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Fatty acid specificity of skin phospholipid subclasses and the response to dietary reduction of EPA and DHA in Atlantic salmon (*Salmo salar*)

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Abbreviation

AA	arachidonic acid
ALA	alpha-linolenic acid
BHT	2,6-Di-t-butyl-p-cresol
BW	body weight
CC	commercial control
DHA	docosahexaenoic acid
EFA	essential fatty acid
EPA	eicosapentaenoic acid
FAMES	fatty acid methyl esters
FM	fish meal
FO	fish oil
FO	fish oil
GC	gas liquid chromatography
LA	linoleic acid
lcPUFA	long chain polyunsaturated fatty acid
LO	linseed oil
MUFA	monounsaturated fatty acid
n-3	omega-3
n-6	omega-6
NC	negative control
OA	oleic acid
OO	oleic oil
PA	phosphatidic acid
PC	phosphatidylcholine
PCA	principle component analysis of data
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PL	phospholipid
PS	phosphatidylserine

PUFA	polyunsaturated fatty acid
RO	rapeseed oil
SBM	soybean meal
SFA	saturated fatty acid
SM	sphingomyelin
TAG	tricylglycerol
TEWL	transepidermal water loss
TLC	thin layer chromatography
VM	vegetable meal
VO	vegetable oil

Abstract

The different phospholipid classes play a critical role in cell membrane structure and function. Skin is the first barrier for the animal body to prevent environmental damage and are in addition to the gills also involved in osmoregulation. However, there are no earlier studies that have investigated the relationship between different dietary levels of either EPA, DHA or a mix of EPA and DHA on the fatty acid composition of Atlantic salmon skin PL subclasses. Therefore, we aimed to find the effects of n-3 PUFA deficiency for the fatty acid composition in Atlantic salmon skin PL subclasses.

The experimental fish was reared from 40 gram and divided into 14 groups. For each group, they were fed with one of 14 different diets. The diets were 12 different diets (0.5%, 1%, 1.5% and 2% of either EPA, DHA or a 1:1 mix of EPA+DHA, respectively) with one negative control diet (0% EPA + DHA) and one diet with a composition close to a commercial diet with 2,2% EPA+DHA. The skin samples was taken when the fish was 200 gram and 400 gram, for each group, the skin sample was analyzed in duplicates (in EPA or DHA group) or triplicates (in EPA+DHA, NC and CC group). TLC was applied in duplicate, to separate the different PL classes. GC was used to determine the fatty acid composition in the PL subclasses. PCA was used to do the statistical analysis.

Each PL subclass contains a unique fatty acid pattern regardless the diet group. But all of PL subclasses were affected by the different fatty acid compositions of the diets. The influence was more pronounced the longer time the fish had been fed the different diets, PS and PI are more conserved than PC and PE. 20:4n-6 is dynamically increased when EPA and DHA decreases in the PE and PI fractions, whereas an increase in 20:3n-6 follows the EPA and DHA decrease in the PS fraction. Moreover, PI fraction contains the highest percentage of 20:4n-6. An increase in short chain MUFAs in the PC and PS fractions was also observed when the EPA and DHA decreased, while the increase of some SFA (12:0, 16:0, 18:0) with EPA and DHA decrease was observed in almost all PL subclasses, except PC fraction. The percentage of DHA was significantly reduced in all Atlantic salmon skin PL classes, while a significant reduction of EPA was detected in the PC and PE fractions. Furthermore, DHA is much better retained in PE and PS group than in PC and PI fractions.

In general, 18:1n-9, some short chain SFA and n-6 PUFA increased with the dietary

decrease in EPA and DHA, whereas n-3 lcPUFA decreased with dietary EPA and DHA decrease.

1. Introduction

1.1 Importance of fish and fish farming

Fish is an important food source for human. Protein from fish is a good protein source to provide essential amino acid for human as other animal protein (livestock and poultry) (Gjedrem et al. 2012). Oils from fish, especially from marine fish, can provide numerous omega-3 polyunsaturated fatty acid (n-3 PUFA), especially the two long-chain omega-3 polyunsaturated fatty acids (lcPUFA), EPA and DHA for human, and these lcPUFA are very helpful to prevent heart disease, brain disease and inflammation (Jacobsen. 2015). Another scientific group from Korean proved that fish skin is an ingredient to make fish meal with additional fish oil and essential amino acid (Cho et al. 2014). On the other hand, fish can convert feed into their biomass more efficiently than terrestrial animals; it means fish can output more with the same input. Thus, fish is an important source to satisfy the demand of food for an increasing human population.

Fish farming becomes more and more important. Because the world population is increasing steadily (fig 1.1), this leads to an increasing demand of seafood, but the production from fishery and fish capture keep stable or even decrease (fig 1.2) (FAO, 2014). Fortunately, worldwide aquaculture industry has grown faster and faster over the last five decades (fig 1.2) (FAO, 2014), and it is predicted to continue expanding. This increasing trend of aquaculture is helpful to meet the demands of seafood for a growing global population.

1.2 Importance and necessity of using less fish ingredients in aquaculture feed

Feed plays a critical role in aquaculture industry. Fish, especially fat species like Atlantic salmon (*Salmo salar*), is rich in n-3 lcPUFA in lipids that have critical roles in animal nutrition, including fish (cell fluidity) and human nutrition (nervous system) (Tocher 2003). Marine fish, especially carnivorous fish species, needs fish meal (FM) and fish oil (FO) in its feed to satisfy the requirements for the protein, energy, essential amino acid and essential fatty acid to regular growth, health and reproduction (Tocher 2003; Tacon 2008). Marine captured fish has been used as the only source of dietary protein and dietary lipids for farmed fish for a long time, due to its high level n-3 PUFA, animal protein, wide existence and attractive price (Turchini et al. 2009). Aquaculture industry has developed very fast to meet the seafood demand of an increasing human population. It accounted less than 5% in 1950

and increased to 45% after 2012 (FAO 2014). However, the aquaculture industry still relies highly on fish meal and fish oil, and the supply of these two ingredients from industrial fisheries cannot satisfy the increasing requirement from the aquaculture industry (Tacon et al. 2008; Turchini et al. 2009). According to some research (Worm et al. 2006; FAO 2014), world food fish aquaculture production expanded at an average annual rate of 6.2% in the period 2000–2012 from 32.4 million tons to 66.6 million tons, while worldwide fish oil production decreased at an average annual rate of 2.6% in the period 1994 – 2009 from 1.50 million tons in 1994 to 1.07 million tons in 2009 and now is quite stable production.

Feed investment is the largest part in aquaculture. For FM, “ the consumption of fish meal in aqua feeds increased over two-fold from 1882 thousand tons in 1995 (27.5% total reported fish meal production of 6852 thousand tons) to 4300 thousand tons in 2005 (68.9% total reported fish meal production of 6242 thousand tones)” (Reviewed by Tacon et al. 2008). In the case of FO, “ the consumption of fish oil in compound aqua feeds increased from 474 thousand tons in 1995 (34.3% total reported fish oil production of 1382 thousand tones) to 843 thousand tons in 2005 (93.7% total reported fish oil production of 900 thousand tones)” (Reviewed by Tacon et al. 2008). By contrast, the average dietary level of fish meal and fish oil in the compound aqua feed has decreased steadily from 1990-2013 (Ytrestøyl et al 2015). For instance, 90% of the salmon feed ingredients were from marine fishery in 1990, whereas in 2013 only around 30%. The main reason for this phenomenon was that it is not enough and then the price of FM and FO is increasing (Fig. 1.3); fish meal prices increased from \$694 per ton to \$1379 per ton between July 2005 and July 2006, and fish oil prices increased from \$894 per ton to \$1700 per ton between March 2007 and March 2008 (Fig. 1.3). This phenomenon will cause the reduction value in the fish products, both economic value and nutritional value. Therefore, it is important and necessary to use less fish ingredients in fish feed

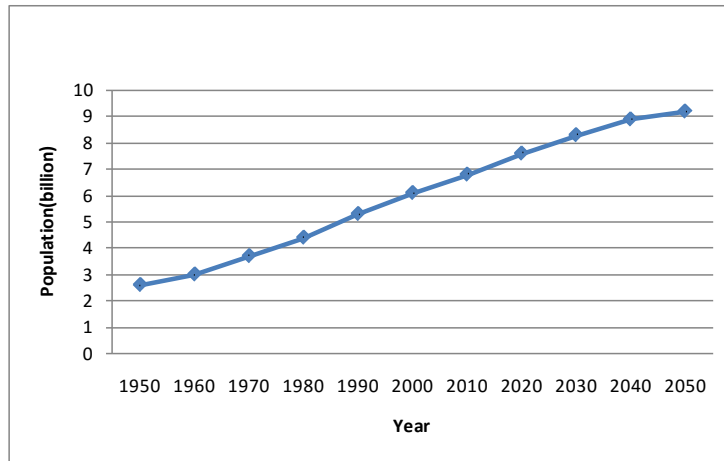


Fig 1.1 The world population from 1950-2050, values from U.S. census bureau, International Database, update at June 2010.

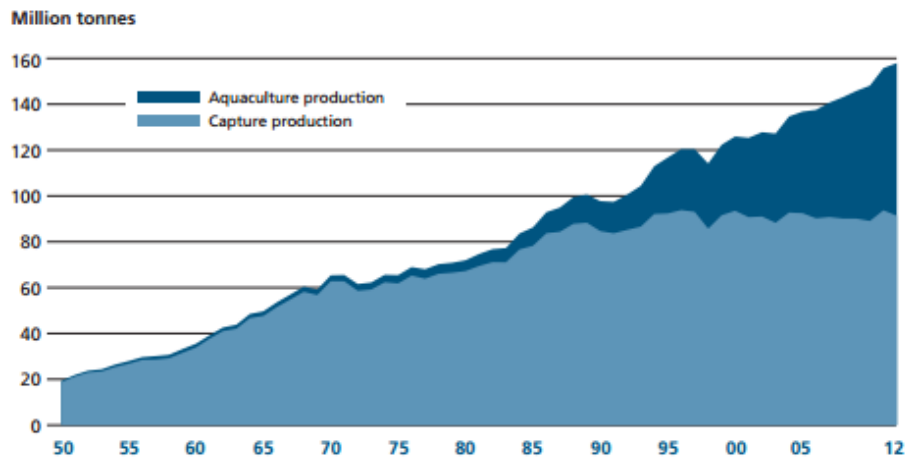


Fig 1.2 World capture fisheries and aquaculture production, figure taken from FAO 2014.

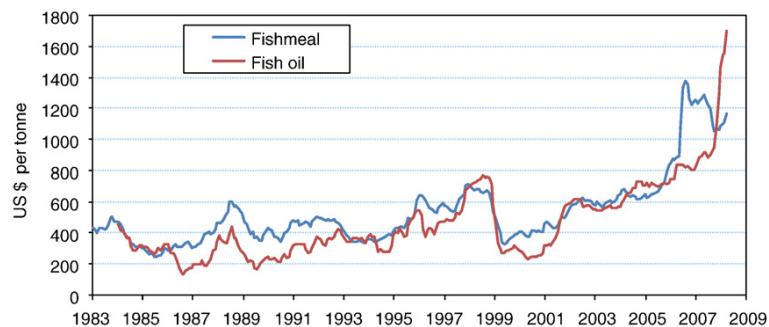


Fig 1.3 International market price for fish oil and fish meal , figure taken from Tacon (2008).

1.3 Replacement of fish ingredients in aquaculture feed

Many methods were tested in trial and some methods have been used in practical terms to replace FM and FO in feed.

First of all, some scientists focus on the utilization of fish by-products as fish meal successfully. For instance, trout offal oil has been tested in diet as an alternative lipids source for Murray cod, *Maccullochella peelii peelii* (Turchini et al. 2003a). Another example, fish

skin supplied with additional fish oils and essential amino acids can be a viable alternative for fish meal formulation (Cho et al. 2014). However, despite the possibility of this alternative source, the production, availability and price of processing fish by-products have not been estimate (Turchini et al. 2009).

Recently, a new alternative lipid source, derived from unicellular algae or pelagic organism containing high amounts of n-3 HUFA, has been tested in trial (Reviewed by Turchini et al. 2009). However, the limiting factor for this new alternative source is the unpredictable supply and high production costs.

The last and the most important, vegetable meal (VM) and vegetable oil (VO) was the best substitute for FM and FO and has been utilized in practical terms, such as soybean meal (SBM) and rapeseed oil (RO) (Øverland et al, 2009; Bell et al. 2001; Regost et al. 2003a; Ng et al. 2007a; Stubhaug et al. 2007). In the case of VO, most VO are poorly source of n-3 fatty acids (only contains some 18:3n-3) and rich sources of n-6 and n-9 fatty acids, mainly linoleic acid (LA; 18:2n-6) and oleic acid (OA; 18:1 n-9), with the exception of linseed oil (LO), which is rich in alpha-linolenic acid (ALA; 18:3 n-3), in comparison to FO. However, VM and VO cannot be the sole protein and lipid source in diet for fish, but a blend of vegetable ingredient and fish ingredient in diet for fish can provide a good growth (Ruyter et al, 2000a; Øverland et al, 2009, Ytrestøyl et al 2015). On the other hand, despite the blend of vegetable ingredients with fish ingredients, the ratio of SFA, MUFA and PUFA will be affected, in comparison to the sole usage of fish ingredients in diets (Torstensen et al. 2005; Francis et al. 2007a).

Until now, fish oil is necessary for Atlantic salmon to provide EPA and DHA. Turchini pointed that fish oil is essential in feed to provide n-3 lcPUFA but a lot of fish oil in feed can be replaced by plant oils (Turchini et al. 2009). Thus, our group is trying to find the minimal level of EPA and DHA in diet for Atlantic salmon.

1.4 Lipids

A common definition of lipids is the hydrophobic compounds that are soluble in organic solvent (Tocher, 2003; Turchini et al, 2009). Lipids are the most efficient energy source for animals, in comparison to protein or carbohydrates, because of energy density (Bureau et al, 2002). Lipids in animals contain many different lipid classes and some of the main lipid

classes are TAG, PL, wax ester and sterol (Tocher 2003; Turchini et al. 2009). Fatty acids are the important compounds of the all type of lipids. For the neutral lipids, TAG, constitute the major neutral lipids and three esterified molecules of fatty acid and one molecule of glycerol while wax esters constitute another class of neutral lipid consisting of a single esterified molecule of fatty acid. Phospholipids are a major class of polar lipids, due to the polar head (fig 1.4), and phosphoglycerides are the most common phospholipids. Phosphoglycerides consist a glycerol with two esterified fatty acid. Sterol, in animal including fish tissues, is existed as cholesterol. “Cholesterol is the most special lipid in animal tissues that contains no fatty acids, but cholesterol is to a relatively large extent esterified to fatty acids in the body” (Tocher 2003). For fish, “lipids are required for a source of energy, a structural component of cell membranes, a carrier of fat-soluble nutrients and a precursor of numerous hormones and eicosanoids” (Higgs & Dong, 2000).

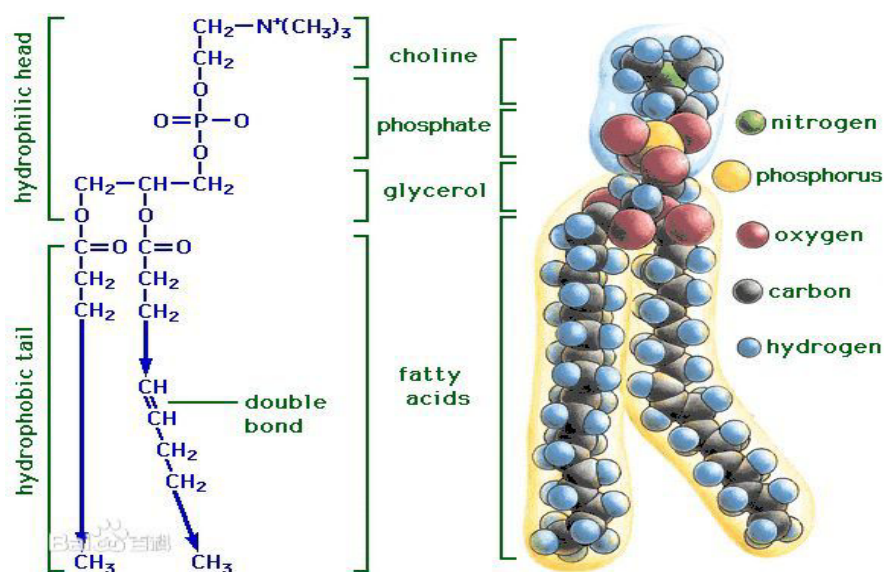


Fig 1.4 The structure of phospholipids. Figure taken from Baidu Baika.

PL are one form of animal lipids, in the term of all lipids including phosphorous and phospholipids are a major class of polar lipids (Tocher et al, 2008). Recent research proved that phospholipids play a critical role in animals. First of all, phospholipids incorporation with glycoprotein to form the bilayer of cell membrane, due to its amphipathic structure (Hazel and Williams, 1990; Tocher et al., 2008). Secondly, phospholipids are an emulsifier in gut to emulsifying lipids and increase the lipid absorption in body (Couttear et al, 1997; Tocher et al, 2008). Thirdly, phospholipids are a critical precursor for a series of metabolism mediators, such as eicosanoids (Tocher et al, 2008). Finally, a lot of researches indicate that

phospholipids have a significant effect on growth, survival and stress resistance in some fish larvae and early juvenile (Cahu et al., 2003; Kanazawa, 1993; Kanazawa et al., 1981; Liu et al., 2002; Tocher et al., 2008, Zhao et al, 2013). According to the investigation of the phospholipids synthesis pathway, many scientists pointed that phospholipids are necessary in the diet, due to their insufficient endogenous production, and provide a better growth for fish compare to fish fed with TAG in diet (Coutteau et al, 1997; Richard et al, 2007; Glencross, 2009). In the case of PL subclasses, phospholipids can be divided into sphingolipid such as sphingomyelin (SM) and phosphoglycerides, due to the different backbone of phosphatidic acid (PA). “In the case of phosphoglycerides, it has a common backbone of PA, formed from L-glycerol 3-phosphate with two fatty acids esterified on positions 1 and 2. Due to the different polar head group, phosphoglycerides can be divided into four different major forms in animal. They are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI), respectively, are formed by the esterification of the “bases” choline, ethanolamine, serine and inositol to the phosphate group of PA” (Tocher et al, 2008). For the bilayer, the different subclasses of phospholipids are combined to form the amphipathic structure, PC and SM are concentrated on the outer leaflet and PE, PS and PI are concentrated on the inner leaflet (Kagan et al, 1984). Some phosphorylated derivative, such as PI, plays an important role in inner-cellular trafficking, cytoskeleton and cell survival (Tocher et al 2008). For PC, numerous studies proved that this phosphoglyceride rich in some liposomes (PC-L) can interact with plasma lipoproteins and have shown an antiatherogenic effect (Hajj Hassan et al. 2005, Jian et al. 1997). In the case of PS, it has an important influence in apoptosis and blood coagulation (Yamaji-Hasegawa & Tsujimoto, 2006).

Current research is most focus on the phospholipids endogenous synthetic pathway, the level of phospholipids in diet and the fatty acid composition in total phospholipids. On the other hand, less research is focused on the fatty acid composition in phospholipids subclasses and the changes of EFA in phospholipids subclasses with the different diet, especially in fish.

1.5 Fatty acids and the five key essential fatty acids

International Union of Physical and Applied Chemists (IUPAC) have defined fatty acid. According to IUPAC standards, “the fatty acid formula is defined with the carbon atoms in

the fatty acid chain, the number of unsaturated bonds (double bonds) within the chain and the position of the first double bonds within the chain” (Rigaudy & Klesney 1979). Fatty acid can be divided into fatty acid without double bonds (e.g. C16:0) called SFA, fatty acid with one single double bond within the chain (e.g. C18:1n-9) called MUFA and fatty acid with two or more double bonds within the chain (e.g. C20:4n-6) called PUFA (Rawn 1989). In the case of PUFA, if the carbon atoms are more than 20 in the fatty acid chain and the double bonds are more than 3, these fatty acids are called lcPUFA (Nichols 2004).

Fatty acids that cannot be biosynthesized *in vivo* are termed essential fatty acid (EFA). According to Glencross (2009), there are five key EFA in vertebrates: LA, ALA, AA, EPA and DHA. In fact, LA and ALA are the absolute EFA while AA, EPA and DHA are the relative EFA. Because LA and ALA are the base of the other n-6 fatty acids and n-3 fatty acids and the biosynthesis *in vivo* of LA and ALA is impossible for vertebrates (Watanabe 1982). All vertebrates, including fish, have the potential to synthesis AA, EPA and DHA from LA and ALA *in vivo* with the process of elongation and desaturation (Nakamura & Nara 2004). “Due to the adaption of lcPUFA-rich environment, numerous species have lost this capacity and require a level of lcPUFA in their diet” (Sargent et al 2002). For example, the most life time of Atlantic salmon is in sea and EPA and DHA are EFA for Atlantic salmon. On the other hand, LA and ALA is not sufficient for some species, thus they require a level of lcPUFA in their diet, for instance, Atlantic salmon requires EPA and DHA in their diet due to the insufficient conversion from ALA to EPA and DHA (Ruyter et al 2000a). Thus, AA, EPA and DHA have been EFA in some species. The deficiency of EFA in fish can lead to poor growth rate, higher sensitivity to stress, diseases and mortality (e.g. fin rot and death after handling) (Castell et al 1972a, b, Watanabe 1982, Sargent et al 1995b, Ruyter et al 2000a).

EFA, especially AA, EPA and DHA, play a vital role in animal growth, reproduction, immunity and product quality (Glencross 2009). DHA provides the greatest EFA value for most species, followed is EPA and AA. In fish, EPA and DHA are more important for cell membrane structure and function. However, AA is more important for terrestrial animal (Sargent et al 1999). “DHA with six double bonds in the fatty acid chain provides a strong but flexible structure. This structure leads DHA to be vital for neural, visual and sperm cell of animals”(Mourente &Tocher 1991, Sargent et al. 1993; Masuda 2003). DHA in retinal cells

can affect the fish feeding behavior and schooling behavior notably (Masuda 2003). AA is a critical n-6 PUFA for mammalian brain, muscle and other organs function (Fan et al 2012; Stroud et al 2009). AA also plays as precursor of a series of highly biologically active lipids such as eicosanoids (Sargent et al 1999). EPA has the similar function to DHA and EPA is important to regulate the action of eicosanoids (Sargent et al 1999).

Due to the competitive interaction of these EFA, it is necessary to investigate the ratio of EPA, DHA and AA when considering their influence for animals. Numerous studies pointed that different ratio of EPA/DHA will leads to different growth rate and the optimal ratio of EPA/DHA is species-dependent (Kalogeropoulos et al 1992; Ibeas et al. 1994, 1996, 1997). A recent research reported that the ratio of EPA/DHA is vital for egg and larval quality, nonspecific immunity and disease resistance and n-3 lcPUFA retention (Reviewed by Qiao et al 2016). Yui et al. (2016) pointed that a too high ratio of plasma EPA: AA or DHA: AA will diminish the protective capacity against brain damage. EPA and AA are opposite when forming eicosanoids: “the eicosanoids formed from EPA will inhibit the biological activity of eicosanoids formed from AA and the ratio of EPA/AA various on species” (Sargent et al 1999). Thus, four important issues have been raised: first, high dietary level of DHA is important for neural development; second, the ratio of DHA/EPA is important for growth; third, the importance of DHA/AA or EPA/AA for brain protective capacity; last, the ratio of EPA/AA is vital to determining eicosanoids action.

1.6 Importance of skin and the importance of fatty acid for skin

Skin is the largest organ and plays a critical role in vertebrates, ranging from fish to human (Schempp et al 2009). There are two major common functions of skin, first, skin is regard as the first biological barrier of animal body to prevent physical, bacterial and chemical damage; second, skin is important to maintenance the homoeostasis (Glover et al 2013). However, the skin functions are different between mammalian and fish. For mammalian, skin has the vital function for minimizing transepidermal water loss (TEWL), sensory activity and hormone metabolism (Rakers et al 2010, Barcelos et al 2015). In the case of fish, “skin plays a more physiological role, it is regarded as a transport epithelium for gases, ions, nitrogenous waste products and nutrients. For fish larval, skin is a surrogate gill and prior to the development of a functional branchical epithelium” (Glover et al 2013). The

skin consists of two components, an epidermis and a dermis (fig 1.5). At the upper epidermis, “mammalian has the stratum corneum to prevent TEWL while the upper strata of fish skin is not cornified, but an alternative permeability barrier could exist and cooperate with gill to do the osmoregulation” (Ghioni et al 1997a, b, c). Fish scale is different to other scaled animals. They are mineralized, not keratinized, and overlain epidermis, except the skin of sharks, in which scales protrude through epidermal surface (Hawkes 1974; Meyer & Seegers 2012).

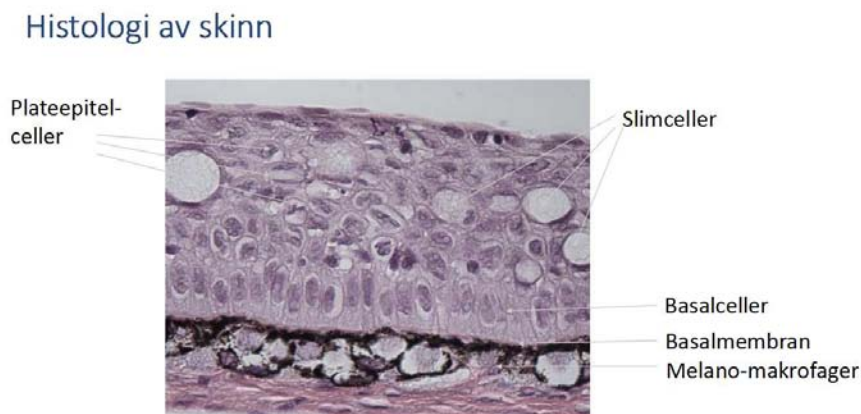


Fig 1.5 The structure of skin. Figure tanken from our trial.

Fatty acids, especially EFA, are crucial for the function of epidermis and the epidermally-derived skin appendage, such as sebaceous glands (Stewart & Downing 1991). According to the review by Lin and Khnykin (2014), fatty acids have multiple functions in epidermis: “in addition to their well-known function in energy generation and storage as well as the structural blocks of bilayer to ensure the integrity and fluidity of cell membrane, fatty acids have four other main effects for epidermis”. First, ceramides, one lipid derived from fatty acids, and other complex lipids play an important role for the permeability barrier. Second, fatty acids are the base of sebum. And then, fatty acids are important to regulate epidermal homeostasis. Finally, fatty acids ensure the structural integrity and barrier functions of stratum corneum. As mentioned above, the permeability barrier related lipids are LA-rich lipids and eicosanoids derived from AA are also important for permeability (Hansen 1986). However, the epidermis cell cannot metabolise LA to AA in mammalian, due to the lack of $\Delta 6$ and $\Delta 5$ desaturases. Thus, AA must be transported from other organs to skin but this mechanism was not investigated in Atlantic salmon (Chapkin & Ziboh 1984). The EFA

deficiency in mammal will increase TEWL and local inflammation. And the components of ceramides or other complex lipids will change from LA to oleic oil (C18:1n-9, OO) (Ghioni et al 1997c). The EFA deficiency in fish will cause fin erosion (March 1993). Recent research pointed that the high level of n-3 lcPUFA and the lower ratio of n-6 PUFA: n-3 PUFA in skin provides a significantly positive effect. The high level of n-3 lcPUFA in skin will decrease the severity of skin photoaging in human middle-age (Latreille et al 2013). And the lower ratio of n-6 PUFA: n-3 PUFA in skin will decrease the TEWL for wistar rats (Barcelos et al 2015).

Until now, the lipid and fatty acid composition in skin is poorly documented in Atlantic salmon (*Salmo salar*). A Korean scientific group investigated four marine fish species: olive flounder (*Paralichthys olivaceus*), black rockfish (*Sebastes schlegeli*), sea bass (*Lateolabrax maculatus*) and red sea bream (*Pagrus major*) and showed that the level of SFA, MUFA and PUFA in fish skin is different depending on fish species (Cho et al 2014).

In the present study, we fed Atlantic salmon with 14 different diets, those diets contain different EPA or DHA or EPA and DHA level. We aimed to find the fatty acid composition in different PL subclasses and the changes of fatty acid composition in Atlantic salmon skin.

2. Methods and Materials

2.1 Chemicals and Equipments

Table 2.1 Chemicals and Equipments

Chloroform	VWR PROLABO CHEMICALS, Fontenay-Sou-Bois, France
Methanol	VWR PROLABO CHEMICALS, Fontenay-Sou-Bois, France
Petroleumseter	SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany
Diethyleter	SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany
Acetic acid	MERCK, Darmstadt, Germany
2-7-Dichlorofluorescin	MERCK, Darmstadt, Germany
Ethanol absolute	VWR PROLABO CHEMICALS, Fontenay-Sou-Bois, France
Benzene	VWR PROLABO CHEMICALS, Fontenay-Sou-Bois, France
Methanolic Hcl, 3N	SUPELCO, PA, USA
2,2-Dimethoxypropane	SIGMA-ALDRICH CHEMIE GmbH, Steinheim, Germany
Hexane	VWR PROLABO CHEMICALS, Fontenay-Sou-Bois, France
TLC Silica Gel 60g 25 glass plate	MERCK, Darmstadt, Germany
GC capillary	SGE Analytical Science Pty Ltd., Victoria, Australia
Hewlett Packard 6890 series gas liquid chromatography system(GC system)	Avondale, PA, USA
Hewlett Packard 7683 series GC injector and auto sampler	Avondale, PA, USA

2.2 Samples, fish farming and feed diet

All of information in this section was provided by Marta Bou Mira.

The experimental fish is Atlantic salmon (*Salmo salar*) that were raised in 33 indoor tanks at *Nofima* institute in *Sunnalsøra*, Norway.

Each tank (1 m² surface area, 0.6 m water depth) was supplied with 15 L seawater min⁻¹ of ca 33 ppt salinity and ambient temperature. Water temperature was measured everyday and

it varied between 6.3 and 13.8 °C. The oxygen saturation level was kept above 85%. For experimental fish, every 70 individuals were stocked in one tank with an average body weight (BW) of 40 gram. Prior to the experiment, fish were fed a commercial diet and treated with light to induce smoltification.

This experiment lasted for 186 days, and the fish grew from 40 g average BW to 400 g average BW. The fish was fed with one of 12 various experimental diets and 2 control diets. The experimental diets were formulated with different levels of EPA, DHA or a 1:1 mixture of EPA plus DHA, defined as 0.5, 1, 1.5 or 2% of the feed dry weight (DW) (Table 2.3). One of the control diets was a negative control diet (NC) which was completely depleted in EPA and DHA. The other control diet resembles to a commercial control diet (CC) which included 2.2% EPA and DHA. All diets were produced by Nofima feed technology centre (Norway). Total lipid content remained constant, on a level of $23.9 \pm 0.65\%$ (mean \pm s.d.) of the feed dry weight. When producing the experimental diets, poultry oil and rapeseed oil was utilized as oil sources, they are naturally absent of EPA and DHA. The fish was fed by automatic disc feeders.

As mentioned above, this experiment has 33 tanks in total, 18 tanks were used for the 0.5, 1 and 1.5% EPA and/or DHA dietary groups (2 tanks per diet) (table 2.2). The rest of tanks were used for the CC, NC and the 2% EPA and/or DHA dietary groups (3 tanks per diet).

Table 2.2 Distribution of tanks over dietary groups

Diet	Amount of tanks
CC ¹	6 (triplicate)
NC ²	
0.5, 1, 1.5% EPA	18 (duplicate)
0.5, 1, 1.5% DHA	
0.5, 1, 1.5% EPA+DHA	
2% EPA	9 (triplicate)
2% DHA	
2% EPA + DHA	

¹CC = commercial control.

²NC = negative control.

After 131 days, when the fish reached an average bodyweight of 200 grams, the first sampling took place. From each tank, 5 fish were randomly selected and anesthetized before

slaughter. Different tissues (including liver, heart, brain, intestine and skin) were collected. The samples were stored at -80 °C. In this thesis, I only analyse the skin samples.

After 186 days, when the fish reached an average bodyweight of 400 grams, another sampling took place. At this time, 8 fish were randomly selected from each tank and slaughter, being the additional 3 fish sampled from each tank used for whole body composition analysis (methods and results are not included in this master thesis).

Table 2.3 FA composition (g per 100 g feed DW) and total lipid content (%) of the experimental diets

	CC ¹	NC ²	0.5% EPA	1% EPA	1.5% EPA	2% EPA	0.5% DHA	1% DHA	1.5% DHA	2% DHA	0.5% EPA+DHA	1% EPA+DHA	1.5% EPA+DHA	2% EPA+DHA
14:00	0.7	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:00	3.0	3.5	3.4	3.2	3.1	3.0	3.4	3.3	3.2	3.1	3.3	3.2	3.3	3.1
18:00	0.8	0.9	0.9	0.8	0.8	0.8	0.9	0.9	0.9	0.9	0.9	0.8	0.9	0.8
20:00	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24:00:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1
Σ Saturated ³	4.6	4.7	4.6	4.4	4.2	4.1	4.7	4.5	4.5	4.4	4.5	4.4	4.5	4.3
16:1n-7	0.8	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.6	0.6	0.6	0.6
18:1(n-7)+(n-9)+(n-11)	7.6	9.4	9.1	8.7	8.4	7.9	9.2	8.7	8.4	8.1	8.9	8.7	8.7	8.3
20:1(n-7)+(n-9)+(n-11)	0.7	0.2	0.2	0.3	0.3	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
22:1(n-7)+(n-9)+(n-11)	0.5	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1
24:1n-9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ Monounsaturated ⁴	9.7	10.3	10.1	9.7	9.4	8.9	10.1	9.7	9.3	9.0	9.8	9.6	9.8	9.4
18:2n-6	4.6	4.8	4.7	4.5	4.3	4.2	4.7	4.5	4.4	4.2	4.6	4.5	4.5	4.3
18:3n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:2n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:3n-6	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ n-6 ⁵	4.9	4.9	4.8	4.6	4.5	4.3	4.8	4.6	4.5	4.3	4.7	4.6	4.7	4.4
18:3n-3	1.1	1.0	1.0	0.9	0.9	0.9	1.0	0.9	0.9	0.9	1.0	0.9	0.9	0.9
18:4n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	n.d.	n.d.	n.d.	n.d.	n.d.
20:4n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:5n-3	1.1	0.0	0.5	1.0	1.5	2.0	0.1	0.2	0.2	0.3	0.3	0.6	0.9	1.2
22:5n-3	0.2	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.2	0.0	0.1	0.1	0.1
22:6n-3	1.1	0.0	0.1	0.3	0.4	0.5	0.5	0.9	1.4	1.9	0.3	0.5	0.9	1.2
Σ n-3 ⁶	3.6	1.1	1.7	2.4	3.0	3.6	1.7	2.2	2.7	3.5	1.7	2.2	3.0	3.6
EPA + DHA	2.2	0.0	0.6	1.2	1.8	2.5	0.6	1.1	1.6	2.3	0.6	1.1	1.8	2.4
Σ Polyunsaturated ⁷	8.7	6.0	6.5	7.0	7.5	8.0	6.5	6.8	7.2	7.8	6.4	6.9	7.6	8.0
Total lipid	25.8	23.4	23.7	23.8	23.7	23.9	23.8	23.6	23.6	23.9	23.3	23.5	24.7	24.4

¹NC = negative control.

²CC = commercial control.

³Includes 15:0, 17:0.

⁴Includes 14:1n-5, 17:1n-7.

⁵Includes 16:2n-6, 20:4n-6, 22:2n-6.

⁶Includes 20:3n-3.

⁷Includes 16:2n-6, 16:3n-4, 20:3n-3, 20:4n-6, 22:2n-6.

2.3 Lipid extraction and gas liquid chromatography

2.3.1 Skin sample homogenization and lipid extraction

Lipids were extracted from homogenized skin samples according to the Folch extraction principles (Folch et al. 1957). Each sample is formed by a pool of 5 fish skin per tank, which were peeled off from fish fillets and kept at -80°C until they were homogenized with dry ice

using a blender. The resulting skin homogenates were collected in plastic bags and stored at -40°C with the bags open to allow the dry ice to evaporate. Once the dry ice was evaporated, the bags were closed and kept at -40°C until the analysis. Firstly, ca 2 grams of skin homogenate (no more than 2 grams) was mixed with 6 ml of NaCl at 0.9% and 50 ml of chloroform-methanol (ratio 2:1) that contained 0.1 ml 0.7mg/L BHT (an antioxidant called 2,6-Di-t-butyl-p-cresol) for 1 minutes. The aim of this step is to extract the total lipid fraction (both polar and unpolar lipids) from skin samples. After that, homogenize the sample again with 6 ml of NaCl at 0.9% to separate the chloroform phase and the water phase (the ratio of chloroform: methanol: water is 86:14:1 and 3:48:47 respectively), the lower phase is chloroform phase (contains lipids) and the upper phase is water phase. Then, the liquid is filtered through cotton filter placed in a funnel to remove the residue (skin residue without lipids and protein). In addition, to avoid losing some lipid groups when extract lipids from skin samples, it is important to keep the ratio between chloroform, methanol and water in the solution equal to 8:4:3. After filtering, keep the samples stay for 1 hour in order to allow the separation of two phases. Then, transfer 20 ml chloroform phase to a pre-weighted beaker for fat content calculation (Section 2.3.2) and 5 ml chloroform to a new glass tube for thin layer chromatography (TLC) (Section 2.3.3).

2.3.2 Fat content calculation

Evaporate the chloroform phase until dryness and dry the rest of water of the lipid in incubator (105°C , 20 min). When evaporation and dryness is finished, wait the beaker reached room temperature to avoid inaccurate results. After that, weigh the beaker and use this formula to calculate the fat content:

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

Where g fat is total weight of the extracted lipids in the beaker, 100 is percentage, I means the weight of the skin sample in grams, U means the pipette chloroform extract (20 ml) in beaker and 37.5 is the total volume of solvent. As noted, the distribution of chloroform after separation is 86 parts in the chloroform phase and 3 parts in the water phase. In the chloroform phase, chloroform and methanol makes in total 100 parts (water is evaporated). In addition, the chloroform phase will contains methanol and some water, thus, the volume of

this phase is not 33.3 ml (chloroform in extract solution is $50 \times \frac{2}{3}$ equal to 33.3 ml), but 37.5 ml ($33.3 \text{ ml} \times \frac{100}{89}$).

2.3.3 Thin layer chromatography (TLC) and gas liquid chromatography (GC)

In this experiment, the whole TLC procedure will be applied in duplicate. In addition, all of the new TLC plates should be washed with pure methanol and marked on the top to determine the migrated direction before using. For the first TLC, the lipid extracts (5 ml) were dried under nitrogen overflow (in order to avoid oxidation), redissolved with several droplets chloroform and applied on the bottom (ca 1-2 cm from the bottom edge to avoid dissolving by mobile phase) of the premade TLC plate. A mixture of petroleum ether, diethyl ether and acetic acid (113:20:1 in volume) as mobile phase was used to separate PL from other lipid classes. Different lipids in the lipid sample ascends at different rates due to the competition of the solute and the mobile phase for binding places on the stationary phase. In this experiment, silica gel is used as the stationary phase and it can be considered polar. Different lipid compounds differ in polarity; the more polar compound has a stronger interaction with the silica and is more capable to dispel the mobile phase from the binding places. Thus, the less polar compound moves higher up the plate (fig 2.1) (Reich & Schibli 2007). The lipid classes were detected by spraying the TLC plate with 2% 2-7-dichlorofluorescein (a fluorogenic dye that measures hydroxyl, peroxy and other reactive oxygen species activity and a highly fluorescent compound which can be detected by fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm respectively) in 96% ethanol and identified with the standards under 366 nm UV light (the PL group is the unmove part in the TLC plate). After detection, the parts corresponding to PL fraction was scraped off to glass tubes and soaked with 2 ml of a polar solvent called Arvidsons (the ratio between chloroform, methanol, acetic acid and water is 50:39:1:10) to eluate PL from silica gel to solvent (Arvidson, 1968). It was suitable to extract phospholipids due to its polarity. In addition, add the acetic acid, mainly to suppress some acids or alkaline substances arising spots tailing. This extraction needs 4 hours or more to react (in freezer, at -40°C) and then centrifuge (ca 2000 rpm/min), add 0.5 ml 0.9% NaCl water before second centrifugation to separated Arvidsion phase and transfer the lower phase to a new glass tube. After that, the second TLC procedure was done. In this procedure, the PL

samples (dry first) were separated by TLC using the mixture of chloroform, methanol, acetic acid and water (100:75:6:2 in volume) as mobile phase. In the second TLC procedure, this mobile phase is a more polar solvent, therefore, it can dispel solutes from the silica binding places easier, and all compounds on the TLC plate will move higher up the plate. The PL group was detected by spraying the TLC plate with 2% 2-7-dichlorofluorescein in 96% ethanol and identified with the standards under UV light. The PL group was separated into several groups, from bottom to top; it was PC, PS, PI and PE. In this experiment, the group of PC, PS, PI and PE was scraped off to glass tubes, applied with 2,2-dimethoxypropane, methanolic HCl and benzene overnight at room temperature to trans-methylation, the methods were described by Mason and Waller(1964), and add 2ml of hexan and 3 mL of NaHCO₃ 36 % to samples at next day. Before trans-methylation, inject 10 μ L tricosylic acid (C 23:0, 0.6176 g/50 ml chloroform) to each tube as an internal standard. After trans-methylation, the liquid was separated into two phase, transfer the upper phase, which contains fatty acid methyl esters (FAMES), into glass tubes and dried with nitrogen overflow. After that, the dryness sample was supplied with 3-5 droplets hexane solvent (combined with 0.1ml 0.7mg/l BHT), transferred to GC glass tube and capped with nitrogen overflow. The GC system was equipped with a flame ionization detector (FID) that monitored the stream (was moved by helium gas) reaching the end of the column at different retention times. Of each sample, 1 μ L was injected by HP 7683 series GC injector into a capillary column (SGE Analytical Science Pty Ltd., Victoria, Australia) of 60 m length, with an internal diameter of 0.25 mm and a 0.25 μ m thick covering film of BPX70 as the stationary phase. The oven temperature was set on a start temperature of 50 °C for 1.2 min; then the temperature was raised to 170 °C with a rate of 4 °C min⁻¹, after that the temperature was reached to 200 °C with a rate of 0.5 °C min⁻¹, and finally reached to 300 °C with a rate of 10 °C min⁻¹. According HP ChemStation software, the area of peak was calculated by integration, enabling the quantification of each fatty acid that was identified by its corresponding retention time.

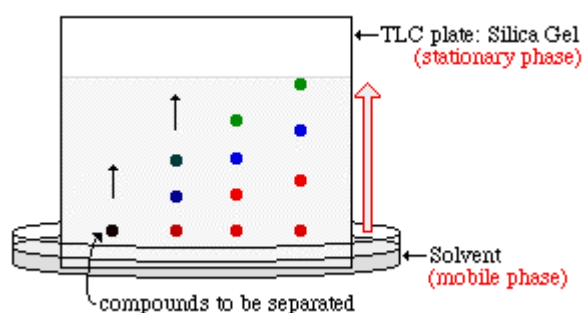


Fig 2.1 An example of TLC, figure taken from Wikipedia.

2.4 Data calculation and statistical analysis

2.4.1 Quantification and percentage of each fatty acid in each group

The total FA content (g) of the lipid extract (section 2.3.2) was calculated:

$$\text{Total FA content(extract)} = \frac{W_{c23} * (A_{\text{sum}} - A_{c23})}{A_{c23}}$$

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 (%). Then, the total FA content per g sample was calculated:

$$\text{Total FA content(sample)} = \frac{\text{total FA content(extract)} * V_{\text{solvent}}}{V_{\text{extract}} * W_s}$$

where V_{solvent} is the volume of the solvent (37.5 ml, details see section 2.3.2), V_{extract} means the volume of samples that taken for GC analysis (5 mL) and W_s is the weight of the sample (g). Now, the quantity of each fatty acid could be calculated, which was expressed in mg per g sample:

$$\text{FA content(mg/g)} = \frac{\text{total FA content (sample)} * A_{\text{FA}}}{A_{\text{sum}} - A_{c23}}$$

where A_{FA} is the area of peak (%) of the related fatty acid. After the quantification of each fatty acid, the percentage of each fatty acid can be calculated:

$$\text{FA content(\%)} = \frac{A_{\text{FA}} * A_{\text{sum}}}{A_{\text{sum}} - A_{c23}}$$

2.4.2 Statistic analysis

To compare the total fat content between the two sampling weights (200g vs. 400g) a student test (t-test) was applied. To find the main influence factors from the multiple factors for each group, principle component analysis of data (PCA) has been applied. Use the

software called Microsoft Excel (version 2007) to calculate the average and standard error mean of each fatty acid. Use the software called The Unscrambler X (version 10.3) to do PCA.

3. Results

3.1 Total fat content.

Skin samples from fish at 200 grams BW and 400 grams BW were analysed for total fat content, in the different experimental groups. According to statistical analysis, there are no significant difference in total fat content between 200g BW and 400g BW. There was a tendency to higher fat content in skin from fish at 400 grams than in skin from fish at 200 grams, 10.6% for 200 grams and 13.6% for 400 grams.

For more details about the data, please see appendix table 1.

3.2 The main fatty acid in different PL groups

A principal component analysis (PCA) in Unscrambler was done in order to get an overview of how the composition of the cell membrane phospholipid classes PC, PE, PI and PS in the skin were affected by the different diets. All diet groups were included in the analyses of fish at both 200 grams and 400 grams (fig 3.2.1, fig 3.2.2) showing that the PC, PE, PI and PS were divided into 4 completely clear groups in the PCA analyses. Therefore, this shows that the fatty acid pattern of each of the different lipid classes is unique to each group (they have distinctive composition regardless of dietary group).

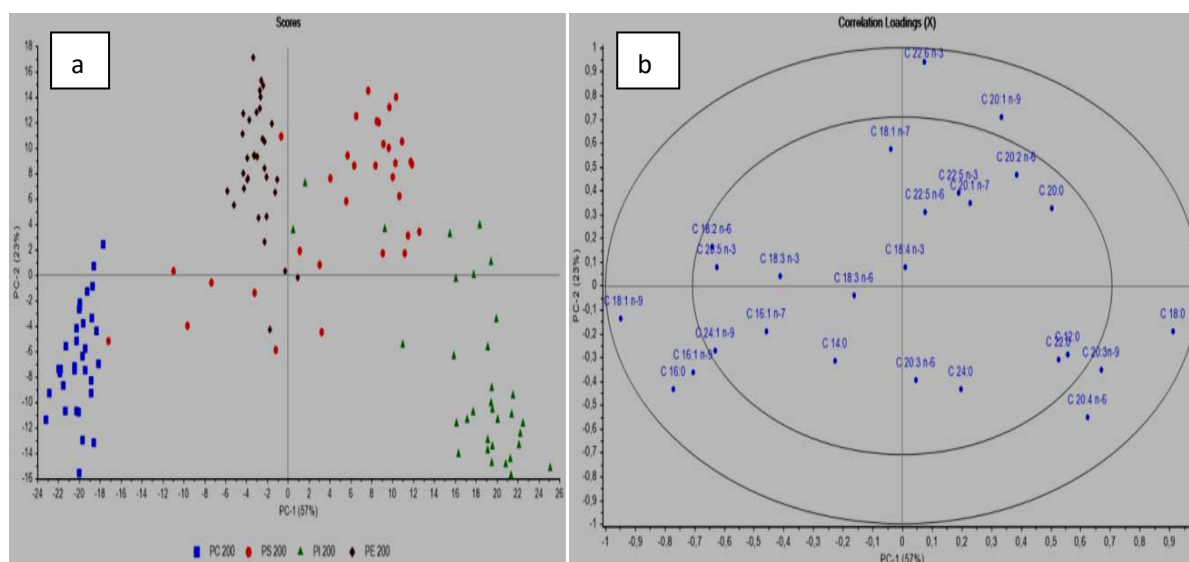


Fig 3.2.1 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 200g sampling Atlantic salmon. Blue spots represent PC group, red spots represent PS group, green spots represent PI group and brown spots represent PE group (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank. PC 1 is separating PC and PE fraction from PS and PI fraction. PC 2 is separating PC and PI fraction from PE and PS fraction.

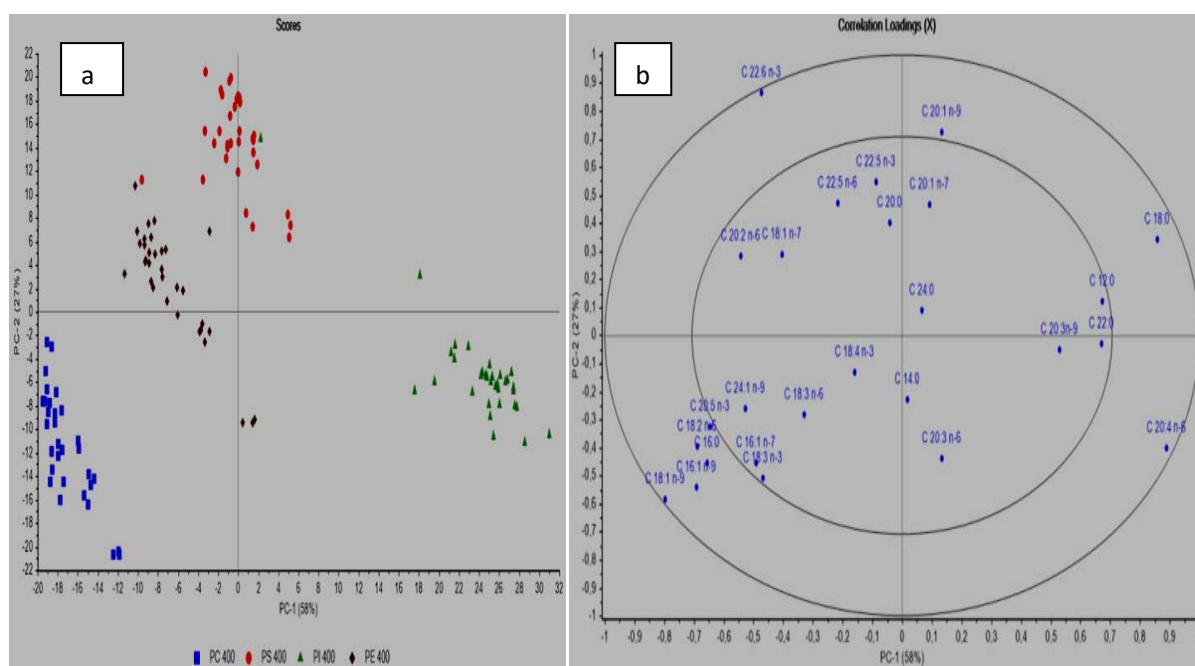


Fig 3.2.2 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 400g sampling Atlantic salmon. Blue spots represent PC group, red spots represent PS group, green spots represent PI group and brown spots represent PE group (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank. PC 1 is separating PC and PE fraction from PS and PI fraction. PC 2 is separating PC and PI fraction from PE and PS fraction.

By plotting the different scores according to principal components, we searched for fatty acids in the data set that are specifically characteristic for and significantly different between the different phospholipid classes. Common features of the various samples in the 4 different lipid classes were looked for (fig 3.2.1b, fig 3.2.2b). In this figure we see that there are 8 fatty acids (fig 3.2.1b) which are grouped clearly in the outer circle which shows fatty acids that are found at a significantly different level between the different lipid classes. Here we have chosen to take out the 8 fatty acids that are distributed significantly different between the PC, PE, PI and PS for presentation in fig 3.2.3. In addition, the pattern in the different PL is the same for all dietary groups and therefore we only choose to present two extreme groups from each size of fish. The PC fraction is characterized by 16:0 and 18:1n-9. The PS fraction is dominated by 18:0 and 22:6n-3. The PI fraction is characterized by 18:0 and 20:4n-6. The PE fraction is dominated by 18:1n-9, and 22:6n-3. In the 400g group, it is almost same as in the 200g group (fig 3.2.4), thus, each PL class is distinct irrespective of size of the fish or dietary group.

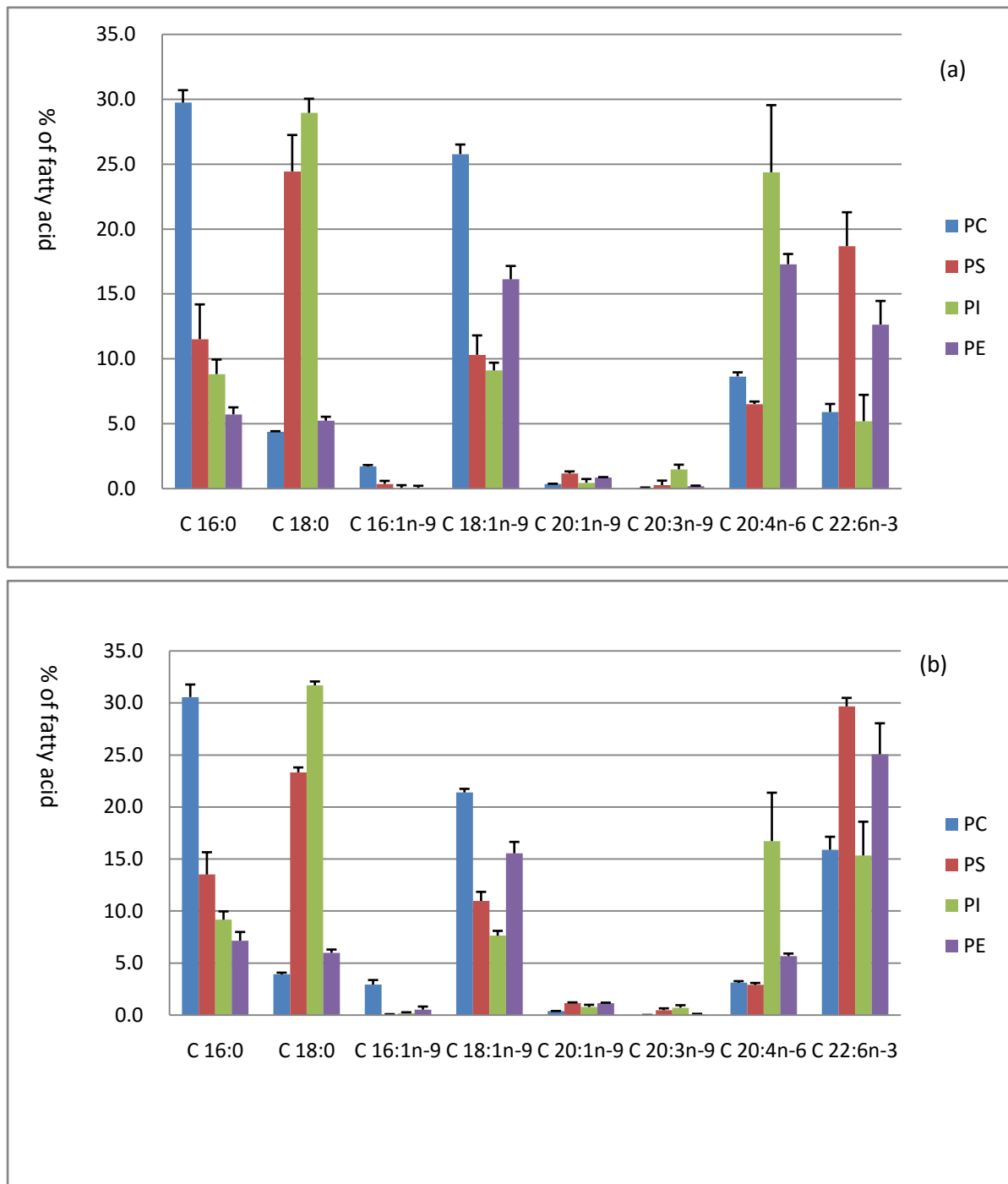


Fig 3.2.3 Level of individual fatty acids in percentage of total fatty acids in the different PLs \pm standard error of mean (sem) in 200g group, n=3 for the NC group (a) and DHA+EPA group (b). Fatty acids are presented as percentage of total fatty acids. Blue bars represent PC group, red bars represent PS group, green bars represent PI group and purple bars representative PE group.

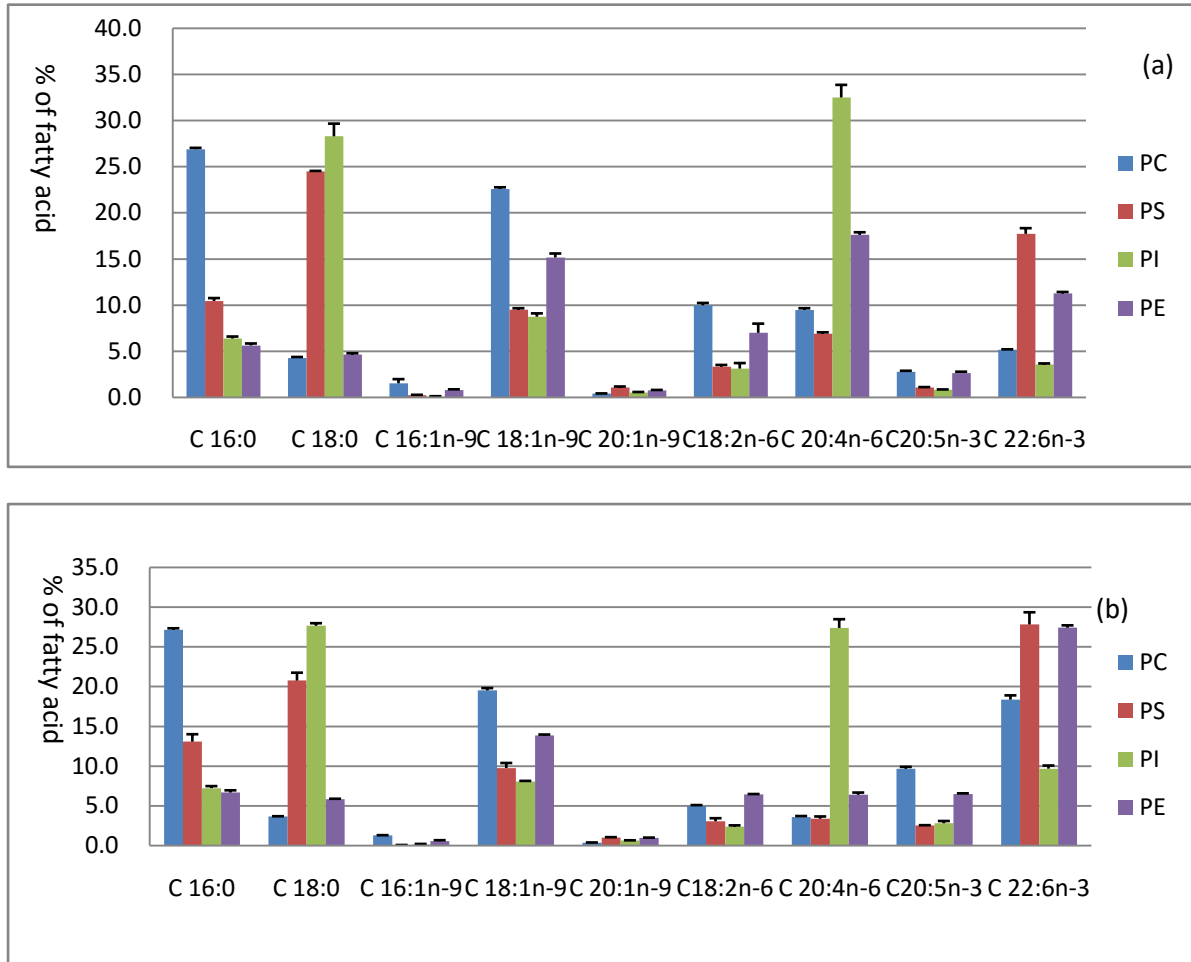


Fig 3.2.4 Level of individual fatty acids in percentage of total fatty acids in the different PLs \pm standard error of mean (sem) in 400g group, $n=3$ for the NC group (a) and DHA+EPA group (b). Fatty acids are presented as percentage of total fatty acids. Blue bars represent PC group, red bars represent PS group, green bars represent PI group and purple bars representative PE group.

3.3 The influence of different dietary n-3 PUFAs on Atlantic salmon skin PL composition

To investigate the specific dietary effects on each PL class, a PCA analysis for each PL class was done. After the PCA analysis, the fatty acids located in the outer circle were chosen as the ones having significant effects to make figures and observe the changing trend with dietary n-3 PUFAs deficiency.

3.3.1 PC group

All dietary groups were included in the analyses of fatty acid composition of skin PC of fish at both 200g group and 400g group. The two first components of PCA accounted for the 82% of variation of 200g PC dataset (fig 3.3.1.1), component 1 explained 58% of variation and separated the samples by the percentage of the dietary n-3 PUFA (0% to 2% of EPA,

DHA or the mix of EPA+DHA groups). Component 2 explained 24% of variation and separated the samples depending on the FA included in the diet (EPA groups, DHA groups and EPA+DHA groups) (fig 3.3.1.1). From these two components, we can determine that the NC group is characterized by 18:1n-9, 18:2n-6, 18:3n-6, 20:3n-6 and 20:4n-6, dietary DHA group is characterized by 22:6n-3 and dietary EPA, EPA+DHA and CC group are characterized by 20:5n-3 and 22:5n-3. For 400g PC (fig 3.3.1.2), the two first components of PCA accounted for the 93% of variation, component 1 explained 63% of variation and separated the samples by the percentage of the dietary n-3 PUFA (0% to 2% of EPA, DHA or the mix of EPA+DHA groups). Component 2 explained 30% of variation and separated the samples depending on the FA included in the diet (EPA groups, DHA groups and EPA+DHA groups). From these two components, we can determine that the NC group is characterized by 18:1n-9, 18:2n-6, 18:3n-6, 20:3n-6 and 20:4n-6, dietary DHA group is characterized by 22:6n-3 and 22:5n-6 and dietary EPA, EPA+DHA and CC group are characterized by 20:5n-3 and 22:5n-3. Fig 3.3.1.1 and fig 3.3.1.2 show that the FA composition in PC group was significantly affected by dietary fatty acid and the dietary effects are more pronounced with time. By plotting the different scores according to principal components, we searched for fatty acids in the data set that are specifically characteristic for and significantly different between the different dietary groups (fig 3.3.1.1b and fig 3.3.1.2b). In these figures, we see that there are 9 fatty acids (18:1n-9, 18:2n-6, 18:3n-6, 20:3n-6, 20:3n-6, 22:5n-6, 20:5n-3, 22:5n-3 and 22:6n-3) which are grouped clearly in the outer circle which shows fatty acids that are found at a significantly different level between the different dietary groups. For dietary EPA group, the decreasing percentage of 18:1n-9 was related to the increase in omega-3 in general and then 18:1n-9 was higher in the NC diet. n-6 fatty acids decreased with increasing dietary EPA whereas EPA, DPA and DHA increased with increasing dietary EPA (fig 3.3.1.3 a, d). The changing degree is larger in 400g PC fraction than in 200g PC fraction, for instance, DHA increased from 5% to 14% in 400g group whereas DHA increased from 7% to 12% in 200g group; 20:4n-6 decreased from 7% to 3% in 200g group whereas 20:4n-6 decreased from 10% to 3% in 400g group. For dietary DHA group, the decreasing percentage of 18:1n-9 was related to the increase in omega-3 in general and then 18:1n-9 was higher in the NC diet. n-6 fatty acids decreased with increasing dietary DHA, EPA increased

slightly with increasing dietary DHA and DHA increased dynamically with increasing dietary DHA. The changing degree is larger in 400g PC fraction than in 200g PC fraction (fig 3.3.1.3 b, e). For instance, DHA increased from 5% to 25% in 400g group whereas DHA increased from 7% to 17% in 200g group; 20:4n-6 decreased from 7% to 4% in 200g group whereas 20:4n-6 decreased from 10% to 4% in 400g group. For dietary EPA+DHA group, the decreasing percentage of 18:1n-9 was related to the increase in omega-3 in general and then 18:1n-9 was higher in the NC diet. n-6 fatty acids decreased with increasing dietary EPA+DHA whereas EPA, DPA and DHA increased with increasing dietary EPA+DHA (fig 3.3.1.3 c, f). The changing degree is larger in 400g PC fraction than in 200g PC fraction. For instance, DHA increased from 5% to 19% in 400g group whereas DHA increase from 6% to 15% in 200g group; 20:4n-6 decreased from 8% to 4% in 200g group whereas 20:4n-6 decreased from 10% to 4% in 400g group.

For more details about the data, please see appendix table 2 and table 3.

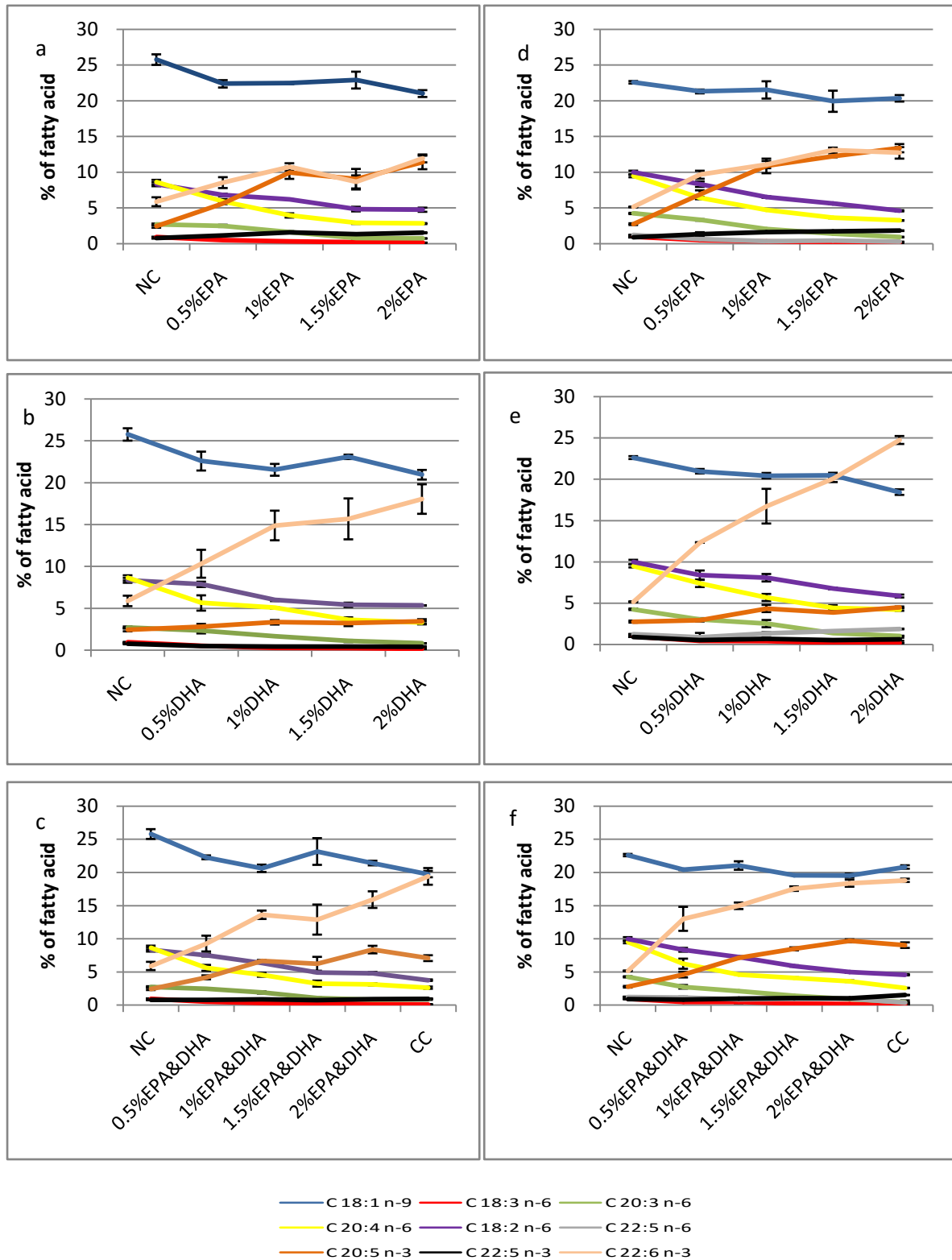


Fig 3.3.1.3 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 200g PC group (a,b,c) and 400g PC group (d,e,f), n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group. Blue line represent 18:1n-9, red line representative 18:3n-6, green line represent 20:3n-6, purple line represent 18:2n-6, yellow line represent 20:4n-6, orange line represent 20:5n-3, black represent 22:5n-3, pink line represent 22:6n-3 and grey line represent 22:5n-6.

3.3.2 PE group

All dietary groups were included in the analyses of fatty acid composition of skin PE of fish at both 200g group and 400g group. The two first components of PCA accounted for the 85% of variation of 200g PE dataset (fig 3.3.2.1), component 1 explained 66% of variation and separated the samples by the percentage of the dietary n-3 PUFA (0% to 2% of EPA, DHA or the mix of EPA+DHA groups). Component 2 explained 19% of variation and separated the samples depending on the FA included in the diet (EPA groups, DHA groups and EPA+DHA groups) (fig 3.3.2.1). From these two components, we can determine that the NC group was characterized by 18:3n-6, 20:3n-6, 20:4n-6 and 22:5n-6, dietary DHA group was characterized by 22:6n-3, dietary EPA and EPA+DHA group were characterized by 16:0 and 18:0 and the CC group was characterized by 20:2n-6. However, the separation in PE 200g group is not very clear, in comparison to the separation in PE 400g group. For 400g PE (fig 3.3.2.2), the two first components of PCA accounted for the 88% of variation, component 1 explained 72% of variation and separated samples by the percentage of the dietary n-3 PUFA (0% to 2% of EPA, DHA or the mix of EPA+DHA groups). Component 2 explained 16% of variation and separated samples depending on the FA included in the diet (EPA groups, DHA groups and EPA+DHA groups). From these two components, we can determine that the NC group was characterized by 20:3n-6 and 20:4n-6, dietary DHA group was characterized by 22:6n-3, dietary EPA+DHA and CC group were characterized by 16:0 and the dietary EPA group was characterized by 20:5n-3 and 22:5n-3. Fig 3.3.2.1 and fig 3.3.2.2 shows that the FA composition in PE group was affected significantly by dietary fatty acid and the dietary effects are more pronounced with time. By plotting the different scores according to principal components, we searched for fatty acids in the data set that are specifically characteristic for and significantly different between the different dietary groups (fig 3.3.2.1b and fig 3.3.2.2b). In these figures, we see that there are 8 fatty acids (16:0, 18:0, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:5n-6 and 22:6n-3) which are grouped clearly in the outer circle which shows fatty acids that are found at a significantly different level between the different dietary groups. For dietary EPA group, 16:0 and 18:0 increased very slightly with increasing dietary EPA; n-6 PUFA, especially 20:4n-6, decreased with increasing dietary EPA; n-3 lCPUFA increased with increasing dietary EPA. The changing degree is larger in 400g group than in 200g group (fig

3.3.2.3a, d). For instance, DHA increased from 11% to 24% in 400g group whereas DHA is increased from 13% to 22% in 200g group; 20:4n-6 is decreased from 16% to 6% in 200g group whereas 20:4n-6 is decreased from 18% to 6% in 400g group. For dietary DHA group, 16:0 and 18:0 increased very slightly with increasing dietary DHA; n-6 PUFA, especially 20:4n-6, decreased with increasing dietary DHA; DHA increased significantly with increasing dietary DHA whereas EPA and DPA increased very slightly with increasing dietary DHA. The changing degree is larger in 400g group than in 200g group (fig 3.3.2.3b, e). For instance, DHA is increased from 11% to 32% in 400g group whereas DHA is increased from 13% to 24% in 200g group; 20:4n-6 is decreased from 16% to 7% in 200g group whereas 20:4n-6 is decreased from 18% to 7% in 400g group. For dietary EPA+DHA group, 16:0 and 18:0 increased very slightly with increasing dietary EPA+DHA; n-6 PUFA, especially 20:4n-6, decreased with increasing dietary EPA+DHA; n-3 lcPUFA increased with increasing dietary EPA+DHA. The changing degree is larger in 400g group than in 200g group (fig 3.3.2.3c, f). For instance, DHA is increased from 11% to 28% in 400g group whereas DHA is increased from 13% to 25% in 200g group; 20:4n-6 is decreased from 16% to 6% in 200g group whereas 20:4n-6 is decreased from 18% to 6% in 400g group. In addition, the PE fraction has a higher percentage of PUFA than the PC fraction.

For more details about the data, please see appendix table 4 and table 5.

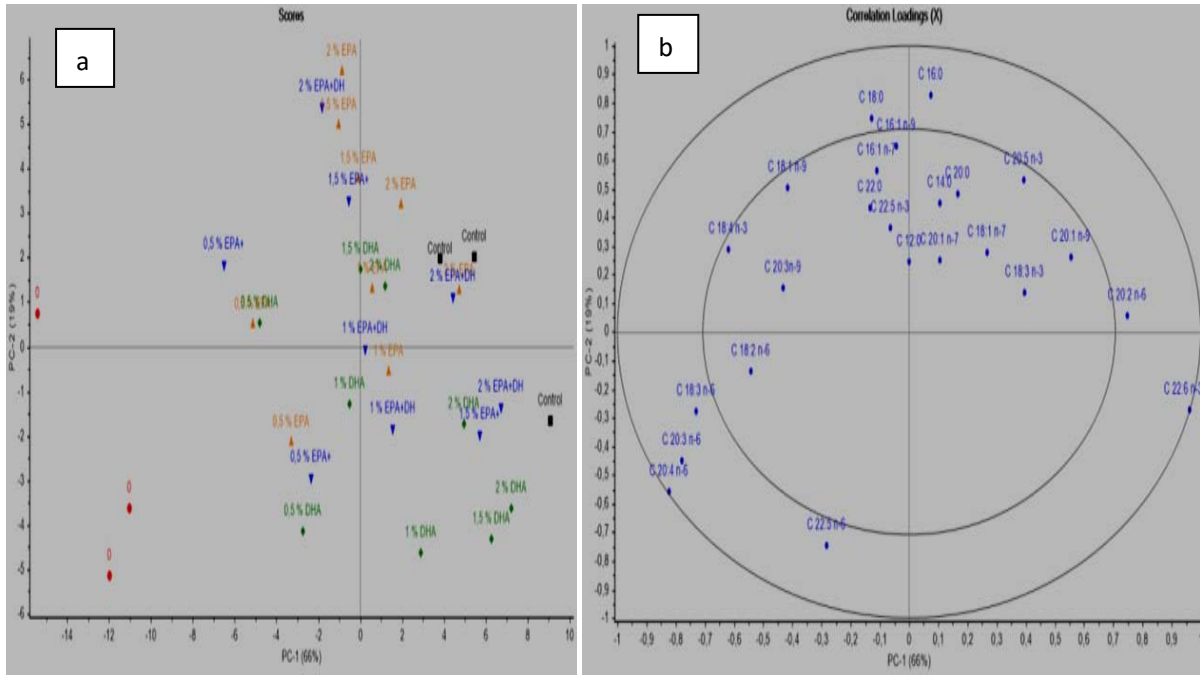


Fig 3.3.2.1 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 200g experimental Atlantic salmon skin PE. EPA diets are shown in orange, DHA diets are shown in green, EPA+DHA diets are shown in blue, CC diets are shown in black and NC diets are shown in red (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank.

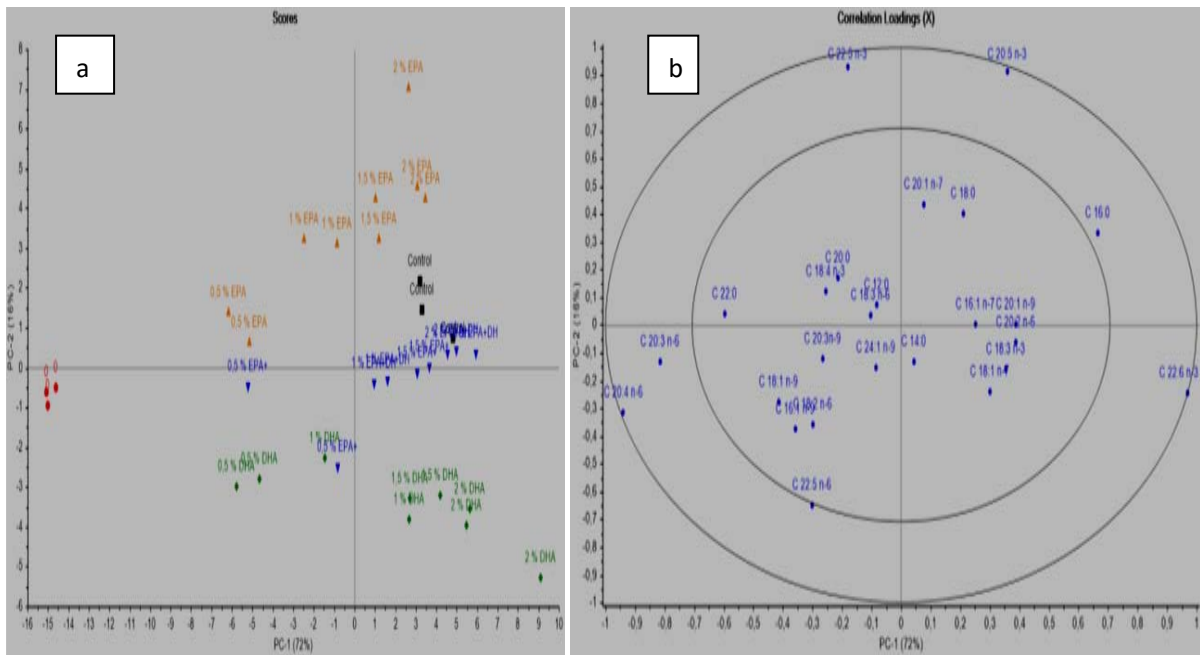


Fig 3.3.2.2 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 400g experimental Atlantic salmon skin PE. EPA diets are shown in orange, DHA diets are shown in green, EPA+DHA diets are shown in blue, CC diets are shown in black and NC diets are shown in red (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank.

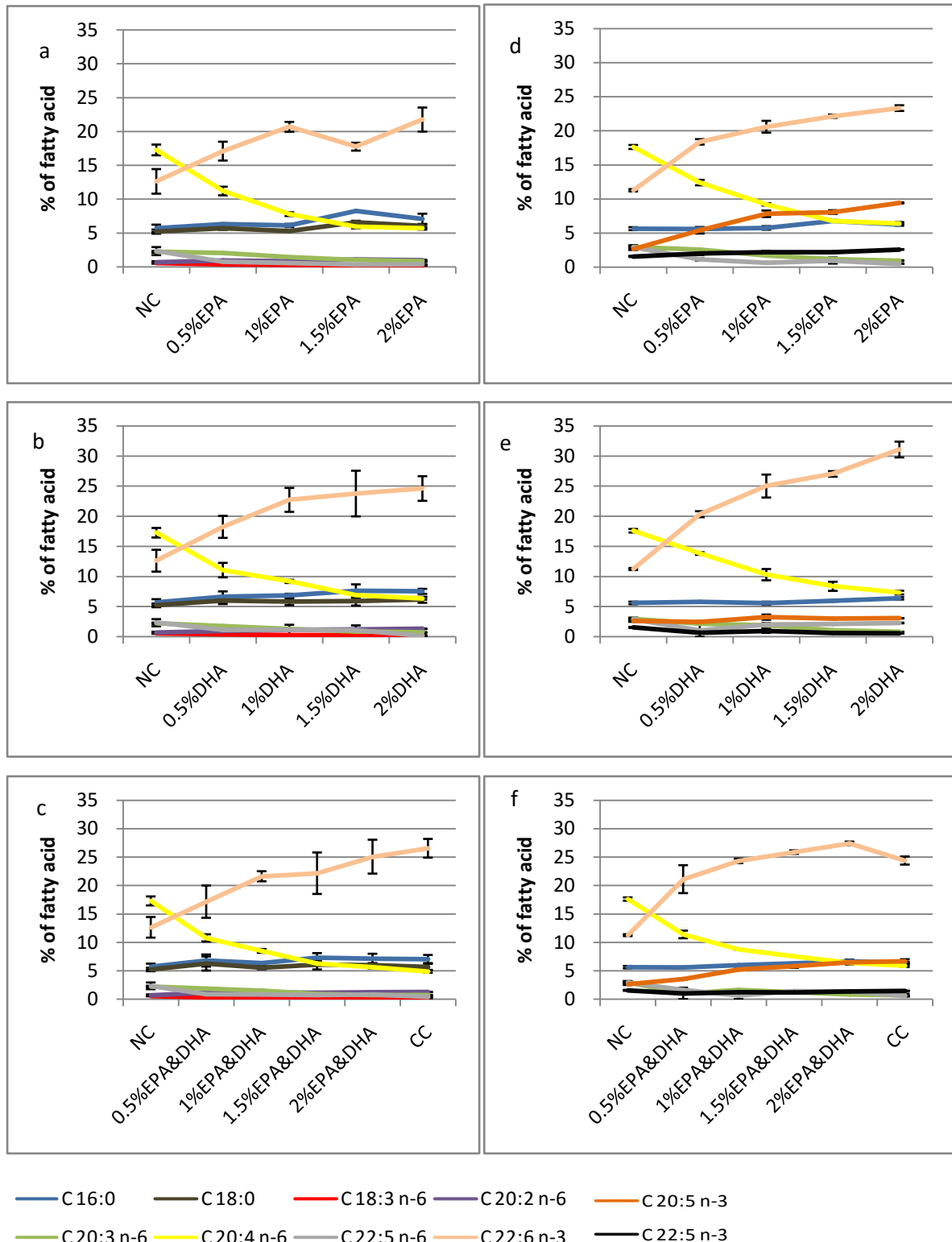


Fig 3.3.2.3 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 200g PE group (a, b, c) and 400g PE group (d, e, f), $n=3$ for the NC, CC, 2% EPA, DHA and DHA+EPA group, $n=2$ for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group. Blue line represent 16:0, brown line represent 18:0, red line represent 18:3n-6, purple line represent 20:2n-6, green line represent 20:3n-6, yellow line represent 20:4n-6, gray represent 22:5n-6, pink line represent 22:6n-3 and black line represent 22:5n-3.

3.3.3 PS group

All dietary groups were included in the analyses of fatty acid composition of skin PS of fish at both 200g group and 400g group. PCA plots show no clear difference between the dietary groups, the groups are more interconnected (fig 3.3.3.1). This may indicate that PS is more conserved, in comparison to PC and PE. For 400g PS (fig 3.3.3.2), the two first components of PCA accounted for the 76% of variation, component 1 explained 59% of variation and separated the samples by the percentage of the dietary n-3 PUFA (0% to 2% of EPA, DHA or the mix of EPA+DHA groups). Component 2 explained 17% of variation and separated the samples depending on the FA included in the diet (EPA groups, DHA groups and EPA+DHA groups). From these two components, we can find that the NC group was characterized by 18:1n-9, 18:2n-6 and 20:3n-6, dietary DHA, EPA+DHA and CC groups were characterized by 22:6n-3 and the dietary EPA group was characterized by 18:0. Fig 3.3.3.2 shows that the FA composition in PS group was significantly affected by dietary fatty acid. By plotting the different scores according to principal components, we searched for fatty acids in the data set that are specifically characteristic for and significantly different between the different dietary groups in 400g group (fig 3.3.3.2). In these figures, we see that there are 5 fatty acids (18:0, 18:2n-6, 18:1n-9, 20:3n-6 and 22:6n-3) which are grouped clearly in the outer circle which shows fatty acids that are found at a significantly different level between the different dietary groups. For dietary EPA group, 18:0 kept stable with increasing dietary EPA; 18:2n-6, 18:1n-9 and 20:3n-6 decreased with increasing dietary EPA; DHA increased significantly with increasing dietary EPA (fig 3.3.3.3a, d). For dietary DHA group, 18:0 decreased very slightly with increasing dietary DHA; 18:1n-9, 18:2n-6 and 20:3n-6 decreased with increasing dietary DHA; DHA increased significantly with increasing dietary DHA (fig 3.3.3.3b, e). For dietary EPA+DHA group, 18:0 decreased with increasing dietary EPA+DHA; 18:2n-6 and 20:3n-6 decreased with increasing dietary EPA+DHA; DHA increased significantly with increasing dietary EPA; 18:1n-9 kept stable with increasing dietary EPA+DHA (fig 3.3.3.3c, f). In addition, PS fraction contained the highest percentage of DHA in all PL classes (18% in the PS fraction of the NC group, 5% in the PC fraction of the NC group, 11% in the PE fraction of the NC group and 3% in the PI fraction of the NC group).

For more details about the data, please see appendix table 6 and table 7.

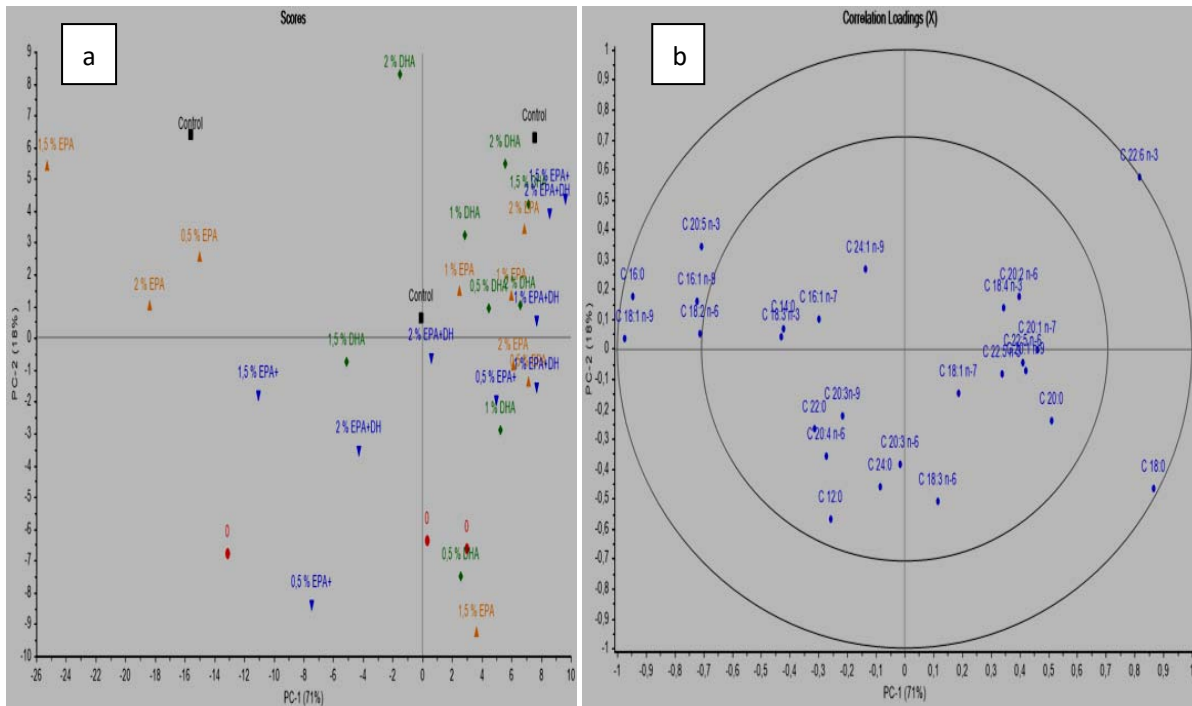


Fig 3.3.3.1 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 200g experimental Atlantic salmon skin PS. EPA diets are shown in orange, DHA diets are shown in green, EPA+DHA diets are shown in blue, CC diets are shown in black and NC diets are shown in red (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank.

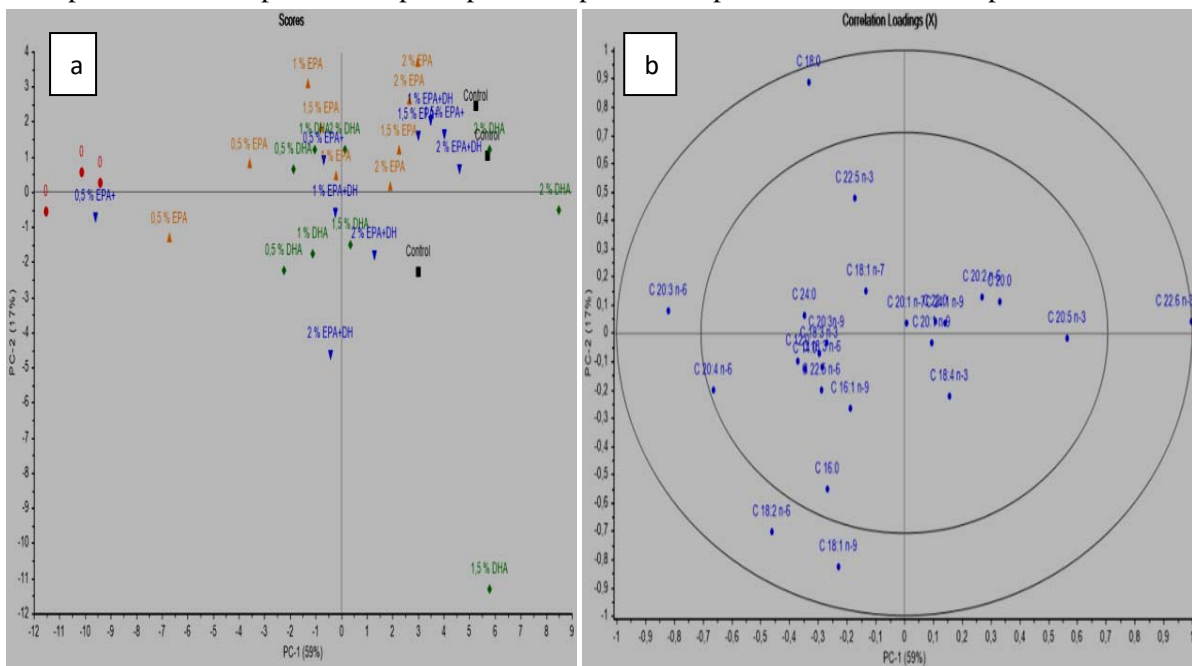


Fig 3.3.3.2 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 400g experimental Atlantic salmon skin PS. EPA diets are shown in orange, DHA diets are shown in green, EPA+DHA diets are shown in blue, CC diets are shown in black and NC diets are shown in red (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank.

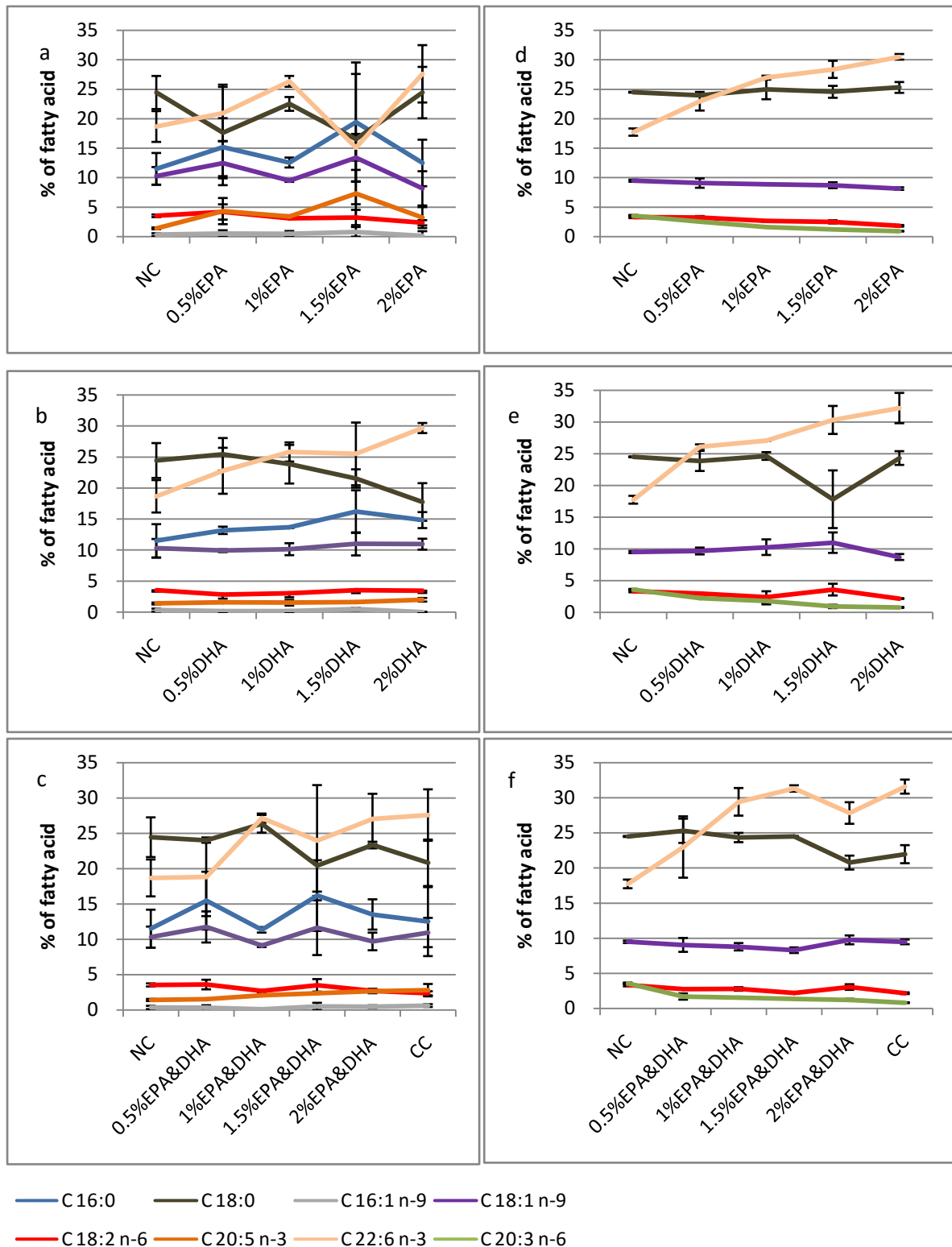


Fig 3.3.3.3 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 200g PS group (a, b, c) and 400g PS group (d, e, f), n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group. Blue line represent 16:0, brown line represent 18:0, gray line represent 16:1n-9, purple line represent 18:1n-9, red line represent 18:2n-6, orange line represent 20:5n-3, pink line represent 22:6n-3 and green line represent 20:3n-6.

3.3.4 PI group

All dietary groups were included in the analyses of fatty acid composition of skin PI of fish at both 200g group and 400g group. PCA plots show no clear difference between the dietary groups, the groups are more interconnected (fig 3.3.4.1), thus, PI is more conserved, in comparison to PE and PC fraction. For 400g PI (fig 3.3.4.2), the two first components of PCA accounted for the 77% of variation, component 1 explained 60% of variation and separated samples by the percentage of the dietary n-3 PUFA (0% to 2% of EPA, DHA or the mix of EPA+DHA groups). Component 2 explained 17% of variation and separated samples depending on the FA included in the diet (EPA groups, DHA groups and EPA+DHA groups). From these two components, we can determine that NC group was characterized by 20:4n-6, dietary DHA and EPA groups were characterized by 22:6n-3 and dietary EPA+DHA and CC groups were characterized by 12:0. Fig 3.3.4.2 shows that the FA composition in PI group was significantly affected by dietary fatty acid. By plotting the different scores according to principal components, we searched for fatty acids in the data set that are specifically characteristic for and significantly different between the different dietary groups in 400g group (fig 3.3.4.1b and fig 3.3.4.2b). In these figures, we see that there are 3 fatty acids (12:0, 20:4n-6 and 22:6n-3) which are grouped clearly in the outer circle which shows fatty acids that are found at a significantly different level between the different dietary groups. For dietary EPA group, 12:0 decreased very slightly with increasing dietary EPA; 20:4n-6 decreased strongly with increasing dietary EPA; DHA increased slightly with increasing dietary EPA (fig 3.3.4.3a, d). For dietary DHA group, 12:0 decreased very slightly with increasing dietary DHA; 20:4n-6 decreased significantly with increasing dietary DHA; DHA increased with increasing dietary DHA. (fig 3.3.4.3b, e). For dietary EPA+DHA group, 12:0 kept stable with increasing dietary EPA+DHA; 20:4n-6 decreased with increasing dietary EPA+DHA; DHA increased slightly with increasing dietary EPA (fig 3.3.4.3c, f). In addition, PI fraction contained the highest percentage of 20:4n-6 in all PL classes (33% in the PI fraction of the NC group, 3% in the PS fraction of the NC group, 17% in the PE fraction of the NC group and 10% in the PC fraction of the NC fraction).

For more details about the data, please see appendix table 8 and table 9.

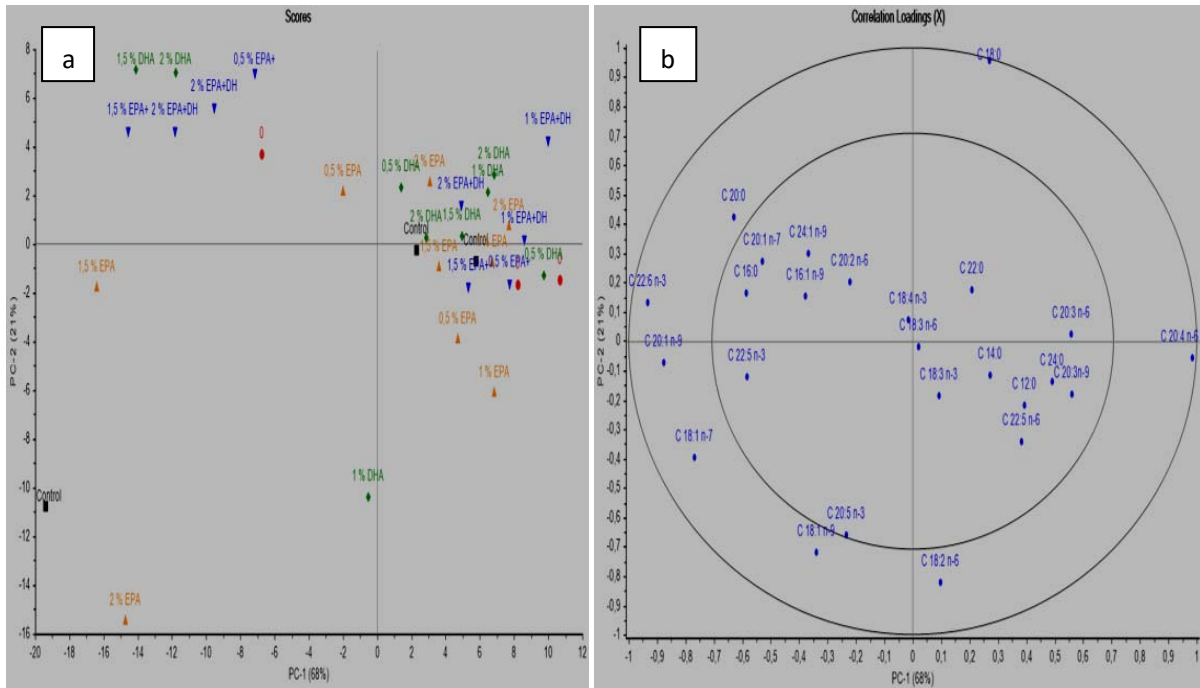


Fig 3.3.4.1 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 200g experimental Atlantic salmon skin PI. EPA diets are shown in orange, DHA diets are shown in green, EPA+DHA diets are shown in blue, CC diets are shown in black and NC diets are shown in red (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank.

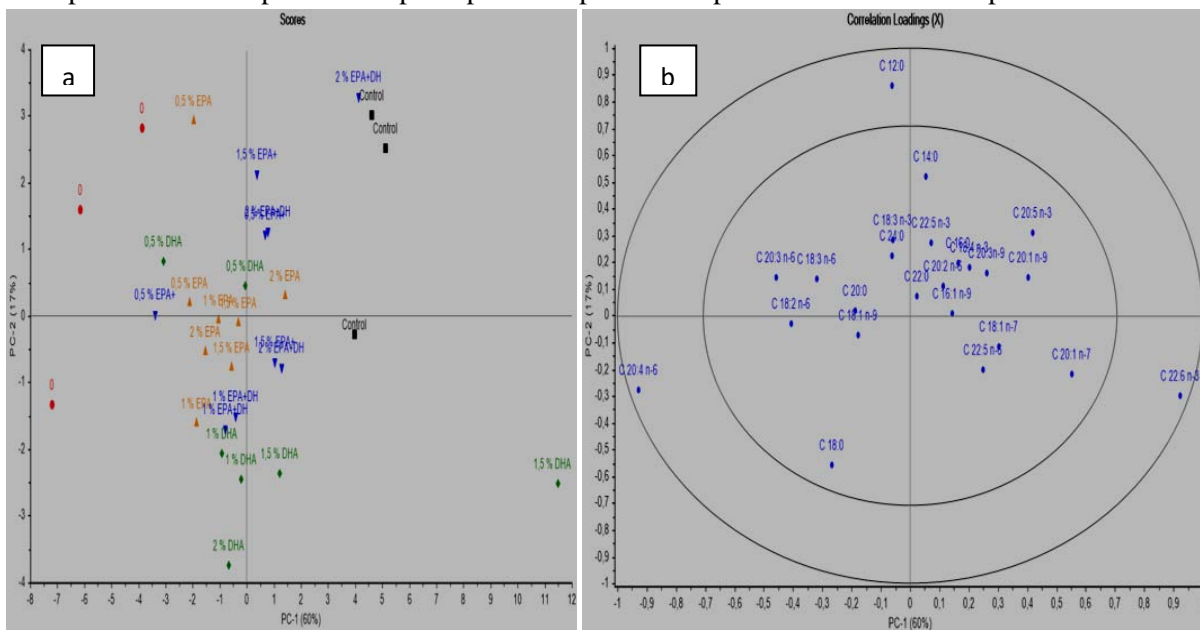


Fig 3.3.4.2 PCA plot (a) and correlation loading plot (b) of the relative fatty acid composition data of diets (NC, 0.5%, 1%, 1.5% and 2% EPA or/and DHA and CC) from 400g experimental Atlantic salmon skin PI. EPA diets are shown in orange, DHA diets are shown in green, EPA+DHA diets are shown in blue, CC diets are shown in black and NC diets are shown in red (a). All 14 dietary groups are represented in the plot. Each spot represent a pooled sample of 5 individual fish per tank.

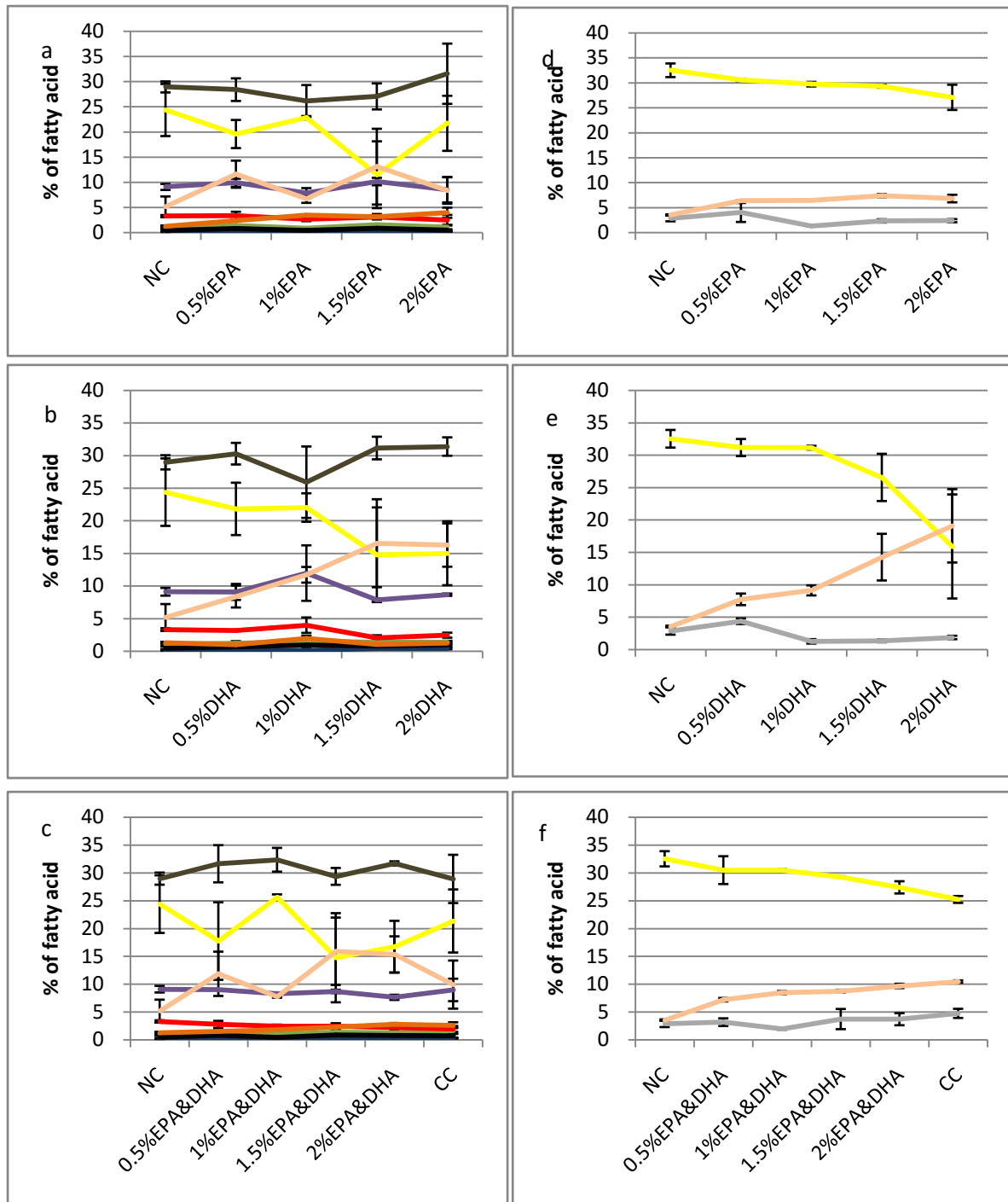


Fig 3.3.4.3 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 200g PI group (a, b, c) and 400g PI group (d, e, f), $n=3$ for the NC, CC, 2% EPA, DHA and DHA+EPA group, $n=2$ for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group. Brown line represent 18:0, blue line represent 20:0, green line represent 18:1n-7, purple line represent 18:1n-9, black line represent 20:1n-9, red line represent 18:2n-6, yellow line represent 20:4n-6, orange line represent 20:5n-3, pink line represent 22:6n-3 and gray line represent 12:0.

4. Discussion and conclusion

Skin is the first biological barrier of animal body to prevent physical, bacterial and chemical damage (Schempp et al 2009). PLs play a vital role in animals, especially in cell membrane (Tocher et al, 2008). Thus, it is important to investigate PLs in skin. However, there are several studies on the effects of dietary fatty acids on the fatty acid composition of different PL classes (Ghioni et al. 1997a, b, Cejas et al. 2003, Loucas et al. 2010, Li et al. 2014, Chap 2016, Thomassen et al. 2016). To the best of our knowledge, there are no earlier studies that have investigated the relationship between dietary fatty acids and fatty acids composition of Atlantic salmon skin PL subclasses.

Our data showed that the fatty acid pattern of each of the different lipid classes in the skin of Atlantic salmon is unique to each group (they have distinctive composition regardless of diet group). The PC fraction is characterized by 16:0 and 18:1n-9 whereas PS fraction is dominated by 18:0 and 22:6n-3 while 18:0 and 20:4n-6 is abundant in PI fraction and the PE fraction is characterized by 18:1n-9 and 22:6n-3. Both PE and PS are particularly rich in DHA. Those results are almost in agreement with the composition of PL classes from rainbow trout skin with Ghioni et al (1997a, b) who pointed that the dominating fatty acids in PC is 16:0 and 18:1n-9; PS is characterized by 18:0 and C22 PUFA; PI having high level of 18:0 and C20 PUFA and PE having low saturated fatty acids and relatively high percentages of all PUFA. These results indicate that the fatty acid pattern of each individual PL class is relatively well conserved between different tissues and species. The little differences found, may be caused by different fish species and that the fish had been fed different diets, this is also proved in rainbow trout and cod that fed with different diets (Bell et al. 1983, Bell et al. 1989, Bell et al. 1990, Bell et al. 1991, Ghioni et al 1997a, b).

Even though the overall fatty acid pattern was conserved within each PL class, every phospholipid classes (PC, PS, PI and PE) were affected by the different fatty acid composition of the diets. The influence was more pronounced the longer time the fish was fed the different diets. However, the major influenced fatty acids in each PL classes are different and the different PL classes show a different resistance for fatty acids composition changing, for instance, PI and PE is more conserved than PE and PC fraction.

In this study, the PC fraction is the most affected by the fatty acid composition of the

diets. This is also proved by Ghioni et al (1997a) that the PC fraction is most influenced by EPA and DHA deficiency in rainbow trout skin cell cultivation. In our trial, in the PC fraction, the 18:1n-9, 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6 increases and 20:5n-3, 22:5n-3, 22:6n-3 decreases with decreasing dietary levels of EPA and DHA. Therefore, n-6 will accumulate with EPA and DHA decrease, it indicates that Atlantic salmon try to compensate for losing these fatty acids in the membrane phospholipids by making the longer chain n-6 fatty acids (18:3n-6, 20:3n-6, 20:4n-6 and 22:5n-6) when the salmon lacks EPA and DHA in the diet. This is agreed with Thomassen et al (2016) who showed that 20:4n-6, 20:3n-9 and 22:5n-6 is a compensation when n-3 EFA deficiency in almost all Atlantic salmon PL subclasses. Besides, 18:1n-9 will increase with EPA and DHA decrease. This may agreement with rainbow trout that the increase of 18:1n-9 is mainly related to PC fraction (Ghioni et al. 1997a), but this may not enough confidence, because our experimental diet contained rapeseed oil (containing high levels of 18:1n-9) and the dietary level of 18:1n-9 and 18:2n-6 was increased when the dietary EPA and DHA was reduced. In Atlantic salmon liver, it was also observed that 18:1n-9 increases with EFA deficiency (Ruyter et al 2000b). On the other hands, we found that in EPA dietary group, DHA will also increase with increasing EPA level in the diet, therefore, it indicates that EPA can be converted efficiently to DHA by Atlantic salmon. This conclusion was agreed with Ruyter et al (2000a) who pointed that dietary EPA and DHA will lead to an increase of DHA in Atlantic salmon liver. Moreover, we found that in DHA dietary group, EPA will also increase slightly with DHA increasing, thus, it may indicate that DHA can reconvert to EPA, it is agreed with the result for Atlantic salmon carcass, liver and blood from Ruyter et al (2000b). Besides, there is another way for EPA increase that the levels of n-3 and n-6 fatty acids were maintained in the phospholipids, whereas they decreased in TAG and transferred from TAG to PL (Xia et al 1993, Ruyter et al 2000b).

In this study, PE fraction have a higher percentage of PUFA (both n-3 and n-6) than PC fraction and PE fraction are more resistant than PC fraction, it was also proved by Bell et al (1995) that PE is more conserved than PC in juvenile herring and the result also proved in rainbow trout skin (Ghioni et al 1997b). In our trial, in the PE fraction, the 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6 increases and 16:0, 18:0, 20:5n-3, 22:5n-3 and 22:6n-3 decreases with

decreasing dietary levels of EPA and DHA. In addition, 20:4n-6 is dynamic increase with n-3 PUFA decrease in PE group, in comparison to PC and PS fraction. For others results, Ghioni et al. (1997a, b) observed that 20:1n-9 declined fast and AA increased dynamically with EPA and DHA deficiency in rainbow trout skin cell PE fraction. Thomassen et al. (2016) observed a high amount of 20:3n-9 in Atlantic salmon liver and intestinal PE, little in heart and muscle PE fraction, with 18:3n-3 deficient in diet (our trial and Ghioni's trial applied 18:3n-3 in diet). Thus, the major influenced fatty acids is different in different fish species, organs and diets.

In this study, the PS fraction is more conserved than PC and PE fraction, our PCA plots for PS fraction show no clear difference between the dietary groups, the groups are more interconnected, meaning not so influenced by the diet, in comparison to PC and PE fraction, it is also proved by Ghioni et al (1997b) that EPA and DHA deficiency have a very little effects on PS fraction for rainbow trout skin. On the other hands, PS contains the highest percentage of DHA in all PL classes. The same results are shown in rainbow trout (Ghioni 1997b). In our trial, in the PS fraction, only 18:1n-9, 18:2n-6, 18:0, and 20:3n-6 increases and 22:6n-3 decreases with decreasing dietary levels of EPA and DHA. Besides, 20:4n-6 is not a major FA that influenced by diet in PS fraction, this is agreed with Ghioni et al (1997a) that they have not detected any 20:4n-6 in rainbow trout skin PS fraction. In PS fraction, only 20:3n-6 will increase significant with n-3 lcPUFA decrease, whereas 18:0 increase very slightly with EPA and DHA decrease, for instance, the percentage of 18:0 in PS fraction was increased from 24.5% to 24.8% with EPA and DHA decrease. This is significant different with the PS fraction in rainbow trout skin cell that 18:0 is increased dramatically and 20:1n-9 decreased dynamically with EPA and DHA deficiency (Ghioni et al. 1997a).

“PI is the major depository for C20 PUFA in fish, especially 20:4n-6” (Bell et al. 1983, Bell et al. 1989, Bell et al. 1990). In our trial, PI fraction in Atlantic salmon skin contains the highest level of 20:4n-6 reinforce this theory. Besides, 20:4n-6 as the most important fatty acid in PI fraction for eicosanoids synthesis, the accumulation of 20:4n-6 (with EPA and DHA deficiency) in PI fraction is larger than in other PL subclasses, it is the same as Ghioni et al (1997b) pointed that the accumulation of 20:4n-6 with EPA and DHA deficiency is greatest in PI fraction than in other PL classes, in rainbow trout skin. In this study, the PI fraction is more conserve than PE and PC fraction, because our PCA plots for PS fraction

show no clear difference between the dietary groups, the groups are more interconnected, in comparison to PC and PE fraction. In PI fraction, only 12:0, 20:4n-6 increases and 22:6n-3 decreases with decreasing dietary levels of EPA and DHA. In PI fraction, 20:4n-6 accumulate significant with n-3 lcPUFA decrease and 12:0 slightly increase with EPA and DHA decrease, this result is agreed with Ghioni et al (1997a) that SFA is slightly elevated in rainbow trout skin PI fraction with DHA deficiency. There are some differences, in rainbow trout skin PI fraction (Ghioni et al. 1997a, b), 20:3n-9 and 22:1n-9 increased significantly with EPA and DHA deficiency (they provided [1-C¹⁴]18:3n-3 in diet), whereas it has not been observed in our trial. However, Thomassen et al (2016) also pointed that 22:5n-6 is increased slightly with n-3 EFA deficiency in Atlantic salmon liver and intestine PI fraction. Thus, it may be caused by the different fish species, the different organs and that the fish fed the different diet.

Moreover, PI and PS fraction are very tiny and difficult to figure out, this is also supported by Loucas et al.(2010) who pointed that the percentage of PI+PS in PL for *Pagrus pagrus* (cultured and wild), *Trachinus draco* and *Trigla lyra* muscle is 1.97%, 0.64%, 6.71% and 8.61% respectively, thus, it may exist some error in the conclusion.

In general, n-6 PUFA will increase with n-3 dietary PUFA decrease in all PL fraction of Atlantic salmon skin. The percentage of DHA was significantly reduced in all Atlantic salmon skin PL classes, while the significant reduction of EPA only detected in PC and PE fraction. This is different in other Atlantic salmon organs (Thomssen et al. 2016). For instance, DHA are reduced in all PL classes in liver and intestine, and in muscle and heart PI and PC fraction, but retained in muscle and heart PE fraction. It represents an organ-specific response to n-3 EFA deficiency and also be supported both in fish and mammalian (Harel & Place 2003, Martin et al. 2011, Lefkowitz et al. 1985; Moussa et al. 1996). Furthermore, DHA are much better retained in PE and PS group, it is the same as the results from Salem et al (1986), Brown (1994) and Ghioni et al. (1997b). In addition, AA constitute an antagonism relationship with EPA and DHA. This result is also supported by Ghioni et al (1997a) whose trial shows a significant decrease in EPA and DHA with the increase of AA in rainbow trout skin PL classes. Besides, Ghioni et al (1997a, b) found that SFA and short chain MUFA is increased with EPA and DHA deficiency, it is also found in Atlantic salmon skin in our trial. However,

this result may not enough confidence, because we provided 18:1n-9 in our experimental diet. On the other hands, a lots of n-6 PUFA increase (only 18:2n-6 is applied in diet) may indicates Atlantic salmon epidermis can enlongate and desaturate 18:2n-6 to n-6 lcPUFA, because Ghioni et al (1997a, b) proved that rainbow trout skin cell can enlongate and desaturate 18:2n-6 to 20:4n-6 but this function have not been found in mammalian (Chapkin and Ziboh 1984).

The fatty acid composition of the different membrane PLs of the skin of Atlantic salmon is to a higher extent influenced by the fatty acid composition of the diets, than for instance brain, liver and intestine (manuscript in preparation Marta Bou). We may predict the effects of the deficiency in EPA and DHA for Atlantic salmon skin, but the physiological consequences for the fish remains to be investigated.

Our research has some imperfections, we just test the level of fatty acids in PL subclasses in Atlantic salmon skin, we do not observe the skin performance in the different dietary groups. From other studies, for instance, Morifuji et al (2015) and Latreille et al (2013), it was pointed out that a high level of n-3 lcPUFA in mammalian skin can reduce the severity of skin photoaging and TEWL, Ghioni et al (1997b) showed that n-3 EFA deficiency will cause the loss of skin integrity in rainbow trout, while Glover et al (2013) showed that EFA in fish skin play a critical role in oxygen uptake and carbondioxide excretion.

Reference

- Arvidson, G. A. E. (1968). Structural and metabolic heterogeneity of rat liver glycerophosphatides. *European Journal of Biochemistry*, 4(4), 478-486.
- Baeverfjord, G., & Krogdahl, Å. (1996). Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *Journal of Fish Diseases*, 19(5), 375-387.
- Baidu Baike. Structure of phospholipids. The available URL:
<http://baike.baidu.com/pic/%E7%A3%B7%E8%84%82/3755114/0/48540923dd54564e24f33aa3b1de9c82d0584f92?fr=lemma&ct=single#aid=0&pic=48540923dd54564e24f33aa3b1de9c82d0584f92>
- Barcelos, R. C., de Mello-Sampayo, C., Antoniazzi, C. T., Segat, H. J., Silva, H., Veit, J. C., ... & Rodrigues, L. M. (2015). Oral supplementation with fish oil reduces dryness and pruritus in the acetone-induced dry skin rat model. *Journal of dermatological science*, 79(3), 298-304.
- Bell, M. V., Simpson, C. M., & Sargent, J. R. (1983). (n-3) and (n-6) polyunsaturated fatty acids in the phosphoglycerides of salt-secreting epithelia from two marine fish species. *Lipids*, 18(10), 720-726.
- Bell, M. V., & Tocher, D. R. (1989). Molecular species composition of the major phospholipids in brain and retina from rainbow trout (*Salmo gairdneri*). Occurrence of high levels of di-(n-3) polyunsaturated fatty acid species. *Biochemical Journal*, 264(3), 909-915.
- Bell, M. V., & Dick, J. R. (1990). Molecular species composition of phosphatidylinositol from the brain, retina, liver and muscle of cod (*Gadus morhua*). *Lipids*, 25(11), 691-694.
- Bell, M. V., & Dick, J. R. (1991). Molecular species composition of the major diacyl glycerophospholipids from muscle, liver, retina and brain of cod (*Gadus morhua*). *Lipids*, 26(8), 565-573.
- Bell, M. V., Batty, R. S., Dick, J. R., Fretwell, K., Navarro, J. C., & Sargent, J. R. (1995). Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids*, 30(5), 443-449.
- Bell, J. G., McEvoy, J., Tocher, D. R., McGhee, F., Campbell, P. J., & Sargent, J. R. (2001). Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *The Journal of*

nutrition, 131(5), 1535-1543.

Bell, J. G., McGhee, F., Campbell, P. J., & Sargent, J. R. (2003). Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil “wash out”. *Aquaculture*, 218(1), 515-528.

Brown, M. F. (1994). Modulation of rhodopsin function by properties of the membrane bilayer. *Chemistry and physics of lipids*, 73(1), 159-180.

Bureau, D. P., Kaushik, S. J., Cho, C. Y. (2002). Bioenergetics. In: Halver JE, Hardy RW (eds) *Fish Nutrition*, 3rd edn. (pp. 2–61). USA: Elsevier Science.

Cahu, C. L., Infante, J. L. Z., & Barbosa, V. (2003). Effect of dietary phospholipid level and phospholipid: neutral lipid value on the development of sea bass (*Dicentrarchus labrax*) larvae fed a compound diet. *British Journal of Nutrition*, 90(01), 21-28.

Castell, J. D., Sinnhuber, R. O., Lee, D. J., & Wales, J. H. (1972a). Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): physiological symptoms of EFA deficiency. *Journal of Nutrition*, 102(1), 87-92.

Castell, J. D., Sinnhuber, R. O., Wales, J. H., & Lee, D. J. (1972b). Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): growth, feed conversion and some gross deficiency symptoms. *Journal of Nutrition*, 102(1), 77-85.

Cejas, J. R., Almansa, E., Tejera, N., Jerez, S., Bolaños, A., & Lorenzo, A. (2003). Effect of dietary supplementation with shrimp on skin pigmentation and lipid composition of red porgy (*Pagrus pagrus*) alevins. *Aquaculture*, 218(1), 457-469.

Chap, H. (2016). Forty five years with membrane phospholipids, phospholipases and lipid mediators: A historical perspective. *Biochimie*.

Chapkin, R. S., & Ziboh, V. A. (1984). Inability of skin enzyme preparations to biosynthesize arachidonic acid from linoleic acid. *Biochemical and biophysical research communications*, 124(3), 784-792.

Cho, J.K., Jin, Y.G., Rha, S.J., Kim, S.J., Hwang, J.H. (2014). Biochemical characteristics of four marine fish skins in Korea. *Food Chemistry*, 159, 200–207.

Choi, S. S., & Regenstein, J. M. (2000). Physicochemical and sensory characteristics of fish gelatin. *Journal of Food Science*, 65(2), 194-199.

- Coutteau, P., Geurden, I., Camara, M. R., Bergot, P., & Sorgeloos, P. (1997). Review on the dietary effects of phospholipids in fish and crustacean larviculture. *Aquaculture*, 155(1), 149-164.
- Fan, Y. Y., Monk, J. M., Hou, T. Y., Callway, E., Vincent, L., Weeks, B., ... & Chapkin, R. S. (2012). Characterization of an arachidonic acid-deficient (Fads1 knockout) mouse model. *Journal of lipid research*, 53(7), 1287-1295.
- FAO. 2014. The State of World Fisheries and Aquaculture. Available URL: <http://www.fao.org/3/a-i3720e.pdf>
- Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol chem*, 226(1), 497-509.
- Francis, D. S., Turchini, G. M., Jones, P. L., & De Silva, S. S. (2007). Dietary lipid source modulates in vivo fatty acid metabolism in the freshwater fish, Murray cod (*Maccullochella peelii peelii*). *Journal of agricultural and food chemistry*, 55(4), 1582-1591.
- Ghioni, C., Bell, J. G., Bell, M. V., & Sargent, J. R. (1997a). Fatty acid composition, eicosanoid production and permeability in skin tissues of rainbow trout (*Oncorhynchus mykiss*) fed a control or an essential fatty acid deficient diet. *Prostaglandins, leukotrienes and essential fatty acids*, 56(6), 479-489.
- Ghioni, C., Bell, J. G., Bell, M. V., & Sargent, J. R. (1997b). Fatty acid composition, eicosanoid production and permeability in skin tissues of rainbow trout (*Oncorhynchus mykiss*) fed a control or an essential fatty acid deficient diet. *Prostaglandins, leukotrienes and essential fatty acids*, 56(6), 479-489.
- Ghioni, C., Tocher, D. R., & Sargent, J. R. (1997c). The effect of culture on morphology, lipid and fatty acid composition, and polyunsaturated fatty acid metabolism of rainbow trout (*Oncorhynchus mykiss*) skin cells. *Fish Physiology and Biochemistry*, 16(6), 499-513.
- Gjedrem, T., Robinson, N., & Rye, M. (2012). The importance of selective breeding in aquaculture to meet future demands for animal protein: a review. *Aquaculture*, 350, 117-129.
- Glencross, B. D. (2009). Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture*, 1(2), 71-124.
- Glover, C. N., Bucking, C., & Wood, C. M. (2013). The skin of fish as a transport epithelium: a review. *Journal of Comparative Physiology B*, 183(7), 877-891.

- Hansen, H. S. (1986). The essential nature of linoleic acid in mammals. *Trends in Biochemical Sciences*, 11(6), 263-265.
- Harel, M., & Place, A. R. (2003). Tissue essential fatty acid composition and competitive response to dietary manipulations in white bass (*Morone chrysops*), striped bass (*M. saxatilis*) and hybrid striped bass (*M. chrysops* × *M. saxatilis*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 135(1), 83-94.
- Hassan, H. H., Blain, S., Boucher, B., Denis, M., Krimbou, L., & Genest, J. (2005). Structural modification of plasma HDL by phospholipids promotes efficient ABCA1-mediated cholesterol release. *Journal of lipid research*, 46(7), 1457-1465.
- Hawkes, J. W. (1974). The structure of fish skin. *Cell and tissue research*, 149(2), 159-172.
- Hazel, J. R., & Williams, E. E. (1990). The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Progress in lipid research*, 29(3), 167-227.
- Higgs, D. A., Dong, F. M. (2000). Lipids and fatty acids. In: Stickney RR (ed.) *Encyclopedia of Aquaculture* (pp. 476–496). New York: John Wiley & Sons.
- Ibeas, C., Izquierdo, M. S., & Lorenzo, A. (1994). Effect of different levels of n-3 highly unsaturated fatty acids on growth and fatty acid composition of juvenile gilthead seabream (*Sparus aurata*). *Aquaculture*, 127(2), 177-188.
- Ibeas, C., Cejas, J., Gomez, T., Jerez, S., & Lorenzo, A. (1996). Influence of dietary n-3 highly unsaturated fatty acids levels on juvenile gilthead seabream (*Sparus aurata*) growth and tissue fatty acid composition. *Aquaculture*, 142(3), 221-235.
- Ibeas, C., Cejas, J. R., Fores, R., Badía, P., Gómez, T., & Hernández, A. L. (1997). Influence of eicosapentaenoic to docosahexaenoic acid ratio (EPADHA) of dietary lipids on growth and fatty acid composition of gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture*, 150(1), 91-102.
- Jacobsen, C.. (2015). *Fish Oils: Composition and Health Effects* (pp. 686-692). Reference Module in Food Science.
- Jian, B., De la Llera-Moya, M., Royer, L., Rothblat, G., Francone, O., & Swaney, J. B. (1997). Modification of the cholesterol efflux properties of human serum by enrichment with phospholipid. *Journal of lipid research*, 38(4), 734-744.

- Kagan, V.E., Tyurin, V.A., Gorbunov, N.V., Prilipko, L.L., Chelomin, V.P. (1984). Are changes in the microviscosity and an asymmetrical distribution of phospholipids in the membrane necessary conditions for signal transmission? A comparison of the mechanisms of signal transmission in plasma membranes of brain synaptosomes and photoreceptor membranes of the retina. *J. Evol. Biochem. Physiol.* 20, 6–11.
- Kalogeropoulos, N., Alexis, M. N., & Henderson, R. J. (1992). Effects of dietary soybean and cod-liver oil levels on growth and body composition of gilthead bream (*Sparus aurata*). *Aquaculture*, 104(3), 293-308.
- Kanazawa, A., Teshima, S. I., Inamori, S., Iwashita, T., & Nagao, A. (1981). Effects of phospholipids on growth, survival rate and incidence of malformation in the larval ayu. *Mem. Fac. Fish. Kagoshima Univ*, 30, 301-309.
- Kanazawa, A. (1993). Essential phospholipids of fish and crustaceans. In: Kaushik, [S.], Luquet, P. (Eds.), *Fish Nutrition in Practice. : IVth International Symposium on Fish Nutrition and Feeding Paris* (pp. 519–530). France: INRA.
- Krogdahl, Å., Bakke - McKellep, A. M., & Baeverfjord, G. (2003). Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition*, 9(6), 361-371.
- Latreille, J., Kesse-Guyot, E., Malvy, D., Andreeva, V., Galan, P., Tschachler, E., ... & Ezzedine, K. (2013). Association between dietary intake of n-3 polyunsaturated fatty acids and severity of skin photoaging in a middle-aged Caucasian population. *Journal of dermatological science*, 72(3), 233-239.
- Lefkowitz, J. B., Flippo, V., Sprecher, H., & Needleman, P. (1985). Paradoxical conservation of cardiac and renal arachidonate content in essential fatty acid deficiency. *Journal of Biological Chemistry*, 260(29), 15736-15744.
- Li, X., Wang, J., Han, T., Hu, S., Jiang, Y., & Wang, C. (2014). Effect of dietary phospholipids levels and sources on growth performance, fatty acid composition of the juvenile swimming crab, *Portunus trituberculatus*. *Aquaculture*, 430, 166-172.
- Lin, M. H., & Khnykin, D. (2014). Fatty acid transporters in skin development, function and disease. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1841(3), 362-368.

- Liu, J.K., Li, W.R., Wang, W.Q., Lei, J.L. (2002). Effects of fish oil, DHA oil and lecithin in microparticulate diets on stress tolerance of larval gilthead seabream (*Sparus aurata*). *Chinese Journal of Oceanology and Limnology*, 20(4), 338-343.
- Loukas, V., Dimizas, C., Sinanoglou, V. J., & Miniadis-Meimaroglou, S. (2010). EPA, DHA, cholesterol and phospholipid content in *Pagrus pagrus* (cultured and wild), *Trachinus draco* and *Trigla lyra* from Mediterranean Sea. *Chemistry and physics of lipids*, 163(3), 292-299.
- March, B. E. (1993). Essential fatty acids in fish physiology. *Canadian journal of physiology and pharmacology*, 71(9), 684-689.
- Martín, M. V., Almansa, E., Cejas, J. R., Bolaños, A., Jerez, S., & Lorenzo, A. (2011). Effects of a diet lacking HUFA on lipid and fatty acid content of intestine and gills of male gilthead seabream (*Sparus aurata* L.) broodstock at different stages of the reproductive cycle. *Fish physiology and biochemistry*, 37(4), 935-949.
- Mason, M. E., & Waller, G. R. (1964). Dimethoxypropane Induced Transesterification of Fats and Oils in Preparation of Methyl Esters for Gas Chromatographic Analysis. *Analytical Chemistry*, 36(3), 583-586.
- Masuda, R. (2003). The critical role of docosahexaenoic acid in marine and terrestrial ecosystems: from bacteria to human behavior. *Big fish bang*, 249-256.
- Meyer, W., & Seegers, U. (2012). Basics of skin structure and function in elasmobranchs: a review. *Journal of fish biology*, 80(5), 1940-1967.
- Morifuji, M., Oba, C., Ichikawa, S., Ito, K., Kawahata, K., Asami, Y., ... & Sugawara, T. (2015). A novel mechanism for improvement of dry skin by dietary milk phospholipids: Effect on epidermal covalently bound ceramides and skin inflammation in hairless mice. *Journal of dermatological science*, 78(3), 224-231.
- Mourente, G., Tocher, D. R., & Sargent, J. R. (1991). Specific accumulation of docosahexaenoic acid (22 : 6n-3) in brain lipids during development of juvenile turbot *Scophthalmus maximus* L. *Lipids*, 26(11), 871-877.
- Moussa, M., Garcia, J., Ghisolfi, J., Périquet, B., & Thouvenot, J. P. (1996). Dietary essential fatty acid deficiency differentially affects tissues of rats. *The Journal of nutrition*, 126(12), 3040.
- Nakamura, M. T., & Nara, T. Y. (2004). Structure, function, and dietary regulation of $\Delta 6$, $\Delta 5$,

- and $\Delta 9$ desaturases. *Annu. Rev. Nutr.*, 24, 345-376.
- Ng, W. K., Tocher, D. R., & Bell, J. G. (2007). The use of palm oil in aquaculture feeds for salmonid species. *European Journal of Lipid Science and Technology*, 109(4), 394-399.
- Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., & Skrede, A. (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)—effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. *Aquaculture*, 288(3), 305-311.
- Qiao, H., Cong, C., Sun, C., Li, B., Wang, J., & Zhang, L. (2016). Effect of culture conditions on growth, fatty acid composition and DHA/EPA ratio of *Phaeodactylum tricornutum*. *Aquaculture*, 452, 311-317.
- Rakers, S., Gebert, M., Uppalapati, S., Meyer, W., Maderson, P., Sell, A. F., ... & Paus, R. (2010). 'Fish matters': the relevance of fish skin biology to investigative dermatology. *Experimental dermatology*, 19(4), 313-324.
- Rawn, J.D. (1989). Neil Patterson Publishers, Burlington. Nichols PD (2004) Sources of long-chain omega-3 oils (pp. 247-251). UK: Biochemistry.
- Regost, C., Arzel, J., Robin, J., Rosenlund, G., & Kaushik, S. J. (2003). Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*): 1. Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture*, 217(1), 465-482.
- Reich, E., & Schibli, A. (2007). *High-performance thin-layer chromatography for the analysis of medicinal plants*. Thieme.
- Rigaudy J, Klesney SP (1979) Nomenclature of Organic Chemistry. Pergamon, Oxford, UK.
- Rinchar, J., Czesny, S., & Dabrowski, K. (2007). Influence of lipid class and fatty acid deficiency on survival, growth, and fatty acid composition in rainbow trout juveniles. *Aquaculture*, 264(1), 363-371.
- Ruyter, B., Rosjo, C., Einen, O., & Thomassen, M. S. (2000a). Essential fatty acids in Atlantic salmon: effects of increasing dietary doses of n-6 and n-3 fatty acids on growth, survival and fatty acid composition of liver, blood and carcass. *Aquaculture Nutrition*, 6(2), 119-128.
- Ruyter, B., Rosjo, C., Einen, O., & Thomassen, M. S. (2000b). Essential fatty acids in Atlantic salmon: effects of increasing dietary doses of n-6 and n-3 fatty acids on growth,

- survival and fatty acid composition of liver, blood and carcass. *Aquaculture Nutrition*, 6(2), 119-128.
- Salem N., Kim H.-Y., Yergey A. A. (1986). Docosahexaenoic acid: membrane function and metabolism. In: Simopoulos A. P., ed. *Health Effects of Polyunsaturated Fatty Acids in Seafoods* (pp. 263-317). New York: Academic Press.
- Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J., & Tocher, D. R. (1993). The metabolism of phospholipids and polyunsaturated fatty acids in fish. *Aquaculture: Fundamental and Applied Research*, 103-124.
- Sargent JR, Tocher DR, Bell JG (2002) The lipids. In: Halver JE, Hardy RW (eds) *Fish Nutrition*, pp. 181–257. Academic Press, Elsevier, San Diego.
- Sargent, J. R. (1995). Polyunsaturated fatty acids and farmed fish. *Fish Oil: Technology, Nutrition and Marketing*, 67-94.
- Schempp, C., Emde, M., & Wölfle, U. (2009). Dermatology in the Darwin anniversary. Part 1: Evolution of the integument. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, 7(9), 750-757.
- Stewart, M. E., & Downing, D. T. (1991). Chemistry and function of mammalian sebaceous lipids. *Skin Lipids: Advances in Lipid Research*, 24, 263-302.
- Stroud, C. K., Nara, T. Y., Roqueta-Rivera, M., Radlowski, E. C., Lawrence, P., Zhang, Y., ... & Haschek, W. M. (2009). Disruption of FADS2 gene in mice impairs male reproduction and causes dermal and intestinal ulceration. *Journal of lipid research*, 50(9), 1870-1880.
- Stubhaug, I., Lie, Ø., & Torstensen, B. E. (2007). Fatty acid productive value and β - oxidation capacity in Atlantic salmon (*Salmo salar* L.) fed on different lipid sources along the whole growth period. *Aquaculture Nutrition*, 13(2), 145-155.
- Tacon, A. G., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*, 285(1), 146-158.
- Thomassen, M. S., Bou, M., Røsjø, C., & Ruyter, B. (2016). Organ and phospholipid class fatty acid specificity in response to dietary depletion of essential n - 3 fatty acids in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition*.
- Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost

- fish. *Reviews in fisheries science*, *11*(2), 107-184.
- Torstensen, B. E., Bell, J. G., Rosenlund, G., Henderson, R. J., Graff, I. E., Tocher, D. R., ... & Sargent, J. R. (2005). Tailoring of Atlantic salmon (*Salmo salar* L.) flesh lipid composition and sensory quality by replacing fish oil with a vegetable oil blend. *Journal of Agricultural and Food Chemistry*, *53*(26), 10166-10178.
- Torstensen, B. E., Frøyland, L., Ørnstrud, R., & Lie, Ø. (2004). Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. *Food Chemistry*, *87*(4), 567-580.
- Turchini, G. M., Gunasekera, R. M., & De Silva, S. S. (2003). Effect of crude oil extracts from trout offal as a replacement for fish oil in the diets of the Australian native fish Murray cod *Maccullochella peelii peelii*. *Aquaculture Research*, *34*(9), 697-708.
- Turchini, G. M., Torstensen, B. E., & Ng, W. K. (2009). Fish oil replacement in finfish nutrition. *Reviews in Aquaculture*, *1*(1), 10-57.
- Verkleij, A. J., Zwaal, R. F. A., Roelofsen, B., Comfurius, P., Kastelijn, D., & Van Deenen, L. L. M. (1973). The asymmetric distribution of phospholipids in the human red cell membrane. A combined study using phospholipases and freeze-etch electron microscopy. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *323*(2), 178-193.
- Watanabe T. (1982) Lipid nutrition in fish. *Comparative Biochemistry and Physiology – Part B* *73*: 3–15.
- Wikipedia. An example of TLC. Available URL: https://en.wikipedia.org/wiki/Thin-layer_chromatography
- Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., ... & Sala, E. (2006). Impacts of biodiversity loss on ocean ecosystem services. *science*, *314*(5800), 787-790.
- Yamaji-Hasegawa, A. & Tsujimoto, M. (2006) Lipid Dynamics and Pathobiology in Membrane Lipid Rafts. Asymmetric Distribution of Phospholipids in Biomembranes. *Biol. Pharm. Bull.* *29*(8) 1547-1553.
- Yoo, S. J., Cho, S. M., Woo, J. W., Kim, S. H., Han, Y. N., Ahn, J. R., ... & Kim, S. B. (2008). Processing and physicochemical properties of collagen from yellowfin tuna (*Thunnus albacares*) abdominal skin. *Korean Journal of Fisheries and Aquatic Sciences*, *41*(6),

427-434.

Ytrestøyl, T., Aas, T. S., & Åsgård, T. (2015). Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. *Aquaculture*, *448*, 365-374.

Yui, K., Imataka, G., Kawasak, Y., & Yamada, H. (2016). Increased ω -3 polyunsaturated fatty acid/arachidonic acid ratios and upregulation of signaling mediator in individuals with autism spectrum disorders. *Life sciences*, *145*, 205-212.

Zhao, J., Ai, Q., Mai, K., Zuo, R., & Luo, Y. (2013). Effects of dietary phospholipids on survival, growth, digestive enzymes and stress resistance of large yellow croaker, *Larmichthys crocea* larvae. *Aquaculture*, *410*, 122-128.

Appendix

Table 1 Total fat content of skin (gram lipid /100 gram skin sample). Data shown as a mean of duplicate for EPA or DHA group (n=2) or triplicate NC, CC and EPA&DHA group (n=3) samples. All data are shown in percentage.

Fat content%	200g		400g	
	MEAN	S.E.M. ³	MEAN	S.E.M. ³
NC ¹	7.6	± 0.59	12.3	± 1.45
0.5%EPA	9.3	± 0.16	12.9	± 2.42
1%EPA	9.6	± 1.74	9.0	± 2.16
1.5%EPA	9.9	± 2.01	15.8	± 1.39
2%EPA	11.8	± 1.78	13.2	± 2.27
0.5%DHA	7.7	± 0.59	10.9	± 0.78
1%DHA	8.9	± 2.15	12.9	± 2.26
1.5%DHA	11.0	± 1.29	16.3	± 1.21
2%DHA	11.2	± 1.42	12.6	± 2.36
0.5%EPA&DHA	14.8	± 5.68	9.3	± 0.06
1%EPA&DHA	11.9	± 2.20	12.1	± 1.50
1.5%EPA&DHA	10.2	± 0.31	18.3	± 2.24
2%EPA&DHA	12.2	± 0.24	16.5	± 3.14
CC ²	12.2	± 0.84	18.6	± 3.10
Average	10.6	± 1.50	13.6	± 1.88

NC¹= negative control

CC²= commercial control

S.E.M.³= standard error mean

Appendix

Table 2 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 200g PC group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PC200	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	7.6 \pm 0.59	9.3 \pm 0.16	9.6 \pm 1.74	9.9 \pm 2.01	11.8 \pm 1.78	12.2 \pm 0.84
C 12:0	0.3 \pm 0.11	0.3 \pm 0.07	0.2 \pm 0.00	0.6 \pm 0.15	0.3 \pm 0.20	0.3 \pm 0.06
C 14:0	0.0 \pm 0.24	0.7 \pm 0.09	0.2 \pm 0.22	0.7 \pm 0.00	0.6 \pm 0.13	1.3 \pm 0.10
C 16:0	29.7 \pm 0.96	30.6 \pm 0.48	29.5 \pm 0.64	33.6 \pm 1.14	31.7 \pm 0.69	28.6 \pm 1.19
C 18:0	4.4 \pm 0.03	4.3 \pm 0.01	3.9 \pm 0.00	4.2 \pm 0.07	4.0 \pm 0.55	3.5 \pm 0.21
C 20:0	0.1 \pm 0.00	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.00	0.1 \pm 0.01	0.1 \pm 0.01
C 22:0	0.0 \pm 0.03	0.1 \pm 0.01	0.1 \pm 0.02	0.0 \pm 0.04	0.1 \pm 0.03	0.1 \pm 0.00
C 24:0	0.1 \pm 0.34	0.0 \pm 0.04	0.1 \pm 0.01	0.1 \pm 0.02	0.1 \pm 0.04	0.1 \pm 0.12
SUM SFA³	35.0 \pm 1.73	36.5 \pm 0.76	34.6 \pm 0.93	39.9 \pm 1.51	37.3 \pm 1.76	34.7 \pm 1.77
C 16:1 n-9	1.7 \pm 0.09	2.6 \pm 0.97	1.4 \pm 0.08	1.6 \pm 0.22	2.1 \pm 0.49	1.9 \pm 0.20
C 16:1 n-7	0.0 \pm 0.59	0.0 \pm 0.00	0.0 \pm 0.00	2.0 \pm 0.07	0.0 \pm 0.53	0.0 \pm 0.59
C 18:1 n-9	25.8 \pm 0.74	22.4 \pm 0.52	22.5 \pm 0.12	22.9 \pm 1.18	21.0 \pm 0.48	19.7 \pm 0.48
C 20:1 n-9	0.4 \pm 0.01	0.4 \pm 0.00	0.3 \pm 0.01	0.4 \pm 0.01	0.3 \pm 0.04	0.6 \pm 0.01
C 22:1 n-7	0.2 \pm 0.02	0.2 \pm 0.01	0.3 \pm 0.01	0.2 \pm 0.05	0.2 \pm 0.02	0.3 \pm 0.02
C 24:1 n-9	0.6 \pm 0.15	0.2 \pm 0.14	0.3 \pm 0.06	0.4 \pm 0.15	0.2 \pm 0.17	0.7 \pm 0.08
SUM MUFA⁴	28.8 \pm 2.37	27.9 \pm 1.84	25.9 \pm 1.13	29.8 \pm 1.93	25.8 \pm 1.95	25.5 \pm 1.56
C 18:2 n-6	8.3 \pm 0.29	6.8 \pm 0.17	6.2 \pm 0.03	4.9 \pm 0.32	4.8 \pm 0.29	3.8 \pm 0.11
C 18:3 n-6	0.9 \pm 0.05	0.5 \pm 0.00	0.3 \pm 0.01	0.2 \pm 0.00	0.2 \pm 0.01	0.1 \pm 0.03
C 18:3 n-3	0.6 \pm 0.04	0.6 \pm 0.00	0.6 \pm 0.02	0.5 \pm 0.05	0.5 \pm 0.05	0.5 \pm 0.03
C 18:4 n-3	0.1 \pm 0.01	0.1 \pm 0.00	0.1 \pm 0.00	0.0 \pm 0.05	0.0 \pm 0.03	0.1 \pm 0.01
C 20:3n-9	0.0 \pm 0.04	0.0 \pm 0.05	0.1 \pm 0.04	0.0 \pm 0.00	0.1 \pm 0.02	0.0 \pm 0.00
C 20:3 n-6	2.7 \pm 0.12	2.5 \pm 0.16	1.6 \pm 0.14	0.8 \pm 0.28	0.8 \pm 0.04	0.9 \pm 0.09
C 20:4 n-6	8.6 \pm 0.31	5.9 \pm 0.35	4.0 \pm 0.30	2.9 \pm 0.10	2.8 \pm 0.07	2.6 \pm 0.17
C 20:5 n-3	2.4 \pm 0.18	5.6 \pm 0.03	9.9 \pm 0.86	9.0 \pm 1.44	11.4 \pm 0.95	7.1 \pm 0.44
C 22:5 n-6	1.3 \pm 0.30	0.5 \pm 0.22	0.3 \pm 0.01	0.1 \pm 0.11	0.2 \pm 0.09	0.3 \pm 0.13
C 22:5 n-3	0.8 \pm 0.05	1.1 \pm 0.04	1.6 \pm 0.07	1.3 \pm 0.16	1.6 \pm 0.07	0.9 \pm 0.06
C 22:6 n-3	5.9 \pm 0.62	8.6 \pm 0.76	10.8 \pm 0.52	8.7 \pm 0.91	12.0 \pm 0.55	19.4 \pm 1.26
SUM PUFA⁵	32.1 \pm 2.04	32.5 \pm 1.82	35.8 \pm 2.04	28.7 \pm 3.49	34.5 \pm 2.25	36.0 \pm 2.40
PC200	NC ¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC ²
Fett % Folch	7.6 \pm 0.59	7.7 \pm 0.59	8.9 \pm 2.15	11.0 \pm 1.29	11.2 \pm 1.42	12.2 \pm 0.84
C 12:0	0.3 \pm 0.11	0.5 \pm 0.13	0.3 \pm 0.08	0.3 \pm 0.11	0.4 \pm 0.04	0.3 \pm 0.06
C 14:0	0.0 \pm 0.24	0.6 \pm 0.11	0.5 \pm 0.05	0.6 \pm 0.04	0.6 \pm 0.11	1.3 \pm 0.10
C 16:0	29.7 \pm 0.96	31.0 \pm 1.90	31.3 \pm 1.11	32.5 \pm 1.80	31.8 \pm 1.26	28.6 \pm 1.19
C 18:0	4.4 \pm 0.03	4.0 \pm 0.14	3.7 \pm 0.14	3.9 \pm 0.19	3.9 \pm 0.14	3.5 \pm 0.21
C 20:0	0.1 \pm 0.00	0.1 \pm 0.00	0.1 \pm 0.00	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.01
C 22:0	0.0 \pm 0.03	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.02	0.1 \pm 0.00
C 24:0	0.1 \pm 0.34	0.4 \pm 0.41	0.0 \pm 0.00	0.6 \pm 0.48	0.1 \pm 0.04	0.1 \pm 0.12
SUM SFA³	35.0 \pm 1.73	37.1 \pm 2.73	36.4 \pm 1.42	38.4 \pm 2.70	37.4 \pm 1.66	34.7 \pm 1.77
C 16:1 n-9	1.7 \pm 0.09	1.8 \pm 0.17	2.7 \pm 0.95	1.5 \pm 0.13	3.1 \pm 0.60	1.9 \pm 0.20
C 16:1 n-7	0.0 \pm 0.59	1.0 \pm 0.99	0.1 \pm 0.12	0.8 \pm 0.85	0.0 \pm 0.47	0.0 \pm 0.59
C 18:1 n-9	25.8 \pm 0.74	22.6 \pm 1.13	21.5 \pm 0.71	23.1 \pm 0.26	21.0 \pm 0.58	19.7 \pm 0.48
C 20:1 n-9	0.4 \pm 0.01	0.4 \pm 0.01	0.4 \pm 0.03	0.4 \pm 0.00	0.4 \pm 0.03	0.6 \pm 0.01
C 22:1 n-7	0.2 \pm 0.02	0.2 \pm 0.02	0.2 \pm 0.01	0.1 \pm 0.02	0.1 \pm 0.00	0.3 \pm 0.02
C 24:1 n-9	0.6 \pm 0.15	0.3 \pm 0.06	0.8 \pm 0.30	0.4 \pm 0.01	0.5 \pm 0.14	0.7 \pm 0.08
SUM MUFA⁴	28.8 \pm 2.37	28.4 \pm 2.70	27.7 \pm 2.34	27.7 \pm 2.39	27.0 \pm 2.02	25.5 \pm 1.56
C 18:2 n-6	8.3 \pm 0.29	7.9 \pm 0.32	6.0 \pm 0.12	5.4 \pm 0.26	5.3 \pm 0.03	3.8 \pm 0.11
C 18:3 n-6	0.9 \pm 0.05	0.5 \pm 0.03	0.3 \pm 0.01	0.3 \pm 0.02	0.2 \pm 0.00	0.1 \pm 0.03
C 18:3 n-3	0.6 \pm 0.04	0.6 \pm 0.05	0.5 \pm 0.06	0.4 \pm 0.03	0.5 \pm 0.02	0.5 \pm 0.03
C 18:4 n-3	0.1 \pm 0.01	0.1 \pm 0.06	0.1 \pm 0.01	0.0 \pm 0.00	0.1 \pm 0.00	0.1 \pm 0.01
C 20:3n-9	0.5 \pm 0.12	0.6 \pm 0.02	0.6 \pm 0.04	0.8 \pm 0.14	0.8 \pm 0.03	0.8 \pm 0.01
C 20:3 n-6	0.0 \pm 0.04	0.1 \pm 0.02	0.1 \pm 0.01	0.1 \pm 0.05	0.1 \pm 0.04	0.0 \pm 0.00
C 20:4 n-6	2.7 \pm 0.12	2.3 \pm 0.33	1.7 \pm 0.03	1.1 \pm 0.13	0.8 \pm 0.02	0.9 \pm 0.09
C 20:5 n-3	8.6 \pm 0.31	5.6 \pm 0.91	5.1 \pm 0.08	3.6 \pm 0.31	3.3 \pm 0.19	2.6 \pm 0.17
C 22:5 n-6	2.4 \pm 0.18	2.8 \pm 0.34	3.3 \pm 0.27	3.2 \pm 0.34	3.4 \pm 0.26	7.1 \pm 0.44
C 22:5 n-3	1.3 \pm 0.30	0.6 \pm 0.63	0.8 \pm 0.55	0.8 \pm 0.61	0.9 \pm 0.47	0.3 \pm 0.13
C 22:6 n-3	0.8 \pm 0.05	0.5 \pm 0.08	0.4 \pm 0.05	0.4 \pm 0.06	0.4 \pm 0.02	0.9 \pm 0.06
SUM PUFA⁵	5.9 \pm 0.62	10.3 \pm 1.67	14.9 \pm 1.77	15.7 \pm 2.44	18.1 \pm 1.77	19.4 \pm 1.26
PC200	NC ¹	0.5%EPA&DH/1%EPA&DHA	1.5%EPA&DH/2%EPA&DHA	CC ²		
Fett % Folch	7.6 \pm 0.59	14.8 \pm 5.68	11.9 \pm 2.20	10.2 \pm 0.31	12.2 \pm 0.24	12.2 \pm 0.84
C 12:0	0.3 \pm 0.11	0.5 \pm 0.06	0.4 \pm 0.01	0.3 \pm 0.10	0.3 \pm 0.05	0.3 \pm 0.06
C 14:0	0.0 \pm 0.24	0.7 \pm 0.08	0.6 \pm 0.01	0.7 \pm 0.13	0.3 \pm 0.22	1.3 \pm 0.10
C 16:0	29.7 \pm 0.96	30.9 \pm 0.99	29.7 \pm 0.56	32.1 \pm 1.94	30.6 \pm 1.21	28.6 \pm 1.19
C 18:0	4.4 \pm 0.03	4.2 \pm 0.17	3.9 \pm 0.10	4.1 \pm 0.31	3.9 \pm 0.15	3.5 \pm 0.21
C 20:0	0.1 \pm 0.00	0.1 \pm 0.02	0.1 \pm 0.01	0.1 \pm 0.02	0.1 \pm 0.00	0.1 \pm 0.01
C 22:0	0.0 \pm 0.03	0.1 \pm 0.03	0.1 \pm 0.01	0.1 \pm 0.02	0.1 \pm 0.00	0.1 \pm 0.00
C 24:0	0.1 \pm 0.34	0.2 \pm 0.10	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.21	0.1 \pm 0.12
SUM SFA³	35.0 \pm 1.73	37.2 \pm 1.59	35.4 \pm 0.78	37.9 \pm 2.61	35.8 \pm 1.89	34.7 \pm 1.77
C 16:1 n-9	1.7 \pm 0.09	1.8 \pm 0.06	1.6 \pm 0.02	2.8 \pm 1.00	2.9 \pm 0.43	1.9 \pm 0.20
C 16:1 n-7	0.0 \pm 0.59	0.8 \pm 0.81	0.8 \pm 0.80	0.1 \pm 0.10	0.0 \pm 0.54	0.0 \pm 0.59
C 18:1 n-9	25.8 \pm 0.74	22.3 \pm 0.29	20.6 \pm 0.56	23.1 \pm 2.01	21.4 \pm 0.34	19.7 \pm 0.48
C 20:1 n-9	0.4 \pm 0.01	0.4 \pm 0.02	0.4 \pm 0.01	0.4 \pm 0.08	0.4 \pm 0.01	0.6 \pm 0.01
C 22:1 n-7	0.2 \pm 0.02	0.2 \pm 0.00	0.2 \pm 0.00	0.2 \pm 0.02	0.2 \pm 0.01	0.3 \pm 0.02
C 24:1 n-9	0.6 \pm 0.15	0.3 \pm 0.27	0.4 \pm 0.06	0.2 \pm 0.18	0.5 \pm 0.02	0.7 \pm 0.08
SUM MUFA⁴	28.8 \pm 2.37	27.8 \pm 1.63	26.0 \pm 1.62	27.9 \pm 4.48	26.6 \pm 2.08	25.5 \pm 1.56
C 18:2 n-6	8.3 \pm 0.29	7.5 \pm 0.21	6.3 \pm 0.04	4.9 \pm 0.08	4.8 \pm 0.19	3.8 \pm 0.11
C 18:3 n-6	0.9 \pm 0.05	0.5 \pm 0.03	0.3 \pm 0.02	0.3 \pm 0.01	0.2 \pm 0.01	0.1 \pm 0.03
C 18:3 n-3	0.6 \pm 0.04	0.6 \pm 0.05	0.5 \pm 0.01	0.4 \pm 0.03	0.5 \pm 0.04	0.5 \pm 0.03
C 18:4 n-3	0.1 \pm 0.01	0.1 \pm 0.00	0.1 \pm 0.05	0.1 \pm 0.01	0.1 \pm 0.03	0.1 \pm 0.01
C 20:3n-9	0.5 \pm 0.12	0.6 \pm 0.01	0.7 \pm 0.00	0.7 \pm 0.06	0.7 \pm 0.02	0.8 \pm 0.01
C 20:3 n-6	0.0 \pm 0.04	0.1 \pm 0.00	0.1 \pm 0.01	0.1 \pm 0.00	0.0 \pm 0.02	0.0 \pm 0.00
C 20:4 n-6	2.7 \pm 0.12	2.4 \pm 0.10	1.9 \pm 0.16	1.1 \pm 0.12	0.8 \pm 0.04	0.9 \pm 0.09
C 20:5 n-3	8.6 \pm 0.31	5.6 \pm 0.45	4.6 \pm 0.29	3.2 \pm 0.45	3.1 \pm 0.15	2.6 \pm 0.17
C 22:5 n-6	2.4 \pm 0.18	4.2 \pm 0.30	6.7 \pm 0.13	6.2 \pm 1.05	8.4 \pm 0.57	7.1 \pm 0.44
C 22:5 n-3	1.3 \pm 0.30	0.6 \pm 0.31	0.9 \pm 0.05	0.5 \pm 0.32	0.5 \pm 0.25	0.3 \pm 0.13
C 22:6 n-3	0.8 \pm 0.05	0.8 \pm 0.01	0.9 \pm 0.07	0.8 \pm 0.12	0.9 \pm 0.07	0.9 \pm 0.06
SUM PUFA⁵	5.9 \pm					

Table 3 Level of individual fatty acids in percentage of total fatty acids in the different dietary group ± standard error of mean (sem) in 400g PC group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PC400	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	12.3 ± 1.45	12.9 ± 2.42	9.0 ± 2.16	15.8 ± 1.39	13.2 ± 2.27	18.6 ± 3.10
C 12:0	0.6 ± 0.06	0.3 ± 0.04	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.17	0.3 ± 0.10
C 14:0	0.5 ± 0.03	0.5 ± 0.07	0.3 ± 0.26	0.5 ± 0.08	0.5 ± 0.02	1.4 ± 0.14
C 16:0	26.9 ± 0.15	26.6 ± 0.27	28.0 ± 0.69	27.0 ± 0.49	28.4 ± 0.66	23.8 ± 0.39
C 18:0	4.3 ± 0.11	4.2 ± 0.12	3.8 ± 0.15	3.9 ± 0.08	4.1 ± 0.06	3.1 ± 0.05
C 20:0	0.1 ± 0.01	0.5 ± 0.40	0.1 ± 0.01	0.3 ± 0.19	0.1 ± 0.02	0.2 ± 0.01
C 22:0	0.2 ± 0.05	0.1 ± 0.06	0.1 ± 0.02	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.02
C 24:0	0.1 ± 0.06	0.2 ± 0.02	0.1 ± 0.03	0.2 ± 0.01	0.2 ± 0.03	0.2 ± 0.03
SUM SFA³	33.0 ± 0.50	32.7 ± 1.02	33.1 ± 1.20	33.1 ± 1.14	34.3 ± 1.04	29.7 ± 0.77
C 16:1 n-9	1.5 ± 0.45	1.3 ± 0.08	1.2 ± 0.05	1.3 ± 0.03	1.3 ± 0.04	1.4 ± 0.08
C 16:1 n-7	0.8 ± 0.42	1.4 ± 0.17	0.6 ± 0.60	0.5 ± 0.54	0.6 ± 0.38	0.8 ± 0.54
C 18:1 n-9	22.6 ± 0.17	21.3 ± 0.27	21.5 ± 1.21	19.9 ± 1.48	20.4 ± 0.45	20.8 ± 0.26
C 20:1 n-9	0.4 ± 0.01	0.5 ± 0.01	0.4 ± 0.00	0.4 ± 0.01	0.4 ± 0.02	0.8 ± 0.03
C 22:1 n-7	0.3 ± 0.01	0.4 ± 0.07	0.3 ± 0.00	0.2 ± 0.05	0.2 ± 0.06	0.4 ± 0.03
C 24:1 n-9	0.6 ± 0.04	0.6 ± 0.23	0.4 ± 0.04	0.6 ± 0.02	0.5 ± 0.06	1.3 ± 0.18
SUM MUFA⁴	28.0 ± 1.29	27.2 ± 1.51	25.4 ± 2.81	24.0 ± 3.00	25.0 ± 1.19	28.2 ± 1.41
C 18:2 n-6	10.0 ± 0.26	8.3 ± 0.37	6.5 ± 0.11	5.6 ± 0.06	4.6 ± 0.09	4.6 ± 0.10
C 18:3 n-6	1.0 ± 0.07	0.5 ± 0.03	0.3 ± 0.00	0.2 ± 0.03	0.2 ± 0.01	0.1 ± 0.02
C 18:3 n-3	0.8 ± 0.09	0.7 ± 0.06	0.6 ± 0.03	0.3 ± 0.29	0.5 ± 0.01	1.0 ± 0.02
C 18:4 n-3	0.1 ± 0.02	0.1 ± 0.00	0.0 ± 0.04	0.0 ± 0.05	0.0 ± 0.03	0.0 ± 0.03
C 20:3 n-9	0.7 ± 0.02	0.9 ± 0.02	0.8 ± 0.04	0.8 ± 0.01	0.7 ± 0.03	0.8 ± 0.04
C 20:3 n-6	0.2 ± 0.02	0.0 ± 0.04	0.1 ± 0.04	0.0 ± 0.04	0.0 ± 0.00	0.0 ± 0.00
C 20:4 n-6	4.3 ± 0.09	3.3 ± 0.11	2.1 ± 0.07	1.4 ± 0.01	1.0 ± 0.04	0.4 ± 0.28
C 20:5 n-3	9.5 ± 0.17	6.4 ± 0.15	4.7 ± 0.00	3.7 ± 0.08	3.3 ± 0.04	2.6 ± 0.01
C 22:5 n-6	2.8 ± 0.12	6.9 ± 0.61	10.9 ± 1.02	12.2 ± 0.14	13.4 ± 0.57	9.0 ± 0.42
C 22:5 n-3	1.2 ± 0.04	0.6 ± 0.10	0.4 ± 0.00	0.4 ± 0.10	0.3 ± 0.04	0.4 ± 0.01
C 22:6 n-3	0.9 ± 0.02	1.3 ± 0.29	1.6 ± 0.09	1.7 ± 0.00	1.8 ± 0.05	1.5 ± 0.02
SUM PUFA⁵	5.2 ± 0.05	9.7 ± 0.56	11.1 ± 0.50	13.1 ± 0.36	12.7 ± 0.80	18.8 ± 0.25
SUM PUFA	36.6 ± 1.02	39.2 ± 2.47	39.5 ± 2.03	40.1 ± 1.34	38.8 ± 1.77	39.6 ± 1.29

PC400	NC ¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC ²
Fett % Folch	12.3 ± 1.45	10.9 ± 0.78	12.9 ± 2.26	16.3 ± 1.21	12.6 ± 2.36	18.6 ± 3.10
C 12:0	0.6 ± 0.06	0.7 ± 0.17	0.2 ± 0.07	0.3 ± 0.01	0.3 ± 0.07	0.3 ± 0.10
C 14:0	0.5 ± 0.03	0.5 ± 0.10	0.2 ± 0.16	0.5 ± 0.04	0.4 ± 0.03	1.4 ± 0.14
C 16:0	26.9 ± 0.15	26.1 ± 0.22	25.6 ± 0.42	26.2 ± 0.50	24.8 ± 1.36	23.8 ± 0.39
C 18:0	4.3 ± 0.11	3.9 ± 0.18	3.8 ± 0.12	3.7 ± 0.01	3.6 ± 0.17	3.1 ± 0.05
C 20:0	0.1 ± 0.01	0.4 ± 0.30	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.01
C 22:0	0.2 ± 0.05	0.1 ± 0.06	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.05	0.2 ± 0.02
C 24:0	0.1 ± 0.06	0.6 ± 0.51	0.2 ± 0.04	0.2 ± 0.02	0.2 ± 0.06	0.2 ± 0.03
SUM SFA³	33.0 ± 0.50	32.8 ± 1.63	30.5 ± 0.92	31.4 ± 0.65	29.9 ± 1.82	29.7 ± 0.77
C 16:1 n-9	1.5 ± 0.45	1.6 ± 0.21	1.3 ± 0.09	1.3 ± 0.07	1.0 ± 0.06	1.4 ± 0.08
C 16:1 n-7	0.8 ± 0.42	1.4 ± 0.10	0.6 ± 0.62	0.7 ± 0.67	0.0 ± 0.40	0.8 ± 0.54
C 18:1 n-9	22.6 ± 0.17	21.0 ± 0.27	20.4 ± 0.33	20.5 ± 0.28	18.4 ± 0.34	20.8 ± 0.26
C 20:1 n-9	0.4 ± 0.01	0.4 ± 0.03	0.5 ± 0.01	0.3 ± 0.21	0.5 ± 0.04	0.8 ± 0.03
C 22:1 n-7	0.3 ± 0.01	0.2 ± 0.05	0.2 ± 0.03	0.2 ± 0.00	0.2 ± 0.02	0.4 ± 0.03
C 24:1 n-9	0.6 ± 0.04	0.4 ± 0.06	0.5 ± 0.04	0.9 ± 0.04	1.0 ± 0.34	1.3 ± 0.18
SUM MUFA⁴	28.0 ± 1.29	26.8 ± 0.87	25.4 ± 1.23	25.5 ± 1.40	22.8 ± 1.29	28.2 ± 1.41
C 18:2 n-6	10.0 ± 0.26	8.4 ± 0.57	8.1 ± 0.45	6.8 ± 0.10	5.9 ± 0.18	4.6 ± 0.10
C 18:3 n-6	1.0 ± 0.07	0.4 ± 0.03	0.4 ± 0.07	0.2 ± 0.02	0.2 ± 0.01	0.1 ± 0.02
C 18:3 n-3	0.8 ± 0.09	0.6 ± 0.07	0.7 ± 0.06	0.6 ± 0.02	0.6 ± 0.02	1.0 ± 0.02
C 18:4 n-3	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.01	0.0 ± 0.03	0.0 ± 0.03
C 20:2 n-6	0.7 ± 0.02	0.8 ± 0.32	1.0 ± 0.05	1.1 ± 0.04	1.1 ± 0.03	0.8 ± 0.04
C 20:3 n-9	0.2 ± 0.02	0.1 ± 0.14	0.1 ± 0.02	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 20:3 n-6	4.3 ± 0.09	3.0 ± 0.14	2.5 ± 0.44	1.4 ± 0.03	1.0 ± 0.03	0.4 ± 0.28
C 20:4 n-6	9.5 ± 0.17	7.4 ± 0.45	5.7 ± 0.43	4.4 ± 0.30	4.2 ± 0.15	2.6 ± 0.01
C 20:5 n-3	2.8 ± 0.12	2.9 ± 0.17	4.3 ± 0.44	3.9 ± 0.01	4.5 ± 0.08	9.0 ± 0.42
C 22:5 n-6	1.2 ± 0.04	0.9 ± 0.53	1.3 ± 0.14	1.6 ± 0.06	1.9 ± 0.04	0.4 ± 0.01
C 22:5 n-3	0.9 ± 0.02	0.5 ± 0.00	0.7 ± 0.16	0.5 ± 0.00	0.6 ± 0.18	1.5 ± 0.02
C 22:6 n-3	5.2 ± 0.05	12.3 ± 0.03	16.7 ± 2.10	20.1 ± 0.42	24.7 ± 0.48	18.8 ± 0.25
SUM PUFA⁵	36.6 ± 1.02	37.8 ± 2.64	41.9 ± 4.42	40.9 ± 1.11	45.0 ± 1.31	39.6 ± 1.29

PC400	NC ¹	0.5%EPA&DH/1%EPA&DHA	1.5%EPA&DH/2%EPA&DHA	CC ²		
Fett % Folch	12.3 ± 1.45	9.3 ± 0.06	12.1 ± 1.50	18.3 ± 2.24	16.5 ± 3.14	18.6 ± 3.10
C 12:0	0.6 ± 0.06	0.4 ± 0.02	0.3 ± 0.07	0.4 ± 0.10	0.4 ± 0.04	0.3 ± 0.10
C 14:0	0.5 ± 0.03	0.5 ± 0.06	0.2 ± 0.21	0.5 ± 0.12	0.4 ± 0.14	1.4 ± 0.14
C 16:0	26.9 ± 0.15	26.0 ± 0.36	27.2 ± 0.00	26.4 ± 0.23	27.1 ± 0.22	23.8 ± 0.39
C 18:0	4.3 ± 0.11	4.3 ± 0.13	3.7 ± 0.02	3.8 ± 0.06	3.6 ± 0.05	3.1 ± 0.05
C 20:0	0.1 ± 0.01	0.3 ± 0.12	0.2 ± 0.14	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.01
C 22:0	0.2 ± 0.05	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.02
C 24:0	0.1 ± 0.06	0.2 ± 0.03	0.1 ± 0.02	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.03
SUM SFA³	33.0 ± 0.50	32.2 ± 0.87	32.2 ± 0.50	31.9 ± 0.59	32.4 ± 0.52	29.7 ± 0.77
C 16:1 n-9	1.5 ± 0.45	1.3 ± 0.02	1.2 ± 0.03	1.3 ± 0.08	1.3 ± 0.01	1.4 ± 0.08
C 16:1 n-7	0.8 ± 0.42	1.3 ± 0.09	0.6 ± 0.59	0.6 ± 0.62	1.2 ± 0.03	0.8 ± 0.54
C 18:1 n-9	22.6 ± 0.17	20.4 ± 0.08	21.0 ± 0.64	19.5 ± 0.02	19.5 ± 0.30	20.8 ± 0.26
C 20:1 n-9	0.4 ± 0.01	0.7 ± 0.27	0.4 ± 0.02	0.4 ± 0.00	0.4 ± 0.01	0.8 ± 0.03
C 22:1 n-7	0.3 ± 0.01	0.2 ± 0.00	0.2 ± 0.01	0.3 ± 0.01	0.2 ± 0.00	0.4 ± 0.03
C 24:1 n-9	0.6 ± 0.04	0.5 ± 0.09	0.5 ± 0.12	0.8 ± 0.09	0.8 ± 0.12	1.3 ± 0.18
SUM MUFA⁴	28.0 ± 1.29	26.7 ± 0.91	24.9 ± 2.33	24.6 ± 0.97	25.2 ± 0.57	28.2 ± 1.41
C 18:2 n-6	10.0 ± 0.26	8.3 ± 0.29	7.3 ± 0.10	5.9 ± 0.11	5.0 ± 0.12	4.6 ± 0.10
C 18:3 n-6	1.0 ± 0.07	0.5 ± 0.04	0.5 ± 0.21	0.2 ± 0.01	0.2 ± 0.02	0.1 ± 0.02
C 18:3 n-3	0.8 ± 0.09	0.7 ± 0.05	0.9 ± 0.20	0.7 ± 0.02	0.5 ± 0.03	1.0 ± 0.02
C 18:4 n-3	0.1 ± 0.02	0.0 ± 0.05	0.0 ± 0.04	0.1 ± 0.02	0.1 ± 0.01	0.0 ± 0.03
C 20:2 n-6	0.7 ± 0.02	1.0 ± 0.11	1.1 ± 0.03	0.9 ± 0.04	0.7 ± 0.09	0.8 ± 0.04
C 20:3 n-9	0.2 ± 0.02	0.1 ± 0.02	0.1 ± 0.08	0.0 ± 0.02	0.0 ± 0.00	0.0 ± 0.00
C 20:3 n-6	4.3 ± 0.09	2.8 ± 0.27	2.1 ± 0.00	1.4 ± 0.04	0.9 ± 0.02	0.4 ± 0.28
C 20:4 n-6	9.5 ± 0.17	6.2 ± 0.76	4.6 ± 0.03	4.1 ± 0.08	3.6 ± 0.11	2.6 ± 0.01
C 20:5 n-3	2.8 ± 0.12	4.6 ± 0.43	7.1 ± 0.06	8.5 ± 0.23	9.7 ± 0.23	9.0 ± 0.42
C 22:5 n-6	1.2 ± 0.04	1.2 ± 0.12	0.9 ± 0.03	1.0 ± 0.01	1.0 ± 0.02	0.4 ± 0.01
C 22:5 n-3	0.9 ± 0.02	0.8 ± 0.11	1.0 ± 0.01	1.1 ± 0.03	1.0 ± 0.03	1.5 ± 0.02
C 22:6 n-3	5.2 ± 0.05	13.0 ± 1.81	15.0 ± 0.49	17.5 ± 0.34	18.4 ± 0.53	18.8 ± 0.25
SUM PUFA⁵	36.6 ± 1.02	39.5 ± 4.25	40.8 ± 1.33	41.5 ± 1.09	41.2 ± 1.30	39.6 ± 1.29

NC¹ means negative control, CC² means commercial control. Sum SFA³ also contains 15:0 and 17:0. Sum MUFA⁴ also contains 14:1n-5, 15:1, 16:1n-5, 17:1n-7, 18:1n-1, 18:1n-7, 20:1n-1, 20:1n-7, 22:1n-1 and 22:1n-9. Sum PUFA⁵ also contains 16:2n-6, 16:2n-3, 20:4n-3, 20:3n-3, 22:2n-6 and 22:4n-6.

Table 4 Level of individual fatty acids in percentage of total fatty acids in the different dietary group ± standard error of mean (sem) in 200g PE group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PE200	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	7.6 ± 0.59	9.3 ± 0.16	9.6 ± 1.74	9.9 ± 2.01	11.8 ± 1.78	12.2 ± 0.84
C 12:0	0.6 ± 0.03	0.8 ± 0.13	0.6 ± 0.18	0.9 ± 0.01	0.5 ± 0.08	0.9 ± 0.19
C 14:0	0.2 ± 0.03	0.1 ± 0.01	0.1 ± 0.11	0.2 ± 0.01	0.3 ± 0.18	0.3 ± 0.13
C 16:0	5.7 ± 0.54	6.3 ± 0.14	6.1 ± 0.24	8.2 ± 0.01	7.1 ± 0.79	7.1 ± 0.71
C 18:0	5.2 ± 0.31	5.7 ± 0.21	5.3 ± 0.03	6.6 ± 0.24	6.0 ± 0.32	5.7 ± 0.54
C 20:0	0.1 ± 0.00	0.1 ± 0.04	0.2 ± 0.05	0.2 ± 0.00	0.2 ± 0.05	0.2 ± 0.00
C 22:0	0.0 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.25	0.0 ± 0.08	0.0 ± 0.12
C 24:0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM SFA³	12.3 ± 1.01	13.5 ± 0.60	12.6 ± 0.67	17.0 ± 0.52	14.6 ± 1.57	14.6 ± 1.80
C 16:1 n-9	0.0 ± 0.21	0.3 ± 0.28	0.0 ± 0.00	0.6 ± 0.05	0.2 ± 0.66	0.3 ± 0.50
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.30	0.0 ± 0.24	0.0 ± 0.20
C 18:1 n-9	16.1 ± 1.03	16.1 ± 0.60	15.2 ± 0.24	16.6 ± 0.66	14.6 ± 1.41	16.7 ± 0.87
C 20:1 n-9	0.8 ± 0.03	1.0 ± 0.01	1.0 ± 0.01	1.1 ± 0.01	0.9 ± 0.10	1.8 ± 0.07
C 22:1 n-7	0.1 ± 0.19	0.2 ± 0.04	0.3 ± 0.03	0.2 ± 0.22	0.4 ± 0.13	0.3 ± 0.10
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	33.0 ± 6.08	26.1 ± 6.78	30.6 ± 1.27	23.1 ± 2.64	24.9 ± 6.69	29.3 ± 6.01
C 18:2 n-6	7.2 ± 0.42	7.1 ± 0.07	7.4 ± 0.07	6.8 ± 0.29	6.6 ± 0.43	6.0 ± 0.27
C 18:3 n-6	0.6 ± 0.02	0.4 ± 0.02	0.3 ± 0.03	0.2 ± 0.00	0.2 ± 0.02	0.3 ± 0.15
C 18:3 n-3	0.3 ± 0.03	0.4 ± 0.02	0.5 ± 0.08	0.4 ± 0.03	0.5 ± 0.19	0.7 ± 0.12
C 18:4 n-3	0.2 ± 0.14	0.2 ± 0.01	0.2 ± 0.00	0.2 ± 0.01	0.1 ± 0.08	0.1 ± 0.05
C 20:2 n-6	0.7 ± 0.17	1.0 ± 0.09	0.9 ± 0.27	1.1 ± 0.01	1.0 ± 0.09	1.3 ± 0.01
C 20:3 n-9	0.2 ± 0.03	0.1 ± 0.02	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.07	0.0 ± 0.06
C 20:3 n-6	2.2 ± 0.13	2.0 ± 0.04	1.4 ± 0.03	1.0 ± 0.00	0.8 ± 0.04	0.7 ± 0.03
C 20:4 n-6	17.3 ± 0.79	11.2 ± 0.64	7.8 ± 0.27	5.9 ± 0.24	5.7 ± 0.20	4.9 ± 0.23
C 20:5 n-3	2.8 ± 0.25	5.5 ± 0.10	7.8 ± 0.80	8.1 ± 0.09	9.4 ± 0.47	6.3 ± 0.34
C 22:5 n-6	2.3 ± 0.59	0.8 ± 0.31	0.6 ± 0.01	0.3 ± 0.02	0.4 ± 0.06	0.4 ± 0.18
C 22:5 n-3	1.4 ± 0.14	1.7 ± 0.00	2.4 ± 0.02	2.1 ± 0.08	2.3 ± 0.11	1.1 ± 0.06
C 22:6 n-3	12.6 ± 1.82	17.1 ± 1.39	20.7 ± 0.73	17.8 ± 0.58	21.8 ± 1.78	26.5 ± 1.64
SUM PUFA⁵	49.0 ± 8.18	53.5 ± 7.58	51.3 ± 2.48	53.7 ± 1.63	54.6 ± 6.25	50.6 ± 4.63
PE200	NC ¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC ²
Fett % Folch	7.6 ± 0.59	7.7 ± 0.59	8.9 ± 2.15	11.0 ± 1.29	11.2 ± 1.42	12.2 ± 0.84
C 12:0	0.6 ± 0.03	0.7 ± 0.04	0.5 ± 0.14	0.8 ± 0.11	0.8 ± 0.08	0.9 ± 0.19
C 14:0	0.2 ± 0.03	0.1 ± 0.13	0.2 ± 0.01	0.1 ± 0.06	0.3 ± 0.07	0.3 ± 0.13
C 16:0	5.7 ± 0.54	6.7 ± 0.89	6.9 ± 0.29	7.6 ± 1.09	7.6 ± 0.42	7.1 ± 0.71
C 18:0	5.2 ± 0.31	6.0 ± 0.55	5.8 ± 0.58	5.9 ± 0.74	6.2 ± 0.50	5.7 ± 0.54
C 20:0	0.1 ± 0.00	0.2 ± 0.03	0.1 ± 0.07	0.2 ± 0.04	0.2 ± 0.00	0.2 ± 0.00
C 22:0	0.0 ± 0.06	0.2 ± 0.22	0.2 ± 0.19	0.2 ± 0.24	0.0 ± 0.11	0.0 ± 0.12
C 24:0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM SFA³	12.3 ± 1.01	14.2 ± 2.00	14.2 ± 1.34	15.3 ± 2.34	15.8 ± 1.44	14.6 ± 1.80
C 16:1 n-9	0.0 ± 0.21	0.3 ± 0.34	0.4 ± 0.44	0.3 ± 0.32	0.2 ± 0.18	0.3 ± 0.50
C 16:1 n-7	0.0 ± 0.00	0.4 ± 0.37	0.2 ± 0.25	0.0 ± 0.00	0.3 ± 0.19	0.0 ± 0.20
C 18:1 n-9	16.1 ± 1.03	17.6 ± 1.09	15.8 ± 1.36	17.2 ± 1.76	17.0 ± 1.07	16.7 ± 0.87
C 20:1 n-9	0.8 ± 0.03	1.1 ± 0.08	1.1 ± 0.07	1.2 ± 0.06	1.3 ± 0.08	1.8 ± 0.07
C 22:1 n-7	0.1 ± 0.19	0.0 ± 0.00	0.1 ± 0.14	0.0 ± 0.00	0.2 ± 0.17	0.3 ± 0.10
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	33.0 ± 6.08	28.0 ± 7.92	27.8 ± 7.00	27.4 ± 7.99	27.4 ± 5.48	29.3 ± 6.01
C 18:2 n-6	7.2 ± 0.42	7.8 ± 0.10	7.4 ± 0.02	7.2 ± 0.05	7.3 ± 0.16	6.0 ± 0.27
C 18:3 n-6	0.6 ± 0.02	0.4 ± 0.01	0.3 ± 0.02	0.3 ± 0.02	0.2 ± 0.01	0.3 ± 0.15
C 18:3 n-3	0.3 ± 0.03	0.4 ± 0.01	0.5 ± 0.11	0.5 ± 0.10	0.5 ± 0.07	0.7 ± 0.12
C 18:4 n-3	0.2 ± 0.14	0.2 ± 0.04	0.0 ± 0.00	0.2 ± 0.01	0.0 ± 0.05	0.1 ± 0.05
C 20:2 n-6	0.7 ± 0.17	1.0 ± 0.09	1.2 ± 0.02	1.2 ± 0.06	1.3 ± 0.04	1.3 ± 0.01
C 20:3 n-9	0.2 ± 0.03	0.2 ± 0.01	0.1 ± 0.03	0.2 ± 0.01	0.2 ± 0.06	0.0 ± 0.06
C 20:3 n-6	2.2 ± 0.13	1.8 ± 0.09	1.3 ± 0.03	0.9 ± 0.07	0.7 ± 0.04	0.7 ± 0.03
C 20:4 n-6	17.3 ± 0.79	11.1 ± 1.20	9.2 ± 0.26	6.9 ± 0.43	6.4 ± 0.20	4.9 ± 0.23
C 20:5 n-3	2.8 ± 0.25	2.9 ± 0.18	3.1 ± 0.38	3.0 ± 0.09	3.2 ± 0.21	6.3 ± 0.34
C 22:5 n-6	2.3 ± 0.59	1.1 ± 0.74	1.2 ± 0.85	1.1 ± 0.83	0.1 ± 0.09	0.4 ± 0.18
C 22:5 n-3	1.4 ± 0.14	0.7 ± 0.03	0.5 ± 0.11	0.5 ± 0.04	0.5 ± 0.04	1.1 ± 0.06
C 22:6 n-3	12.6 ± 1.82	18.3 ± 1.83	22.7 ± 1.99	23.8 ± 3.79	24.6 ± 2.04	26.5 ± 1.64
SUM PUFA⁵	49.0 ± 8.18	50.6 ± 9.08	53.0 ± 7.91	50.6 ± 9.53	50.3 ± 5.65	50.6 ± 4.63
PE200	NC ¹	0.5%EPA&DH/	1%EPA&DHA	1.5%EPA&DHA	2%EPA&DHA	CC ²
Fett % Folch	7.6 ± 0.59	14.8 ± 5.68	11.9 ± 2.20	10.2 ± 0.31	12.2 ± 0.24	12.2 ± 0.84
C 12:0	0.6 ± 0.03	0.5 ± 0.14	0.8 ± 0.44	0.6 ± 0.10	0.5 ± 0.50	0.9 ± 0.19
C 14:0	0.2 ± 0.03	0.2 ± 0.07	0.2 ± 0.04	0.2 ± 0.01	0.2 ± 0.12	0.3 ± 0.13
C 16:0	5.7 ± 0.54	6.8 ± 1.06	6.4 ± 0.13	7.3 ± 0.80	7.2 ± 0.84	7.1 ± 0.71
C 18:0	5.2 ± 0.31	6.3 ± 1.24	5.5 ± 0.28	6.1 ± 0.86	6.0 ± 0.32	5.7 ± 0.54
C 20:0	0.1 ± 0.00	0.2 ± 0.01	0.1 ± 0.07	0.1 ± 0.03	0.2 ± 0.02	0.2 ± 0.00
C 22:0	0.0 ± 0.06	0.1 ± 0.09	0.0 ± 0.00	0.3 ± 0.26	0.0 ± 0.05	0.0 ± 0.12
C 24:0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM SFA³	12.3 ± 1.01	14.5 ± 2.68	13.2 ± 1.13	15.0 ± 2.12	14.3 ± 1.94	14.6 ± 1.80
C 16:1 n-9	0.0 ± 0.21	0.2 ± 0.21	0.2 ± 0.22	0.3 ± 0.26	0.5 ± 0.30	0.3 ± 0.50
C 16:1 n-7	0.0 ± 0.00	0.3 ± 0.31	0.0 ± 0.00	0.3 ± 0.32	0.0 ± 0.30	0.0 ± 0.20
C 18:1 n-9	16.1 ± 1.03	18.0 ± 0.38	16.7 ± 0.62	16.5 ± 1.78	15.6 ± 1.09	16.7 ± 0.87
C 20:1 n-9	0.8 ± 0.03	1.1 ± 0.07	1.1 ± 0.02	1.2 ± 0.10	1.1 ± 0.04	1.8 ± 0.07
C 22:1 n-7	0.1 ± 0.19	0.5 ± 0.16	0.2 ± 0.18	0.1 ± 0.13	0.4 ± 0.05	0.3 ± 0.10
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	33.0 ± 6.08	27.9 ± 8.33	26.0 ± 6.16	27.2 ± 8.51	26.0 ± 5.69	29.3 ± 6.01
C 18:2 n-6	7.2 ± 0.42	7.5 ± 0.20	7.7 ± 0.23	6.5 ± 0.00	6.7 ± 0.12	6.0 ± 0.27
C 18:3 n-6	0.6 ± 0.02	0.4 ± 0.02	0.3 ± 0.02	0.3 ± 0.01	0.2 ± 0.01	0.3 ± 0.15
C 18:3 n-3	0.3 ± 0.03	0.4 ± 0.07	0.4 ± 0.06	0.5 ± 0.12	0.4 ± 0.03	0.7 ± 0.12
C 18:4 n-3	0.2 ± 0.14	0.2 ± 0.04	0.1 ± 0.07	0.2 ± 0.04	0.1 ± 0.06	0.1 ± 0.05
C 20:2 n-6	0.7 ± 0.17	1.1 ± 0.02	1.2 ± 0.01	1.1 ± 0.03	1.2 ± 0.09	1.3 ± 0.01
C 20:3 n-9	0.2 ± 0.03	0.2 ± 0.00	0.1 ± 0.07	0.1 ± 0.02	0.1 ± 0.06	0.0 ± 0.06
C 20:3 n-6	2.2 ± 0.13	1.9 ± 0.09	1.5 ± 0.12	0.9 ± 0.11	0.7 ± 0.03	0.7 ± 0.03
C 20:4 n-6	17.3 ± 0.79	10.8 ± 0.63	8.5 ± 0.34	6.3 ± 0.37	5.7 ± 0.26	4.9 ± 0.23
C 20:5 n-3	2.8 ± 0.25	4.1 ± 0.30	5.6 ± 0.12	5.5 ± 0.54	6.6 ± 0.37	6.3 ± 0.34
C 22:5 n-6	2.3 ± 0.59	0.9 ± 0.41	0.8 ± 0.50	0.7 ± 0.46	0.8 ± 0.36	0.4 ± 0.18
C 22:5 n-3	1.4 ± 0.14	1.2 ± 0.02	1.1 ± 0.10	1.1 ± 0.13	1.2 ± 0.14	1.1 ± 0.06
C 22:6 n-3	12.6 ± 1.82	17.2 ± 2.84	21.6 ± 0.89	22.2 ± 3.65	25.1 ± 2.98	26.5 ± 1.64
SUM PUFA⁵	49.0 ± 8.18	51.4 ± 8.88	53.8 ± 7.22	51.3 ± 10.05	54.4 ± 7.43	50.6 ± 4.63

NC¹ means negative control, CC² means commercial control. Sum SFA³ also contains 15:0 and 17:0. Sum MUFA⁴ also contains 14:1n-5, 15:1, 16:1n-5, 17:1n-7, 18:1n-1, 18:1n-7, 20:1n-1, 20:1n-7, 22:1n-1 and 22:1n-9. Sum PUFA⁵ also contains 16:2n-6, 16:2n-3, 20:4n-3, 20:3n-3, 22:2n-6 and 22:4n-6.

Table 5 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 400g PE group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PE400	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	12.3 ± 1.45	12.9 ± 2.42	9.0 ± 2.16	15.8 ± 1.39	13.2 ± 2.27	18.6 ± 3.10
C 12:0	1.2 ± 0.26	1.7 ± 0.02	1.1 ± 0.05	1.1 ± 0.22	1.4 ± 0.04	1.7 ± 0.23
C 14:0	0.1 ± 0.12	0.3 ± 0.09	0.3 ± 0.09	0.3 ± 0.01	0.2 ± 0.07	0.3 ± 0.02
C 16:0	5.6 ± 0.21	5.6 ± 0.22	5.7 ± 0.26	6.8 ± 0.13	6.2 ± 0.19	6.4 ± 0.17
C 18:0	4.7 ± 0.13	5.6 ± 0.16	5.4 ± 0.36	5.4 ± 0.56	10.1 ± 2.64	4.4 ± 1.06
C 20:0	1.1 ± 0.50	0.1 ± 0.07	0.1 ± 0.01	1.1 ± 0.99	0.2 ± 0.01	0.3 ± 0.06
C 22:0	0.9 ± 0.37	0.2 ± 0.00	0.2 ± 0.07	0.1 ± 0.08	0.1 ± 0.04	0.0 ± 0.00
C 24:0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM SFA³	15.2 ± 2.13	13.9 ± 0.72	13.4 ± 0.95	16.3 ± 2.94	18.9 ± 3.30	14.3 ± 1.86
C 16:1 n-9	0.8 ± 0.07	0.7 ± 0.02	0.2 ± 0.18	0.2 ± 0.22	0.5 ± 0.06	0.5 ± 0.05
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.12	0.0 ± 0.00
C 18:1 n-9	15.2 ± 0.43	14.6 ± 0.09	14.8 ± 0.03	13.6 ± 1.03	12.3 ± 1.14	15.7 ± 0.46
C 20:1 n-9	0.8 ± 0.03	1.0 ± 0.03	0.8 ± 0.01	0.9 ± 0.04	0.9 ± 0.01	1.8 ± 0.04
C 22:1 n-7	0.0 ± 0.00	0.1 ± 0.13	0.1 ± 0.15	0.3 ± 0.06	0.3 ± 0.03	0.5 ± 0.02
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	18.9 ± 0.81	19.1 ± 0.51	25.1 ± 7.02	23.1 ± 7.33	16.1 ± 2.21	22.3 ± 0.74
C 18:2 n-6	7.0 ± 1.00	7.5 ± 0.12	7.2 ± 0.13	7.6 ± 0.68	6.4 ± 0.06	5.2 ± 0.05
C 18:3 n-6	0.0 ± 0.21	0.0 ± 0.00	0.1 ± 0.15	0.1 ± 0.10	0.3 ± 0.05	0.0 ± 0.00
C 18:3 n-3	0.3 ± 0.02	0.4 ± 0.02	0.4 ± 0.02	0.3 ± 0.04	0.4 ± 0.01	0.6 ± 0.03
C 18:4 n-3	0.0 ± 0.06	0.0 ± 0.00	0.1 ± 0.06	0.0 ± 0.00	0.1 ± 0.06	0.0 ± 0.00
C 20:2 n-6	0.9 ± 0.26	1.0 ± 0.06	0.9 ± 0.04	0.9 ± 0.17	1.0 ± 0.05	1.0 ± 0.03
C 20:3n-9	0.2 ± 0.13	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.09	0.1 ± 0.05	0.3 ± 0.10
C 20:3 n-6	3.0 ± 0.15	2.6 ± 0.04	1.7 ± 0.01	1.2 ± 0.05	0.9 ± 0.06	0.7 ± 0.24
C 20:4 n-6	17.6 ± 0.29	12.4 ± 0.40	9.2 ± 0.17	6.8 ± 0.08	6.4 ± 0.19	5.9 ± 0.19
C 20:5 n-3	2.6 ± 0.14	5.4 ± 0.47	7.8 ± 0.47	8.1 ± 0.27	9.4 ± 0.08	6.7 ± 0.36
C 22:5 n-6	2.9 ± 0.30	1.1 ± 0.16	0.6 ± 0.02	0.9 ± 0.44	0.5 ± 0.02	0.5 ± 0.03
C 22:5 n-3	1.6 ± 0.09	2.0 ± 0.26	2.2 ± 0.18	2.2 ± 0.07	2.6 ± 0.05	1.5 ± 0.00
C 22:6 n-3	11.3 ± 0.17	18.4 ± 0.40	20.6 ± 0.87	22.1 ± 0.24	23.3 ± 0.41	24.4 ± 0.71
SUM PUFA⁵	55.5 ± 3.54	57.6 ± 2.31	54.6 ± 5.15	54.2 ± 5.33	58.2 ± 2.11	53.7 ± 2.55
PE400	NC¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC²
Fett % Folch	12.3 ± 1.45	10.9 ± 0.77	12.9 ± 2.26	16.3 ± 1.21	12.6 ± 2.36	18.6 ± 3.10
C 12:0	1.2 ± 0.26	1.3 ± 0.15	0.8 ± 0.13	2.1 ± 1.61	0.7 ± 0.20	1.7 ± 0.23
C 14:0	0.1 ± 0.12	0.3 ± 0.03	0.3 ± 0.13	0.2 ± 0.07	0.3 ± 0.02	0.3 ± 0.02
C 16:0	5.6 ± 0.21	5.8 ± 0.06	5.6 ± 0.26	6.0 ± 0.12	6.4 ± 0.20	6.4 ± 0.17
C 18:0	4.7 ± 0.13	5.3 ± 0.04	5.2 ± 0.02	5.8 ± 0.59	6.0 ± 0.26	4.4 ± 1.06
C 20:0	1.1 ± 0.50	0.1 ± 0.01	0.1 ± 0.09	0.2 ± 0.02	0.1 ± 0.08	0.3 ± 0.06
C 22:0	0.9 ± 0.37	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.07	0.0 ± 0.00
C 24:0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM SFA³	15.2 ± 2.13	13.5 ± 0.36	12.6 ± 0.83	15.0 ± 2.53	14.3 ± 0.93	14.3 ± 1.86
C 16:1 n-9	0.8 ± 0.07	0.9 ± 0.12	0.6 ± 0.14	0.6 ± 0.02	0.5 ± 0.04	0.5 ± 0.05
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.18	0.2 ± 0.13	0.0 ± 0.00
C 18:1 n-9	15.2 ± 0.43	14.8 ± 0.16	14.4 ± 0.07	14.5 ± 0.48	14.0 ± 0.40	15.7 ± 0.46
C 20:1 n-9	0.8 ± 0.03	0.9 ± 0.01	1.0 ± 0.01	0.8 ± 0.25	1.1 ± 0.05	1.8 ± 0.04
C 22:1 n-7	0.0 ± 0.00	0.1 ± 0.09	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.07	0.5 ± 0.02
C 24:1 n-9	0.0 ± 0.00	1.0 ± 0.99	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	18.9 ± 0.81	20.1 ± 1.71	19.0 ± 0.44	20.3 ± 2.29	18.9 ± 0.86	22.3 ± 0.74
C 18:2 n-6	7.0 ± 1.00	7.5 ± 0.33	7.8 ± 0.14	7.4 ± 0.06	7.3 ± 0.15	5.2 ± 0.05
C 18:3 n-6	0.0 ± 0.21	0.4 ± 0.01	0.0 ± 0.00	0.1 ± 0.12	0.2 ± 0.01	0.0 ± 0.00
C 18:3 n-3	0.3 ± 0.02	0.4 ± 0.08	0.5 ± 0.01	0.5 ± 0.02	0.5 ± 0.03	0.6 ± 0.03
C 18:4 n-3	0.0 ± 0.06	0.1 ± 0.01	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 20:2 n-6	0.9 ± 0.26	0.4 ± 0.44	1.1 ± 0.03	1.2 ± 0.10	1.2 ± 0.01	1.0 ± 0.03
C 20:3n-9	0.2 ± 0.13	0.5 ± 0.38	0.1 ± 0.06	0.1 ± 0.02	0.2 ± 0.05	0.3 ± 0.10
C 20:3 n-6	3.0 ± 0.15	2.2 ± 0.03	1.9 ± 0.40	1.1 ± 0.05	0.8 ± 0.03	0.7 ± 0.24
C 20:4 n-6	17.6 ± 0.29	13.8 ± 0.21	10.3 ± 0.93	8.4 ± 0.76	7.3 ± 0.31	5.9 ± 0.19
C 20:5 n-3	2.6 ± 0.14	2.5 ± 0.15	3.3 ± 0.43	3.0 ± 0.10	3.1 ± 0.03	6.7 ± 0.36
C 22:5 n-6	2.9 ± 0.30	1.1 ± 1.05	2.0 ± 0.03	2.1 ± 0.08	2.3 ± 0.06	0.5 ± 0.03
C 22:5 n-3	1.6 ± 0.09	0.7 ± 0.04	1.0 ± 0.32	0.6 ± 0.03	0.6 ± 0.04	1.5 ± 0.00
C 22:6 n-3	11.3 ± 0.17	20.4 ± 0.49	25.0 ± 1.90	27.0 ± 0.46	31.1 ± 1.31	24.4 ± 0.71
SUM PUFA⁵	55.5 ± 3.54	57.2 ± 3.90	59.1 ± 4.68	56.8 ± 1.96	59.9 ± 2.35	53.7 ± 2.55
PE400	NC¹	0.5%EPA&DH/1%EPA&DHA	1.5%EPA&DH/2%EPA&DHA	CC²		
Fett % Folch	12.3 ± 1.45	9.3 ± 0.06	12.1 ± 1.50	18.3 ± 2.24	16.5 ± 3.14	18.6 ± 3.10
C 12:0	1.2 ± 0.26	1.6 ± 0.29	0.9 ± 0.51	1.1 ± 0.47	0.9 ± 0.25	1.7 ± 0.23
C 14:0	0.1 ± 0.12	0.3 ± 0.07	0.2 ± 0.01	0.4 ± 0.01	0.2 ± 0.05	0.3 ± 0.02
C 16:0	5.6 ± 0.21	5.6 ± 0.12	6.0 ± 0.05	6.3 ± 0.05	6.7 ± 0.28	6.4 ± 0.17
C 18:0	4.7 ± 0.13	5.2 ± 0.05	5.4 ± 0.05	5.8 ± 0.04	5.8 ± 0.07	4.4 ± 1.06
C 20:0	1.1 ± 0.50	0.1 ± 0.08	0.2 ± 0.00	0.1 ± 0.00	0.2 ± 0.02	0.3 ± 0.06
C 22:0	0.9 ± 0.37	0.1 ± 0.12	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 24:0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM SFA³	15.2 ± 2.13	13.5 ± 0.88	13.2 ± 0.69	14.2 ± 0.61	14.3 ± 0.73	14.3 ± 1.86
C 16:1 n-9	0.8 ± 0.07	0.7 ± 0.06	0.3 ± 0.31	0.5 ± 0.15	0.6 ± 0.12	0.5 ± 0.05
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.20	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	15.2 ± 0.43	14.6 ± 0.24	14.5 ± 0.19	14.4 ± 0.51	13.9 ± 0.12	15.7 ± 0.46
C 20:1 n-9	0.8 ± 0.03	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.02	1.0 ± 0.03	1.8 ± 0.04
C 22:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.03	0.5 ± 0.02
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.7 ± 0.71	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	18.9 ± 0.81	19.3 ± 0.58	25.0 ± 7.27	19.4 ± 1.24	18.5 ± 0.45	22.3 ± 0.74
C 18:2 n-6	7.0 ± 1.00	7.8 ± 0.26	7.5 ± 0.13	6.8 ± 0.05	6.4 ± 0.05	5.2 ± 0.05
C 18:3 n-6	0.0 ± 0.21	0.2 ± 0.17	0.3 ± 0.02	0.2 ± 0.02	0.0 ± 0.05	0.0 ± 0.00
C 18:3 n-3	0.3 ± 0.02	0.6 ± 0.09	0.5 ± 0.05	0.4 ± 0.01	0.5 ± 0.04	0.6 ± 0.03
C 18:4 n-3	0.0 ± 0.06	0.0 ± 0.00	0.1 ± 0.06	0.1 ± 0.06	0.0 ± 0.03	0.0 ± 0.00
C 20:2 n-6	0.9 ± 0.26	1.0 ± 0.00	1.2 ± 0.04	1.1 ± 0.06	1.1 ± 0.05	1.0 ± 0.03
C 20:3n-9	0.2 ± 0.13	1.1 ± 0.95	0.1 ± 0.06	0.2 ± 0.02	0.0 ± 0.00	0.3 ± 0.10
C 20:3 n-6	3.0 ± 0.15	1.0 ± 1.01	1.6 ± 0.01	1.2 ± 0.01	0.8 ± 0.01	0.7 ± 0.24
C 20:4 n-6	17.6 ± 0.29	11.4 ± 0.67	8.8 ± 0.06	7.6 ± 0.06	6.4 ± 0.28	5.9 ± 0.19
C 20:5 n-3	2.6 ± 0.14	3.5 ± 0.10	5.2 ± 0.12	5.8 ± 0.30	6.5 ± 0.09	6.7 ± 0.36
C 22:5 n-6	2.9 ± 0.30	1.6 ± 0.21	0.6 ± 0.65	1.4 ± 0.02	1.4 ± 0.03	0.5 ± 0.03
C 22:5 n-3	1.6 ± 0.09	1.0 ± 0.18	1.2 ± 0.03	1.2 ± 0.04	1.4 ± 0.07	1.5 ± 0.00
C 22:6 n-3	11.3 ± 0.17	21.1 ± 2.46	24.3 ± 0.39	25.9 ± 0.31	27.4 ± 0.30	24.4 ± 0.71
SUM PUFA⁵	55.5 ± 3.54	57.1 ± 6.62	55.4 ± 4.55	58.2 ± 1.40	59.3 ± 1.36	53.7 ± 2.55

NC¹ means negative control, CC² means commercial control. Sum SFA³ also contains 15:0 and 17:0. Sum MUFA⁴ also contains 14:1n-5, 15:1, 16:1n-5, 17:1n-7, 18:1n-1, 18:1n-7, 20:1n-1, 20:1n-7, 22:1n-1 and 22:1n-9. Sum PUFA⁵ also contains 16:2n-6, 16:2n-3, 20:4n-3, 20:3n-3, 22:2n-6 and 22:4n-6.

Table 6 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 200g PS group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PS200	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	7.6 ± 0.59	9.3 ± 0.16	9.6 ± 1.74	9.9 ± 2.01	11.8 ± 1.78	12.2 ± 0.84
C 12:0	1.2 ± 1.47	0.5 ± 0.06	1.5 ± 0.77	2.4 ± 1.21	1.8 ± 0.53	0.7 ± 0.02
C 14:0	0.1 ± 0.08	0.3 ± 0.17	0.4 ± 0.23	0.6 ± 0.16	0.5 ± 0.12	0.5 ± 0.22
C 16:0	11.5 ± 2.68	15.2 ± 4.92	12.6 ± 0.84	19.4 ± 10.11	12.5 ± 3.94	12.5 ± 4.88
C 18:0	24.4 ± 2.81	17.6 ± 7.75	22.5 ± 1.17	16.5 ± 11.04	24.4 ± 4.35	20.8 ± 3.29
C 20:0	0.6 ± 0.01	0.4 ± 0.15	0.6 ± 0.06	0.3 ± 0.24	0.7 ± 0.15	0.8 ± 0.14
C 22:0	0.3 ± 0.70	0.3 ± 0.06	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.02	0.2 ± 0.07
C 24:0	0.1 ± 0.32	0.1 ± 0.08	0.1 ± 0.07	0.3 ± 0.34	0.1 ± 0.07	0.1 ± 0.05
SUM SFA³	39.1 ± 8.32	35.1 ± 13.59	38.8 ± 3.29	42.5 ± 25.40	41.1 ± 9.52	37.3 ± 8.96
C 16:1 n-9	0.4 ± 0.23	0.5 ± 0.54	0.5 ± 0.48	0.8 ± 0.82	0.1 ± 0.79	0.6 ± 0.19
C 16:1 n-7	0.0 ± 0.00	0.6 ± 0.55	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	10.3 ± 1.50	12.5 ± 3.75	9.5 ± 0.22	13.4 ± 4.01	8.2 ± 2.90	11.0 ± 2.07
C 20:1 n-9	1.2 ± 0.15	1.1 ± 0.21	1.1 ± 0.04	1.2 ± 0.80	1.2 ± 0.16	2.6 ± 0.72
C 22:1 n-7	0.0 ± 0.03	0.1 ± 0.08	0.1 ± 0.04	1.0 ± 1.02	0.1 ± 0.08	0.2 ± 0.11
C 24:1 n-9	0.0 ± 0.02	0.1 ± 0.05	0.1 ± 0.06	0.1 ± 0.11	0.1 ± 0.11	0.1 ± 0.08
SUM MUFA⁴	14.4 ± 2.41	17.0 ± 5.37	13.5 ± 1.07	18.7 ± 7.44	12.0 ± 4.36	19.2 ± 4.58
C 18:2 n-6	3.5 ± 0.22	4.2 ± 1.30	3.1 ± 0.09	3.2 ± 1.30	2.4 ± 0.44	2.4 ± 0.29
C 18:3 n-6	0.5 ± 0.06	0.4 ± 0.10	0.2 ± 0.02	1.1 ± 0.84	0.2 ± 0.08	0.3 ± 0.10
C 18:3 n-3	0.2 ± 0.09	0.4 ± 0.21	0.2 ± 0.01	0.6 ± 0.07	0.2 ± 0.04	0.3 ± 0.02
C 18:4 n-3	0.2 ± 0.06	0.2 ± 0.02	0.2 ± 0.01	0.0 ± 0.00	0.1 ± 0.04	0.1 ± 0.04
C 20:2 n-6	1.1 ± 0.22	0.9 ± 0.28	1.2 ± 0.02	0.7 ± 0.01	1.1 ± 0.12	0.9 ± 0.16
C 20:3 n-9	0.3 ± 0.34	0.5 ± 0.10	0.3 ± 0.01	0.2 ± 0.03	0.3 ± 0.11	0.4 ± 0.07
C 20:3 n-6	2.6 ± 0.13	2.5 ± 0.11	1.5 ± 0.06	1.0 ± 0.02	0.8 ± 0.06	0.8 ± 0.10
C 20:4 n-6	6.5 ± 0.19	5.5 ± 1.10	3.1 ± 0.09	2.8 ± 0.70	2.2 ± 0.56	2.0 ± 0.21
C 20:5 n-3	1.4 ± 0.15	4.3 ± 2.22	3.4 ± 0.20	7.3 ± 4.02	3.2 ± 1.77	2.8 ± 0.88
C 22:5 n-6	4.5 ± 1.23	1.6 ± 0.49	1.0 ± 0.04	0.3 ± 0.05	0.7 ± 0.17	0.7 ± 0.29
C 22:5 n-3	3.2 ± 0.65	3.2 ± 0.78	4.9 ± 0.39	2.5 ± 0.77	4.9 ± 0.93	2.0 ± 0.32
C 22:6 n-3	18.7 ± 2.61	21.0 ± 4.80	26.3 ± 0.92	15.1 ± 2.20	27.6 ± 4.86	27.6 ± 3.64
SUM PUFA⁵	42.9 ± 6.13	45.1 ± 11.76	45.8 ± 1.90	36.1 ± 11.21	44.4 ± 9.45	40.6 ± 6.23

PS200	NC ¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC ²
Fett % Folch	7.6 ± 0.59	7.7 ± 0.59	8.9 ± 2.15	11.0 ± 1.29	11.2 ± 1.42	12.2 ± 0.84
C 12:0	1.2 ± 1.47	1.6 ± 0.66	1.4 ± 0.81	1.8 ± 1.32	1.6 ± 0.45	0.7 ± 0.02
C 14:0	0.1 ± 0.08	0.3 ± 0.03	0.4 ± 0.45	0.3 ± 0.23	0.3 ± 0.16	0.5 ± 0.22
C 16:0	11.5 ± 2.68	13.2 ± 0.59	13.7 ± 0.13	16.2 ± 3.43	14.8 ± 1.29	12.5 ± 4.88
C 18:0	24.4 ± 2.81	25.4 ± 2.62	23.8 ± 3.11	21.5 ± 1.48	17.8 ± 3.03	20.8 ± 3.29
C 20:0	0.6 ± 0.01	0.7 ± 0.18	0.4 ± 0.16	0.5 ± 0.03	0.4 ± 0.08	0.8 ± 0.14
C 22:0	0.3 ± 0.70	0.1 ± 0.13	0.3 ± 0.02	0.5 ± 0.18	0.6 ± 0.21	0.2 ± 0.07
C 24:0	0.1 ± 0.32	0.5 ± 0.38	0.1 ± 0.09	0.1 ± 0.08	0.3 ± 0.10	0.1 ± 0.05
SUM SFA³	39.1 ± 8.32	43.1 ± 5.18	41.3 ± 5.03	42.1 ± 7.04	37.3 ± 5.59	37.3 ± 8.96
C 16:1 n-9	0.4 ± 0.23	0.2 ± 0.16	0.2 ± 0.18	0.5 ± 0.21	0.0 ± 0.09	0.6 ± 0.19
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	10.3 ± 1.50	9.9 ± 0.24	10.2 ± 0.96	11.0 ± 1.86	11.0 ± 0.88	11.0 ± 2.07
C 20:1 n-9	1.2 ± 0.15	1.2 ± 0.04	1.1 ± 0.02	1.0 ± 0.16	1.0 ± 0.09	2.6 ± 0.72
C 22:1 n-7	0.0 ± 0.03	0.3 ± 0.32	0.1 ± 0.09	0.0 ± 0.00	0.0 ± 0.07	0.2 ± 0.11
C 24:1 n-9	0.0 ± 0.02	0.2 ± 0.08	0.1 ± 0.06	0.1 ± 0.07	0.1 ± 0.07	0.1 ± 0.08
SUM MUFA⁴	14.4 ± 2.41	14.6 ± 1.38	14.2 ± 1.85	14.7 ± 2.65	14.0 ± 1.74	19.2 ± 4.58
C 18:2 n-6	3.5 ± 0.22	2.8 ± 0.63	3.1 ± 0.64	3.5 ± 0.46	3.5 ± 0.35	2.4 ± 0.29
C 18:3 n-6	0.5 ± 0.06	0.3 ± 0.02	0.3 ± 0.10	0.2 ± 0.05	0.2 ± 0.07	0.3 ± 0.10
C 18:3 n-3	0.2 ± 0.09	0.1 ± 0.11	0.1 ± 0.04	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.02
C 18:4 n-3	0.2 ± 0.06	0.1 ± 0.07	0.1 ± 0.07	0.1 ± 0.07	0.1 ± 0.04	0.1 ± 0.04
C 20:2 n-6	1.1 ± 0.22	1.0 ± 0.10	1.2 ± 0.02	1.2 ± 0.15	1.2 ± 0.02	0.9 ± 0.16
C 20:3 n-9	0.3 ± 0.34	0.3 ± 0.01	0.3 ± 0.05	0.5 ± 0.22	0.6 ± 0.23	0.4 ± 0.07
C 20:3 n-6	2.6 ± 0.13	1.7 ± 0.20	1.2 ± 0.21	0.9 ± 0.05	0.9 ± 0.11	0.8 ± 0.10
C 20:4 n-6	6.5 ± 0.19	4.3 ± 0.48	3.6 ± 0.60	3.2 ± 0.18	2.9 ± 0.19	2.0 ± 0.21
C 20:5 n-3	1.4 ± 0.15	1.6 ± 0.02	1.5 ± 0.46	1.6 ± 0.05	2.0 ± 0.29	2.8 ± 0.88
C 22:5 n-6	4.5 ± 1.23	1.9 ± 1.31	1.8 ± 1.23	1.7 ± 1.32	1.7 ± 0.89	0.7 ± 0.29
C 22:5 n-3	3.2 ± 0.65	1.4 ± 0.22	1.1 ± 0.13	1.0 ± 0.21	1.0 ± 0.14	2.0 ± 0.32
C 22:6 n-3	18.7 ± 2.61	22.8 ± 3.70	25.8 ± 1.54	25.5 ± 5.05	29.7 ± 0.81	27.6 ± 3.64
SUM PUFA⁵	42.9 ± 6.13	39.3 ± 7.56	40.6 ± 5.10	40.2 ± 7.89	44.5 ± 3.20	40.6 ± 6.23

PS200	NC ¹	0.5%EPA&DH/1%EPA&DHA	1.5%EPA&DHA	2%EPA&DHA	CC ²
Fett % Folch	7.6 ± 0.59	14.8 ± 5.68	11.9 ± 2.20	10.2 ± 0.31	12.2 ± 0.84
C 12:0	1.2 ± 1.47	2.7 ± 0.07	0.7 ± 0.01	2.0 ± 1.32	1.3 ± 0.77
C 14:0	0.1 ± 0.08	0.7 ± 0.18	0.2 ± 0.01	0.5 ± 0.36	0.2 ± 0.24
C 16:0	11.5 ± 2.68	15.5 ± 4.09	11.3 ± 0.39	16.2 ± 5.00	13.5 ± 2.14
C 18:0	24.4 ± 2.81	24.0 ± 0.35	26.3 ± 1.25	20.4 ± 3.67	23.3 ± 0.47
C 20:0	0.6 ± 0.01	0.5 ± 0.01	0.3 ± 0.29	0.5 ± 0.10	0.5 ± 0.03
C 22:0	0.3 ± 0.70	0.7 ± 0.17	0.4 ± 0.01	0.4 ± 0.13	0.4 ± 0.24
C 24:0	0.1 ± 0.32	0.4 ± 0.19	0.1 ± 0.01	0.2 ± 0.04	0.1 ± 0.05
SUM SFA³	39.1 ± 8.32	45.8 ± 5.52	40.7 ± 2.05	41.1 ± 10.77	41.3 ± 4.59
C 16:1 n-9	0.4 ± 0.23	0.3 ± 0.33	0.1 ± 0.11	0.5 ± 0.51	0.5 ± 0.19
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	10.3 ± 1.50	11.7 ± 2.20	9.1 ± 0.21	11.6 ± 3.88	9.7 ± 1.25
C 20:1 n-9	1.2 ± 0.15	1.1 ± 0.21	1.3 ± 0.03	1.1 ± 0.21	1.2 ± 0.07
C 22:1 n-7	0.0 ± 0.03	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.1 ± 0.04
C 24:1 n-9	0.0 ± 0.02	0.0 ± 0.00	0.1 ± 0.00	0.2 ± 0.05	0.1 ± 0.05
SUM MUFA⁴	14.4 ± 2.41	16.0 ± 3.13	12.9 ± 0.57	16.1 ± 5.05	14.4 ± 2.40
C 18:2 n-6	3.5 ± 0.22	3.6 ± 0.68	2.7 ± 0.19	3.5 ± 0.88	2.7 ± 0.32
C 18:3 n-6	0.5 ± 0.06	0.3 ± 0.02	0.3 ± 0.06	0.2 ± 0.02	0.1 ± 0.06
C 18:3 n-3	0.2 ± 0.09	0.2 ± 0.07	0.4 ± 0.24	0.2 ± 0.04	0.2 ± 0.02
C 18:4 n-3	0.2 ± 0.06	0.1 ± 0.09	0.2 ± 0.04	0.2 ± 0.02	0.2 ± 0.07
C 20:2 n-6	1.1 ± 0.22	0.6 ± 0.45	1.3 ± 0.04	1.1 ± 0.12	0.6 ± 0.43
C 20:3 n-9	0.3 ± 0.34	0.7 ± 0.32	0.4 ± 0.08	0.4 ± 0.11	0.5 ± 0.18
C 20:3 n-6	2.6 ± 0.13	1.4 ± 0.36	1.5 ± 0.17	1.0 ± 0.05	0.8 ± 0.02
C 20:4 n-6	6.5 ± 0.19	3.7 ± 0.06	3.1 ± 0.25	2.9 ± 0.24	2.3 ± 0.15
C 20:5 n-3	1.4 ± 0.15	1.5 ± 0.04	2.1 ± 0.02	2.3 ± 0.03	2.6 ± 0.10
C 22:5 n-6	4.5 ± 1.23	1.5 ± 0.83	1.4 ± 0.79	1.0 ± 1.04	1.3 ± 0.66
C 22:5 n-3	3.2 ± 0.65	2.0 ± 0.43	2.4 ± 0.14	1.9 ± 0.64	2.3 ± 0.29
C 22:6 n-3	18.7 ± 2.61	18.8 ± 5.55	27.1 ± 0.65	24.0 ± 7.84	27.0 ± 3.56
SUM PUFA⁵	42.9 ± 6.13	34.9 ± 9.00	43.4 ± 2.73	39.5 ± 11.34	41.2 ± 5.94

NC¹ means negative control, CC² means commercial control. Sum SFA³ also contains 15:0 and 17:0. Sum MUFA⁴ also contains 14:1n-5, 15:1, 16:1n-5, 17:1n-7, 18:1n-1, 18:1n-7, 20:1n-1, 20:1n-7, 22:1n-1 and 22:1n-9. Sum PUFA⁵ also contains 16:2n-6, 16:2n-3, 20:4n-3, 20:3n-3, 22:2n-6 and 22:4n-6.

Appendix

Table 7 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 400g PS group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PS400	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	12.3 \pm 1.45	12.9 \pm 2.42	9.0 \pm 2.16	15.8 \pm 1.39	13.2 \pm 2.27	18.6 \pm 3.10
C 12:0	2.2 \pm 0.64	3.6 \pm 0.14	1.1 \pm 0.48	1.7 \pm 0.88	1.5 \pm 0.33	1.9 \pm 0.70
C 14:0	0.5 \pm 0.23	0.1 \pm 0.05	0.0 \pm 0.04	0.1 \pm 0.01	0.2 \pm 0.03	0.5 \pm 0.28
C 16:0	10.4 \pm 0.32	12.7 \pm 2.06	11.4 \pm 0.03	11.7 \pm 0.72	10.8 \pm 0.31	10.5 \pm 0.33
C 18:0	24.5 \pm 0.07	24.0 \pm 0.09	25.0 \pm 1.66	24.6 \pm 1.01	25.3 \pm 0.92	22.0 \pm 1.29
C 20:0	0.4 \pm 0.07	0.3 \pm 0.01	0.7 \pm 0.02	0.5 \pm 0.00	0.6 \pm 0.03	0.8 \pm 0.06
C 22:0	0.2 \pm 0.12	0.0 \pm 0.00	0.4 \pm 0.09	0.4 \pm 0.02	0.4 \pm 0.03	0.4 \pm 0.03
C 24:0	2.5 \pm 1.55	1.1 \pm 1.12	0.1 \pm 0.10	0.2 \pm 0.00	0.3 \pm 0.04	0.1 \pm 0.06
SUM SFA³	41.5 \pm 3.04	42.7 \pm 3.57	39.7 \pm 2.65	40.1 \pm 2.67	40.0 \pm 1.92	37.2 \pm 2.87
C 16:1 n-9	0.2 \pm 0.05	0.4 \pm 0.11	0.3 \pm 0.08	0.2 \pm 0.02	0.2 \pm 0.04	0.3 \pm 0.12
C 16:1 n-7	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00
C 18:1 n-9	9.5 \pm 0.17	9.1 \pm 0.79	8.9 \pm 0.13	8.7 \pm 0.50	8.2 \pm 0.21	9.5 \pm 0.34
C 20:1 n-9	1.1 \pm 0.12	1.2 \pm 0.17	1.4 \pm 0.34	1.1 \pm 0.12	0.6 \pm 0.30	2.0 \pm 0.11
C 22:1 n-7	0.1 \pm 0.04	0.1 \pm 0.12	0.3 \pm 0.18	0.1 \pm 0.02	0.0 \pm 0.03	0.1 \pm 0.06
C 24:1 n-9	0.1 \pm 0.09	0.1 \pm 0.06	0.0 \pm 0.00	0.1 \pm 0.06	0.1 \pm 0.09	0.2 \pm 0.33
SUM MUFA⁴	13.2 \pm 0.69	13.3 \pm 2.34	12.7 \pm 1.05	12.1 \pm 0.87	10.3 \pm 0.99	14.5 \pm 1.17
C 18:2 n-6	3.3 \pm 0.18	3.3 \pm 0.24	2.7 \pm 0.14	2.5 \pm 0.27	1.8 \pm 0.14	2.2 \pm 0.12
C 18:3 n-6	0.3 \pm 0.10	0.4 \pm 0.09	0.1 \pm 0.10	0.2 \pm 0.01	0.1 \pm 0.05	0.1 \pm 0.05
C 18:3 n-3	0.1 \pm 0.04	0.2 \pm 0.03	0.3 \pm 0.02	0.2 \pm 0.01	0.1 \pm 0.06	0.2 \pm 0.07
C 18:4 n-3	0.0 \pm 0.00	0.0 \pm 0.00	0.1 \pm 0.06	0.0 \pm 0.05	0.0 \pm 0.00	0.0 \pm 0.00
C 20:2 n-6	0.6 \pm 0.12	0.7 \pm 0.01	0.9 \pm 0.12	1.1 \pm 0.01	1.0 \pm 0.13	0.8 \pm 0.12
C 20:3 n-9	0.4 \pm 0.21	0.5 \pm 0.20	0.7 \pm 0.40	0.3 \pm 0.00	0.1 \pm 0.07	0.0 \pm 0.00
C 20:3 n-6	3.6 \pm 0.11	2.6 \pm 0.02	1.6 \pm 0.04	1.3 \pm 0.02	0.9 \pm 0.06	0.8 \pm 0.06
C 20:4 n-6	6.9 \pm 0.15	4.4 \pm 0.09	3.3 \pm 0.14	2.9 \pm 0.11	2.8 \pm 0.02	2.9 \pm 0.22
C 20:5 n-3	1.1 \pm 0.03	1.8 \pm 0.21	2.8 \pm 0.03	3.1 \pm 0.23	3.4 \pm 0.19	3.1 \pm 0.23
C 22:5 n-6	3.9 \pm 1.17	0.7 \pm 0.54	1.2 \pm 0.02	0.9 \pm 0.10	0.8 \pm 0.03	1.1 \pm 0.38
C 22:5 n-3	3.7 \pm 0.20	3.8 \pm 0.10	4.7 \pm 0.08	4.5 \pm 0.05	5.5 \pm 0.34	2.8 \pm 0.11
C 22:6 n-3	17.7 \pm 0.62	23.0 \pm 1.57	27.0 \pm 0.31	28.4 \pm 1.46	30.5 \pm 0.47	31.6 \pm 1.00
SUM PUFA⁵	42.0 \pm 3.09	41.4 \pm 3.30	45.2 \pm 1.49	45.6 \pm 2.55	47.3 \pm 1.70	45.9 \pm 2.53
PS400	NC¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC²
Fett % Folch	12.3 \pm 1.45	10.9 \pm 0.77	12.9 \pm 2.26	16.3 \pm 1.21	12.6 \pm 2.36	18.6 \pm 3.10
C 12:0	2.2 \pm 0.64	1.3 \pm 1.34	2.8 \pm 0.14	1.5 \pm 0.91	1.2 \pm 0.29	1.9 \pm 0.70
C 14:0	0.5 \pm 0.23	0.6 \pm 0.13	0.4 \pm 0.28	0.3 \pm 0.06	0.3 \pm 0.13	0.5 \pm 0.28
C 16:0	10.4 \pm 0.32	11.7 \pm 0.33	12.2 \pm 1.10	13.3 \pm 1.65	12.1 \pm 0.97	10.5 \pm 0.33
C 18:0	24.5 \pm 0.07	23.8 \pm 1.59	24.6 \pm 0.60	17.8 \pm 4.54	24.3 \pm 1.08	22.0 \pm 1.29
C 20:0	0.4 \pm 0.07	0.2 \pm 0.23	0.4 \pm 0.10	0.5 \pm 0.07	0.5 \pm 0.04	0.8 \pm 0.06
C 22:0	0.2 \pm 0.12	0.3 \pm 0.03	0.3 \pm 0.30	0.3 \pm 0.06	0.6 \pm 0.15	0.4 \pm 0.03
C 24:0	2.5 \pm 1.55	0.1 \pm 0.10	0.0 \pm 0.00	0.1 \pm 0.05	0.0 \pm 0.00	0.1 \pm 0.06
SUM SFA³	41.5 \pm 3.04	38.8 \pm 4.07	41.5 \pm 2.73	34.6 \pm 7.40	40.2 \pm 2.98	37.2 \pm 2.87
C 16:1 n-9	0.2 \pm 0.05	0.3 \pm 0.05	0.4 \pm 0.06	0.5 \pm 0.02	0.2 \pm 0.15	0.3 \pm 0.12
C 16:1 n-7	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00
C 18:1 n-9	9.5 \pm 0.17	9.7 \pm 0.54	10.3 \pm 1.23	11.0 \pm 1.61	8.7 \pm 0.47	9.5 \pm 0.34
C 20:1 n-9	1.1 \pm 0.12	1.1 \pm 0.03	1.4 \pm 0.25	1.1 \pm 0.22	1.2 \pm 0.07	2.0 \pm 0.11
C 22:1 n-7	0.1 \pm 0.04	0.0 \pm 0.04	0.0 \pm 0.00	0.1 \pm 0.08	0.0 \pm 0.00	0.1 \pm 0.06
C 24:1 n-9	0.1 \pm 0.09	0.3 \pm 0.01	0.0 \pm 0.00	0.1 \pm 0.05	0.0 \pm 0.00	0.2 \pm 0.33
SUM MUFA⁴	13.2 \pm 0.69	13.2 \pm 0.78	13.8 \pm 1.87	14.5 \pm 2.48	11.6 \pm 0.96	14.5 \pm 1.17
C 18:2 n-6	3.3 \pm 0.18	3.0 \pm 0.02	2.4 \pm 0.90	3.6 \pm 0.93	2.2 \pm 0.06	2.2 \pm 0.12
C 18:3 n-6	0.3 \pm 0.10	0.3 \pm 0.02	0.3 \pm 0.11	0.2 \pm 0.03	0.0 \pm 0.16	0.1 \pm 0.05
C 18:3 n-3	0.1 \pm 0.04	0.3 \pm 0.06	0.4 \pm 0.13	0.1 \pm 0.10	0.1 \pm 0.07	0.2 \pm 0.07
C 18:4 n-3	0.0 \pm 0.00	0.1 \pm 0.09	0.0 \pm 0.00	0.1 \pm 0.09	0.1 \pm 0.08	0.0 \pm 0.00
C 20:2 n-6	0.6 \pm 0.12	1.0 \pm 0.11	0.6 \pm 0.03	0.8 \pm 0.12	0.5 \pm 0.24	0.8 \pm 0.12
C 20:3 n-9	0.4 \pm 0.21	0.2 \pm 0.16	0.2 \pm 0.17	0.3 \pm 0.06	0.0 \pm 0.09	0.0 \pm 0.00
C 20:3 n-6	3.6 \pm 0.11	2.3 \pm 0.05	1.8 \pm 0.53	0.9 \pm 0.26	0.8 \pm 0.07	0.8 \pm 0.06
C 20:4 n-6	6.9 \pm 0.15	4.8 \pm 0.08	3.7 \pm 0.49	4.2 \pm 0.48	3.0 \pm 0.15	2.9 \pm 0.22
C 20:5 n-3	1.1 \pm 0.03	1.4 \pm 0.11	1.5 \pm 0.68	2.4 \pm 1.14	2.3 \pm 0.36	3.1 \pm 0.23
C 22:5 n-6	3.9 \pm 1.17	3.7 \pm 0.07	2.8 \pm 0.16	3.1 \pm 0.24	3.3 \pm 0.16	1.1 \pm 0.38
C 22:5 n-3	3.7 \pm 0.20	1.5 \pm 0.05	1.4 \pm 0.46	1.5 \pm 0.50	1.1 \pm 0.08	2.8 \pm 0.11
C 22:6 n-3	17.7 \pm 0.62	26.1 \pm 0.36	27.1 \pm 0.07	30.3 \pm 2.21	32.2 \pm 2.39	31.6 \pm 1.00
SUM PUFA⁵	42.0 \pm 3.09	44.7 \pm 1.25	42.3 \pm 3.84	47.8 \pm 6.32	45.8 \pm 3.96	45.9 \pm 2.53
PS400	NC¹	0.5%EPA&DH/1%EPA&DHA	1.5%EPA&DH/2%EPA&DHA	CC²		
Fett % Folch	12.3 \pm 1.45	9.3 \pm 0.06	12.1 \pm 1.50	18.3 \pm 2.24	16.5 \pm 3.14	18.6 \pm 3.10
C 12:0	2.2 \pm 0.64	2.4 \pm 0.30	0.5 \pm 0.48	1.3 \pm 0.42	2.5 \pm 0.22	1.9 \pm 0.70
C 14:0	0.5 \pm 0.23	1.4 \pm 0.43	0.4 \pm 0.14	0.3 \pm 0.18	0.2 \pm 0.08	0.5 \pm 0.28
C 16:0	10.4 \pm 0.32	13.5 \pm 3.90	11.5 \pm 1.16	10.0 \pm 0.10	13.1 \pm 0.94	10.5 \pm 0.33
C 18:0	24.5 \pm 0.07	25.3 \pm 1.72	24.3 \pm 0.66	24.5 \pm 0.09	20.8 \pm 0.99	22.0 \pm 1.29
C 20:0	0.4 \pm 0.07	0.7 \pm 0.27	0.5 \pm 0.05	0.5 \pm 0.01	0.4 \pm 0.03	0.8 \pm 0.06
C 22:0	0.2 \pm 0.12	0.4 \pm 0.36	0.1 \pm 0.14	0.2 \pm 0.20	0.4 \pm 0.13	0.4 \pm 0.03
C 24:0	2.5 \pm 1.55	1.4 \pm 1.36	0.2 \pm 0.04	0.1 \pm 0.10	1.2 \pm 0.61	0.1 \pm 0.06
SUM SFA³	41.5 \pm 3.04	45.9 \pm 8.58	38.2 \pm 2.97	37.9 \pm 1.49	39.6 \pm 3.12	37.2 \pm 2.87
C 16:1 n-9	0.2 \pm 0.05	0.7 \pm 0.41	0.5 \pm 0.32	0.2 \pm 0.02	0.0 \pm 0.08	0.3 \pm 0.12
C 16:1 n-7	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00
C 18:1 n-9	9.5 \pm 0.17	9.0 \pm 0.99	8.8 \pm 0.52	8.3 \pm 0.40	9.8 \pm 0.63	9.5 \pm 0.34
C 20:1 n-9	1.1 \pm 0.12	1.3 \pm 0.17	1.2 \pm 0.09	1.2 \pm 0.01	1.0 \pm 0.04	2.0 \pm 0.11
C 22:1 n-7	0.1 \pm 0.04	0.1 \pm 0.13	0.2 \pm 0.19	0.1 \pm 0.13	0.2 \pm 0.08	0.1 \pm 0.06
C 24:1 n-9	0.1 \pm 0.09	0.1 \pm 0.07	0.3 \pm 0.09	0.1 \pm 0.09	0.4 \pm 0.08	0.2 \pm 0.33
SUM MUFA⁴	13.2 \pm 0.69	14.0 \pm 3.15	12.8 \pm 1.47	11.6 \pm 1.07	13.4 \pm 1.10	14.5 \pm 1.17
C 18:2 n-6	3.3 \pm 0.18	2.8 \pm 0.09	2.8 \pm 0.25	2.2 \pm 0.11	3.0 \pm 0.40	2.2 \pm 0.12
C 18:3 n-6	0.3 \pm 0.10	0.0 \pm 0.04	0.2 \pm 0.00	0.1 \pm 0.02	0.2 \pm 0.05	0.1 \pm 0.05
C 18:3 n-3						

Appendix

Table 8 Level of individual fatty acids in percentage of total fatty acids in the different dietary group \pm standard error of mean (sem) in 200g PI group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PI200	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	7.6 ± 0.59	9.3 ± 0.16	9.6 ± 1.74	9.9 ± 2.01	11.8 ± 1.78	12.2 ± 0.84
C 12:0	5.2 ± 1.66	1.3 ± 0.13	2.3 ± 0.20	3.8 ± 0.43	1.9 ± 0.32	2.0 ± 0.71
C 14:0	1.0 ± 0.28	0.1 ± 0.13	0.6 ± 0.10	0.3 ± 0.27	0.2 ± 0.12	0.3 ± 0.09
C 16:0	8.8 ± 1.12	9.9 ± 0.59	8.8 ± 1.83	12.6 ± 0.47	10.1 ± 1.21	8.5 ± 1.88
C 18:0	29.0 ± 1.09	28.4 ± 2.24	26.2 ± 3.16	27.1 ± 2.58	31.6 ± 5.98	28.9 ± 4.33
C 20:0	0.1 ± 0.19	0.3 ± 0.08	0.1 ± 0.10	0.3 ± 0.25	0.3 ± 0.01	0.3 ± 0.06
C 22:0	1.2 ± 0.29	0.9 ± 0.12	0.8 ± 0.22	0.5 ± 0.12	1.1 ± 0.41	0.6 ± 0.22
C 24:0	0.8 ± 0.28	0.2 ± 0.23	1.1 ± 1.08	0.0 ± 0.00	0.2 ± 0.12	0.3 ± 0.11
SUM SFA³	47.8 ± 5.32	42.4 ± 3.74	41.0 ± 6.95	46.0 ± 4.78	46.9 ± 8.64	42.4 ± 7.77
C 16:1 n-9	0.0 ± 0.25	0.2 ± 0.20	0.1 ± 0.13	1.5 ± 0.28	0.1 ± 0.12	0.5 ± 0.30
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	9.1 ± 0.59	9.9 ± 0.79	7.9 ± 0.99	10.2 ± 0.73	8.5 ± 2.53	9.0 ± 2.02
C 18:1 n-7	1.1 ± 0.28	1.4 ± 0.16	0.9 ± 0.04	1.6 ± 0.12	1.1 ± 0.51	1.7 ± 0.52
C 20:1 n-9	0.4 ± 0.28	0.8 ± 0.25	0.4 ± 0.02	0.9 ± 0.23	0.5 ± 0.21	0.7 ± 0.33
C 22:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.17	0.0 ± 0.13
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	10.9 ± 1.79	13.0 ± 1.63	12.6 ± 4.28	15.3 ± 1.95	11.3 ± 4.12	12.8 ± 3.67
C 18:2 n-6	3.3 ± 0.21	3.4 ± 0.77	2.7 ± 0.37	3.1 ± 0.36	2.5 ± 1.05	2.0 ± 0.53
C 18:3 n-6	0.1 ± 0.05	0.2 ± 0.20	0.2 ± 0.02	0.1 ± 0.10	0.1 ± 0.04	0.2 ± 0.12
C 18:3 n-3	0.0 ± 0.00	0.3 ± 0.05	0.0 ± 0.00	0.4 ± 0.20	0.1 ± 0.11	1.3 ± 0.71
C 18:4 n-3	0.0 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.9 ± 0.58
C 20:2 n-6	1.5 ± 0.20	1.1 ± 0.19	0.7 ± 0.02	1.0 ± 0.24	0.7 ± 0.39	0.8 ± 0.16
C 20:3 n-9	1.5 ± 0.36	0.6 ± 0.01	0.6 ± 0.05	0.7 ± 0.11	0.9 ± 0.27	0.7 ± 0.14
C 20:3 n-6	2.3 ± 0.07	1.9 ± 0.19	1.7 ± 0.22	1.2 ± 0.09	1.0 ± 0.09	1.4 ± 0.27
C 20:4 n-6	24.4 ± 5.17	19.6 ± 2.79	22.8 ± 0.33	11.5 ± 6.63	21.7 ± 5.47	21.3 ± 5.66
C 20:5 n-3	1.3 ± 0.14	2.4 ± 0.17	3.5 ± 0.01	3.2 ± 0.53	4.0 ± 1.02	2.6 ± 0.53
C 22:5 n-6	0.6 ± 0.20	0.2 ± 0.20	1.2 ± 0.69	0.0 ± 0.00	0.1 ± 0.10	0.0 ± 0.07
C 22:5 n-3	0.6 ± 0.63	1.5 ± 0.53	1.1 ± 0.11	2.6 ± 1.51	1.5 ± 0.27	0.7 ± 0.18
C 22:6 n-3	5.2 ± 2.03	11.6 ± 2.69	6.7 ± 0.78	13.1 ± 7.52	8.4 ± 13.29	9.9 ± 4.32
SUM PUFA⁵	40.9 ± 9.79	43.4 ± 8.36	44.4 ± 5.39	37.6 ± 17.66	41.4 ± 23.29	42.4 ± 14.14

PI200	NC ¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC ²
Fett % Folch	7.6 ± 0.59	7.7 ± 0.59	8.9 ± 2.15	11.0 ± 1.29	11.2 ± 1.42	12.2 ± 0.84
C 12:0	5.2 ± 1.66	3.6 ± 1.89	2.1 ± 0.35	2.5 ± 2.00	1.4 ± 0.11	2.0 ± 0.71
C 14:0	1.0 ± 0.28	1.3 ± 0.02	0.0 ± 0.00	0.4 ± 0.39	0.3 ± 0.02	0.3 ± 0.09
C 16:0	8.8 ± 1.12	9.7 ± 0.29	8.4 ± 0.65	10.1 ± 0.20	10.8 ± 0.62	8.5 ± 1.88
C 18:0	29.0 ± 1.09	30.3 ± 1.66	25.9 ± 5.48	31.1 ± 1.73	31.4 ± 1.41	28.9 ± 4.33
C 20:0	0.1 ± 0.19	0.2 ± 0.02	0.1 ± 0.11	0.4 ± 0.21	0.4 ± 0.13	0.3 ± 0.06
C 22:0	1.2 ± 0.29	0.6 ± 0.61	1.1 ± 0.05	0.7 ± 0.34	0.6 ± 0.11	0.6 ± 0.22
C 24:0	0.8 ± 0.28	0.5 ± 0.18	0.4 ± 0.40	0.4 ± 0.36	0.2 ± 0.13	0.3 ± 0.11
SUM SFA³	47.8 ± 5.32	47.1 ± 5.59	39.4 ± 7.49	47.0 ± 5.52	46.8 ± 2.73	42.4 ± 7.77
C 16:1 n-9	0.0 ± 0.25	0.7 ± 0.52	0.1 ± 0.13	0.3 ± 0.25	0.5 ± 0.11	0.5 ± 0.30
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	9.1 ± 0.59	9.1 ± 1.23	12.0 ± 4.26	7.9 ± 0.22	8.7 ± 0.17	9.0 ± 2.02
C 18:1 n-7	1.1 ± 0.28	1.2 ± 0.32	1.5 ± 0.46	1.4 ± 0.31	1.4 ± 0.23	1.7 ± 0.52
C 20:1 n-9	0.4 ± 0.28	0.7 ± 0.09	1.0 ± 0.57	0.8 ± 0.50	1.0 ± 0.28	0.7 ± 0.33
C 22:1 n-7	0.0 ± 0.00	0.6 ± 0.60	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.13
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.15	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	10.9 ± 1.79	12.4 ± 2.96	14.6 ± 5.43	10.7 ± 1.70	11.9 ± 1.00	12.8 ± 3.67
C 18:2 n-6	3.3 ± 0.21	3.2 ± 0.01	4.0 ± 1.18	2.0 ± 0.40	2.4 ± 0.38	2.0 ± 0.53
C 18:3 n-6	0.1 ± 0.05	0.2 ± 0.18	0.2 ± 0.17	0.4 ± 0.15	0.1 ± 0.04	0.2 ± 0.12
C 18:3 n-3	0.0 ± 0.00	0.1 ± 0.13	0.2 ± 0.22	0.1 ± 0.07	0.2 ± 0.10	1.3 ± 0.71
C 18:4 n-3	0.0 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.06	0.0 ± 0.03	0.9 ± 0.58
C 20:2 n-6	1.5 ± 0.20	1.0 ± 0.50	0.9 ± 0.27	1.3 ± 0.13	0.9 ± 0.12	0.8 ± 0.16
C 20:3 n-9	1.5 ± 0.36	0.9 ± 0.31	1.4 ± 0.49	0.9 ± 0.54	0.5 ± 0.15	0.7 ± 0.14
C 20:3 n-6	2.3 ± 0.07	1.8 ± 0.10	1.4 ± 0.10	1.0 ± 0.23	0.9 ± 0.13	1.4 ± 0.27
C 20:4 n-6	24.4 ± 5.17	21.8 ± 4.01	22.0 ± 2.18	14.8 ± 7.26	15.0 ± 4.90	21.3 ± 5.66
C 20:5 n-3	1.3 ± 0.14	1.0 ± 0.04	1.9 ± 0.42	1.0 ± 0.11	1.3 ± 0.29	2.6 ± 0.53
C 22:5 n-6	0.6 ± 0.20	0.2 ± 0.21	0.3 ± 0.30	0.3 ± 0.29	0.4 ± 0.24	0.0 ± 0.07
C 22:5 n-3	0.6 ± 0.63	0.2 ± 0.16	0.8 ± 0.35	0.4 ± 0.09	0.6 ± 0.04	0.7 ± 0.18
C 22:6 n-3	5.2 ± 2.03	8.4 ± 1.68	11.7 ± 1.20	16.5 ± 6.74	16.3 ± 3.32	9.9 ± 4.32
SUM PUFA⁵	40.9 ± 9.79	39.4 ± 7.70	45.0 ± 7.05	39.3 ± 16.27	39.2 ± 9.96	42.4 ± 14.14

PI200	NC ¹	0.5%EPA&DHA	1%EPA&DHA	1.5%EPA&DHA	2%EPA&DHA	CC ²
Fett % Folch	7.6 ± 0.59	14.8 ± 5.68	11.9 ± 2.20	10.2 ± 0.31	12.2 ± 0.24	12.2 ± 0.84
C 12:0	5.2 ± 1.66	1.4 ± 0.69	2.1 ± 1.20	2.7 ± 2.03	1.6 ± 0.57	2.0 ± 0.71
C 14:0	1.0 ± 0.28	0.2 ± 0.24	0.5 ± 0.48	0.8 ± 0.31	0.2 ± 0.16	0.3 ± 0.09
C 16:0	8.8 ± 1.12	10.0 ± 0.39	8.4 ± 0.19	9.8 ± 1.50	9.2 ± 0.80	8.5 ± 1.88
C 18:0	29.0 ± 1.09	31.6 ± 3.34	32.3 ± 2.14	29.4 ± 1.51	31.7 ± 0.37	28.9 ± 4.33
C 20:0	0.1 ± 0.19	0.4 ± 0.13	0.3 ± 0.11	0.4 ± 0.17	0.2 ± 0.20	0.3 ± 0.06
C 22:0	1.2 ± 0.29	0.7 ± 0.18	0.8 ± 0.05	0.6 ± 0.24	0.7 ± 1.05	0.6 ± 0.22
C 24:0	0.8 ± 0.28	0.2 ± 0.21	0.5 ± 0.03	0.2 ± 0.05	0.4 ± 0.15	0.3 ± 0.11
SUM SFA³	47.8 ± 5.32	45.9 ± 5.52	46.2 ± 4.33	45.4 ± 5.89	45.4 ± 3.89	42.4 ± 7.77
C 16:1 n-9	0.0 ± 0.25	0.3 ± 0.28	0.4 ± 0.21	0.7 ± 0.34	0.2 ± 0.10	0.5 ± 0.30
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	9.1 ± 0.59	9.1 ± 0.05	8.3 ± 0.02	8.7 ± 0.30	7.6 ± 0.46	9.0 ± 2.02
C 18:1 n-7	1.1 ± 0.28	1.4 ± 0.33	1.0 ± 0.10	1.4 ± 0.28	1.3 ± 0.11	1.7 ± 0.52
C 20:1 n-9	0.4 ± 0.28	0.8 ± 0.31	0.4 ± 0.00	0.9 ± 0.42	0.8 ± 0.21	0.7 ± 0.33
C 22:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.13
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.06	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	10.9 ± 1.79	12.0 ± 1.40	10.6 ± 0.52	12.3 ± 1.67	10.2 ± 1.67	12.8 ± 3.67
C 18:2 n-6	3.3 ± 0.21	2.8 ± 0.62	2.4 ± 0.30	2.5 ± 0.52	2.2 ± 0.46	2.0 ± 0.53
C 18:3 n-6	0.1 ± 0.05	0.4 ± 0.21	0.0 ± 0.00	0.1 ± 0.00	0.2 ± 0.13	0.2 ± 0.12
C 18:3 n-3	0.0 ± 0.00	0.2 ± 0.21	0.2 ± 0.04	0.2 ± 0.01	0.3 ± 0.10	1.3 ± 0.71
C 18:4 n-3	0.0 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.07	0.0 ± 0.15	0.9 ± 0.58
C 20:2 n-6	1.5 ± 0.20	0.9 ± 0.30	1.2 ± 0.11	0.6 ± 0.60	1.2 ± 0.11	0.8 ± 0.16
C 20:3 n-9	1.5 ± 0.36	0.4 ± 0.09	1.1 ± 0.19	0.4 ± 0.09	0.7 ± 0.24	0.7 ± 0.14
C 20:3 n-6	2.3 ± 0.07	2.0 ± 0.07	1.8 ± 0.08	1.1 ± 0.26	1.0 ± 0.17	1.4 ± 0.27
C 20:4 n-6	24.4 ± 5.17	17.7 ± 6.96	25.6 ± 0.50	14.7 ± 8.00	16.7 ± 4.65	21.3 ± 5.66
C 20:5 n-3	1.3 ± 0.14	1.5 ± 0.30	1.8 ± 0.20	2.3 ± 0.54	2.8 ± 0.24	2.6 ± 0.53
C 22:5 n-6	0.6 ± 0.20	0.2 ± 0.21	0.0 ± 0.00	0.2 ± 0.19	0.4 ± 0.24	0.0 ± 0.07
C 22:5 n-3	0.6 ± 0.63	1.3 ± 0.59	0.6 ± 0.02	1.2 ± 0.53	1.2 ± 0.25	0.7 ± 0.18
C 22:6 n-3	5.2 ± 2.03	11.9 ± 3.98	7.8 ± 0.19	15.9 ± 6.05	15.3 ± 3.25	9.9 ± 4.32
SUM PUFA⁵	40.9 ± 9.79	40.4 ± 14.30	42.9 ± 1.76	40.1 ± 17.22	42.3 ± 10.30	42.4 ± 14.14

NC¹ means negative control, CC² means commercial control. Sum SFA³ also contains 15:0 and 17:0. Sum MUFA⁴ also contains 14:1n-5, 15:1, 16:1n-5, 17:1n-7, 18:1n-1, 18:1n-7, 20:1n-1, 20:1n-7, 22:1n-1 and 22:1n-9. Sum PUFA⁵ also contains 16:2n-6, 16:2n-3, 20:4n-3, 20:3n-3, 22:2n-6 and 22:4n-6.

Table 9 Level of individual fatty acids in percentage of total fatty acids in the different dietary group ± standard error of mean (sem) in 400g PI group, n=3 for the NC, CC, 2% EPA, DHA and DHA+EPA group, n=2 for 0.5%, 1%, 1.5% EPA, DHA and EPA+DHA group.

PI400	NC ¹	0.5%EPA	1%EPA	1.5%EPA	2%EPA	CC ²
Fett % Folch	12.3 ± 1.45	12.9 ± 2.42	9.0 ± 2.16	15.8 ± 1.39	13.2 ± 2.27	18.6 ± 3.10
C 12:0	2.9 ± 0.58	4.1 ± 1.90	1.3 ± 0.03	2.4 ± 0.33	2.4 ± 0.31	4.8 ± 0.82
C 14:0	0.2 ± 0.29	0.4 ± 0.20	0.4 ± 0.05	0.3 ± 0.12	0.4 ± 0.07	0.7 ± 0.28
C 16:0	6.4 ± 0.22	6.9 ± 0.12	7.4 ± 0.48	7.0 ± 0.08	6.6 ± 0.27	6.3 ± 0.30
C 18:0	28.3 ± 1.37	27.4 ± 0.25	29.9 ± 1.25	30.4 ± 0.93	27.0 ± 1.55	27.2 ± 0.59
C 20:0	0.3 ± 0.08	0.1 ± 0.08	0.4 ± 0.03	0.3 ± 0.14	0.1 ± 0.05	0.1 ± 0.06
C 22:0	0.9 ± 0.27	0.6 ± 0.07	0.6 ± 0.04	0.5 ± 0.01	1.3 ± 0.49	0.7 ± 0.12
C 24:0	0.2 ± 0.05	0.1 ± 0.15	0.2 ± 0.16	0.3 ± 0.02	0.1 ± 0.09	0.4 ± 0.14
SUM SFA³	40.7 ± 3.44	40.5 ± 2.85	41.3 ± 2.17	41.8 ± 1.71	39.3 ± 3.73	41.2 ± 2.56
C 16:1 n-9	0.1 ± 0.05	0.1 ± 0.11	0.2 ± 0.24	0.1 ± 0.11	0.2 ± 0.04	0.2 ± 0.05
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	8.7 ± 0.36	9.1 ± 1.05	8.9 ± 0.16	8.4 ± 0.29	7.1 ± 0.57	8.9 ± 0.31
C 20:1 n-9	0.5 ± 0.06	0.8 ± 0.16	1.0 ± 0.25	0.6 ± 0.11	0.7 ± 0.04	1.1 ± 0.04
C 22:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.05	0.1 ± 0.05
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	10.5 ± 0.70	10.9 ± 1.32	11.8 ± 1.18	10.2 ± 0.63	9.4 ± 0.96	11.7 ± 0.73
C 18:2 n-6	3.1 ± 0.60	2.5 ± 0.28	2.1 ± 0.05	2.0 ± 0.14	2.0 ± 0.18	0.9 ± 0.51
C 18:3 n-6	0.1 ± 0.00	0.0 ± 0.04	0.1 ± 0.12	0.1 ± 0.03	0.1 ± 0.05	0.0 ± 0.02
C 18:3 n-3	1.0 ± 0.56	0.1 ± 0.10	0.2 ± 0.05	0.2 ± 0.01	0.3 ± 0.06	0.3 ± 0.04
C 18:4 n-3	0.0 ± 0.03	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.06	0.2 ± 0.10
C 20:2 n-6	1.0 ± 0.45	0.3 ± 0.02	0.6 ± 0.23	0.3 ± 0.02	0.2 ± 0.11	0.8 ± 0.27
C 20:3 n-9	0.5 ± 0.02	0.3 ± 0.27	0.6 ± 0.04	0.5 ± 0.05	1.5 ± 0.73	1.0 ± 0.29
C 20:3 n-6	2.4 ± 0.06	2.4 ± 0.05	1.9 ± 0.05	1.7 ± 0.06	1.1 ± 0.15	1.7 ± 0.04
C 20:4 n-6	32.5 ± 1.36	30.6 ± 0.23	29.7 ± 0.46	29.3 ± 0.28	27.1 ± 2.52	25.2 ± 0.62
C 20:5 n-3	0.8 ± 0.03	1.5 ± 0.06	2.1 ± 0.24	2.9 ± 0.05	3.0 ± 0.33	3.2 ± 0.08
C 22:5 n-6	0.7 ± 0.12	0.1 ± 0.15	0.1 ± 0.06	0.1 ± 0.01	0.6 ± 0.30	0.1 ± 0.05
C 22:5 n-3	0.7 ± 0.05	1.1 ± 0.18	1.2 ± 0.06	1.2 ± 0.01	2.2 ± 0.53	1.1 ± 0.05
C 22:6 n-3	3.6 ± 0.12	6.4 ± 0.07	6.5 ± 0.10	7.4 ± 0.32	6.9 ± 0.76	10.5 ± 0.22
SUM PUFA⁵	47.1 ± 3.84	45.9 ± 1.79	45.2 ± 1.62	46.1 ± 1.24	45.5 ± 6.18	45.2 ± 2.45

PI400	NC ¹	0.5%DHA	1%DHA	1.5%DHA	2%DHA	CC ²
Fett % Folch	12.3 ± 1.45	10.9 ± 0.77	12.9 ± 2.26	16.3 ± 1.21	12.6 ± 2.36	18.6 ± 3.10
C 12:0	2.9 ± 0.58	4.4 ± 0.43	1.3 ± 0.34	1.3 ± 0.21	1.9 ± 0.26	4.8 ± 0.82
C 14:0	0.2 ± 0.29	0.4 ± 0.33	0.2 ± 0.01	0.3 ± 0.04	0.4 ± 0.04	0.7 ± 0.28
C 16:0	6.4 ± 0.22	6.6 ± 0.23	6.1 ± 0.15	6.7 ± 0.11	9.9 ± 1.52	6.3 ± 0.30
C 18:0	28.3 ± 1.37	27.9 ± 0.12	28.3 ± 0.00	27.9 ± 0.59	23.4 ± 1.92	27.2 ± 0.59
C 20:0	0.3 ± 0.08	0.2 ± 0.02	0.1 ± 0.06	0.2 ± 0.18	0.3 ± 0.22	0.1 ± 0.06
C 22:0	0.9 ± 0.27	0.5 ± 0.04	0.8 ± 0.16	0.7 ± 0.01	0.7 ± 0.13	0.7 ± 0.12
C 24:0	0.2 ± 0.05	0.3 ± 0.02	0.2 ± 0.19	0.1 ± 0.13	0.0 ± 0.00	0.4 ± 0.14
SUM SFA³	40.7 ± 3.44	40.9 ± 1.21	37.2 ± 0.98	38.2 ± 1.61	38.2 ± 4.89	41.2 ± 2.56
C 16:1 n-9	0.1 ± 0.05	0.3 ± 0.02	0.1 ± 0.08	0.1 ± 0.04	0.3 ± 0.09	0.2 ± 0.05
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	8.7 ± 0.36	8.1 ± 0.60	8.9 ± 0.60	8.2 ± 0.19	8.8 ± 0.88	8.9 ± 0.31
C 20:1 n-9	0.5 ± 0.06	0.9 ± 0.08	0.8 ± 0.09	0.9 ± 0.13	1.1 ± 0.23	1.1 ± 0.04
C 22:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.05
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	10.5 ± 0.70	10.3 ± 1.01	10.8 ± 0.92	10.7 ± 0.98	12.7 ± 2.08	11.7 ± 0.73
C 18:2 n-6	3.1 ± 0.60	2.2 ± 0.11	2.3 ± 0.06	2.3 ± 0.17	2.2 ± 0.17	0.9 ± 0.51
C 18:3 n-6	0.1 ± 0.00	0.1 ± 0.04	0.0 ± 0.00	0.0 ± 0.02	0.0 ± 0.00	0.0 ± 0.02
C 18:3 n-3	1.0 ± 0.56	0.2 ± 0.02	0.3 ± 0.01	0.3 ± 0.05	0.4 ± 0.05	0.3 ± 0.04
C 18:4 n-3	0.0 ± 0.03	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.10
C 20:2 n-6	1.0 ± 0.45	0.2 ± 0.21	0.4 ± 0.00	0.7 ± 0.01	0.7 ± 0.19	0.8 ± 0.27
C 20:3 n-9	0.5 ± 0.02	0.5 ± 0.04	0.6 ± 0.14	0.5 ± 0.04	0.6 ± 0.10	1.0 ± 0.29
C 20:3 n-6	2.4 ± 0.06	2.1 ± 0.29	2.1 ± 0.13	1.6 ± 0.05	1.0 ± 0.16	1.7 ± 0.04
C 20:4 n-6	32.5 ± 1.36	31.2 ± 1.32	31.1 ± 0.29	26.6 ± 3.64	15.9 ± 8.02	25.2 ± 0.62
C 20:5 n-3	0.8 ± 0.03	0.8 ± 0.06	1.0 ± 0.06	1.1 ± 0.03	2.0 ± 0.65	3.2 ± 0.08
C 22:5 n-6	0.7 ± 0.12	0.6 ± 0.25	0.4 ± 0.00	1.0 ± 0.49	1.7 ± 0.84	0.1 ± 0.05
C 22:5 n-3	0.7 ± 0.05	0.6 ± 0.25	0.6 ± 0.04	0.4 ± 0.11	0.9 ± 0.23	1.1 ± 0.05
C 22:6 n-3	3.6 ± 0.12	7.7 ± 0.89	9.1 ± 0.77	14.3 ± 3.60	19.1 ± 5.66	10.5 ± 0.22
SUM PUFA⁵	47.1 ± 3.84	46.4 ± 3.59	48.5 ± 1.86	48.9 ± 8.50	44.6 ± 16.20	45.2 ± 2.45

PI400	NC ¹	0.5%EPA&DH/1%EPA&DHA	1.5%EPA&DH/2%EPA&DHA	CC ²
Fett % Folch	12.3 ± 1.45	9.3 ± 0.06	12.1 ± 1.50	18.3 ± 2.24
C 12:0	2.9 ± 0.58	3.2 ± 0.69	2.0 ± 0.13	3.7 ± 1.81
C 14:0	0.2 ± 0.29	0.6 ± 0.24	0.2 ± 0.16	0.8 ± 0.58
C 16:0	6.4 ± 0.22	6.7 ± 0.78	6.8 ± 0.12	6.7 ± 0.45
C 18:0	28.3 ± 1.37	27.5 ± 0.38	29.6 ± 0.92	28.1 ± 0.31
C 20:0	0.3 ± 0.08	0.1 ± 0.07	0.2 ± 0.06	0.1 ± 0.07
C 22:0	0.9 ± 0.27	0.8 ± 0.28	0.4 ± 0.03	0.7 ± 0.13
C 24:0	0.2 ± 0.05	0.1 ± 0.13	0.3 ± 0.05	0.1 ± 0.11
SUM SFA³	40.7 ± 3.44	39.8 ± 2.88	40.4 ± 1.68	40.9 ± 3.75
C 16:1 n-9	0.1 ± 0.05	0.3 ± 0.24	0.1 ± 0.10	0.3 ± 0.01
C 16:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 18:1 n-9	8.7 ± 0.36	8.4 ± 0.18	8.8 ± 0.04	7.7 ± 0.25
C 20:1 n-9	0.5 ± 0.06	1.0 ± 0.31	0.4 ± 0.33	0.9 ± 0.09
C 22:1 n-7	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.01
C 24:1 n-9	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
SUM MUFA⁴	10.5 ± 0.70	11.0 ± 1.10	10.4 ± 0.54	10.4 ± 0.91
C 18:2 n-6	3.1 ± 0.60	2.5 ± 0.23	2.3 ± 0.02	1.9 ± 0.02
C 18:3 n-6	0.1 ± 0.00	0.2 ± 0.18	0.2 ± 0.04	0.2 ± 0.19
C 18:3 n-3	1.0 ± 0.56	0.3 ± 0.12	0.2 ± 0.01	0.2 ± 0.00
C 18:4 n-3	0.0 ± 0.03	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
C 20:2 n-6	1.0 ± 0.45	0.6 ± 0.22	0.6 ± 0.26	0.4 ± 0.06
C 20:3 n-9	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.00	0.5 ± 0.05
C 20:3 n-6	2.4 ± 0.06	2.3 ± 0.32	2.0 ± 0.16	1.6 ± 0.00
C 20:4 n-6	32.5 ± 1.36	30.5 ± 2.50	30.4 ± 0.19	29.2 ± 0.12
C 20:5 n-3	0.8 ± 0.03	1.5 ± 0.44	1.5 ± 0.17	2.3 ± 0.39
C 22:5 n-6	0.7 ± 0.12	0.2 ± 0.16	0.2 ± 0.08	0.4 ± 0.08
C 22:5 n-3	0.7 ± 0.05	0.6 ± 0.02	0.7 ± 0.02	0.8 ± 0.17
C 22:6 n-3	3.6 ± 0.12	7.2 ± 0.31	8.5 ± 0.28	8.7 ± 0.25
SUM PUFA⁵	47.1 ± 3.84	46.7 ± 4.80	47.6 ± 1.64	46.7 ± 1.62

NC¹ means negative control, CC² means commercial control. Sum SFA³ also contains 15:0 and 17:0. Sum MUFA⁴ also contains 14:1n-5, 15:1, 16:1n-5, 17:1n-7, 18:1n-1, 18:1n-7, 20:1n-1, 20:1n-7, 22:1n-1 and 22:1n-9. Sum PUFA⁵ also contains 16:2n-6, 16:2n-3, 20:4n-3, 20:3n-3, 22:2n-6 and 22:4n-6.



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