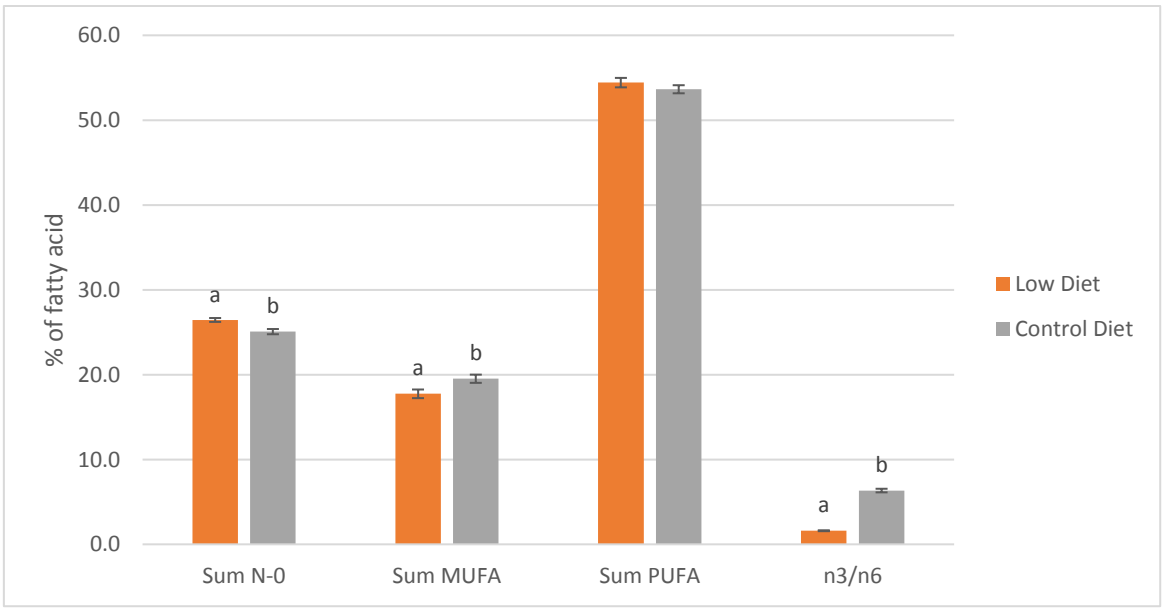


Figure 4.1.b Effect of diet on the sum N-0, sum MUFA, sum PUFA and n-3/n-6 ratio in Erythrocytes (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences ($P \leq 0.05$) between the different dietary groups.

As visible in the **Figure 4.1.b** diets with different level of EPA and DHA influenced the fatty acid composition of Salmon erythrocytes to some extent. Sum N-0 and sum of MUFA are significantly different between both groups and reflect that of a diet. There was no significant difference between dietary groups in percentage of sum PUFA. The n-3/n-6 ratio was significantly different between both dietary groups, as follows 1,6 in LD and 6,3 in CC group as expected.

4.2. Fatty Acid Composition of Individual Phospholipid Classes

The results show that each phospholipid class is characteristically abundant with certain fatty acids regardless of dietary groups. PC contains higher levels of C16:0, C 18:1(n-9) and C22:6(n-3); PS C18:0 and C22:6(n-3); PI C18:0 and C20:4(n-6) and PE C 22:6(n-3). For a better overview refer to the tables in **Appendix**. Although both PS and PI contain C18:0, PI (36 % of TFA) is more abundant with this fatty acid than PS (27 % of TFA). DHA is occurring in all phospholipid classes in order as follows PE>PS>PC>PI. EPA is also occurring in all phospholipid classes in different order PC>PE>PS>PI with lower levels in LD compared with CC. This analogy is also observed in DHA levels. Significant difference in percentages of those characteristic fatty acids have been observed between LD and CC and therefore presented and discussed further (**Figure 4.2.a**; **Figure 4.2.b**.). When observing Sum of N-0 in all phospholipid fractions it can be seen they are distributed as follows PI>PS>PC>PE and how inclusion of dietary EPA and DHA is decreasing, the values of those fatty acids are increasing (**Figure.4.2.1**; **Figure 4.2.2**; **Figure 4.2.3** and **Figure 4.2.4**). Also from figures mentioned above it can be observed that ratio of n-3/n-6 was pointedly reduced in PS and PI compared with other phospholipid fractions. This is consistent with the observation that both PS and PI have higher levels of C18:2(n-6) and C20:4(n-6).

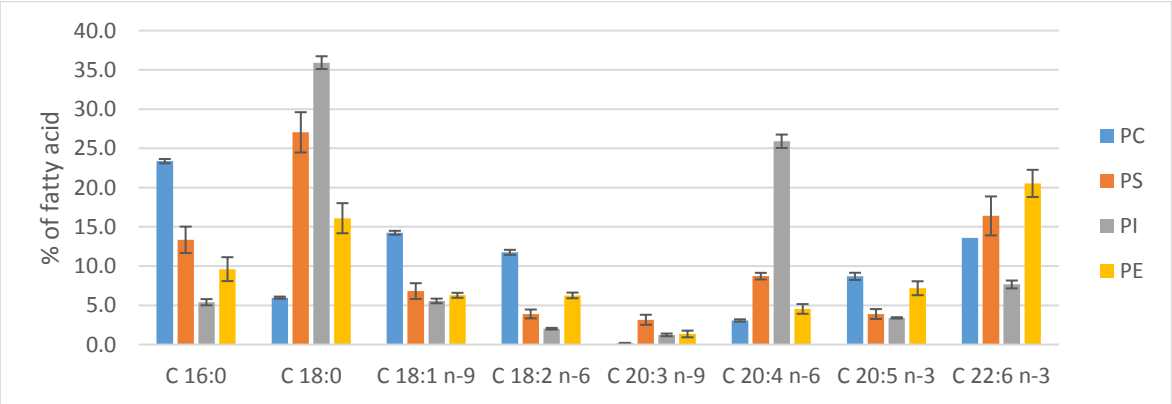


Figure 4.2.a Levels of selected fatty acids in relevant phospholipid classes (% of total fatty acids) in fish fed low diet (0.2 % EPA and DHA). Data are expressed as mean ± standard error mean (S.E.M.)

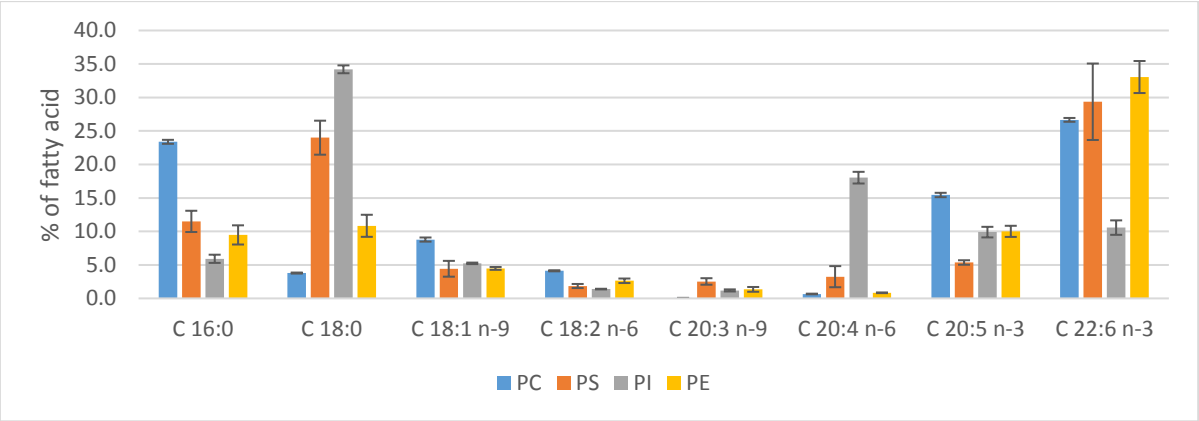


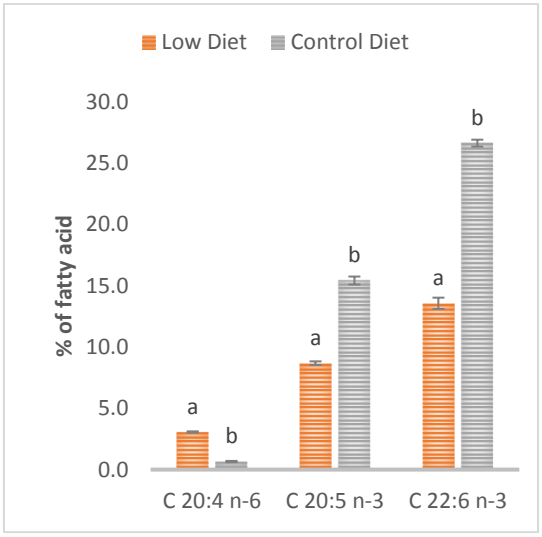
Figure 4.2.b Levels of selected fatty acids in relevant phospholipid classes (% of total fatty acids) in fish fed commercial control diet (1.7 % EPA and DHA). Data are expressed as mean ± standard error mean (S.E.M.)

4.2.1. PC

As mentioned above the most abundant fatty acids in PC fraction are C16:0, C18:1(n-9) and C22:6(n-3). Different dietary EPA and DHA inclusions influenced levels of those fatty acids as it can be seen in **Figure 4.2.a** and **Figure 4.2.b.** Any significant difference in levels of C16:0 is not observed between LD and CC. Significant difference was observed between CC and LD in content of C 18:1(n-9), increasing as inclusion of EPA and DHA in diet is decreasing, from 8,8 % in CC group to 14,2 % of TFA in LD group. As expected, levels of DHA were significantly lower in LD group than in CC group (**Table 4.2.1.**). Also, the lowest level of 20:3(n-9) is observed in this fraction in LD group.

Table 4.2.1. Effect of diet on the level of selected fatty acids in PC (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences (P ≤ 0.05) between the different dietary groups.

FATTY ACID (% OF TFA)	*LOW DIET* *0,2% EPA + DHA*			*CONTROL DIET* *1,7% EPA+DHA*		
C 16:0	23.37	±	0.27	23.38	±	0.30
C18:1 N-7	1.32	±	0.04[a]	1.76	±	0.04[b]
C 18:0	5.98	±	0.14[a]	3.78	±	0.08[b]
C 18:1 N-9	14.24	±	0.25[a]	8.79	±	0.31[b]
C 20:3 N-9	0.17	±	0.02[a]	0.07	±	0.01[b]
C 18:2 N-6	11.75	±	0.32[a]	4.12	±	0.08[b]
C 20:3 N-6	3.26	±	0.09[a]	0.51	±	0.03[b]
C 20:4 N-6	3.07	±	0.05[a]	0.66	±	0.05[b]
C 20:5 N-3	8.69	±	0.15[a]	15.44	±	0.33[b]
C 22:6 N-3	13.57	±	0.46[a]	26.65	±	0.28[b]



The ratio between C20:4(n-6) and C20:5(n-3) in LD group is 0,4 while in CC group is 0,5.

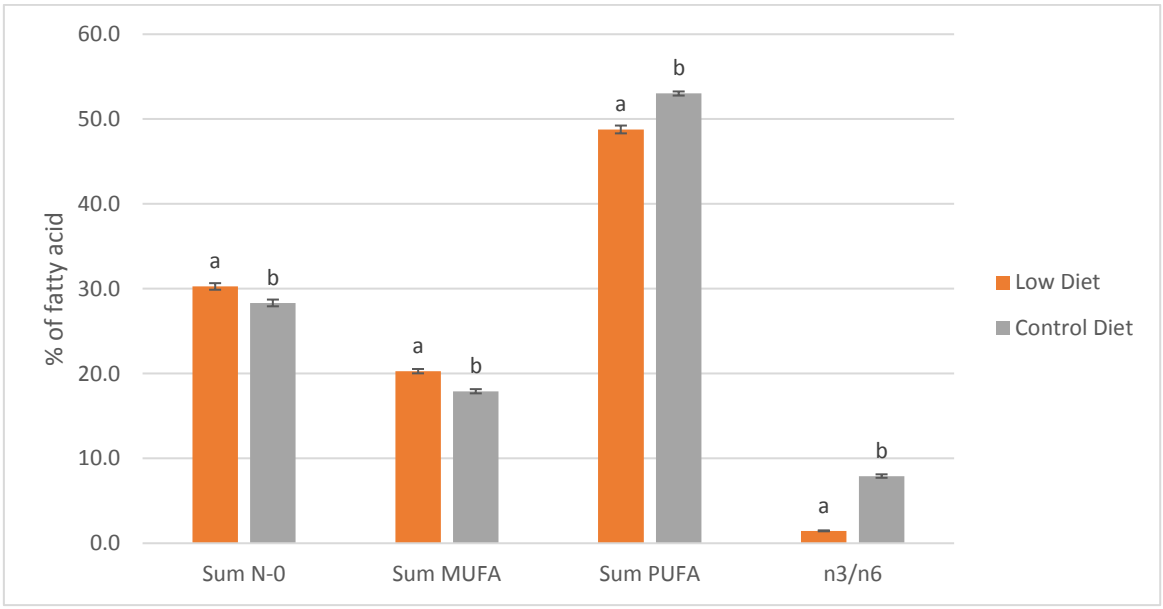


Figure 4.2.1. Effect of diet on the level of sum N-0, sum MUFA, sum PUFA and n-3/n-6 ratio in PC (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences (P ≤ 0.05) between the different dietary groups.

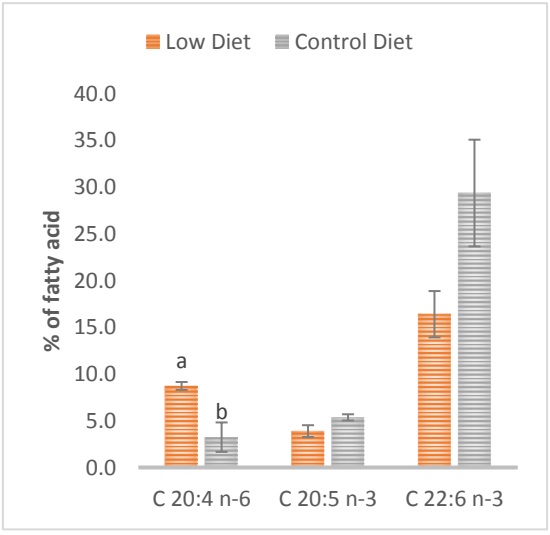
Significant difference is observed between LD and CC in sum N-0, sum PUFA and sum of MUFA and as well in ratio between n-3 and n-6 fatty acids (**Figure 4.2.1.**). As expected the ratio n-3/n-6 was highest in CC group (7,9) and lowest in LD group (1,5). Sum of MUFA is increased in LD group and it does not follow the pattern observed in other phospholipid fractions (being lower in LD than CC group which reflects the diet). Consequently, the sum of PUFA is significantly decreased in LD group.

4.2.2. PS

Effects of the diet on most abundant fatty acids in PS, C18:0 and C22:6(n-3), are shown in **Figure 4.2.a** and Figure **4.2.b.** Accordingly to the analogy mentioned above, the levels of C18 are higher in LD group comparing with CC group. DHA level in PS was lower as inclusion of EPA and DHA was lower in the diet. Also, the highest level of 20:3(n-9) is observed in this fraction comparing to others and as well in higher amount in LD than CC group. Significant differences were observed between LD and CC group in levels of C18:2(n-6) and C20:4(n-6), as well C 20:3(n-6) as expected.

Table 4.2.2. Effect of diet on the level of selected fatty acids in PS (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences ($P \leq 0.05$) between the different dietary groups.

FATTY ACID (% OF TFA)	*LOW DIET* ***0,2% EPA + DHA***	*CONTROL DIET* ***1,7% EPA+DHA***
C 16:0	13.34 ± 1.68	11.50 ± 1.59
C18:1 N-7	0.50 ± 0.21	0.78 ± 0.25
C 18:0	27.04 ± 2.57	23.99 ± 2.54
C 18:1 N-9	6.82 ± 1.00	4.42 ± 1.18
C 20:3 N-9	3.16 ± 0.64	2.53 ± 0.49
C 18:2 N-6	3.90 ± 0.56[a]	1.85 ± 0.31[b]
C 20:3 N-6	2.23 ± 0.57[a]	0.20 ± 0.09[b]
C 20:4 N-6	8.72 ± 0.42[a]	3.24 ± 1.57[b]
C 20:5 N-3	3.89 ± 0.63	5.36 ± 0.33
C 22:6 N-3	16.39 ± 2.48	29.36 ± 5.71



The ratio between C20:4(n-6) and C20:5(n-3) in LD group is 2,2 while in CC group is 0,6.

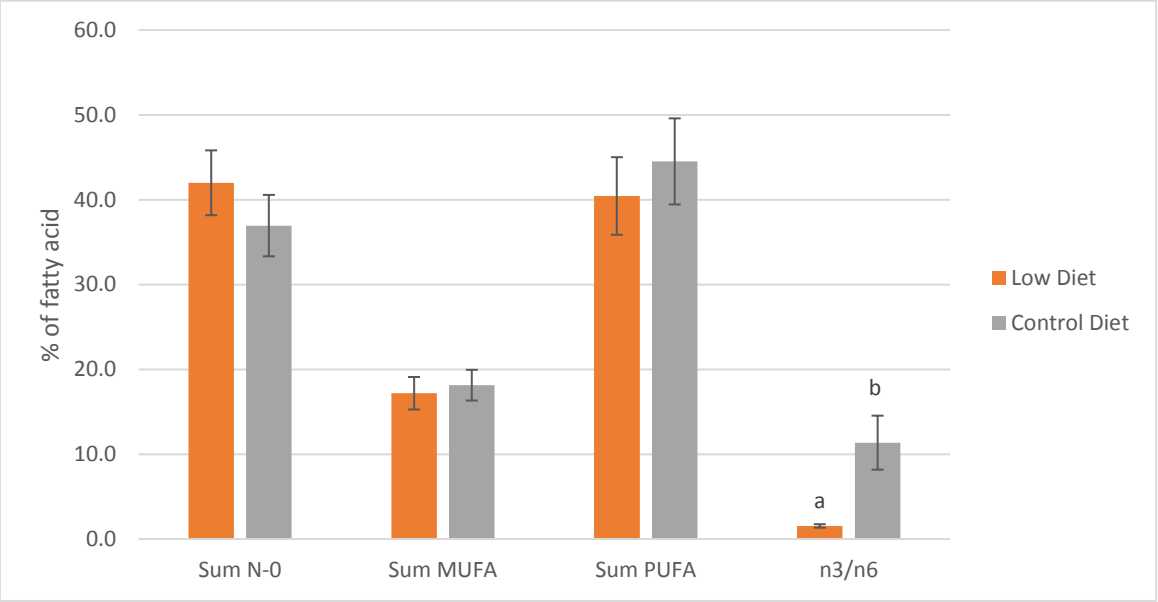


Figure 4.2.2. Effect of diet on the level of sum N-0, sum MUFA, sum PUFA and n-3/n-6 ratio in PS (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences ($P \leq 0.05$) between the different dietary groups.

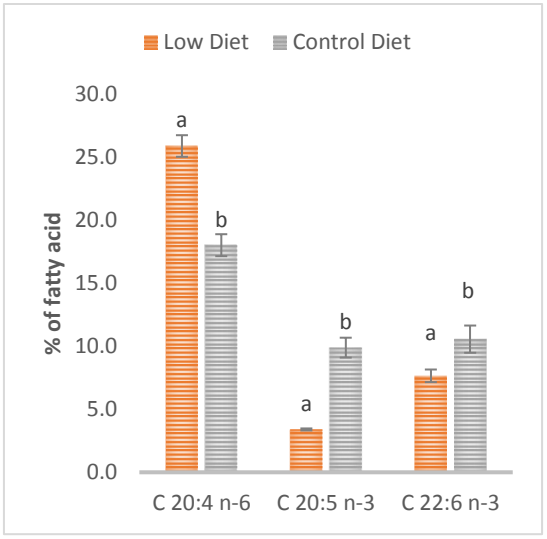
Effect of diet on the level sum N-0, sum MUFA, sum PUFA and n-3/n-6 ratio in PS shown in **Figure 4.2.2.** is significantly different between both diet groups solely in n-3/n-6 ratio, as follows 1,5 in LD and 11,36 in CC group.

4.2.3. PI

PI contains high amount of C18:0 and to the great extent, DHA in PI was replaced with C20:4(n-6). Both fatty acids are elevated when the inclusion of EPA and DHA in dietary lipid is decreased (**Figure 4.2.a** and **Figure 4.2.b**). From all the phospholipid classes PI is least abundant with EPA and DHA and most with AA, which is also reflected on n-3/n-6 ratio.

Table 4.2.3. Effect of diet on the level of selected fatty acids in PI (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences (P ≤ 0.05) between the different dietary groups.

FATTY ACID (% OF TFA)	*LOW DIET* *0,2% EPA + DHA*			*CONTROL DIET* *1,7% EPA+DHA*		
C 16:0	5.40	±	0.39	5.91	±	0.61
C18:1 N-7	0.69	±	0.07[a]	1.18	±	0.04[b]
C 18:0	35.92	±	0.81	34.20	±	0.59
C 18:1 N-9	5.56	±	0.30	5.23	±	0.11
C 20:3 N-9	1.24	±	0.17	1.19	±	0.16
C 18:2 N-6	2.02	±	0.11[a]	1.39	±	0.05[b]
C 20:3 N-6	2.76	±	0.19[a]	1.78	±	0.09[b]
C 20:4 N-6	25.90	±	0.86[a]	18.03	±	0.87[b]
C 20:5 N-3	3.40	±	0.08[a]	9.89	±	0.79[b]
C 22:6 N-3	7.66	±	0.50[a]	10.57	±	1.08[b]



The ratio between C20:4(n-6) and C20:5(n-3) in LD group is 7,6 while in CC group is 1,8.

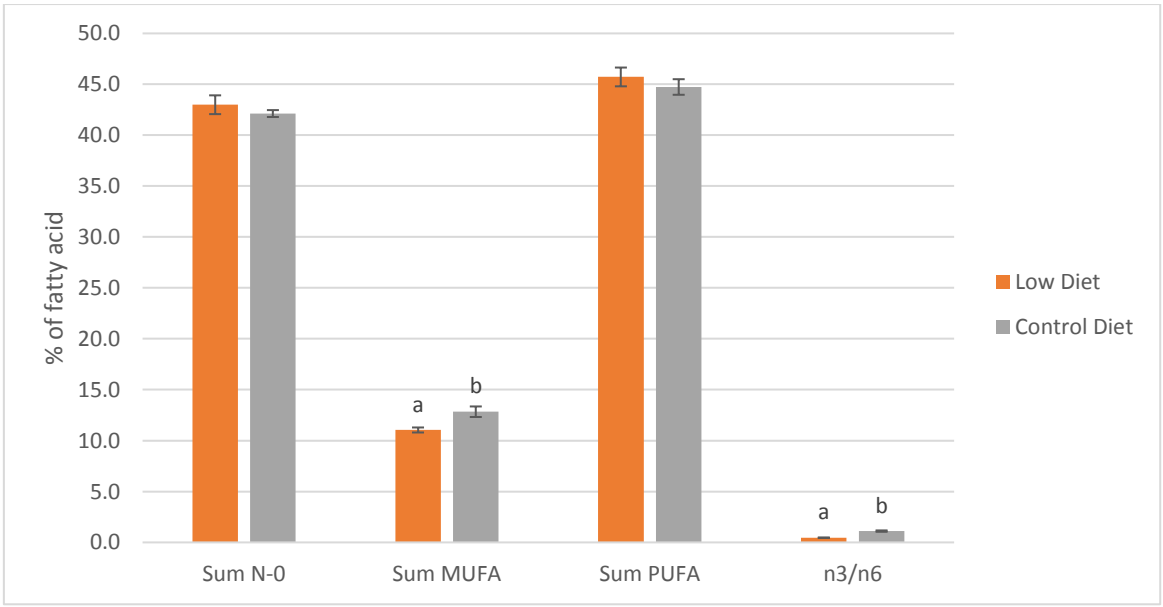


Figure 4.2.3. Effect of diet on the level of sum N-0, sum MUFA, sum PUFA and n-3/n-6 ratio in PI (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences (P ≤ 0.05) between the different dietary groups.

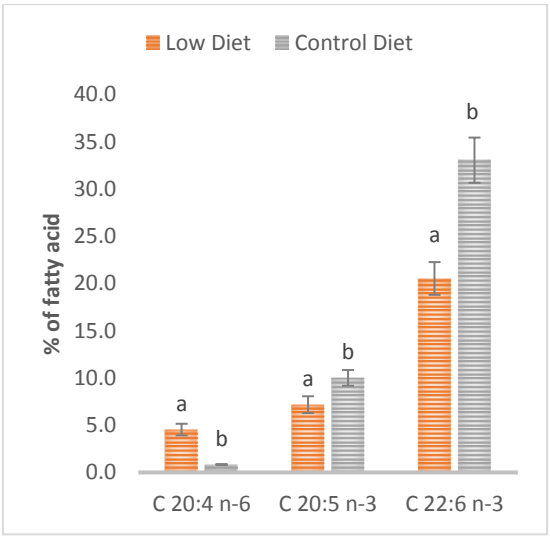
Considering sum N-0 no significant difference is between LD and CC group. Sum of MUFA is significantly different in both diet groups, 11 % in LD and 12,8 % of TFA in CC. There is no significant difference between LD and CC regarding the sum of PUFAs. However, n-3/n-6 ratio in both diet groups is low, in LD only 0,5 and in CC 1,1.

4.2.4. PE

From all phospholipid classes, PE is most abundant with C 22:6(n-3). Values of DHA are decreasing as the levels of dietary EPA and DHA (**Figure 4.2.a** and Figure **4.2.b**).

Table 4.2.4. Effect of diet on the level of selected fatty acids in PE (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences ($P \leq 0.05$) between the different dietary groups.

FATTY ACID (% OF TFA)	*LOW DIET* *0,2% EPA + DHA*			*CONTROL DIET* *1,7% EPA+DHA*		
C 16:0	9.61	±	1.52	9.47	±	1.43
C18:1 N-7	1.19	±	0.05[a]	1.87	±	0.08[b]
C 18:0	16.10	±	1.92[a]	10.84	±	1.65[b]
C 18:1 N-9	6.28	±	0.31[a]	4.47	±	0.21[b]
C 20:3 N-9	1.35	±	0.43	1.35	±	0.36
C 18:2 N-6	6.26	±	0.36[a]	2.63	±	0.34[b]
C 20:3 N-6	4.22	±	0.50[a]	0.62	±	0.05[b]
C 20:4 N-6	4.54	±	0.62[a]	0.84	±	0.05[b]
C 20:5 N-3	7.18	±	0.89[a]	10.01	±	0.83[b]
C 22:6 N-3	20.53	±	1.73[a]	33.05	±	2.39[b]



The ratio between C20:4(n-6) and C20:5(n-3) in LD group is 0,6 while in CC group is 0,08.

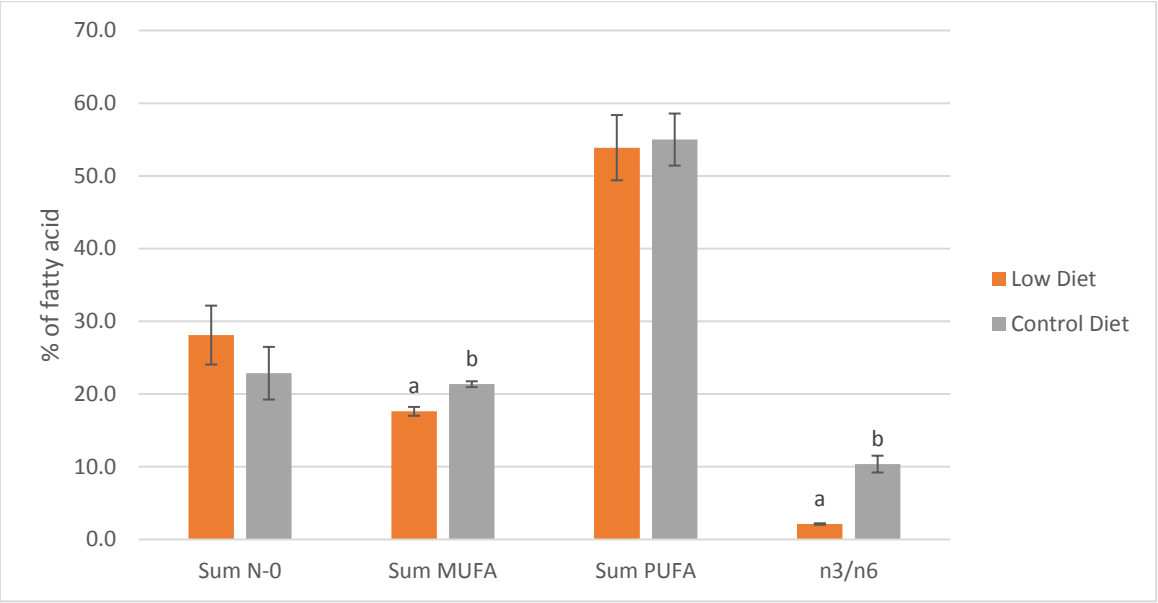


Figure 4.2.4. Effect of diet on the level of sum N-0, sum MUFA, sum PUFA and n-3/n-6 ratio in PE (% of total fatty acids). Data are expressed as mean ± standard error mean (S.E.M.), n=15. Different letters indicate significant differences ($P \leq 0.05$) between the different dietary groups.

There is no significant difference between sum N-0 and sum of PUFAs between diet groups. Sum of MUFA are significantly different between LD and CC group. Ratio of n-3 and n-6 is significantly different between both diet groups. Value for those ratios are, in LD 2,1 and in CC 10,4.

5. Discussion and Conclusion

Fatty acid composition of total lipid fraction as well as the fatty acid profile of individual phospholipid classes (PC, PS, PI, and PE) in Atlantic salmon erythrocytes are influenced by different dietary inclusions of EPA and DHA combined. Erythrocytes are better biomarker of the PUFA status as they reflect longer-term intake than circulating lipids in plasma or serum (Arab, 2003). However, the life span of fish erythrocytes is 80-500 days (Fischer et al., 1998) so this could explain why there was no considerable effect of five pre-diets on fatty acid composition. Thus, it was chosen to focus on the significant effect of main diets.

Significant differences are observed in concentrations of most fatty acids of erythrocytes between fish fed low diet - 0,2 % EPA and DHA (LD) and commercial control diet - 1,7 % EPA and DHA (CC), with a different response among individual phospholipid classes (PC, PS, PI and PE). As level of inclusion of EPA and DHA in the diet is lower the same fatty acids are decreasing in total fatty acid profile of erythrocytes and erythrocyte phospholipid classes.

Dietary level of EPA and DHA also significantly influenced n-3/n-6 ratio. Mentioned ratio was significantly reduced in PS and PI in fish fed LD while in fish fed CC diet all PL classes, with exclusion of PI, still more or less maintain characteristic ratio for marine species (Henderson and Tocher, 1987). It is unavoidable to alter the n-3/n-6 ratio when using vegetable oils and plant proteins in fish diet as an alternative to fish oil (FO). Torstensen et al. (2011) reported increased percentages of C18:3(n-3), C18:2(n-6) and 18:1(n-9) and decrease in EPA and DHA in Atlantic salmon fed with high levels of FO alternative. Consequently, high levels of n-6 fatty acids decreased the n-3/n-6 ratio.

Decreasing the inclusion of EPA and DHA in the diet and simultaneously elevating pro-inflammatory n-6, in this study significantly 20-carbon (n-6), fatty acids, in the individual phospholipid classes as well total lipid fraction in erythrocytes, may disturb the balance of inflammatory system.

Observing sum of N-0, MUFA and PUFA in general, it can be summarised that erythrocytes as well as all the individual phospholipid classes excluding PC reflect that of a diet. It seems that in PC as a compensation to reduced level of EPA and DHA, MUFAs are incorporated rather than C20:4(n-6). This analogy is also reported in muscle PC of Atlantic salmon upon the depletion of n-3 essential fatty acids by Thomassen et al. (2016).

Further, mead acid (C20:3 n-9) was detected in erythrocytes as well in all phospholipid fractions, with higher levels in LD than CC, being most abundant in PS.

Sargent et al. (1989) suggested that the ratio of C20:3(n-9)/C20:5(n-3) in polar lipids from the liver in rainbow trout might be an indicator of EFA deficiency if it is greater than 0.4, although mentioned ratio in this study was higher than 0.4 in both dietary groups (results not shown) by further observation it can be seen that C20:3(n-9) detected in erythrocytes and relevant phospholipid classes originates via desaturation and elongation of the high dietary level of C18:1 (n-9). Thus, mead acid cannot be defined as an indicator of EFA deficiency in this study; this is consistent with findings of Sissener et al. (2016) in the erythrocytes in Atlantic salmon in seawater.

Although arachidonic acid (AA) was present in all phospholipid fractions, the highest concentration is observed in PI fraction. This is consistent with Thomassen et al. (2016) founding in liver, muscle, heart and intestine in Atlantic salmon fry and as well reported in other fish species and mammals (Bell et all, 1985; Bell et al., 1995). The presence of increased levels of AA could be explained by Δ 6- and Δ 5- desaturase activity; elongation, and desaturation of C18:2(n-6) or as a compensation for the reduction in EPA and DHA. Fish fed LD with inclusion of 0,2 % EPA and DHA significantly elevated levels of C20:4(n-6) leading to higher ratio between C20:4(n-6) and C20:5(n-3) and consequently lower between n-3 and n-6 PUFA. The same analogy was recognised in juvenile turbot (Bell et al., 1995) and Atlantic salmon during the seawater production cycle (Sissener et al., 2016). In number of fish species (Anderson et al., 1981; Tocher and Sargent, 1987; Bell et al.,1994) as well in human (Lands et al.,1992) and rats (Lands et al.,1992) the competition between C20:4(n-6) and C20:5(n-3) for the cytosolic enzymes was observed and reported a preference for C20:4(n-6) as a substrate for eicosanoid production, even though dietary EPA was present.

As already mentioned in this study, three PUFAs: EPA, DHA and AA have important biological activities in fish as in other vertebrates, maintaining the structure and by that fluidity of membranes, and acting as precursors for highly biologically active compounds (eicosanoids). Studies on this matter therefore suggest that ratio between C20:4(n-6) and C20:5(n-3) should be of relevance when observing changes in lipid metabolism during smoltification process and transfer to the sea water in Atlantic salmon. The optimal ratio of C20:4(n-6) and C20:5(n-3) in mammals is not known, on the other hand recommended dietary ratio of n-6/n-3 PUFA is 5:1 and probably resembles approximately optimal ratio between C20:4(n-6) and C20:5(n-3) in tissue. Knowledge about this in fish is even more vague, nonetheless fish tissue ratio between C20:4(n-6) and C20:5(n-3) is significantly lower than that of mammals (Bell et al, 1997.; Sargent et al, 1999.).

Since EPA, DHA and AA, as well as they precursors or products, compete for the same enzymes in biochemical and physiological reactions requirement for any of these fatty acids cannot be considered solely. Therefore, it is suggested that in addition to EPA and DHA requirement, dietary requirement of AA should be as well established and EPA/DHA/AA ratio should be observed (Sargent et al, 1999).

In this study erythrocytes are shown to be a good indicator of how certain fatty acids are incorporated to phospholipid fractions under dietary influence, however to obtain better results, it is suggested that blood samples should be taken more frequently and in living fish. Using method called cannulation, improved in the recent years, blood samples could be taken more frequently while fish is swimming and moving around without any negative consequences on fish welfare (Djordjevic et al, 2012).

AA derived eicosanoids in general seem to be produced in response to stressful situations, both at a cellular and whole body level, however, to which extent potent eicosanoid formation in erythrocytes influenced fish health, i.e. inflammatory status in fish subjected in this study is not known and it could be only of speculative nature. Nevertheless, this could be a possible matter of research in future.

On the other hand, in same fish subjected in this study, group of researchers revealed that diet of 1% EPA and DHA or lower (0,2 % EPA and DHA) had negative influence on fish health. It has been reported that fish fed mentioned above levels EPA and DHA combined died in significantly greater number (50 % and 60 %) than fish fed 1,7 % EPA and DHA (CC) when subjected to stress. Also, low levels of EPA and DHA in the diet resulted in reduced levels of astaxanthin in the muscle, increased amount of fat in the liver, enlargement of intervertebral spaces in the spine and histological changes in the middle intestine (Ruyter et al, 2015).

Keeping all the information obtained in this study on mind, it can be concluded that inclusion of 0,2 % EPA and DHA (0.4 % of total fatty acids) in the diet is not sufficient to maintain DHA status in erythrocytes and individual phospholipid classes from erythrocyte membrane (PC, PS, PI, and PE) during the sea water production cycle despite the presence of dietary 18:3(n-3).

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8. Appendix

8.1. The fatty acid composition of erythrocytes

Table 8.1.a Fatty acid profile of Salmon erythrocytes (% of total fatty acids) fed with main diet (1 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	1% Main Diet																
	Pre-Diet 0%			**Pre-Diet 2% DHA**			**Pre-Diet 2% EPA**			**Pre-Diet 2% EPA +DHA**			**Com cont**		
C 14:0	**0.73**	±	0.12	**0.75**	±	0.03	**0.82**	±	0.20	**0.75**	±	0.07	**0.48**	±	0.25
C 16:0	**17.06**	±	0.20	**16.86**	±	0.20	**16.60**	±	0.25	**16.73**	±	0.20	**16.50**	±	0.40
C 18:0	**5.89**	±	0.40	**6.11**	±	0.17	**5.76**	±	0.52	**7.55**	±	0.10	**6.86**	±	0.27
C 22:0	**1.47**	±	0.09	**1.48**	±	0.17	**1.78**	±	0.14	**1.59**	±	0.22	**1.79**	±	0.10
Sum N-0	**25.93**	±	0.28	**26.40**	±	0.16	**25.91**	±	0.86	**26.89**	±	0.02	**26.29**	±	0.76
C 16:1 n-7	**1.58**	±	0.03	**1.28**	±	0.06	**1.27**	±	0.15	**0.64**	±	0.53	**1.28**	±	0.20
C 18:1 n-11	**0.51**	±	0.02	**0.46**	±	0.04	**0.48**	±	0.06	**0.38**	±	0.04	**0.43**	±	0.05
C 18:1 n-9	**11.20**	±	0.27	**11.71**	±	0.85	**11.40**	±	1.45	**9.90**	±	1.08	**10.29**	±	1.16
C 18:1 n-7	**1.78**	±	0.12	**1.50**	±	0.03	**1.63**	±	0.05	**1.51**	±	0.06	**1.56**	±	0.05
C 20:1 n-11	**0.29**	±	0.06	**0.43**	±	0.09	**0.31**	±	0.05	**0.22**	±	0.03	**0.31**	±	0.05
C 20:1 n-9	**2.41**	±	0.20	**2.58**	±	0.20	**2.81**	±	0.17	**2.49**	±	0.03	**1.92**	±	0.97
C 22:1 n-11	**1.33**	±	0.25	**1.39**	±	0.11	**1.49**	±	0.12	**1.23**	±	0.26	**1.37**	±	0.22
C 22:1 n-9	**0.29**	±	0.04	**0.29**	±	0.01	**0.29**	±	0.02	**0.22**	±	0.10	**0.32**	±	0.04
SUM MUFA	**20.00**	±	0.54	**20.69**	±	0.64	**20.94**	±	2.45	**17.05**	±	0.93	**18.12**	±	2.28
C 18:2 n-6	**6.22**	±	0.27	**6.39**	±	0.05	**6.25**	±	0.52	**5.88**	±	0.32	**6.18**	±	0.47
C 18:3 n-6	**0.16**	±	0.00	**0.18**	±	0.03	**0.10**	±	0.05	**0.00**	±	0.00	**0.08**	±	0.05
C 18:3 n-3	**2.90**	±	0.32	**2.69**	±	0.12	**2.79**	±	0.33	**1.24**	±	0.78	**2.73**	±	0.25
C 20:4 n-3	**0.22**	±	0.01	**0.28**	±	0.02	**0.33**	±	0.02	**0.23**	±	0.00	**0.28**	±	0.03
C 20:1 n-9	**2.41**	±	0.20	**2.58**	±	0.20	**2.81**	±	0.17	**2.49**	±	0.03	**1.92**	±	0.97
C 20:2 n-6	**0.87**	±	0.06	**0.82**	±	0.04	**0.95**	±	0.06	**1.16**	±	0.15	**0.94**	±	0.05
C 20:3 n-9	**0.09**	±	0.05	**0.11**	±	0.01	**0.11**	±	0.06	**0.46**	±	0.09	**0.26**	±	0.05
C 20:3 n-6	**1.15**	±	0.09	**1.21**	±	0.02	**1.28**	±	0.05	**1.04**	±	0.12	**1.26**	±	0.05
C 20:4 n-6	**2.77**	±	0.32	**2.96**	±	0.14	**2.52**	±	0.27	**3.38**	±	0.04	**3.04**	±	0.11
C 20:3 n-3	**0.59**	±	0.03	**0.51**	±	0.04	**0.60**	±	0.03	**0.69**	±	0.02	**0.62**	±	0.05
C 20:5 n-3	**10.94**	±	0.14	**12.02**	±	1.39	**12.56**	±	0.33	**11.96**	±	1.16	**13.06**	±	0.49
C 22:5 n-3	**3.17**	±	0.16	**3.29**	±	0.14	**3.55**	±	0.21	**3.71**	±	0.51	**3.65**	±	0.17
C 22:6 n-3	**22.59**	±	1.03	**21.01**	±	1.45	**20.73**	±	2.18	**25.46**	±	0.99	**22.33**	±	1.68
SUM PUFA	**51.87**	±	1.12	**51.75**	±	0.61	**52.09**	±	1.82	**55.14**	±	1.31	**54.43**	±	1.41
n3/n6	**3.57**	±	0.03	**3.38**	±	0.07	**3.60**	±	0.29	**3.75**	±	0.19	**3.69**	±	0.29
Others	**1.60**	±	0.24	**2.53**	±	0.43	**2.52**	±	0.54	**0.66**	±	0.15	**1.33**	±	0.44

Table 8.1.b Fatty acid profile of Salmon erythrocytes (% of total fatty acids) fed with low diet (0,2 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	Low diet														
	Pre-Diet 0%			**Pre-diet 2% DHA**			**Pre-Diet 2 % EPA**			**Pre-Diet 2 % EPA+DHA**			**Com cont**		
C 14:0	**0.17**	±	0.09	**0.16**	±	0.08	**0.19**	±	0.10	**0.24**	±	0.12	**0.29**	±	0.04
C 16:0	**15.11**	±	0.72	**16.06**	±	0.25	**15.64**	±	0.16	**16.37**	±	0.12	**16.25**	±	0.32
C 18:0	**6.81**	±	0.51	**7.58**	±	0.54	**7.68**	±	0.24	**7.73**	±	0.10	**7.01**	±	0.44
C 22:0	**2.33**	±	0.16	**2.25**	±	0.10	**2.56**	±	0.17	**2.45**	±	0.08	**2.21**	±	0.07
Sum N-0	**25.60**	±	0.65	**26.62**	±	0.57	**26.64**	±	0.51	**27.13**	±	0.16	**26.25**	±	0.37
C 16:1 n-7	**0.83**	±	0.12	**0.79**	±	0.19	**0.46**	±	0.23	**0.66**	±	0.04	**0.70**	±	0.04
C 18:1 n-11	**0.03**	±	0.03	**0.00**	±	0.00	**0.03**	±	0.03	**0.00**	±	0.00	**0.02**	±	0.02
C 18:1 n-9	**13.95**	±	0.26	**13.09**	±	1.29	**12.72**	±	0.73	**13.03**	±	0.62	**14.08**	±	1.00
C 18:1 n-7	**1.41**	±	0.01	**1.40**	±	0.03	**1.34**	±	0.06	**1.31**	±	0.01	**1.38**	±	0.01
C 20:1 n-11	**0.42**	±	0.05	**0.48**	±	0.14	**0.44**	±	0.03	**0.59**	±	0.02	**0.55**	±	0.10
C 20:1 n-9	**1.08**	±	0.06	**1.08**	±	0.03	**1.01**	±	0.06	**1.06**	±	0.07	**1.15**	±	0.05
C 22:1 n-11	**0.00**	±	0.00	**0.12**	±	0.06	**0.14**	±	0.08	**0.11**	±	0.11	**0.09**	±	0.05
C 22:1 n-9	**0.03**	±	0.03	**0.06**	±	0.06	**0.13**	±	0.02	**0.00**	±	0.00	**0.09**	±	0.05
SUM MUFA	**19.07**	±	1.09	**17.51**	±	1.74	**16.63**	±	0.97	**17.17**	±	0.75	**18.33**	±	1.23
C 18:2 n-6	**9.71**	±	0.30	**9.36**	±	0.97	**10.25**	±	0.27	**10.23**	±	0.35	**10.50**	±	1.10
C 18:3 n-6	**0.40**	±	0.13	**0.32**	±	0.08	**0.28**	±	0.02	**0.42**	±	0.03	**0.37**	±	0.09
C 18:3 n-3	**3.12**	±	0.08	**2.86**	±	0.37	**3.21**	±	0.13	**2.91**	±	0.08	**3.28**	±	0.20
C 20:4 n-3	**0.00**	±	0.00	**0.00**	±	0.00	**0.15**	±	0.15	**0.00**	±	0.00	**0.04**	±	0.04
C 20:1 n-9	**1.08**	±	0.06	**1.08**	±	0.03	**1.01**	±	0.06	**1.06**	±	0.07	**1.15**	±	0.05
C 20:2 n-6	**1.53**	±	0.13	**1.32**	±	0.09	**1.29**	±	0.10	**1.47**	±	0.01	**1.32**	±	0.08
C 20:3 n-9	**0.12**	±	0.07	**0.21**	±	0.06	**0.20**	±	0.02	**0.34**	±	0.03	**0.09**	±	0.06
C 20:3 n-6	**3.34**	±	0.23	**3.28**	±	0.06	**3.28**	±	0.06	**3.72**	±	0.18	**3.22**	±	0.24
C 20:4 n-6	**4.96**	±	0.50	**5.49**	±	0.36	**5.34**	±	0.12	**5.60**	±	0.04	**5.00**	±	0.17
C 20:3 n-3	**0.86**	±	0.09	**0.77**	±	0.10	**0.83**	±	0.06	**0.65**	±	0.08	**0.77**	±	0.12
C 20:5 n-3	**8.48**	±	0.51	**9.48**	±	0.37	**10.21**	±	0.59	**9.63**	±	0.37	**9.10**	±	0.22
C 22:5 n-3	**3.64**	±	0.08	**3.70**	±	0.28	**3.85**	±	0.13	**3.66**	±	0.12	**3.47**	±	0.37
C 22:6 n-3	**15.51**	±	1.01	**17.48**	±	1.52	**15.84**	±	0.50	**15.90**	±	1.12	**15.72**	±	1.88
SUM PUFA	**52.67**	±	1.93	**54.98**	±	1.23	**55.61**	±	0.69	**55.05**	±	1.04	**53.81**	±	1.11
n3/n6	**1.56**	±	0.03	**1.71**	±	0.15	**1.63**	±	0.07	**1.51**	±	0.10	**1.59**	±	0.22
Others	**3.47**	±	1.82	**1.76**	±	0.10	**1.81**	±	0.37	**1.28**	±	0.30	**1.70**	±	0.24

Table 8.1.c Fatty acid profile of Salmon erythrocytes (% of total fatty acids) fed with control diet (1,7 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	Control diet														
	Pre-Diet 0%			**Pre-diet 2% DHA**			**Pre-Diet 2 % EPA**			**Pre-Diet 2 % EPA+DHA**			**Com cont**		
C 14:0	**0.93**	±	0.07	**0.80**	±	0.14	**0.43**	±	0.22	**1.30**	±	0.94	**0.90**	±	0.08
C 16:0	**16.17**	±	0.31	**15.92**	±	0.18	**16.79**	±	0.26	**15.69**	±	0.51	**15.94**	±	0.23
C 18:0	**4.74**	±	0.47	**5.62**	±	0.37	**6.15**	±	0.10	**5.75**	±	0.33	**5.70**	±	0.07
C 22:0	**1.03**	±	0.03	**1.11**	±	0.03	**0.99**	±	0.11	**1.16**	±	0.06	**1.03**	±	0.03
Sum N-0	**24.11**	±	0.69	**24.48**	±	0.06	**25.79**	±	0.18	**25.78**	±	1.05	**25.19**	±	0.75
C 16:1 n-7	**1.71**	±	0.22	**1.48**	±	0.20	**1.29**	±	0.10	**1.23**	±	0.15	**1.60**	±	0.02
C 18:1 n-11	**0.60**	±	0.04	**0.67**	±	0.10	**0.47**	±	0.02	**0.59**	±	0.04	**0.65**	±	0.03
C 18:1 n-9	**9.21**	±	1.04	**9.08**	±	0.88	**8.57**	±	1.32	**8.29**	±	0.82	**8.60**	±	0.24
C 18:1 n-7	**1.77**	±	0.04	**1.94**	±	0.10	**1.70**	±	0.06	**1.82**	±	0.14	**1.92**	±	0.02
C 20:1 n-11	**0.14**	±	0.08	**0.16**	±	0.03	**0.19**	±	0.02	**0.06**	±	0.06	**0.16**	±	0.01
C 20:1 n-9	**3.54**	±	0.05	**3.95**	±	0.11	**3.31**	±	0.39	**3.81**	±	0.29	**3.86**	±	0.14
C 22:1 n-11	**1.94**	±	0.07	**2.08**	±	0.25	**1.60**	±	0.26	**1.99**	±	0.16	**1.97**	±	0.10
C 22:1 n-9	**0.54**	±	0.12	**0.43**	±	0.04	**0.39**	±	0.05	**0.37**	±	0.03	**0.32**	±	0.02
SUM MUFA	**20.09**	±	1.34	**20.40**	±	1.58	**18.22**	±	0.48	**19.03**	±	1.44	**19.86**	±	0.18
C 18:2 n-6	**4.26**	±	0.52	**4.02**	±	0.20	**3.90**	±	0.39	**3.65**	±	0.15	**3.75**	±	0.13
C 18:3 n-6	**0.27**	±	0.15	**0.00**	±	0.00	**0.03**	±	0.03	**0.19**	±	0.19	**0.00**	±	0.00
C 18:3 n-3	**0.96**	±	0.17	**0.99**	±	0.09	**0.92**	±	0.07	**0.92**	±	0.06	**1.00**	±	0.01
C 20:4 n-3	**0.38**	±	0.05	**0.27**	±	0.02	**0.42**	±	0.03	**0.33**	±	0.05	**0.30**	±	0.01
C 20:1 n-9	**3.54**	±	0.05	**3.95**	±	0.11	**3.31**	±	0.39	**3.81**	±	0.29	**3.86**	±	0.14
C 20:2 n-6	**0.67**	±	0.04	**0.80**	±	0.04	**0.70**	±	0.02	**0.71**	±	0.06	**0.82**	±	0.01
C 20:3 n-9	**0.12**	±	0.06	**0.20**	±	0.02	**0.21**	±	0.03	**0.15**	±	0.08	**0.15**	±	0.08
C 20:3 n-6	**0.59**	±	0.06	**0.56**	±	0.02	**0.63**	±	0.04	**0.56**	±	0.05	**0.53**	±	0.04
C 20:4 n-6	**1.68**	±	0.39	**1.80**	±	0.03	**2.09**	±	0.22	**1.80**	±	0.06	**1.78**	±	0.05
C 20:3 n-3	**0.13**	±	0.07	**0.25**	±	0.01	**0.22**	±	0.02	**0.27**	±	0.01	**0.30**	±	0.02
C 20:5 n-3	**13.72**	±	0.73	**14.55**	±	0.39	**13.61**	±	1.23	**13.30**	±	0.51	**14.01**	±	0.55
C 22:5 n-3	**3.44**	±	0.25	**3.96**	±	0.21	**3.90**	±	0.22	**3.67**	±	0.32	**3.66**	±	0.10
C 22:6 n-3	**26.70**	±	0.63	**26.79**	±	1.38	**28.39**	±	0.70	**26.10**	±	0.47	**27.49**	±	0.16
SUM PUFA	**53.17**	±	0.80	**54.22**	±	1.58	**55.02**	±	0.62	**52.02**	±	0.99	**53.79**	±	0.94
n3/n6	**6.10**	±	0.81	**6.34**	±	0.34	**6.41**	±	0.76	**6.18**	±	0.35	**6.66**	±	0.05
Others	**2.13**	±	0.37	**1.68**	±	0.45	**2.13**	±	0.05	**3.12**	±	0.79	**2.39**	±	0.62

8.2. The fatty acid composition of PC

Table 8.2.a Fatty acid profile of Salmon erythrocytes PC (% of total fatty acids) fed with main diet (1 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

1% MAIN DIET

	Pre-Diet 0%	Pre-Diet 2% DHA	Pre-Diet 2% EPA	Pre-Diet 2% EPA +DHA	Com cont
C 14:0	**0.74** ± 0.04	**0.70** ± 0.06	**0.81** ± 0.10	**0.70** ± 0.11	**0.74** ± 0.06
C 16:0	**24.82** ± 0.74	**23.38** ± 0.51	**23.29** ± 0.74	**24.28** ± 0.01	**24.42** ± 0.67
C 18:0	**4.76** ± 0.15	**4.88** ± 0.29	**4.85** ± 0.31	**5.36** ± 0.46	**5.18** ± 0.06
C 22:0	**0.15** ± 0.01	**0.16** ± 0.01	**0.16** ± 0.02	**0.19** ± 0.02	**0.12** ± 0.01
SUM N-0	**30.86** ± 0.79	**29.44** ± 0.26	**29.50** ± 0.93	**30.89** ± 0.35	**30.85** ± 0.66
C 16:1 N-7	**1.35** ± 0.07	**1.35** ± 0.21	**1.21** ± 0.10	**1.07** ± 0.15	**1.23** ± 0.14
C 18:1 N-11	**0.00** ± 0.00	**0.00** ± 0.00	**0.00** ± 0.00	**0.00** ± 0.00	**0.20** ± 0.10
C 18:1 N-9	**11.79** ± 1.08	**11.57** ± 0.20	**12.22** ± 1.12	**10.81** ± 0.92	**10.54** ± 0.31
C 18:1 N-7	**1.55** ± 0.05	**1.31** ± 0.07	**1.59** ± 0.02	**1.55** ± 0.11	**1.58** ± 0.01
C 20:1 N-11	**0.30**	**0.40** ± 0.07	**0.26** ± 0.02	**0.21** ± 0.03	**0.26** ± 0.02
C 20:1 N-9	**2.02** ± 0.13	**2.10** ± 0.32	**2.42** ± 0.11	**2.33** ± 0.13	**2.31** ± 0.07
C 22:1 N-11	**1.16** ± 0.06	**1.21** ± 0.10	**1.35** ± 0.08	**1.24** ± 0.01	**1.28** ± 0.06
C 22:1 N-9	**N.D.**	**N.D.**	**0.27** ± 0.27	**0.70** ± 0.01	**0.73** ± 0.01
SUM MUFA	**18.66** ± 1.01	**18.45** ± 0.48	**20.19** ± 0.74	**18.99** ± 0.94	**19.17** ± 0.32
C 18:2 N-6	**6.74** ± 0.19	**7.04** ± 0.33	**6.89** ± 0.38	**6.75** ± 0.36	**6.96** ± 0.37
C 18:3 N-6	**0.15** ± 0.01	**0.17** ± 0.03	**0.12** ± 0.01	**0.09** ± 0.00	**0.12** ± 0.01
C 18:3 N-3	**2.47** ± 0.17	**2.35** ± 0.19	**2.48** ± 0.18	**2.35** ± 0.20	**2.44** ± 0.08
C 20:2 N-6	**0.78** ± 0.04	**0.86** ± 0.10	**0.93** ± 0.09	**0.86** ± 0.05	**0.79** ± 0.05
C 20:3 N-9	**0.10** ± 0.01	**0.10** ± 0.01	**0.11** ± 0.03	**0.14** ± 0.06	**0.14** ± 0.03
C 20:3 N-6	**1.15** ± 0.05	**1.34** ± 0.02	**1.35** ± 0.08	**1.18** ± 0.03	**1.28** ± 0.05
C 20:4 N-6	**1.55** ± 0.08	**1.78** ± 0.15	**1.46** ± 0.04	**1.39** ± 0.01	**1.40** ± 0.04
C 20:3 N-3	**0.30** ± 0.02	**0.28** ± 0.03	**0.34** ± 0.02	**0.33** ± 0.01	**0.27** ± 0.01
C 20:5 N-3	**11.65** ± 1.33	**13.49** ± 1.33	**13.36** ± 0.69	**12.93** ± 0.36	**13.63** ± 0.03
C 22:5 N-3	**3.28** ± 0.15	**3.29** ± 0.30	**3.72** ± 0.20	**3.42** ± 0.19	**3.30** ± 0.07
C 22:6 N-3	**20.68** ± 0.81	**20.14** ± 2.19	**18.80** ± 1.24	**19.59** ± 0.85	**18.68** ± 1.27
SUM PUFA	**49.16** ± 2.12	**51.16** ± 0.39	**49.98** ± 0.40	**49.36** ± 0.80	**49.40** ± 0.87
N3/N6	**3.64** ± 0.19	**3.49** ± 0.19	**3.56** ± 0.20	**3.71** ± 0.28	**3.60** ± 0.29
OTHERS	**1.14** ± 0.01	**1.12** ± 0.10	**1.57** ± 0.16	**1.66** ± 0.06	**1.67** ± 0.10

Table 8.2.b Fatty acid profile of Salmon erythrocytes PC (% of total fatty acids) fed with low diet (0,2 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

LOW DIET

	Pre-Diet 0%			Pre-diet 2% DHA			Pre-Diet 2 % EPA			Pre-Diet 2 % EPA+DHA			Com cont		
C 14:0	**0.33**	±	0.02	**0.30**	±	0.02	**0.30**	±	0.02	**0.27**	±	0.03	**0.33**	±	0.02
C 16:0	**22.23**	±	0.28	**23.30**	±	0.84	**23.58**	±	0.46	**24.19**	±	0.51	**23.54**	±	0.60
C 18:0	**5.51**	±	0.14	**5.99**	±	0.24	**6.23**	±	0.19	**6.53**	±	0.39	**5.62**	±	0.18
C 22:0	**0.24**	±	0.02	**0.23**	±	0.01	**0.24**	±	0.02	**0.21**	±	0.02	**0.25**	±	0.02
SUM N-0	**28.67**	±	0.22	**30.18**	±	1.05	**30.75**	±	0.68	**31.66**	±	0.90	**30.09**	±	0.70
C 16:1 N-7	**0.79**	±	0.04	**0.73**	±	0.03	**0.78**	±	0.13	**0.66**	±	0.04	**0.98**	±	0.09
C 18:1 N-11	**0.01**	±	0.01	**N.D.**			**N.D.**			**N.D.**			**N.D.**		
C 18:1 N-9	**14.70**	±	0.64	**14.35**	±	0.23	**13.47**	±	0.50	**13.84**	±	0.87	**14.83**	±	0.37
C 18:1 N-7	**1.39**	±	0.00	**1.44**	±	0.09	**1.34**	±	0.06	**1.30**	±	0.05	**1.12**	±	0.12
C 20:1 N-11	**0.41**	±	0.03	**0.44**	±	0.11	**0.44**	±	0.04	**0.53**	±	0.03	**0.53**	±	0.08
C 20:1 N-9	**0.87**	±	0.04	**0.87**	±	0.07	**0.88**	±	0.06	**0.94**	±	0.06	**0.85**	±	0.03
C 22:1 N-11	**1.63**	±	0.08	**1.42**	±	0.10	**1.59**	±	0.01	**1.61**	±	0.08	**1.63**	±	0.11
C 22:1 N-9	**0.09**	±	0.00	**0.10**	±	0.01	**0.10**	±	0.01	**0.11**	±	0.02	**0.05**	±	0.03
SUM MUFA	**20.60**	±	0.53	**20.37**	±	0.28	**19.71**	±	0.65	**20.13**	±	1.02	**20.60**	±	0.43
C 18:2 N-6	**11.36**	±	0.23	**10.80**	±	0.90	**12.22**	±	0.14	**12.03**	±	0.46	**12.33**	±	1.30
C 18:3 N-6	**0.30**	±	0.02	**0.33**	±	0.08	**0.33**	±	0.04	**0.45**	±	0.02	**0.36**	±	0.09
C 18:3 N-3	**3.17**	±	0.07	**2.87**	±	0.32	**3.28**	±	0.07	**2.85**	±	0.11	**3.38**	±	0.21
C 20:2 N-6	**1.07**	±	0.04	**1.00**	±	0.04	**1.00**	±	0.07	**0.88**	±	0.05	**1.14**	±	0.07
C 20:3 N-9	**0.10**	±	0.02	**0.16**	±	0.06	**0.20**	±	0.01	**0.24**	±	0.05	**0.12**	±	0.01
C 20:3 N-6	**3.39**	±	0.08	**3.15**	±	0.21	**3.10**	±	0.07	**3.54**	±	0.23	**3.13**	±	0.36
C 20:4 N-6	**3.23**	±	0.03	**3.19**	±	0.11	**2.97**	±	0.14	**3.08**	±	0.11	**2.88**	±	0.10
C 20:3 N-3	**0.33**	±	0.02	**0.30**	±	0.03	**0.32**	±	0.01	**0.26**	±	0.01	**0.32**	±	0.04
C 20:5 N-3	**8.57**	±	0.21	**8.71**	±	0.21	**8.77**	±	0.24	**8.76**	±	0.76	**8.62**	±	0.21
C 22:5 N-3	**3.28**	±	0.08	**3.11**	±	0.01	**3.20**	±	0.05	**2.92**	±	0.21	**2.98**	±	0.32
C 22:6 N-3	**14.57**	±	0.28	**14.62**	±	0.51	**13.26**	±	0.67	**12.35**	±	1.45	**13.07**	±	1.61
SUM PUFA	**49.83**	±	0.39	**48.65**	±	1.36	**49.02**	±	0.68	**47.65**	±	1.87	**48.73**	±	0.49
N3/N6	**1.51**	±	0.03	**1.58**	±	0.10	**1.44**	±	0.05	**1.34**	±	0.16	**1.44**	±	0.23
OTHERS	**1.51**	±	0.12	**1.73**	±	0.02	**1.84**	±	0.19	**1.86**	±	0.01	**1.37**	±	0.22

Table 8.2.c Fatty acid profile of Salmon erythrocytes PC (% of total fatty acids) fed with control diet (1,7 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

CONTROL DIET

	Pre-Diet 0%			Pre-diet 2% DHA			Pre-Diet 2 % EPA			Pre-Diet 2 % EPA+DHA			Com cont		
C 14:0	**1.06**	±	0.01	**0.97**	±	0.06	**0.96**	±	0.08	**0.29**	±	0.29	**0.39**	±	0.39
C 16:0	**24.00**	±	1.25	**23.71**	±	0.09	**24.14**	±	0.58	**22.99**	±	0.12	**22.35**	±	0.30
C 18:0	**3.48**	±	0.11	**4.03**	±	0.35	**3.88**	±	0.14	**3.69**	±	0.03	**3.81**	±	0.01
C 22:0	**N.D.**			**N.D.**			**0.02**	±	0.02	**N.D.**			**N.D.**		
SUM N-0	**28.92**	±	1.32	**29.12**	±	0.26	**29.53**	±	0.62	**27.45**	±	0.23	**27.08**	±	0.74
C 16:1 N-7	**1.54**	±	0.00	**1.28**	±	0.13	**1.14**	±	0.12	**1.16**	±	0.07	**1.29**	±	0.01
C 18:1 N-11	**0.00**	±	0.00	**0.14**	±	0.11	**0.23**	±	0.02	**0.29**	±	0.02	**0.29**	±	0.03
C 18:1 N-9	**10.01**	±	0.68	**9.00**	±	1.01	**8.54**	±	0.90	**8.32**	±	0.35	**8.53**	±	0.34
C 18:1 N-7	**1.64**	±	0.10	**1.74**	±	0.04	**1.62**	±	0.08	**1.82**	±	0.03	**1.91**	±	0.05
C 20:1 N-11	**0.28**	±	0.04	**0.24**	±	0.09	**0.17**	±	0.02	**0.16**	±	0.01	**0.17**	±	0.01
C 20:1 N-9	**2.90**	±	0.27	**3.40**	±	0.04	**2.71**	±	0.35	**3.11**	±	0.04	**3.34**	±	0.14
C 22:1 N-11	**0.83**	±	0.01	**0.84**	±	0.02	**0.73**	±	0.08	**0.83**	±	0.04	**0.77**	±	0.04
C 22:1 N-9	**N.D.**			**0.57**	±	0.46	**1.00**	±	0.16	**1.24**	±	0.03	**1.21**	±	0.08
SUM MUFA	**17.72**	±	0.57	**17.89**	±	0.35	**17.12**	±	0.27	**18.01**	±	0.40	**18.77**	±	0.68
C 18:2 N-6	**4.35**	±	0.31	**4.02**	±	0.06	**4.07**	±	0.24	**4.10**	±	0.06	**4.10**	±	0.17
C 18:3 N-6	**0.08**	±	0.01	**0.04**	±	0.03	**0.06**	±	0.00	**0.05**	±	0.00	**0.05**	±	0.00
C 18:3 N-3	**1.06**	±	0.09	**0.88**	±	0.05	**0.84**	±	0.03	**0.93**	±	0.01	**0.98**	±	0.02
C 20:2 N-6	**0.64**	±	0.07	**0.73**	±	0.10	**0.49**	±	0.04	**0.58**	±	0.03	**0.65**	±	0.03
C 20:3 N-9	**0.07**	±	0.06	**0.04**	±	0.03	**0.08**	±	0.01	**0.07**	±	0.01	**0.08**	±	0.01
C 20:3 N-6	**0.61**	±	0.09	**0.47**	±	0.02	**0.54**	±	0.05	**0.51**	±	0.04	**0.43**	±	0.03
C 20:4 N-6	**0.80**	±	0.19	**0.61**	±	0.00	**0.75**	±	0.16	**0.59**	±	0.02	**0.58**	±	0.02
C 20:3 N-3	**0.12**	±	0.01	**0.13**	±	0.00	**0.11**	±	0.01	**0.13**	±	0.01	**0.15**	±	0.00
C 20:5 N-3	**15.53**	±	0.33	**15.94**	±	0.32	**14.60**	±	1.37	**15.57**	±	0.38	**15.74**	±	0.35
C 22:5 N-3	**3.08**	±	0.26	**3.52**	±	0.24	**3.69**	±	0.12	**3.82**	±	0.22	**3.63**	±	0.08
C 22:6 N-3	**26.13**	±	0.10	**25.61**	±	0.41	**27.23**	±	0.86	**27.02**	±	0.26	**26.75**	±	0.70
SUM PUFA	**52.70**	±	0.65	**52.29**	±	0.19	**52.72**	±	0.80	**53.62**	±	0.31	**53.40**	±	0.21
N3/N6	**7.18**	±	0.58	**7.89**	±	0.17	**7.92**	±	0.69	**8.16**	±	0.04	**8.19**	±	0.41
OTHERS	**1.05**	±	0.07	**1.31**	±	0.33	**1.66**	±	0.02	**1.71**	±	0.04	**1.93**	±	0.09

8.3. The fatty acid composition of PS

Table 8.3.a Fatty acid profile of Salmon erythrocytes PS (% of total fatty acids) fed with main diet (1 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

1% MAIN DIET

	Pre-Diet 0%	Pre-Diet 2% DHA	Pre-Diet 2% EPA	Pre-Diet 2% EPA +DHA	Com cont
C 14:0	**N.D.**	**0.46**	**N.D.**	**0.18**	**0.27**
C 16:0	**11.64**	**16.92**	**12.33 ± 0.32**	**10.77**	**13.39**
C 18:0	**18.92**	**19.17**	**11.98 ± 0.75**	**24.12**	**9.05**
C 22:0	**0.87**	**0.69**	**1.06 ± 0.06**	**1.47**	**1.36**
SUM N-0	**32.55**	**38.22**	**26.19 ± 1.47**	**38.30**	**24.33**
C 16:1 N-7	**0.19**	**0.46**	**N.D.**	**0.28**	**1.45**
C 18:1 N-11	**0.19**	**0.12**	**N.D.**	**0.18**	**0.36**
C 18:1 N-9	**4.42**	**5.64**	**6.81 ± 0.44**	**6.26**	**6.78**
C 18:1 N-7	**1.18**	**1.21**	**2.35 ± 0.06**	**1.29**	**1.09**
C 20:1 N-11	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
C 20:1 N-9	**4.67**	**3.91**	**7.05 ± 1.32**	**4.88**	**3.98**
C 22:1 N-11	**1.99**	**1.73**	**0.82 ± 0.34**	**1.47**	**1.72**
C 22:1 N-9	**0.93**	**0.75**	**N.D.**	**0.83**	**1.18**
SUM MUFA	**15.31**	**15.83**	**19.85 ± 2.45**	**17.95**	**19.81**
C 18:2 N-6	**3.11**	**3.91**	**3.41 ± 0.04**	**3.13**	**3.26**
C 18:3 N-6	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
C 18:3 N-3	**0.93**	**1.15**	**0.70 ± 0.00**	**0.92**	**1.27**
C 20:4 N-3	**0.19**	**N.D.**	**N.D.**	**0.28**	**N.D.**
C 20:2 N-6	**0.87**	**0.69**	**0.00 ± 0.08**	**0.74**	**0.63**
C 20:3 N-9	**1.37**	**1.50**	**2.82 ± 0.41**	**1.93**	**2.44**
C 20:3 N-6	**1.00**	**1.15**	**1.76 ± 0.72**	**1.10**	**1.00**
C 20:4 N-6	**1.74**	**1.67**	**2.23 ± 0.26**	**9.57**	**2.08**
C 20:3 N-3	**1.18**	**0.86**	**0.00 ± 0.49**	**1.01**	**0.54**
C 20:5 N-3	**6.16**	**5.93**	**4.58 ± 0.44**	**5.62**	**10.31**
C 22:5 N-3	**4.92**	**4.60**	**2.58 ± 0.32**	**3.31**	**3.71**
C 22:6 N-3	**31.49**	**25.33**	**10.92 ± 1.30**	**17.49**	**33.02**
SUM PUFA	**51.96**	**45.64**	**31.24 ± 1.61**	**43.54**	**55.81**
N3/N6	**6.32**	**4.87**	**2.54 ± 0.20**	**1.96**	**7.01**
OTHERS	**1.87**	**1.84**	**5.87 ± 0.68**	**2.95**	**1.09**

Table 8.3.b Fatty acid profile of Salmon erythrocytes PS (% of total fatty acids) fed with low diet (0,2 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	LOW DIET				
	Pre-Diet 0%	**Pre-Diet 2% DHA**	**Pre-Diet 2% EPA**	**Pre-Diet 2 % EPA+DHA**	**Com cont**
C 14:0	**0.15**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
C 16:0	**8.94**	**14.22**	**12.98**	**11.49**	**19.09**
C 18:0	**18.40**	**24.10**	**31.10**	**29.39**	**32.20**
C 22:0	**0.88**	**1.00**	**N.D.**	**2.39**	**N.D.**
SUM N-0	**28.74**	**39.32**	**45.87**	**44.76**	**51.29**
C 16:1 N-7	**0.29**	**0.72**	**N.D.**	**N.D.**	**N.D.**
C 18:1 N-11	**0.37**	**0.00**	**N.D.**	**N.D.**	**N.D.**
C 18:1 N-9	**6.38**	**6.98**	**4.82**	**5.37**	**10.54**
C 18:1 N-7	**0.73**	**1.00**	**N.D.**	**0.75**	**N.D.**
C 20:1 N-11	**0.22**	**0.00**	**N.D.**	**N.D.**	**N.D.**
C 20:1 N-9	**1.83**	**3.08**	**1.71**	**3.43**	**7.41**
C 22:1 N-11	**2.05**	**1.54**	**N.D.**	**2.09**	**N.D.**
C 22:1 N-9	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
SUM MUFA	**14.52**	**20.66**	**11.84**	**16.71**	**22.22**
C 18:2 N-6	**3.52**	**4.62**	**2.53**	**3.13**	**5.70**
C 18:3 N-6	**0.22**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
C 18:3 N-3	**1.03**	**1.09**	**N.D.**	**0.75**	**N.D.**
C 20:4 N-3	**0.95**	**N.D.**	**1.63**	**N.D.**	**N.D.**
C 20:2 N-6	**0.66**	**1.09**	**N.D.**	**0.75**	**N.D.**
C 20:3 N-9	**1.47**	**4.53**	**1.80**	**3.73**	**4.27**
C 20:3 N-6	**3.08**	**2.54**	**2.86**	**2.69**	**N.D.**
C 20:4 N-6	**9.46**	**8.97**	**9.71**	**7.76**	**7.69**
C 20:3 N-3	**0.66**	**0.63**	**N.D.**	**0.75**	**N.D.**
C 20:5 N-3	**6.09**	**4.44**	**3.26**	**2.54**	**3.13**
C 22:5 N-3	**4.03**	**2.54**	**4.24**	**3.73**	**N.D.**
C 22:6 N-3	**24.78**	**13.50**	**18.04**	**15.67**	**9.97**
SUM PUFA	**55.13**	**39.95**	**42.28**	**38.34**	**26.50**
N3/N6	**2.13**	**1.25**	**1.80**	**1.57**	**0.98**
OTHERS	**2.20**	**3.35**	**5.31**	**3.43**	**N.D.**

Table 8.3.c Fatty acid profile of Salmon erythrocytes PS (% of total fatty acids) fed with control diet (1,7 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	CONTROL DIET						
	Pre-Diet 0%	**Pre- diet 2% DHA**	**Pre-Diet 2 % EPA**			**Pre-Diet 2 % EPA+DHA**	**Com cont**
C 14:0	**N.D.**	**N.D.**	**0.23**	±	0.19	**N.D.**	**0.16**
C 16:0	**17.08**	**15.49**	**9.98**	±	0.83	**7.97**	**8.47**
C 18:0	**33.06**	**30.70**	**19.30**	±	0.54	**20.10**	**21.50**
C 22:0	**N.D.**	**N.D.**	**0.39**	±	0.32	**0.82**	**1.20**
SUM N-0	**50.14**	**46.20**	**30.90**	±	1.05	**30.48**	**33.09**
C 16:1 N-7	**N.D.**	**N.D.**	**0.13**	±	0.11	**0.11**	**0.24**
C 18:1 N-11	**N.D.**	**N.D.**	**0.16**	±	0.13	**0.11**	**0.16**
C 18:1 N-9	**8.82**	**7.32**	**3.03**	±	0.18	**2.02**	**2.32**
C 18:1 N-7	**N.D.**	**N.D.**	**1.33**	±	0.03	**0.98**	**1.04**
C 20:1 N-11	**N.D.**	**N.D.**	**1.59**	±	1.30	**N.D.**	**N.D.**
C 20:1 N-9	**9.92**	**12.68**	**5.24**	±	0.30	**4.97**	**5.12**
C 22:1 N-11	**N.D.**	**N.D.**	**1.21**	±	0.12	**1.53**	**1.20**
C 22:1 N-9	**N.D.**	**N.D.**	**1.23**	±	0.05	**1.53**	**1.20**
SUM MUFA	**21.77**	**24.51**	**17.65**	±	1.02	**13.05**	**14.23**
C 18:2 N-6	**2.76**	**2.82**	**1.58**	±	0.01	**1.09**	**1.28**
C 18:3 N-6	**N.D.**	**N.D.**	**N.D.**			**N.D.**	**N.D.**
C 18:3 N-3	**N.D.**	**N.D.**	**0.42**	±	0.03	**0.27**	**0.32**
C 20:4 N-3	**N.D.**	**N.D.**	**0.32**	±	0.27	**0.11**	**N.D.**
C 20:2 N-6	**N.D.**	**N.D.**	**0.63**	±	0.04	**0.66**	**0.64**
C 20:3 N-9	**3.03**	**4.51**	**2.31**	±	0.35	**1.09**	**1.92**
C 20:3 N-6	**N.D.**	**N.D.**	**0.19**	±	0.16	**0.38**	**0.40**
C 20:4 N-6	**8.27**	**8.17**	**0.69**	±	0.07	**0.60**	**1.04**
C 20:3 N-3	**N.D.**	**N.D.**	**0.46**	±	0.00	**0.55**	**0.48**
C 20:5 N-3	**5.79**	**6.48**	**5.45**	±	0.11	**4.92**	**4.08**
C 22:5 N-3	**N.D.**	**N.D.**	**4.68**	±	0.10	**5.57**	**4.88**
C 22:6 N-3	**11.30**	**11.83**	**36.10**	±	1.26	**41.73**	**39.09**
SUM PUFA	**28.10**	**29.30**	**50.63**	±	0.55	**56.09**	**52.36**
N3/N6	**1.55**	**1.67**	**15.43**	±	1.11	**19.50**	**14.60**
OTHERS	**N.D.**	**N.D.**	**2.51**	±	0.24	**2.51**	**2.96**

8.4. The fatty acid composition of PI

Table 8.4.a Fatty acid profile of Salmon erythrocytes PI (% of total fatty acids) fed with main diet (1 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	1% MAIN DIET			
	Pre-Diet 0%	**Pre-Diet 2% DHA**	**Pre-Diet 2% EPA**	**Pre-Diet 2% EPA +DHA**	**Com cont**
C 14:0	**0.04 ± 0.03**	N.D.	**0.10 ± 0.08**	**N.D.**	**0.21 ± 0.12**
C 16:0	**8.55 ± 0.01**	**7.92 ± 0.33**	**9.17 ± 0.00**	**9.04**	**9.94 ± 2.06**
C 18:0	**27.14 ± 1.21**	**27.41 ± 0.71**	**27.71 ± 0.17**	**23.95**	**27.06 ± 1.80**
C 22:0	**0.35 ± 0.06**	**0.40 ± 0.11**	**0.60 ± 0.10**	**0.45**	**0.77 ± 0.29**
SUM N-0	**36.66 ± 1.20**	**36.21 ± 0.22**	**38.41 ± 0.56**	**34.15**	**39.07 ± 0.99**
C 16:1 N-7	**0.22 ± 0.00**	**0.10 ± 0.08**	**0.10 ± 0.08**	**0.33**	**0.23 ± 0.04**
C 18:1 N-11	**0.37 ± 0.01**	**0.36 ± 0.01**	**0.28 ± 0.06**	**0.50**	**0.14 ± 0.11**
C 18:1 N-9	**6.72 ± 0.04**	**7.02 ± 0.33**	**6.30 ± 0.56**	**6.40**	**7.02 ± 0.36**
C 18:1 N-7	**1.05 ± 0.03**	**1.10 ± 0.01**	**1.02 ± 0.07**	**1.16**	**0.96 ± 0.04**
C 20:1 N-11	**N.D.**	**N.D.**	**N.D.**	**1.45**	N.D.
C 20:1 N-9	**2.44 ± 0.11**	**2.75 ± 0.21**	**3.08 ± 0.13**	**2.68**	**2.77 ± 0.21**
C 22:1 N-11	**0.99 ± 0.16**	**1.21 ± 0.01**	**1.12 ± 0.01**	**0.95**	**1.17 ± 0.04**
C 22:1 N-9	**0.59 ± 0.01**	**0.70 ± 0.00**	**0.70 ± 0.06**	**0.62**	**0.53 ± 0.03**
SUM MUFA	**13.31 ± 0.35**	**14.34 ± 0.81**	**16.03 ± 1.38**	**15.48**	**14.80 ± 1.20**
C 18:2 N-6	**2.41 ± 0.09**	**2.28 ± 0.05**	**2.48 ± 0.12**	**2.48**	**3.21 ± 0.54**
C 18:3 N-6	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
C 18:3 N-3	**0.81 ± 0.04**	**0.77 ± 0.01**	**0.66 ± 0.04**	**0.87**	**0.88 ± 0.09**
C 20:4 N-3	**0.19 ± 0.02**	**0.22 ± 0.02**	**0.20 ± 0.03**	**0.62**	**0.07 ± 0.06**
C 20:2 N-6	**0.48 ± 0.01**	**0.51 ± 0.02**	**0.48 ± 0.06**	**0.58**	**0.42 ± 0.00**
C 20:3 N-9	**0.54 ± 0.08**	**0.58 ± 0.10**	**1.04 ± 0.24**	**1.16**	**1.14 ± 0.45**
C 20:3 N-6	**2.10 ± 0.04**	**2.36 ± 0.11**	**1.98 ± 0.06**	**1.82**	**2.27 ± 0.25**
C 20:4 N-6	**19.61 ± 1.13**	**19.69 ± 0.73**	**18.52 ± 0.91**	**16.72**	**17.92 ± 1.87**
C 20:3 N-3	**0.52 ± 0.04**	**0.53 ± 0.04**	**0.53 ± 0.02**	**0.62**	**0.44 ± 0.01**
C 20:5 N-3	**6.73 ± 0.21**	**7.34 ± 0.01**	**6.86 ± 0.12**	**6.98**	**7.12 ± 0.69**
C 22:5 N-3	**2.37 ± 0.26**	**2.74 ± 0.12**	**2.38 ± 0.09**	**2.64**	**2.43 ± 0.03**
C 22:6 N-3	**14.03 ± 1.20**	**12.25 ± 0.03**	**10.96 ± 0.92**	**15.65**	**11.06 ± 0.17**
SUM PUFA	**49.57 ± 0.83**	**49.04 ± 0.53**	**45.29 ± 1.95**	**48.97**	**45.96 ± 2.17**
N3/N6	**1.00 ± 0.11**	**0.96 ± 0.04**	**0.92 ± 0.01**	**1.27**	**0.92 ± 0.04**
OTHERS	**1.28 ± 0.04**	**1.34 ± 0.15**	**3.45 ± 1.69**	**0.95**	**2.07 ± 0.50**

Table 8.4.b Fatty acid profile of Salmon erythrocytes PI (% of total fatty acids) fed with low diet (0,2 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	LOW DIET											
	Pre-Diet 0%			**Pre-diet 2% DHA**			**Pre-Diet 2 % EPA**			**Pre-Diet 2 % EPA+DHA**	**Com cont**	
C 14:0	**0.08**	±	0.03	**0.09**	±	0.08	**0.04**	±	0.03	**0.09**	**N.D.**	
C 16:0	**4.99**	±	0.49	**5.22**	±	0.03	**4.39**	±	0.01	**4.70**	**7.33**	± 0.14
C 18:0	**33.11**	±	0.12	**38.82**	±	0.26	**36.69**	±	0.51	**36.41**	**34.81**	± 1.82
C 22:0	**0.49**	±	0.02	**0.96**	±	0.02	**0.95**	±	0.03	**1.41**	**0.24**	± 0.19
SUM N-0	**39.39**	±	0.69	**46.50**	±	0.42	**42.97**	±	0.85	**44.03**	**42.59**	± 1.31
C 16:1 N-7	**0.10**	±	0.02	**0.10**	±	0.02	**0.04**	±	0.03	**0.09**	**N.D.**	
C 18:1 N-11	**0.11**	±	0.00	**N.D.**			**0.04**	±	0.03	**N.D.**	N.D.	
C 18:1 N-9	**5.80**	±	0.03	**4.31**	±	0.10	**5.75**	±	0.20	**5.55**	**6.39**	± 0.69
C 18:1 N-7	**0.66**	±	0.01	**0.58**	±	0.02	**0.59**	±	0.02	**0.56**	**1.00**	± 0.21
C 20:1 N-11	**N.D.**			**N.D.**			**N.D.**			**N.D.**	**N.D.**	
C 20:1 N-9	**1.40**	±	0.17	**2.31**	±	0.11	**1.98**	±	0.22	**2.35**	**1.44**	± 0.15
C 22:1 N-11	**1.31**	±	0.17	**1.02**	±	0.04	**1.07**	±	0.07	**0.94**	**1.51**	± 0.18
C 22:1 N-9	**0.08**	±	0.06	**N.D.**			**N.D.**			**N.D.**	**N.D.**	
SUM MUFA	**10.57**	±	0.35	**10.35**	±	0.12	**11.30**	±	0.04	**12.04**	**11.45**	± 0.60
C 18:2 N-6	**2.13**	±	0.03	**1.78**	±	0.08	**1.90**	±	0.06	**1.88**	**2.35**	± 0.36
C 18:3 N-6	**N.D.**			**N.D.**			**N.D.**			**N.D.**	**N.D.**	
C 18:3 N-3	**0.57**	±	0.02	**0.41**	±	0.03	**0.49**	±	0.00	**0.47**	**0.64**	± 0.09
C 20:4 N-3	**N.D.**			**N.D.**			**N.D.**			**N.D.**	**0.34**	± 0.28
C 20:2 N-6	**0.51**	±	0.00	**0.48**	±	0.04	**0.46**	±	0.03	**0.47**	**0.60**	± 0.09
C 20:3 N-9	**0.81**	±	0.11	**1.62**	±	0.16	**1.44**	±	0.03	**1.98**	**0.70**	± 0.11
C 20:3 N-6	**3.05**	±	0.24	**2.06**	±	0.10	**2.61**	±	0.06	**2.92**	**3.22**	± 0.45
C 20:4 N-6	**28.83**	±	0.53	**26.06**	±	0.77	**27.06**	±	0.62	**24.84**	**22.19**	± 0.44
C 20:3 N-3	**0.68**	±	0.04	**0.62**	±	0.05	**0.65**	±	0.02	**0.56**	**0.82**	± 0.06
C 20:5 N-3	**3.57**	±	0.14	**3.41**	±	0.03	**3.16**	±	0.24	**3.48**	**3.43**	± 0.12
C 22:5 N-3	**2.09**	±	0.32	**1.71**	±	0.07	**1.89**	±	0.05	**1.69**	**2.27**	± 0.04
C 22:6 N-3	**8.06**	±	0.82	**6.17**	±	0.02	**6.89**	±	0.56	**7.15**	**9.76**	± 0.05
SUM PUFA	**49.79**	±	1.04	**42.85**	±	0.27	**45.46**	±	0.96	**43.65**	**45.83**	± 0.60
N3/N6	**0.43**	±	0.04	**0.40**	±	0.01	**0.41**	±	0.00	**0.44**	**0.60**	± 0.01
OTHERS	**1.34**	±	0.07	**1.99**	±	0.40	**1.63**	±	0.34	**2.16**	**0.85**	± 0.33

Table 8.4.c Fatty acid profile of Salmon erythrocytes PI (% of total fatty acids) fed with control diet (1,7 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

CONTROL DIET

	Pre-Diet 0%	Pre-diet 2% DHA	Pre-Diet 2 % EPA	Pre-Diet 2 % EPA+DHA	Com cont
C 14:0	**0.16 ± 0.02**	**N.D.**	**0.06 ± 0.05**	**N.D.**	**0.07 ± 0.02**
C 16:0	**7.80 ± 0.30**	**9.83**	**5.65 ± 0.05**	**4.49 ± 0.18**	**4.44 ± 0.06**
C 18:0	**31.86 ± 0.72**	**32.33**	**36.57 ± 0.92**	**34.69 ± 0.17**	**34.39 ± 0.14**
C 22:0	**0.64 ± 0.22**	**0.48**	**0.42 ± 0.34**	**0.64 ± 0.08**	**0.69 ± 0.05**
SUM N-0	**41.83 ± 0.93**	**43.33**	**43.44 ± 0.21**	**41.61 ± 0.21**	**41.28 ± 0.10**
C 16:1 N-7	**0.15 ± 0.07**	**N.D.**	**0.09 ± 0.07**	**0.13 ± 0.00**	**0.15 ± 0.00**
C 18:1 N-11	**0.27 ± 0.01**	**N.D.**	**0.15 ± 0.12**	**0.33 ± 0.01**	**0.35 ± 0.01**
C 18:1 N-9	**5.67 ± 0.11**	**4.50**	**5.02 ± 0.06**	**5.23 ± 0.11**	**5.36 ± 0.02**
C 18:1 N-7	**1.12 ± 0.12**	**1.04**	**1.27 ± 0.07**	**1.22 ± 0.03**	**1.14 ± 0.02**
C 20:1 N-11	**0.00 ± 0.00**	**4.22**	**0.00 ± 0.00**	**0.00 ± 0.00**	**0.00 ± 0.00**
C 20:1 N-9	**3.21 ± 0.06**	**3.88**	**3.27 ± 0.10**	**2.95 ± 0.05**	**2.72 ± 0.00**
C 22:1 N-11	**0.68 ± 0.14**	**0.00**	**0.24 ± 0.19**	**0.56 ± 0.02**	**0.47 ± 0.11**
C 22:1 N-9	**0.73 ± 0.06**	**0.00**	**0.36 ± 0.29**	**0.56 ± 0.02**	**0.50 ± 0.05**
SUM MUFA	**13.90 ± 0.00**	**16.61**	**11.72 ± 0.89**	**12.17 ± 0.04**	**12.01 ± 0.17**
C 18:2 N-6	**1.45 ± 0.15**	**1.66**	**1.39 ± 0.03**	**1.34 ± 0.07**	**1.26 ± 0.00**
C 18:3 N-6	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
C 18:3 N-3	**0.16 ± 0.13**	**N.D.**	**0.15 ± 0.12**	**0.33 ± 0.02**	**0.30 ± 0.00**
C 20:4 N-3	**N.D.**	**N.D.**	**0.09 ± 0.07**	**0.10 ± 0.05**	**0.17 ± 0.02**
C 20:2 N-6	**0.32 ± 0.11**	**N.D.**	**0.15 ± 0.12**	**0.30 ± 0.02**	**0.30 ± 0.00**
C 20:3 N-9	**1.35 ± 0.18**	**2.42**	**1.02 ± 0.19**	**0.91 ± 0.06**	**0.99 ± 0.05**
C 20:3 N-6	**1.39 ± 0.17**	**1.59**	**1.94 ± 0.08**	**1.90 ± 0.17**	**1.90 ± 0.07**
C 20:4 N-6	**15.85 ± 0.59**	**12.25**	**18.29 ± 0.44**	**19.83 ± 0.66**	**20.11 ± 0.98**
C 20:3 N-3	**0.16 ± 0.13**	**0.00**	**0.06 ± 0.05**	**0.06 ± 0.06**	**0.07 ± 0.06**
C 20:5 N-3	**6.66 ± 0.85**	**6.09**	**11.87 ± 0.60**	**11.05 ± 0.28**	**11.33 ± 0.57**
C 22:5 N-3	**2.35 ± 0.00**	**2.98**	**2.20 ± 0.00**	**2.34 ± 0.11**	**2.30 ± 0.01**
C 22:6 N-3	**15.70 ± 1.93**	**14.19**	**8.32 ± 0.08**	**8.72 ± 0.10**	**8.65 ± 0.21**
SUM PUFA	**44.11 ± 0.89**	**38.76**	**44.55 ± 0.73**	**46.07 ± 0.22**	**46.53 ± 0.29**
N3/N6	**1.32 ± 0.11**	**1.50**	**1.04 ± 0.00**	**0.97 ± 0.04**	**0.98 ± 0.08**
OTHERS	**2.15 ± 0.16**	**1.25**	**1.12 ± 0.69**	**2.16 ± 0.14**	**2.17 ± 0.03**

8.5. The fatty acid composition of PE

Table 8.5.a Fatty acid profile of Salmon erythrocytes PE (% of total fatty acids) fed with main diet (1 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

	1% MAIN DIET														
	Pre-Diet 0%			**Pre-Diet 2% DHA**			**Pre-Diet 2% EPA**			**Pre-Diet 2% EPA +DHA**			**Com cont**		
C 14:0	**0.09**	±	0.00	**0.14**	±	0.03	**0.09**	±	0.01	**0.00**			**0.15**	±	0.08
C 16:0	**7.18**	±	0.51	**6.80**	±	0.22	**6.57**	±	0.36	**6.11**			**9.54**	±	1.15
C 18:0	**10.96**	±	0.54	**9.52**	±	0.58	**10.10**	±	0.63	**12.97**			**14.61**	±	0.78
C 22:0	**0.60**	±	0.08	**0.43**	±	0.11	**0.29**	±	0.04	**0.35**			**0.47**	±	0.14
SUM N-0	**19.62**	±	0.12	**16.80**	±	0.69	**18.54**	±	1.20	**20.31**			**25.75**	±	1.61
C 16:1 N-7	**0.18**	±	0.04	**0.22**	±	0.00	**0.25**	±	0.03	**0.14**			**0.21**	±	0.02
C 18:1 N-11	**0.18**	±	0.04	**0.16**	±	0.01	**0.13**	±	0.02	**0.07**			**0.04**	±	0.02
C 18:1 N-9	**4.77**	±	0.23	**4.50**	±	0.29	**4.94**	±	0.05	**4.39**			**4.69**	±	0.17
C 18:1 N-7	**2.71**	±	0.55	**2.51**	±	0.18	**1.85**	±	0.38	**1.41**			**1.26**	±	0.09
C 20:1 N-11	**0.00**	±	0.00	**0.00**	±	0.00	**0.00**	±	0.00	**0.00**			**0.00**	±	0.00
C 20:1 N-9	**4.88**	±	0.15	**5.09**	±	0.37	**4.88**	±	0.13	**4.36**			**4.67**	±	0.23
C 22:1 N-11	**3.07**	±	0.27	**3.89**	±	0.17	**3.94**	±	0.42	**3.16**			**3.13**	±	0.38
C 22:1 N-9	**1.46**	±	0.14	**1.72**	±	0.05	**1.52**	±	0.08	**1.12**			**1.16**	±	0.12
SUM MUFA	**18.91**	±	0.61	**18.18**	±	1.74	**19.62**	±	0.72	**15.74**			**17.57**	±	0.64
C 18:2 N-6	**3.95**	±	0.41	**4.00**	±	0.31	**4.05**	±	0.15	**3.55**			**3.65**	±	0.01
C 18:3 N-6	N.D.			N.D.			N.D.			N.D.			N.D.		
C 18:3 N-3	**1.13**	±	0.18	**1.32**	±	0.05	**1.19**	±	0.06	**0.98**			**0.99**	±	0.03
C 20:4 N-3	**0.20**	±	0.01	**0.19**	±	0.01	**0.13**	±	0.04	**0.07**			**0.04**	±	0.02
C 20:2 N-6	**1.38**	±	0.05	**1.31**	±	0.06	**1.26**	±	0.06	**1.05**			**0.76**	±	0.09
C 20:3 N-9	**0.84**	±	0.04	**0.69**	±	0.18	**0.41**	±	0.07	**0.32**			**0.47**	±	0.13
C 20:3 N-6	**1.44**	±	0.11	**1.56**	±	0.03	**1.68**	±	0.14	**1.34**			**1.41**	±	0.11
C 20:4 N-6	**2.38**	±	0.12	**2.23**	±	0.02	**2.14**	±	0.01	**2.21**			**2.11**	±	0.04
C 20:3 N-3	**1.84**	±	0.02	**1.82**	±	0.07	**1.90**	±	0.11	**1.93**			**1.42**	±	0.09
C 20:5 N-3	**9.09**	±	0.11	**11.01**	±	0.43	**10.71**	±	0.57	**9.52**			**8.88**	±	0.60
C 22:5 N-3	**5.11**	±	0.23	**6.00**	±	0.13	**6.66**	±	0.64	**6.40**			**5.67**	±	0.14
C 22:6 N-3	**34.21**	±	0.95	**32.46**	±	1.67	**32.63**	±	0.45	**36.09**			**31.02**	±	0.94
SUM PUFA	**61.27**	±	0.95	**62.37**	±	0.67	**63.00**	±	0.77	**63.71**			**56.47**	±	1.97
N3/N6	**5.43**	±	0.25	**5.61**	±	0.31	**5.56**	±	0.11	**6.42**			**5.80**	±	0.08
OTHERS	**2.08**	±	0.03	**2.06**	±	0.01	**2.29**	±	0.10	**2.21**			**3.44**	±	1.13

Table 8.5.b Fatty acid profile of Salmon erythrocytes PE (% of total fatty acids) fed with low diet (0,2 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

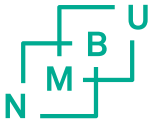
LOW DIET

	Pre-Diet 0%	Pre-diet 2% DHA	Pre-Diet 2 % EPA	Pre-Diet 2 % EPA+DHA	Com cont
C 14:0	**0.02 ± 0.02**	**N.D.**	**N.D.**	**N.D.**	**0.16 ± 0.09**
C 16:0	**5.75 ± 0.21**	**5.57**	**6.20**	**15.45**	**14.01 ± 0.50**
C 18:0	**11.45 ± 0.45**	**9.63**	**13.24**	**22.68**	**21.66 ± 1.44**
C 22:0	**0.18 ± 0.01**	**0.12**	**0.46**	**2.52**	**2.17 ± 0.19**
SUM N-0	**17.91 ± 0.52**	**15.77**	**20.49**	**42.00**	**40.33 ± 1.64**
C 16:1 N-7	**0.10 ± 0.01**	**0.14**	**0.08**	**0.00**	**0.21 ± 0.05**
C 18:1 N-11	N.D.	N.D.	N.D.	N.D.	N.D.
C 18:1 N-9	**5.62 ± 0.07**	**5.98**	**6.11**	**6.89**	**6.89 ± 0.83**
C 18:1 N-7	**1.23 ± 0.09**	**1.21**	**1.26**	**1.18**	**1.11 ± 0.13**
C 20:1 N-11	**0.02 ± 0.02**	**0.08**	**0.00**	**0.00**	**0.00 ± 0.00**
C 20:1 N-9	**2.37 ± 0.05**	**2.07**	**2.78**	**4.54**	**4.92 ± 0.18**
C 22:1 N-11	**5.56 ± 0.33**	**5.32**	**5.90**	**2.52**	**2.02 ± 0.20**
C 22:1 N-9	**0.20 ± 0.01**	**0.21**	**0.21**	**0.00**	**0.00 ± 0.00**
SUM MUFA	**15.95 ± 0.33**	**16.10**	**17.50**	**18.98**	**19.39 ± 0.81**
C 18:2 N-6	**5.71 ± 0.12**	**6.08**	**6.83**	**5.71**	**6.86 ± 1.06**
C 18:3 N-6	**0.02 ± 0.02**	**0.06**	**0.00**	**0.00**	**0.00 ± 0.00**
C 18:3 N-3	**1.23 ± 0.04**	**1.34**	**1.39**	**1.18**	**1.33 ± 0.12**
C 20:4 N-3	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
C 20:2 N-6	**2.11 ± 0.06**	**1.91**	**2.28**	**0.67**	**0.59 ± 0.02**
C 20:3 N-9	**0.22 ± 0.03**	**0.14**	**0.59**	**2.69**	**2.69 ± 0.11**
C 20:3 N-6	**5.38 ± 0.22**	**5.48**	**5.65**	**2.86**	**2.60 ± 0.21**
C 20:4 N-6	**6.01 ± 0.06**	**6.41**	**5.90**	**3.19**	**2.44 ± 0.25**
C 20:3 N-3	**2.61 ± 0.10**	**2.24**	**2.66**	**1.51**	**1.34 ± 0.10**
C 20:5 N-3	**8.92 ± 0.35**	**10.15**	**9.91**	**4.70**	**4.35 ± 0.36**
C 22:5 N-3	**6.73 ± 0.18**	**6.68**	**6.37**	**3.70**	**4.00 ± 0.36**
C 22:6 N-3	**25.58 ± 1.11**	**25.76**	**19.52**	**15.45**	**15.77 ± 1.33**
SUM PUFA	**65.51 ± 0.44**	**67.35**	**61.69**	**38.97**	**40.14 ± 1.34**
N3/N6	**2.21 ± 0.08**	**2.18**	**1.84**	**2.14**	**2.12 ± 0.31**
OTHERS	**2.35 ± 0.28**	**2.65**	**2.32**	**2.52**	**4.75 ± 0.30**

Table 8.5.c Fatty acid profile of Salmon erythrocytes PE (% of total fatty acids) fed with control diet (1,7 % EPA and DHA) including pre- diets. Data are expressed as mean ± standard deviation

CONTROL DIET

	Pre-Diet 0%	**Pre-diet 2% DHA**	**Pre-Diet 2 % EPA**	**Pre-Diet 2 % EPA+DHA**	**Com cont**
C 14:0	**0.36 ± 0.06**	**0.44**	**0.29 ± 0.07**	**0.11 ± 0.02**	**0.12 ± 0.01**
C 16:0	**15.84 ± 0.63**	**16.27**	**7.07 ± 0.47**	**6.63 ± 0.21**	**6.37 ± 0.22**
C 18:0	**19.11 ± 0.88**	**16.57**	**8.21 ± 0.09**	**7.49 ± 0.19**	**7.35 ± 0.22**
C 22:0	**1.70 ± 0.13**	**2.51**	**0.18 ± 0.15**	**0.09 ± 0.09**	**0.21 ± 0.02**
SUM N-0	**39.75 ± 0.11**	**38.46**	**17.80 ± 1.36**	**15.10 ± 0.41**	**14.90 ± 0.04**
C 16:1 N-7	**0.31 ± 0.02**	**0.00**	**0.21 ± 0.03**	**0.23 ± 0.01**	**0.19 ± 0.01**
C 18:1 N-11	**0.10 ± 0.08**	**0.30**	**0.34 ± 0.08**	**0.24 ± 0.00**	**0.25 ± 0.00**
C 18:1 N-9	**4.98 ± 0.12**	**5.92**	**4.18 ± 0.32**	**4.24 ± 0.08**	**3.89 ± 0.08**
C 18:1 N-7	**1.60 ± 0.12**	**1.77**	**1.95 ± 0.14**	**2.13 ± 0.07**	**1.73 ± 0.03**
C 20:1 N-11	**0.00 ± 0.00**	**0.00**	**0.00 ± 0.00**	**0.00 ± 0.00**	**0.00 ± 0.00**
C 20:1 N-9	**6.57 ± 0.71**	**6.36**	**7.74 ± 0.07**	**7.52 ± 0.17**	**6.82 ± 0.02**
C 22:1 N-11	**1.19 ± 0.10**	**1.04**	**2.57 ± 0.04**	**2.76 ± 0.13**	**2.47 ± 0.05**
C 22:1 N-9	**1.45 ± 0.16**	**1.33**	**3.30 ± 0.08**	**3.43 ± 0.22**	**2.76 ± 0.01**
SUM MUFA	**20.74 ± 0.91**	**22.63**	**21.93 ± 0.58**	**22.07 ± 0.40**	**19.70 ± 0.05**
C 18:2 N-6	**2.97 ± 0.02**	**4.29**	**1.22 ± 1.00**	**2.86 ± 0.06**	**2.52 ± 0.02**
C 18:3 N-6	**0.00 ± 0.00**	**0.00**	**0.00 ± 0.00**	**0.00 ± 0.00**	**0.00 ± 0.00**
C 18:3 N-3	**0.51 ± 0.03**	**0.74**	**0.52 ± 0.03**	**0.52 ± 0.02**	**0.50 ± 0.01**
C 20:4 N-3	**N.D.**	**N.D.**	**N.D.**	**0.07 ± 0.07**	**0.17 ± 0.01**
C 20:2 N-6	**0.77 ± 0.00**	**0.59**	**0.99 ± 0.02**	**1.07 ± 0.01**	**1.02 ± 0.03**
C 20:3 N-9	**2.73 ± 0.18**	**3.40**	**0.75 ± 0.04**	**0.64 ± 0.03**	**0.59 ± 0.00**
C 20:3 N-6	**0.51 ± 0.06**	**0.30**	**0.75 ± 0.04**	**0.72 ± 0.03**	**0.60 ± 0.03**
C 20:4 N-6	**0.76 ± 0.17**	**0.59**	**0.91 ± 0.06**	**0.92 ± 0.05**	**0.85 ± 0.02**
C 20:3 N-3	**0.41 ± 0.02**	**0.44**	**0.72 ± 0.02**	**0.79 ± 0.00**	**0.78 ± 0.04**
C 20:5 N-3	**5.75 ± 0.12**	**7.40**	**11.59 ± 0.10**	**11.72 ± 0.43**	**11.44 ± 0.05**
C 22:5 N-3	**3.69 ± 0.07**	**3.11**	**6.27 ± 0.11**	**6.15 ± 0.35**	**6.02 ± 0.22**
C 22:6 N-3	**23.51 ± 1.00**	**21.00**	**35.23 ± 1.95**	**37.00 ± 0.99**	**40.50 ± 0.24**
SUM PUFA	**39.25 ± 1.09**	**38.75**	**58.48 ± 2.93**	**62.14 ± 0.37**	**64.67 ± 0.02**
N3/N6	**6.85 ± 0.18**	**5.72**	**15.06 ± 2.86**	**10.08 ± 0.25**	**11.94 ± 0.03**
OTHERS	**4.91 ± 0.17**	**5.47**	**3.20 ± 1.01**	**1.99 ± 0.07**	**2.11 ± 0.00**



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