Sustainable production of high quality Atlantic salmon fillets

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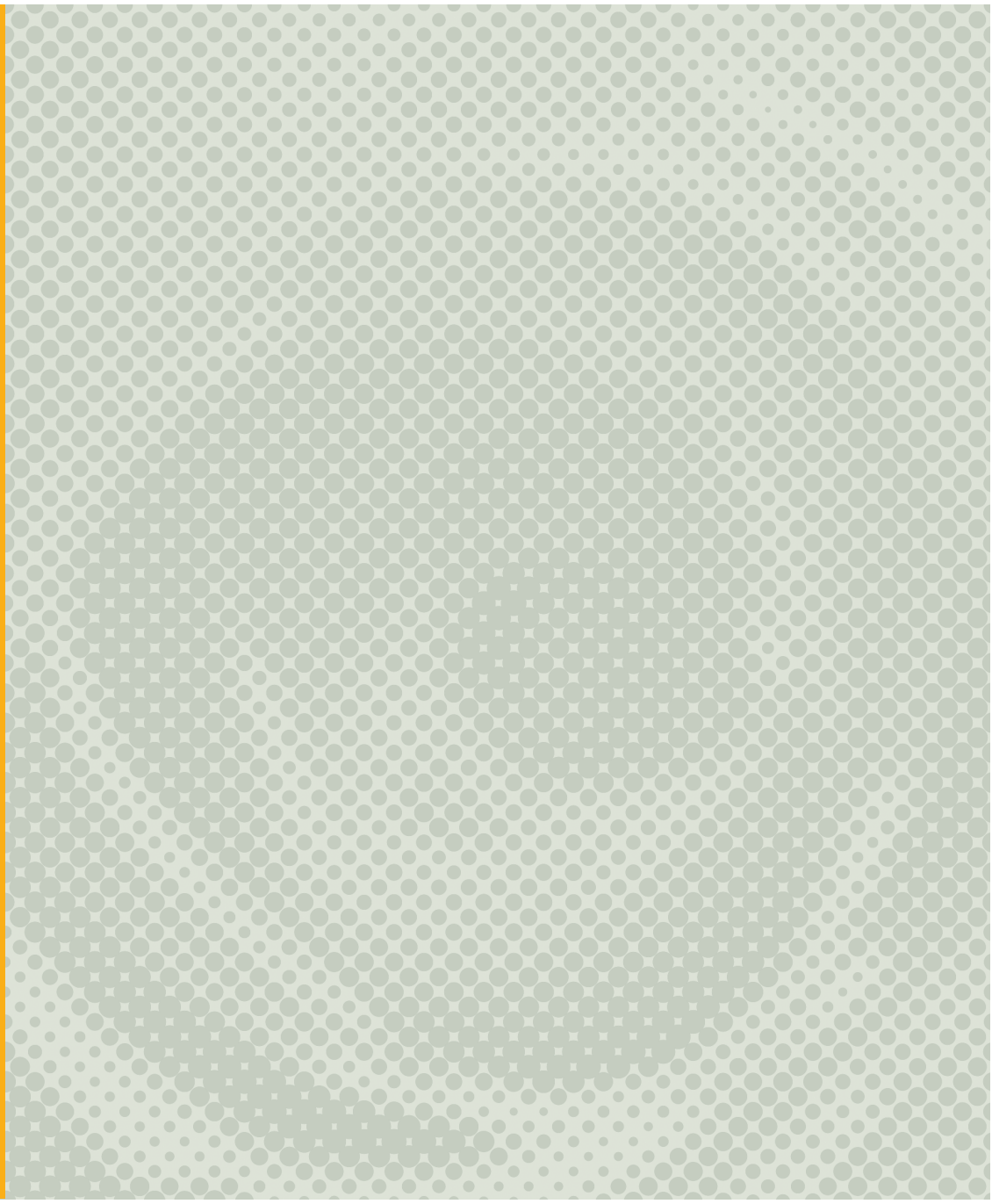

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SUMMARY

The aim of study was to identify the effect of Atlantic salmon (*Salmo salar)* energy status in the late summer on fat accumulation in the autumn, and to investigate whether it is possible to improve utilization of limited dietary marine oils by feeding salmon high content of omega-3 rich oils during the season where salmon are accumulating fat before the winter.

The present study was conducted with Atlantic salmon (1+ smolt) transferred to sea in July 2010. In May 2011, 1950 fish with an initial mean body weight of 1 kg were pit-tagged and distributed into three net pens. During the period from May to August three different pre-diets were used: *FPD*, 34% fat; *MPD*, 18% fat, 100% ration and *LPD*, 18% fat, 50% ration of the MPD. In August 2011 the fish were redistributed into eight net pens, four net pens for each of two main diets: *VO*, a standard diet with a lipid fraction composed by 70% rapeseed oil and 30% South American marine fish oil, and *MO* — a standard diet with a lipid fraction composed by 70% South American marine fish oil and 30% rapeseed oil. From November to the termination of the experiment in December 2011, the group that had been given the MO diet was switched to the VO diet. The sampling points were August (before the start of fat accumulation), October (the period of intensive fat accumulation) and December (no fat accumulation). Length, whole body, gutted body, fillet and organ weights were recorded. Proximate and fatty acid analyses were performed on fillet segments, viscera, liver and heart.

The aim of pre-dietary treatment was to produce salmon with different fat content and growth potential. The FPD group with initially high body weight and muscle fat content had lowest growth and the fat accumulation rate during the experimental period compared to the MPD and LPD groups. Viscera mass index significantly decreased in the FPD fish and increased in LPD fish. The development of lipid content in viscera, liver and heart showed the same tendency as development of lipid content in skeletal muscles. During the experimental period the fat accumulation rate in viscera and liver was the lowest in the FPD group and highest in the LPD group. Liver mass index increased significantly during the experimental period in the LPD fish that indicates high intensity of metabolic processes in this group. Heart mass index and the rate of fat accumulation in heart were the highest in the LPD group as well.

The main dietary treatment that started in August had no effect on body weight, but the slaughter yield, in December showed significant lower for the MO group. The main dietary treatment influenced viscera and heart mass index, and viscera and liver fat content.
The most important result of the main-dietary treatment was the variation in fatty acid composition of organs and tissues in Atlantic salmon. The special focus was on polyunsaturated fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA+DHA percentage in muscle lipids significantly increased in the MO group compared to the VO group after 10 weeks of feeding the main dietary treatment. After the switching the MO diet to VO diet, which had the low content of EPA and DHA, the percentage of these fatty acids decreased insignificant in muscle fat, that indicates the high intensity of fatty acids retention in muscles of Atlantic salmon in autumn. The other examined tissues changed the fatty acid profile accordant with fatty acid composition of the diet. It is suggested that improved sustainable utilization of marine fish oil may be achieved through feeding fish lower levels during periods where the fish is utilizing lipid for energy production and elevated levels in periods with high fat retention; i.e. using elevated levels of dietary fish oil during the autumn for Atlantic salmon.

Keywords: Atlantic salmon, Fat accumulation, Fatty acids, Fish quality, EPA, DHA, Sustainable production.

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1. INTRODUCTION

The world population exceeded seven billion people in 2012 and current projections show a continued increase to 7.5—10.5 billion people by 2050 (United Nations, 2011). State of World Fisheries and Aquaculture 2012 (FAO 2012) reveals that the sector produced a record 128 million tonnes of fish for human food - an average of 18.4 kg per person - providing more than 4.3 billion people with about 15 percent of their animal protein intake. While capture fisheries production remains stable, aquaculture production is one of the fastest-growing animal food-producing sectors. Hence, additional supply of food fish will have to come from aquaculture to maintain at least the current level of per-capita consumption of aquatic foods. According to Tacon and Metian (2008), the finfish and crustacean aquaculture sector is highly dependent upon marine capture fisheries for sourcing key dietary nutrient inputs, including fish meal and fish oil. Meeting the future demand for food from aquaculture will largely depend on the availability of quality feeds in the requisite quantities, without increasing the use of wild fish resources as ingredients in the feed.

In the world aquaculture production of diadromous fish, Atlantic salmon is the dominating fish specie, with a total production of 1.61 million tons in 2011. Norway is the world-leading producer and exporter of salmon, with a total production of one billion tons in 2012 (Norwegian Seafood Council). Consuming salmon is considered to be healthy because it contains high content of quality proteins, omega-3 fatty acids, vitamins and minerals (U.S. Department of Agriculture, Agricultural Research Service, 2012).

Lipid is the preferred dietary non-protein energy source of Atlantic salmon, because of their limited ability to utilize digestible carbohydrates. This bias likely stems from the fact that salmonids in the wild derive most of their energy needs from the high levels of protein and lipid in their prey. In the seawater salmon naturally consume large amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and have insufficient conversion of EPA to DHA from other fatty acids to meet the requirements of these species for growth. Therefore, salmon required dietary DHA and EPA, although the specific requirement is not determinate for various life cycle stages. The fatty acid profile of salmon muscle tends to reflect the profile in the diet (Bell et al., 2003a), but the extent to which this occurs can depend on many factors, such as concentration and profile of fatty acids in the

feed (Tocher et al., 2003), specific tissue and lipid fractions (Aursand et al., 1994), water temperature and physiological state (Shearer et al., 1994).

Fish oil is the traditional source of lipid for salmon feeds, because it is a rich source of the dietary essential fatty acids. According to Tacon and Metian (2008), salmon is the largest consumer of fish oil among all farmed fish species with and estimated consumption of 40-43% percentage on dry feed basis. The global supply of marine lipids is already insufficient to meet the traditional inclusion of oils in salmon feed. The challenge of finding environmentally and economically sustainable sources of fish-feed ingredients raises questions about the future suitability and availability of fish oil. As the demand for fish oil exceeds relative to supply, the price increases, making other lipid sources economically competitive, including vegetable oils. The trend toward an increasing demand for fish oil in a market of static or dwindling supply further supports the need to investigate the suitability of dietary non-fish lipid sources for the rational utilization and sustainable production of salmon rich in omega-3 according to physiological stages of fish and consumer demands.

Objectives

The main focus of the present study was to elucidate the impact of seasonal endogenous rhythms of Atlantic salmon on lipid accumulation and fatty acid deposition in different organs and tissues and the possibility of influencing these processes by altering the feed composition according to season.

Specific aims:

- Influence the energy status before autumn fat accumulation and evaluate the importance of energy status for the growth and fat accumulation in Atlantic salmon.
- Elucidate fatty acid composition in various tissues when feeding salmon diets with high and low EPA and DHA content in the period of high fat retention (i.e. Autumn) and estimate stability of the fatty acid composition.

2. THEORETICAL BACKGROUND

2.1 Fish quality

"Tell me what you eat, and I will tell you what you are." This phrase belongs to a famous French epicure and gastronome of 18-th century Jean Anthelme Brillat-Savarin and can be used as an epigraph not only to this work, but also to the papers about food quality in general. Consequently, balanced nutrition is an important condition for quality of life, health and well-being. Food should therefore be tasty, appetizing, easy to prepare, in addition to being healthy and easy to digest. Fish products meet these and other expectations.

Definition and aspects

There are many definitions of quality. International Organization for Standardization defines quality as "the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs" (ISO, 1986). The total quality of seafood includes two aspects: primary quality and secondary quality of the product. The primary quality depends on production and processing of marine organisms. The secondary quality relates mainly to market and customer. There are many important conditions that determine the superior final product with high biological, sensory, nutritional (fat, protein, vitamins and minerals), technological, hygienic and ethical quality (Nortvedt et al., 2007).

The fish quality, especially its nutritional side, is tightly related to biological conditions of fish; i.e. the species, sex, size, health status of fish and the season of slaughtering directly define its muscle composition (Haard, 1992). Not less important factor is the diet and feeding regime that affect directly the chemical composition and sensory properties of flesh and indirectly the health of fish.

Nutritional quality of Atlantic salmon

Fish is a good source of highly digestible protein with advantageous amino acid composition for the human health. Additionally fish is the main source of long-chained polyunsaturated omega-3 fatty acids (PUFA) eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) for which substantial scientific support for health benefit exists.

Since the human body cannot produce omega-3 fatty acids in the amounts necessary for good health, it is essential to consume PUFAs through the diet. Dietary recommendation for Scandinavian adults and children over 3 years is 450 mg per day (Nordic Council of Ministers, 2004). According to U.S. Department of Agriculture, Agricultural Research Service (2012), cooked salmon fillets provides 2.2 g / 100 g (wild salmon) and 1.9 g / 100 g (farmed salmon) of omega-3, making Atlantic salmon is the one of the best sources of EPA and DHA. This means that according to European standards, eating one salmon meal per week supplies the biological requirement in PUFAs.

Atlantic salmon is also a good source of key micronutrients (calcium, iron, magnesium, selenium, zinc) and vitamins (A, B, D, E) (U.S. Department of Agriculture, Agricultural Research Service, 2012). Salmon meet therefore the high living standards of the modern world and can be considered a healthy food.

2.2 Fish lipids

Lipids and proteins are the major organic constituents of fish. Lipids are chemical compounds used primarily for energy storage, membrane structure, isolation and hormones (Watanabe, 1982). Fish species can be divided into 3 groups by fat content in muscle: lean, middle-fatty and fatty fish (Opplysningsutvalget, 1987). Salmonids belong to middle-fatty or fatty fish that have low fat level in the liver and relatively high, uneven distribution of fat in the muscles (Lynum, 2005).

The main classes of fish lipids

Lipids in fish, including salmon, can be divided into two groups, polar and neutral lipids. Polar lipids are composed principally of phospholipids (PL) and they are essential components of biological membranes. Phosphatidylcholine (PC) is dominating among PL (Tocher,2003).

Neutral lipids are composed principally of triacylglycerols (TAG). TAG is the primary storage molecule in fish and the dominant lipid class in the major of tissues in salmon. The ratio between TAG and PC in selected tissues of Atlantic salmon is shown in Table 2.1. Increase in total flesh lipid in salmon is negatively correlated with PL content and positively correlated with TAG content in the flesh (Bell et al., 1998)

Table 2.1 Lipid content (% of total lipid depot) and proportion of triacylglycerol (TAG) and phosphatidylcholine (PC) in lipids (% of total lipid) of belly flap, red and white muscle, viscera and liver from farmed Atlantic salmon (Aursand et al., 1994).

	Belly flap	Red muscle	White muscle	Viscera	Liver
Lipid content	13.7	7.8	35.4	11.7	0.4
TAG	98.8	96.0	93.3	93.1	9.4
PC	1.2	3.0	4.4	2.7	43.3

Metabolism and deposition of dietary lipids in Atlantic salmon

There are four tissues playing plain the major role in the lipid homeostasis: gastro-intestinal tract, liver, muscle tissue and adipose tissue, and blood and lymphatic vessels connecting them.

Lipid digestion and absorption take place in pyloric caeca. The salmon bile salt-activated lipase from pancreatic tissue is probably capable to complete hydrolysis of TG to free fatty acids (FA) and glycerol (Olsen and Ringoe, 1997). Dietary phosphoglycerides are digested by pancreatic phospholipases to 1-acyl lysoglycerophospholipids and free FA. The intestinal mucosal cells absorb the products of lipid digestion (Henderson and Tocher, 1987). In the intestinal cells absorbed products are re-esterified to TG and phospholipids (Henderson and Tocher, 1987).

Lipid transport starts with export of re-esterified products from the intestine. They are then transported to skeletal muscles and to the liver as chylomicrons via the blood or lymphatic system (Henderson and Tocher, 1987). From the liver, lipids in the form of very low-density lipoproteins (VLDL) are transported to peripheral tissues, Plasma VLDL levels in fish are therefore directly related to their ability to store lipid in specific storage sites as opposed to the liver (Babin and Vernier, 1989).

The tissue for the long-term storage of lipid in Atlantic salmon is the adipose tissue. Fat inclusions of adipose cells are composed mostly of neutral fat (Napolitano, 1965, reviewed by Aursand et al., 1994). Energy stored in the form TAG in adipose tissue can be used in the high-energy demand periods (Jezierska et al., 1982).

According to Aursand et al. (1994), 56.9% of body lipids in Atlantic salmon are stored in the edible parts (white and red muscle, belly flap). In the skeletal muscle tissue, at the termination of muscle fibers, the connective tissue elements combine to form connective tissue sheets (myosepta). These bands of connective tissue (myofibrils) infiltrate the muscle tissue. It is within myosepta that large numbers of adipocytes are located (Ackman

and Zhou, 1994). The bands of myosepta are relatively narrow in the white muscle close to the backbone and become lager as they approach the subdermal fat, red muscle and belly flap (Fig. 2.1).

The fat content in fillet decreases in the cranial-caudal direction and from the belly part to the dorsal part (Bell et al., 1998; Katikou et al., 2001) (Fig. 2.2)

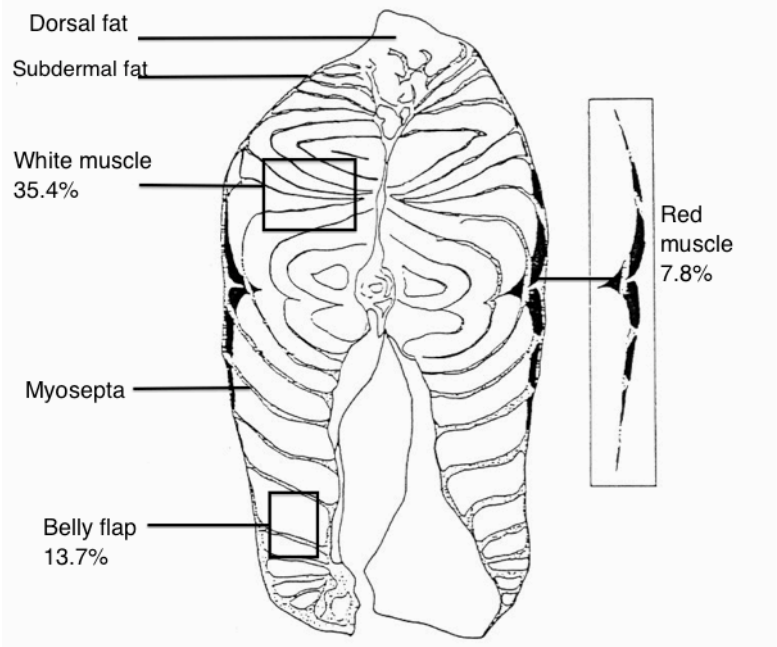


Figure 2.1 Cross section of Atlantic salmon body showing the major fat depot tissues in skeletal muscle, lipid content (% of total lipid depot) of the edible parts of salmon (Aursand et al., 1994, reviewed by Ackman & Zhou, 1994).

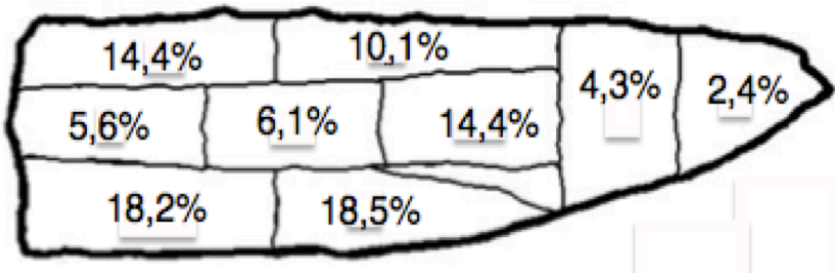


Figure 2.2 Lipid distribution within Atlantic salmon fillet (Katikou et al., 2001).

Visceral adipose tissue of Atlantic salmon is another lipid storage site accounted for from 12% (Aursand et al., 1994) to 40% (Morgan et al., 2002) of the total body lipids. Previously the visceral lipid was supposed to be a more mobile fat depot compared skeletal lipids, i.e. the turnover of lipid in viscera was considered to be higher than in skeletal muscle (Jezierska et al., 1982). An experiment performed by Einen et al. (1998) did not confirm

this suggestion. We need therefore more information about discrimination between lipid depots in the fish metabolism.

Seasonal variations in fat content of Atlantic salmon in seawater phase

In general, feed ration variations could alter the whole body weight and the fat content of salmonids (Shearer, 1994; Einen et al., 1998). In addition, the fat accumulation in Atlantic salmon, and distribution between and within tissues is a dynamic process depended strongly on the season (Henderson and Tocher, 1987). The light-dark cycle and temperature induce metabolic changes in salmonids and influence their feeding rate and utilization, growth rate, energy retention and deposition (Smith et al., 1993).

Energy and fat retention and muscle fat content were significantly reduced in 1+ smolts from their sea transfer in May until July in the experiment performed by Alne et al. in 2006 (Fig. 2.3). During the spring the fish degrades deposited fat and converts this to accessible energy.

a) b)

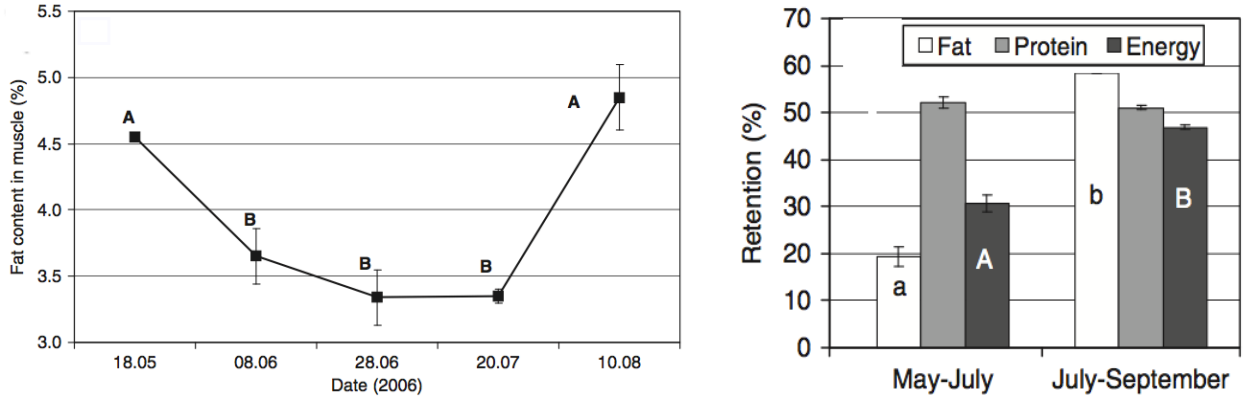


Figure 2.3 Changes in the muscle fat content (a) and retention of nutrients (b) for 1+ Atlantic salmon after sea transfer (Alne et al. 2011). Significant differences between sampling dates and periods are indicated by different letters on the curves/bars. The variation between net-pens within sampling dates/periods is given as the standard error of the mean.

Aksnes et al. (1986) reported significant fat accumulation during the autumn in immature 1+ salmon in their third year in seawater. Likewise, the most substantial fat increase was observed from July to November in 0+ and 1+ smolts after transferring into the seawater by Mørkøre and Rørvik (2001) and Alne et al. (2011). The accumulation of fat is also rapid in 0+ Atlantic salmon during the second autumn in the sea (Mørkøre and Rørvik, 2001; Roth et al., 2005). The results obtained by Alne et al. 2011 from two commercial sites, show

that salmon with low fat content in the spring accumulated fat during the autumn (Fig. 2.4). Fat deposition during autumn is controlled endogenously (Shearer, 1994). In the late summer the day length declines and salmon start fat accumulation in fillet, viscera and carcass due to the evolutionary genetic program of preparation for the cold winter season (Duncan et al., 2002).

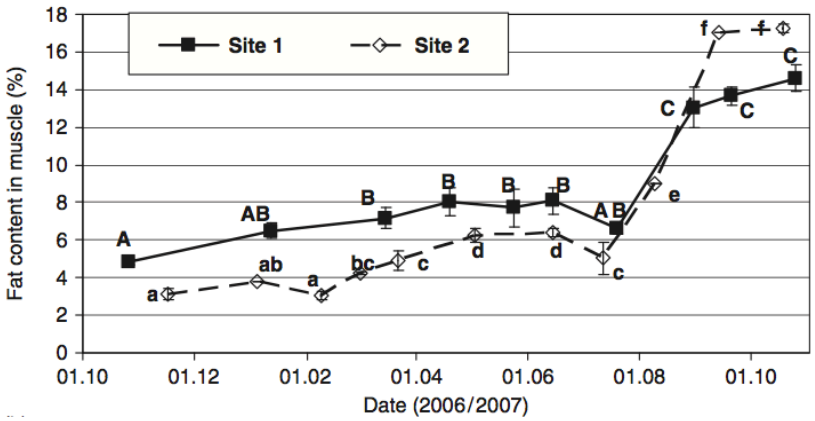


Figure 2.4 Changes in the muscle fat content for 0+ Atlantic salmon throughout a year (October 2006—October 2007) in the sea at two commercial farms in Norway (Alne et al., 2011). Upper case letters show significant differences between samplings at Site1 (solid line) and lower case letters show significant differences between samplings at Site 2 (broken line). The variation between net pens within sampling dates is given as the standard error of the mean.

The fat content in salmon has a tendency to decrease during the winter and early spring (Mørkøre and Rørvik, 2001; Einen et al., 1998). The drop in fat content my be caused by decreased ability to ingest sufficient feed, to store lipids or maintain their energy balance, or the muscle growth dominates over fat accumulation. During experimental starvation of fish from late January to late April (Einen et al., 1998), it was found that both protein and fat could be important sources of energy in the periods of energy deficiency. The muscle lipids were observed as the predominant energy source, followed by visceral and liver fat.
The environmental conditions including temperature and photoperiod show considerable variations along the Norwegian coast (Fig. 2.5). The seasonal fat deposition patterns in salmon therefore differ between geographical regions in Norway. In the Autumn, Northern salmon is leaner and will, in case of sufficient feed access, compensate the energy deficiency with high growth rate and high rate of fat accumulation that can influence negatively the flesh quality. Therefore, biological, seasonal and geographical factors should be considered in the process of diet composition and planning of feeding regimes for Atlantic salmon in different geographical localities.

a)

b)

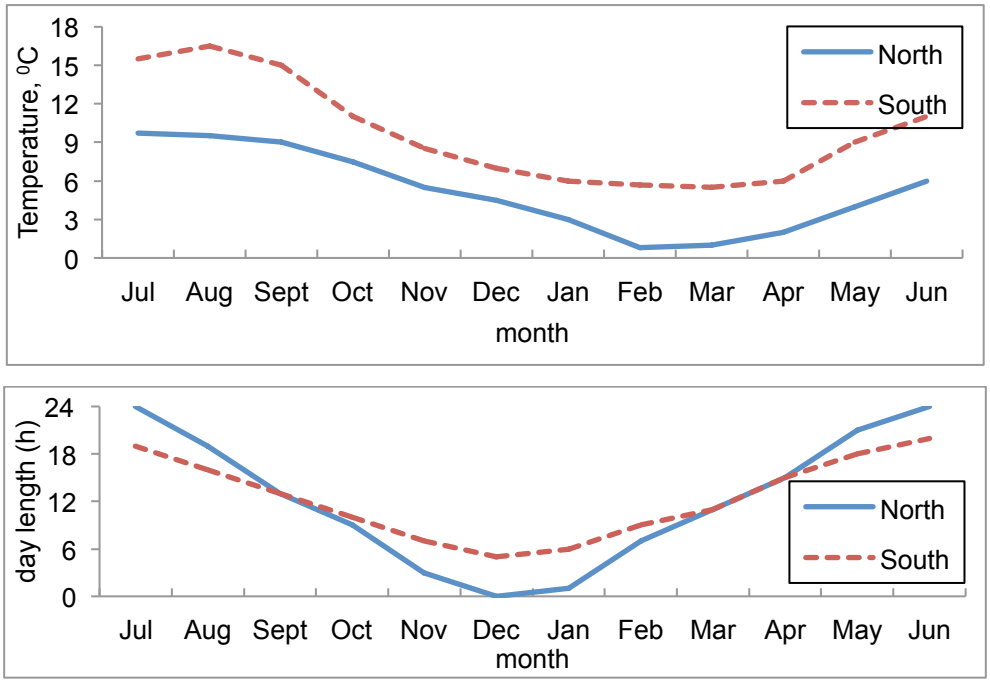


Figure 2.5 Water temperature (a) and day length (b) in Northern and Southern Norway (Rørvik, 2007).

Metabolic fates of dietary fatty acids in Atlantic salmon

Fatty acids may undergo one or several different metabolic processes in fish body depending on FA structure, nutritional state and cell type. FAs can be oxidized to provide energy, incorporated into structural phospholipids or they may be deposited as storage lipids (Kiessling & Kiessling, 1993; Henderson, 1996; Røsjø et al., 2000; Bell et al., 2001).

Fatty acid catabolism is the major source of energy. The process is termed β-oxidation. Several tissues including liver, heart, red and white muscles play significant role in FA-oxidation in Atlantic salmon (Frøyland et al., 2000). The process occurs in two cell organelles, mitochondria and peroxisomes.

Peroxisomes are incapable of producing ATP. They can only chain-shorten fatty acids and are not able to fully degrade the fatty acids into acetyl-CoA units (Wanders et al., 2001). The peroxisome works therefore primarily as chain-shortener for long and very long fatty acids (Reddy and Hashimoto, 2001). After the FAs are degraded in the peroxisomes, they can be completely oxidized in the mitochondria.

Mitochondrial β-oxidation is known to oxidize short (< C8), medium (C8-C12) and long (C14-C20) fatty acid chains (Reddy and Hashimoto, 2001). It takes place in the matrix

within the inner mitochondrial membrane (Mathews et al., 2000). FAs are activated to fatty acyl-CoA that undergoes four main steps of β-oxidation (dehydration, hydration, dehydrogenation and thiolytic cleavage) that results in FADH2, NADH and acetyl-CoA. Acetyl-CoA will further be processed in the tricarboxylic acid cycle (Mathews et al., 2000).
Results from several studies suggest that some substrates are preferred to others in mitochondrial β-oxidation. It has been found that saturated and monounsaturated FAs (16:0, 16:1, 18:1n-9, 22:1n-11) are preferred over PUFAs (Kiessling & Kiessling, 1993; Sidell et al., 1995; Røsjø et al., 2000; Bell et al., 2001).
FAs that are not oxidized can be incorporated into PL and TAG or undergo other metabolic pathways of biosynthesis (Fig. 2.6).

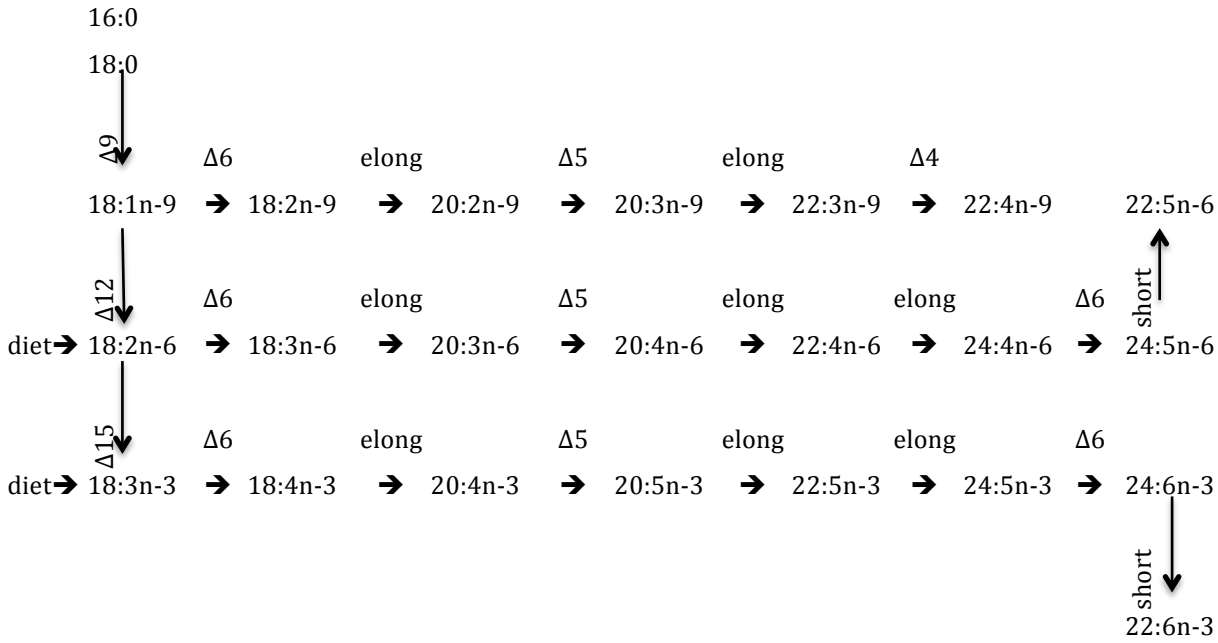


Figure 2.6 Pathways of biosynthesis of C20 and C22 PUFA from n-3, n-6 and n-9 C18 precursors. Δ (5, 6, 9, 12, 15), fatty acyl desaturases; elong, fatty acyl elongases; short, chain shortening (Bell et al., 1986).

The saturated FAs 16:0 and 18:0 are known to be synthesized *de novo* in fish and these FAs can again be metabolized to 16:1n-7 and 18:1n-9, respectively. In fish dietary C18- FAs may be elongated or desaturated to C20- and C22- FAs and the ability to do this varies between species (Ruyter and Thomassen, 1999). Salmon like all vertebrates lack Δ12 and Δ15 (ω3) desaturses to form 18:2n-6 and 18:3n-3 from 18:1n-9 and these two FAs are essential.
Salmonids produce 20:5n-3 and 22:6n-3 from 18:3n-3, and 20:4n-6 from 18:2n-6 (Ruyter and Thomassen, 1999; Sargent et al., 2002) but have only limited ability to carry out the

conversions above due to specific deficiencies in desaturases and elongases. Therefore, it is necessary to supplement the diet with certain amounts of 20:5n-3 and 22:6n-3 to meet their optimal EFAs requirement (Bell et al., 2001). Cell cultures from Atlantic salmon have been found to have a better ability to elongate and desaturate 18:4n-3 to 20:5n-3 than cell culture from turbot (Ghioni et al., 1999). It has been suggested that anadromous fish has a better ability to elongate and desaturate than marine fish, due to the FAs composition of the natural diet of the marine fish (Sargent et al., 2002).

2.3 Lipid sources in feeds for finfish

Salmonids have a limited ability to utilize carbohydrates as an energy source. Dietary lipids play a more important role in providing energy and in sparing dietary protein (Watanabe, 1982). In addition to providing energy, the dietary lipids must supply the essential fatty acids required for normal growth and development.

Lipid and fatty acid requirement

The dietary essential FA needs of salmonids largely reflect the lipid composition of their respective natural prey. The dietary lipid and FA needs of salmon vary in relation to the stage of life history. In seawater phase they consume large amounts of EPA and DHA and little 18:3n-3 in their natural diets (Sargent et al., 1995) and have little or no requirement to metabolize 18:3n-3 to EPA. Hence, there is insufficient conversion of EPA to DHA to meet the requirements of these species. In the diet, then, PUFA must be supplied.

The known dietary lipid and fatty acid requirements for some salmonids are provided in Table 2.2.

Marine and plant lipid sources in feeds for salmonids

Fish oil is the traditional source of lipid for fish feeds. Marine species commonly used for production of fish oil used in Norwegian salmon feed production are Anchoveta, Atlantic herring, Sandeel and Gulf menhaden (Bendiksen et al., 2011).

Table 2.2 Recommended dietary levels (g/kg dry weight basis and percentage of dietary lipid where established) of lipid and fatty acids for maximum growth and feed efficiency in salmonids*.

Species/ Life History Stage	Lipid (g/kg)	Fatty Acid					
		18:3n-3		18:2n-6	n-3 PUFAs		20:4n-6
		(g/kg)	(%)	(g/kg)	(g/kg)	(%)	(g/kg)
Rainbow trout							
FW (juv-ad)	150-230	8.3-16.6	≥20, ≤80	<10	20-30	≥10, ≤40	R
Chinook salmon							
FW (juv)	>63-200	R	R	≤26	R	R	
SW (p/juv, <500 g)	150-200	R	R		R	R	
Coho salmon							
FW (juv)	160-180	10-25	10-25, <40	≤10	R	R	
FW (mat)		R				R	R
Chum salmon							
FW (juv)	55-109	10		10		10	
SW (juv)		10		10		10	
Atlantic salmon							
FW (juv, 80 g)	240	R			R		
SW (p/juv – ad >200 g)	≥330	R			R		
Arctic char							
FW (juv)	200	10-20	20-40	≤7			
Brown trout							
SW (p/juv, 1600 g)	290						

(Cho and Cowey, 1991; Arzel et al., 1994; Yang and Dick, 1994; Yang et al., 1994; Higgs et al., 1995; Grisdale-Helland and Helland, 1997; Hillestad et al., 1998)
FW, freshwater; SW, sea water; R, required; jiv, juvenile; p/juv, postjuveniles; mat, maturating; ad, adult.
* It is assumed that the dietary levels and sources of the other energy-yielding nutrients, viz., protein and carbohydrate, are optimal and that the digestibility of lipid is ≥90%.

The increasing global production of farmed fish, the trend toward increasing the percentage of lipids in feeds for salmon and static or dwindling supply of fish oil in a market have changed the ingredient composition in aquaculture industry towards use of plant based ingredients (Fig. 2.7).

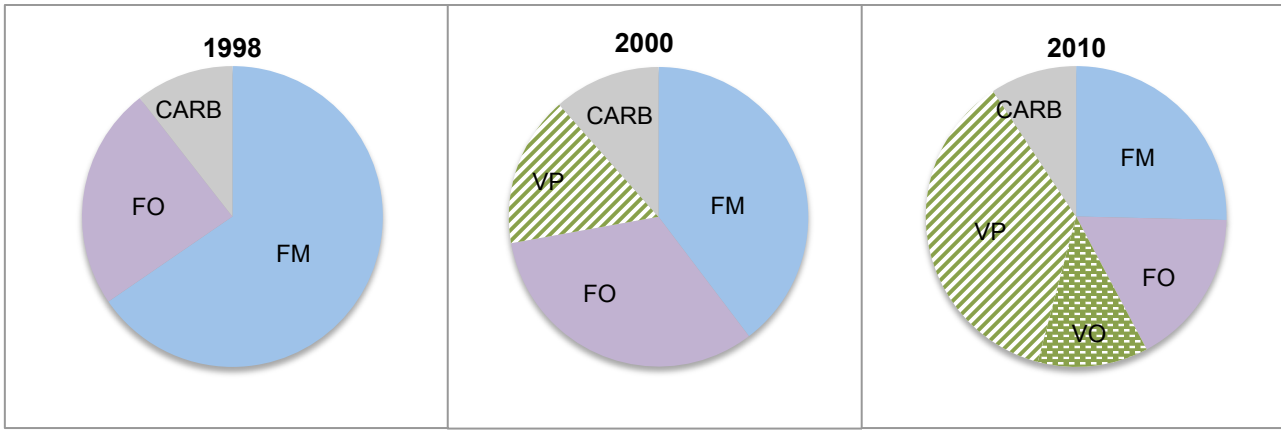


Figure 2.7 Use of fish and plant ingredients in Norwegian aquaculture in 1998, 2000 and 2010 (% used of total feed sold from three feed companies) (Bendiksen et al., 2011).
FM, fish meal; FO, fish oil; VO, vegetable oil; VP, vegetable protein; CARB, carbohydrates.

The major types of vegetable oils produced in 2007-2008 and used in feeds for salmonids were soybean, rapeseed and sunflower oil (Gunstone, 2011).

Marine fish oils are rich in n-3 FA (EPA, DHA) and vegetable oils typically have a high content of n-6 PUFA. Both animal and plant lipid sources can vary in FA-profiles not only between species but also within the species (Tab. 2.3).

Table 2.3 Fatty acid compositions of fish oils and plant oils commonly used in aquaculture production.

Fatty acid	Anchovy	Herring	Capelin	Menhaden	Rapeseed oil	Soybean oil	Sunflower oil	Corn oil
14:0	6.5-9.0	4.6-8.4	6.2-7.0	7.2-12.1	0.2			
16:0	17.0-19.4	10.1-18.6	10.0	15.3-25.6	2.8-5.9	7.0-12.0	3.0-10.0	8.0-19.0
18:0	4.2	1.4	1.2	4.2	1.0-2.4	20-5.0	1.0-10.0	0.5-4.0
20:0					0.4-0.6			
16:1	9.0-13.0	6.2-12.0	10-14.3	9.3-15.8	0.1-0.6	0.5	1.0	0.5
18:1n-9	10-22.0	6.2-12.0	14-15.0	8.3-13.8	53.4-64.6	19-30.0	14.0-65.0	19-50.0
20:1	0.9-1.0	7.3-25.2	17.0	n.d.-1.0				
20:1n-9					0.7-1.6			
22:1n-9					0.2-0.8			
22:1n-11	1.0-2.1	6.9-30.6	15.4	n.d.-1.4				
18:2n-6	2.8	0.1-0.6	0.7	0.7-2.8	18.8-22.9	45-58.0	20.0-75.0	34-62.0
18:3n-3	1.8	n.d.-2.0	0.2	0.8-2.3	7.6-12.9	4.0-10.0	0.7	2.0
20:5n-3	7.6-22.0	3.9-15.2	6.1-8.0	11.1-16.3				
22:5n-3	1.6-2.0	0.8	0.6	2.0				
22:6n-3	9.0-12.7	2.0-7.8	3.7-6.0	4.6-13.8				

(U.S. Department of Agriculture, Agricultural Research Service, 2012)

Effect of dietary lipids on body composition

Tissue FA composition of fishes largely reflects the diet (Shearer, 1994), and thus fishes fed marine-derived oils contain substantial amounts of EPA and DHA compared with those fed diets with high inclusion of plant oils (Berge et al., 2009). Considerable changes occur in the FA-profiles of the fish tissues with more pronounced effect in the storage lipids compared with the membrane lipids. Main effect is a reduction in n-3 FA, mainly EPA and DHA, and an increase in linoleic acid (18:2n-6) with increasing proportions of plant oils in the diet (Bransden et al., 2003). Salmon fed plant oils-based diets for the majority of the production cycle have been fed finishing diets with high content of marine oil to restore the FA profile (Bell et al., 2003, 2004).

2.4 Sustainability

Sustainability is a way to secure future needs by maintaining the diversity of resources in order to be able to cope with and adapt to future conditions.

Marine fish oils become a limiting factor for the growing fish farming industry in the coming years due to limitations in global supply. Global fish oil production from marine capture fisheries have been decreasing at average rates of 2.6 percent in period since 1994 to 2009 (FAO, 2012).

Future development of aquaculture will rely on development of additional sustainable raw materials. Vegetable oils can partially replace fish oils in salmon feeds (Rosenlund et al., 2001) and the use of plant oils in fish feed would improve the sustainability of production of farmed carnivorous fish, such as Atlantic salmon (Tacon and Metian, 2008).

The alternative way is the rational utilization of marine oil.

3. MATERIALS AND METHODS

3.1 Fish and experimental design

The study was performed in 2011 at Nofima Sea Water Research Station at Averøy, on the west coast of Norway (62 ^{o}N). In July 2010, 15 800 Atlantic salmon (*Salmo salar L.*) were transferred to sea (1+), with an average weight of 62 g. The fish originally came from "Urke fiskeoppdrett" and were vaccinated including PD.

At start up of the experiment in May 2011, 1950 fish were randomly selected from the original population. The average weight of the fish was approximately 1 kg. The weight and length were recorded and pit tags were inserted into the body cavity of each individual fish before they were distributed into three net pens (7x7x7 m; 650 fish in each net pen). From May to August the fish were fed two different pre-diets with 34 or 17.5% fat or the lean diet at half ration. The aim by providing the fish these treatments was to produce three groups of salmon with high, medium and lean muscle in August, before the fish were entering the fat accumulation period.

In August the fish were redistributed into eight net pens (5x5x5 m; 150 fish in each net pen), four net pens for each of two dietary treatments (Fig. 3.1). The composition of the main diets was the same and based on a commercial formulation, but the oil source differed. For the diet Rapeseed 70 (VO), the oil fraction was composed by 70% rapeseed oil and 30% South American fish oil, while the diet Marine 70 (MO) was composed by 30% rapeseed oil and 70% South American fish oil. A schematic presentation of the total experimental design is given in Figure 3.1, while distribution of net pens in the experiment with the main diets is shown in Figure 3.2. Composition of the pre-diets and main diets are given in Table 3.2 and 3.2, respectively.

During the period when the fish were fed the main diet, all net pens were located at the same pier to minimize environmental effect. The net pens were divided into two blocks depending on the position on the pier (Fig. 3.2). Salmon from block 1 was analyzed in October and December; salmon from block 2 was analyzed in December only in order to minimize handling stress of fish.

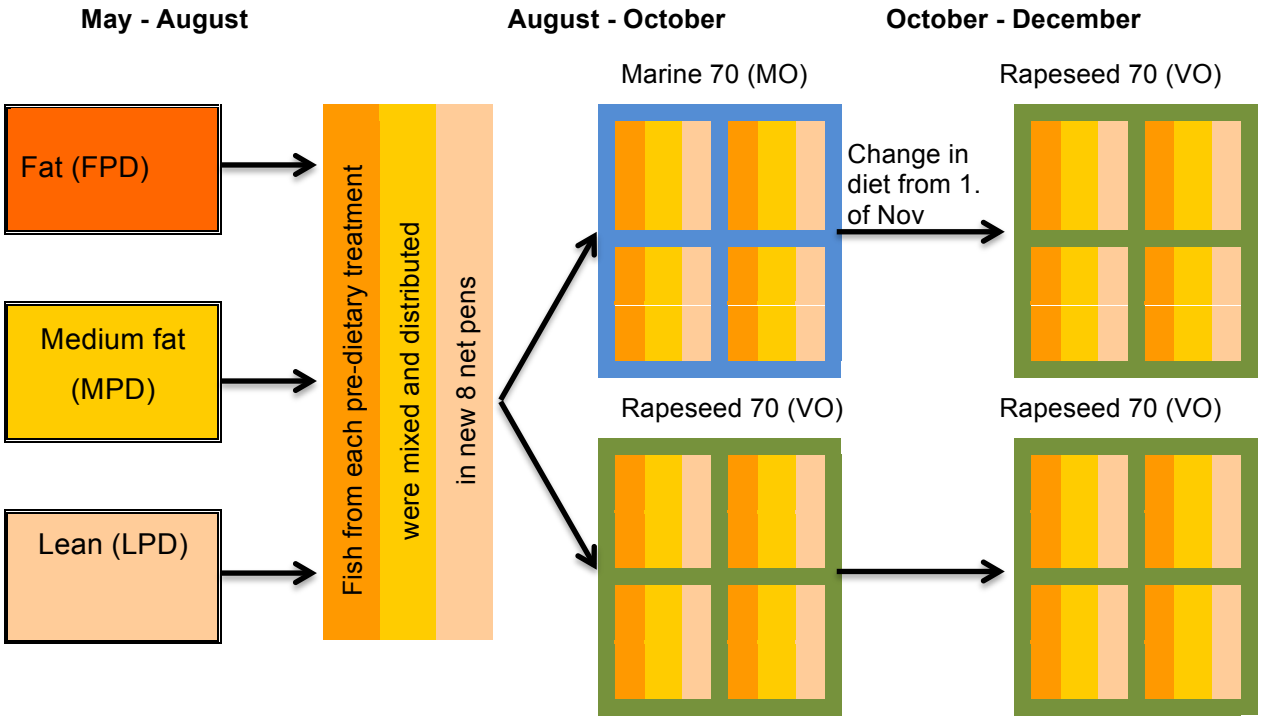


Figure 3.1 Overview of the experimental setup.

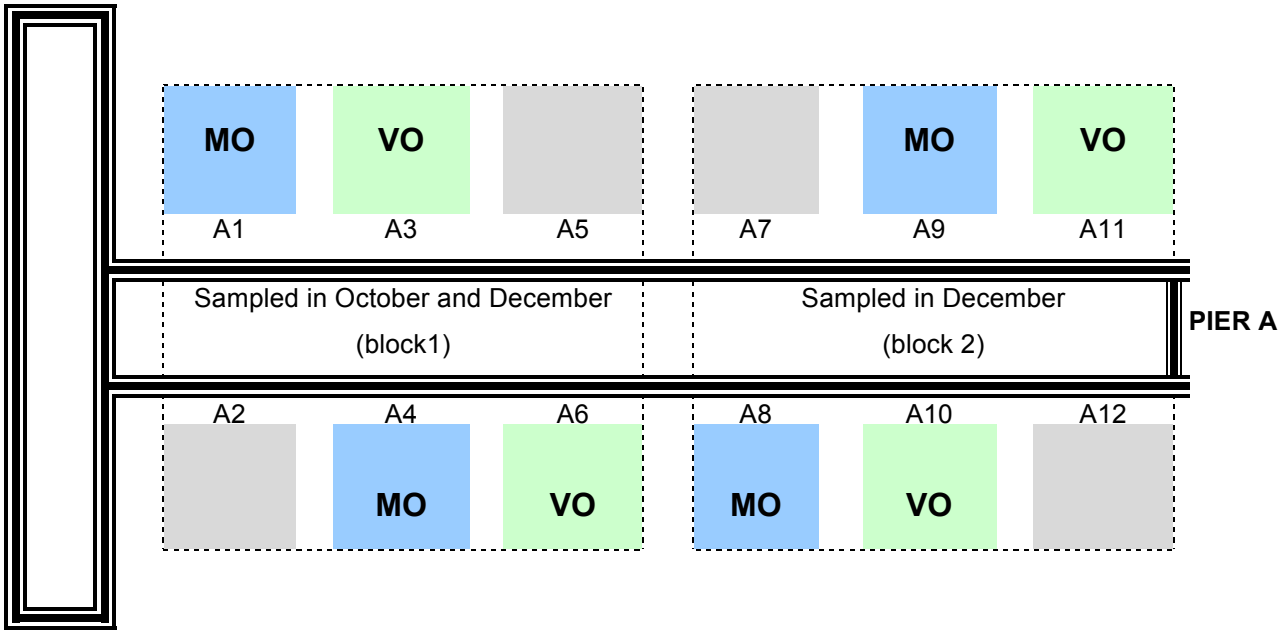


Figure 3.2 Overview of the net pens, where A1-A12 is the number of net pen and MO, VO represents the two main diets. The gray squares in the figure are net pens provided with diets that were not included in the present study.

3.2 Diets and feeding

The fish were fed in excess 3-4 times a day during the whole experimental period, except for the group fed the lean pre-diet at half ration and in periods of starvation prior to handling. All net pens were equipped with a system for collecting uneated pellets, as described by Einen et al. (1998). The group fed the lean pre-diet at half ration was fed 1-2 times a day half amount of the feed provided the full fed group on the lean diet, calculated based on the feed consumed the day before. The fish fed the lean diet at half ration were fed less frequently to ensure that all fish got access to feed and to avoid strong competition between individual fish. The diameter of pellets was 7mm for all type of feed.

Pre-diets (May — August)

The aim of pre-dietary treatment was to produce salmon with different fat content and growth potential. There were three pre-diets:

- fat (FPD) — a commercial salmon feed with a fat level of 34%;
- medium fat (MPD) — a commercial cod feed with a fat level of 18%, coated with astaxanthin (100% ration);
- lean (LPD) — a commercial cod feed with a fat level of 18%, coated with astaxanthin (1/2 ration of that the MPD group consumed the day before).

The macronutrient composition of the pre-diets is shown in Tab. 3.1.

Table 3.1 Macronutrients in the pre-diets.

Macronutrients in the pre-diets[1]	FPD	MPD, LPD
Crude protein Kjeldahl N*6.25 (%)	33.5	49.9
Ash (%)	4.6	7.2
Lipid (%)[2]	34.1	17.5
Starch (%)	9.3	6.2
Total dry matter (%)[3]	93.4	91.7
Free astaxanthin (mg/kg)[4]	46	<1

[1] The feed have been was analyzed by Nofima BioLab, Bergen (www.nofima.no/ingrediens)
[2] Amount of marine oils were similar in both diets.
[3] Vitamins and minerals were not analyzed, but included in the total dry matter.
[4] Analysis was done prior to astaxanthin coating of the diets.

Main diets (August — December)

There were 2 main diets:

- Rapeseed 70% (VO) — a standard diet with a lipid fraction composed by 70% rapeseed oil and 30% South American marine fish oil,

- Marine70% (MO) — a standard diet with a lipid fraction composed by 70% South American marine fish oil and 30% rapeseed oil.

Fish were fed these two diets in the period from August to November. From 1. November to the termination of the experiment in December, the group that had been given the MO diet, were switched to the VO diet.

The macronutrients composition of the main diets is shown in Tab. 3.2.

Table 3.2 Macronutrients in the main diets.

Macronutrients in the pre-diets[1]	Marine70	Rapeseed70
Crude protein Kjeldahl N*6.25 (%)	41.7	41.8
Ash (%)	4.9	4.9
Lipid (%)[2]	31.7	32.4
Starch (%)	6.8	6.4
Total dry matter (%)[3]	93.8	94
Free astaxanthin (mg/kg)	52	51

[1] The feed have been was analyzed by Nofima BioLab, Bergen (www.nofima.no/ingrediens).
[2] The analysis was performed prior to 2.5% fat coating of the diets
[3] Vitamins and minerals were not analyzed, but included in the total dry matter.

The fatty acid compositions of the pre-diets and the main diets are shown in Tab. 3.3.

Table 3.3 Fatty acid compositions (% of total fatty acids) of the experimental feeds.

	FPD	MPD, LPD	Rapeseed70	Marine70
C 14:0	3.8	6.2	2.4	4.9
C 15:0	0.3	0.5	0.3	0.3
C 16:0	9.5	16.2	8.5	12.7
C 17:0	0.1	0.6	0.4	0.9
C 18:0	2.3	2.2	2.7	3.3
C 22:0	0.0	0.1	0.9	0.8
C 16:1n-7	3.9	7.3	2.9	6.0
C 18:1n-7	2.7	2.5	0.2	0.1
C 18:1n-9	32.4	14.0	41.7	26.6
C 20:1n-9	6.6	4.5	1.5	1.4
C 20:1n-11	1.3	2.2	0.8	0.5
C 22:1n-9	0.8	0.6	0.5	0.3
C 22:1n-11	7.5	6.7	0.9	1.4
C 24:1n-9	0.4	0.7	0.3	0.3
C 16:3n-4	0.3	0.7	0.5	1.0
C 18:2n-6	10.6	5.1	13.8	8.1
C 18:3n-3	4.6	1.6	6.4	3.4
C 20:4n-3	0.5	0.6	0.0	1.8
C 20:4n-6	0.2	0.6	0.4	0.8
C 20:5n-3	3.5	9.8	4.6	10.2
C 22:5n-3	0.4	1.2	0.6	1.3
C 22:6n-3	3.3	9.9	3.4	7.3
Total EPA/DHA	6.8	19.7	8.0	17.5
Total n-0	16.7	26.4	15.1	22.8
Total n-3	13.2	23.2	15.3	24.4
Total n-6	11.3	6.5	14.5	9.4

Fatty acids <0.3 are not shown in the table.

3.3 Samplings and recordings

Seawater temperature

Seawater temperature was registered at 3 meters depth, every day through out the whole experimental period (Fig. 3.3).

In the period May-August the average temperature was 10.7^{0}C, while in August-December it was 10.9^{0}C. The coldest month was December with an average temperature of 6.9^{0}C whereas September was the warmest month with an average temperature of 14.1^{0}C (Tab. 3.4).

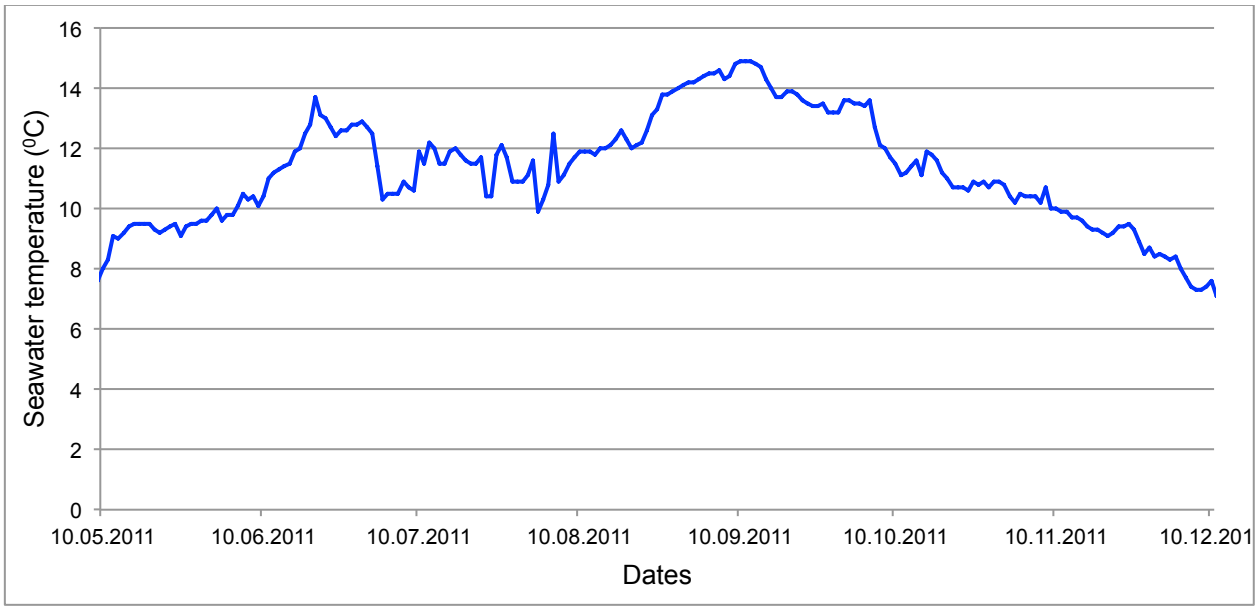


Figure 3.3 Seawater temperature (^{0}C) at 3 m, from the start to the end of experimental period.

Table 3.4 Average seawater temperature (^{0}C) at 3 m in the period from May to December 2011.

	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean t (^{0}C)	8.77	11.60	11.31	12.26	14.07	11.62	9.60	6.87

Sampling

Samples of the fish were taken four times during the experiment: at starting point in May, entering autumn in August, during autumn in October and at termination in December (Tab. 3.5). Certain numbers of fish from each net pen, representing the average with regard to body weight were randomly sampled for analysis at the sampling points.

Table 3.5 Overview of the sampling dates, number of sampled net pens, number of fish weighed and measured and number of fish taken out for further analysis.

Date	Number of net pens sampled	Number of fish (tot.)	Fish per net (average)	Fish sampled per net	Fish sampled (tot.)
10.-13. May	3	1950	650	10	30
9.-11. Aug	3	1929	643	20	60
18.-19. Oct	4	600	150	30	120
6.-9. Dec	8	960	120	30	240

All fish were taken up from the net pen in batches of 15-20 fish and anesthetized in seawater with MS 222 (Metacaine 0.1 g L-1; Alpharma, Animal Health Ltd, Hampshire, UK).

The individual pit tag number, body weight and length were recorded, thereafter the fish were returned to the net pen or taken out for further analysis.

The fish sampled for analysis were killed by percussive stunning and cut through the gills. The fish were bled in a tank with seawater for 20 minutes. For each of sampled fish, fork length, exsanguinated, gutted weight, viscera, liver and heart weights were recorded. After the manual filleting the fillet weight was recorded. The left fillets, liver, heart and intestine were packed in boxes with ice, and sent to Nofima laboratory, Ås, for storage at -20^{0}C prior to analyses.

3.4 Chemical analyses

Preparation of samples for chemical analyses

The fat content and the fatty acid composition were analyzed in muscle, visceral, liver and heart tissues. The muscle tissue was taken from the dorsal part of the left fillet under the dorsal fin (Fig. 3.4).

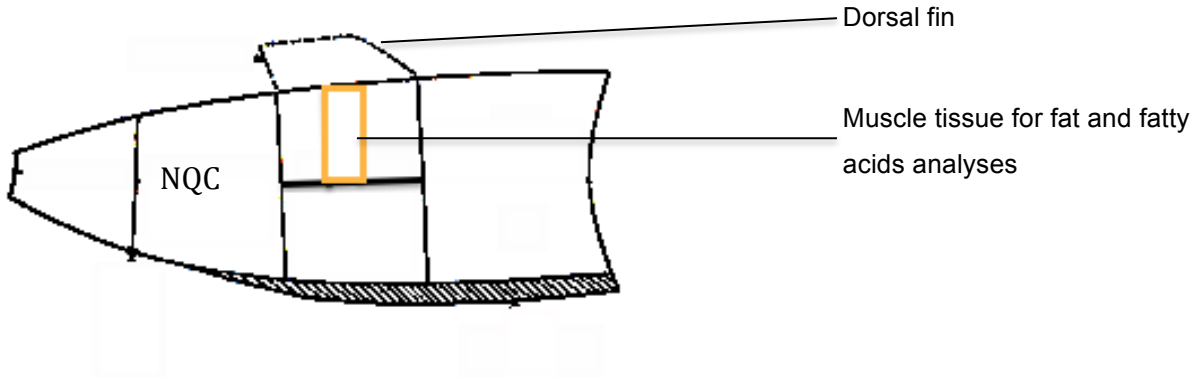


Figure 3.4 The part of the left fillet taken for analyses.

Muscle tissue and organs of 10 fish from each of three "pre-diet" treatments were sampled for analyses at each sampling time. The numbers of net pens analyzed were 3, 12 (two net pens from each main diet VO and MO; in total n=120 salmon) and 24 (four net pens from each main diet VO and MO; in total n=240 salmon) in August, October and December, respectively (Table 3.5)

Organs (viscera, liver, heart) and muscle tissue were homogenized into pooled samples (one sample was made of 10 organs/tissues, except heart (five hearts per sample), presented 1 pre-diet group of fish from one of net-pens), transferred to separate tubes (2 tubes for each sample), corked and frozen at -20^{0}C immediately to prevent oxidation.

Total lipid and fatty acid analyses

The total lipids were extracted according to method of Folch et al. (1957) and the total lipid content was determined gravimetrically (see Attachment for details).

Fat from the tissues was methylated over night with 0.2 ml of dimethoxypropane, 2 ml of methanolic HCL and 2 ml of benzene at room temperature, as described by Mason & Waller (1964) and Hoshi et al. (1973). Following, 2 ml of hexane was added and the samples were neutralized by 3 ml of 6 % $NaHCO_3$ solution. The test tubes were then corked, mixed, separated into two layers and kept at -40 °Cover night for further separation. The top layer consisting of hexane, benzene and methylated lipids was then transferred to new test tubes, dried at 60 °C with nitrogen flushing and re-dissolved in hexane. Fatty acids were separated and determined in a gas chromatograph (Hewlett Packard 6890 GC system) with a split injector SGE capillary column (length 60 m, diameter 0.25 mm and film thickness of 0.25 µm). The carrier gas was helium and the pre-selected oven program had a temperature regime that consisted of a raise from 50 to 170 °C at the rate of 4 °C/min, a raise to 200 °C at the rate of 0.5 °C/min, a raise to 300 °C at the rate of 10°C/min. The results were analyzed using Aligent ChemStation software and the relative quantity of each FA is given as the percentage of total FAs. This was found by measuring the area under the chromatograph peak for each FA.

3.5 Calculations and statistics

Calculations

Monitoring of data and calculations was carried out using Microsoft® Excel ® 2007 (Microsoft, Redmond, WA, USA). The following calculations are used in this thesis.

Condition factor (CF) was used to measure the condition of the fish and was calculated as followed:

$$CF = BW / L^3 \times 100 \quad (1)$$

BW = Whole body weight (g)
L = Fork length (cm)

Slaughter yield was calculated as:

$$\text{Slaughter yield (\%)} = GW / BW \times 100\% \quad (2)$$

BW = Whole body weight (g)

GW = Gutted body weight (g)
100 = %

To measure the relative fillets/viscera/liver/heart weight, the mass index was used:

$$\text{Mass index (\%)} = W / BW \times 100 \quad (3)$$

W = Organ/fillet weight (g)
BW = Whole body weight (g)
100 = %

The total lipid content was calculated using following equation:

$$\text{Total lipid content (\%)} = (\text{fat} * 100) / ((I * U)/TV) \quad (4)$$

fat = Evaporated sample in breaker (g)
100 = %
I = Weighted out sample
U = Transferred lipid/chloroform extract in ml
TV = The solvents total volume.

Statistical analysis

Data from the trial were statistically analyzed by analysis of variance (ANOVA) using the general linear model (GLM) statement of the Statistical Analysis Software (SAS) release 9.0 for Windows (SAS Institute Inc. Cary, NC, USA). A total of three pre-dietary and two main-dietary treatments were tested. In the statistical model, whole and gutted body weight, condition factor, slaughter yield, fillet and organs mass indexes, fat content of tissues, FA compositions of tissues and blood plasma components were used as dependent variables. Sampling date, pre-diet and main-diet were used as class variables.

The pdiffstatement was used to detect statistical differences between treatments. R^2 expresses the proportion of the variance explained by the model and equals the between-group sum-of-squares divided by the total sum-of-squares (type III). The level of significance was indicated at $P \leq 0.05$ and $P \leq 0.1$ was considered as a trend. The results are presented as lsmeans ± standard error of the mean (SE).

The main GLM model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

Y_{ijk} = response variable
μ = general mean
α_i = effect of sample time i
β_j = effect of dietary treatment j
ε_{ijk} = random fault

4. RESULTS

4.1 Body measurements

The initial mean body weight of the salmon used in the trial was 1.10 kg in May when the pre-dietary feeding started, while the initial condition factor was 1.1. The average slaughter yield was 90.3% relative to whole body weight.

Body weight, condition factor and slaughter yield for the salmon sampled in October and December are presented in table 4.1 for the pre-dietary groups fed the MO or VO diets.

Table 4.1 Average weight, condition factor and slaughter yield of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO, VO). Results are shown for salmon sampled in October and December (LSmeans ± SE). Different superscript letters indicate significant differences (*P < 0.05*) between groups within the same sampling time.

		October			December		
		FPD	MPD	LPD	FPD	MPD	LPD
Whole body weight (g)	MO	3955.5 ± 105.3^{a}	3991.0 ± 123.8^{a}	3550.0 ± 86.5^{b}	4593.3 ± 87.5^{b}	4985.0 ± 98.4^{a}	4356.3 ± 91.2^{b}
	VO	3928.5 ± 88.6^{a}	3965.0 ± 126.8^{a}	3554.8 ± 68.3^{b}	4772.9 ± 119.0^{ab}	4747.8 ± 100.1^{ab}	4476.2 ± 91.2^{b}
Condition factor	MO	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4± 0.0^{b}	1.4 ± 0.0^{ab}	1.5 ± 0.0^{a}
	VO	1.4± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0^{ab}	1.4 ± 0.0^{b}	1.4 ± 0.0^{ab}
Slaughter yield (% BW)	MO	89.8 ± 0.2^{b}	90.4 ± 0.2^{ab}	90.1 ±0.23^{ab}	89.8 ± 0.2^{c}	89.7 ± 0.2^{c}	89.4 ± 0.1^{c}
	VO	90.1 ± 0.2^{ab}	90.5 ± 0.3^{a}	90.3 ± 0.2^{ab}	90.3 ± 0.2^{b}	90.8 ± 0.2^{a}	90.0 ± 0.2^{bc}

Body weight

In the period from August to December the body weight was significantly affected by the pre-dietary treatment (Fig. 4.1.a). The body weight in August was 2.7 kg for the FPD group and 2.5 kg for the MPD group. The LPD group had significantly lowest body weight, averaging 1.8 kg (*P < 0.0001*).

The whole body weight of salmon from the LPD group was still significantly lowest in October (3.6 kg, *P < 0.002*) and December (4.4 kg, *P = 0.05*) in comparison to the FPD (3.9 and 4.7 kg in October and December, respectively) and MPD (4.0 and 4.9 kg in October and December, respectively) groups.

No effect of the main diets on body weight was detected during the experimental period. The MO and VO groups had a body weights of 3.83 and 3.82 kg, respectively, in October, and 4.64 and 4.67 kg, respectively, in December.

Condition factor (CF)

As shown in figure 4.1.b, the pre-diets were shown to have a significant effect on CF in August (*P < 0.0001*). At this sampling, the LPD group had significantly lower CF (1.0 ± 0.01) than the FPD and MPD groups (1.2 ± 0.02 for the both) (fig. 4.1.b). No significant differences between pre-dietary groups were detected in October. In December there was a trend (*P = 0.11*) towards a higher CF for the LPD group (1.45 ± 0.01) compared to the FPD (1.41 ± 0.01) and MPD (1.42 ± 0.01) groups.

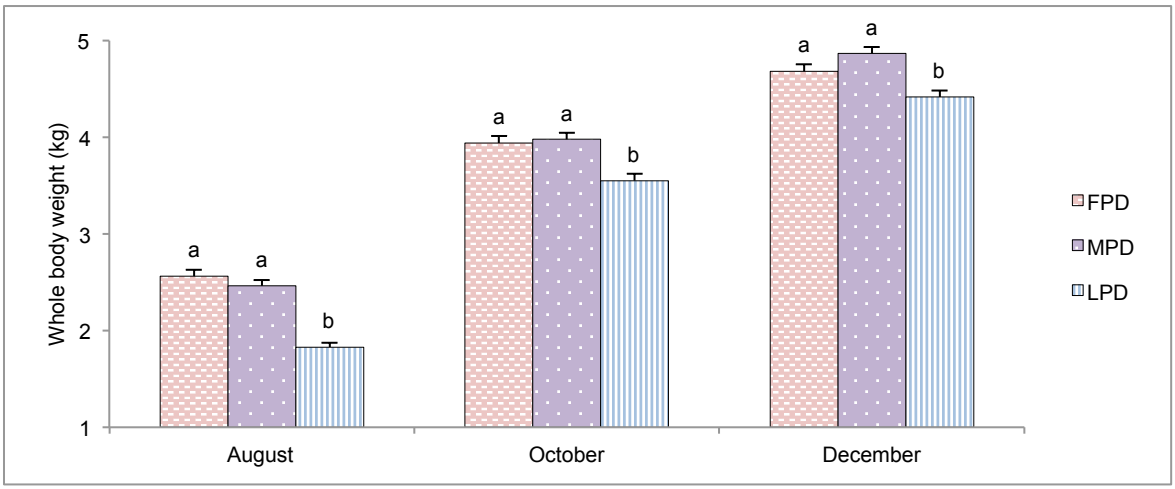
No effect of the main diets on CF was detected during the experimental period. Both the MO and VO groups had a significant increase of CF in during the period October-December (*P < 0.0001*); from 1.39 to 1.43 for MO and from 1.40 to 1.42 for VO.

Slaughter yield

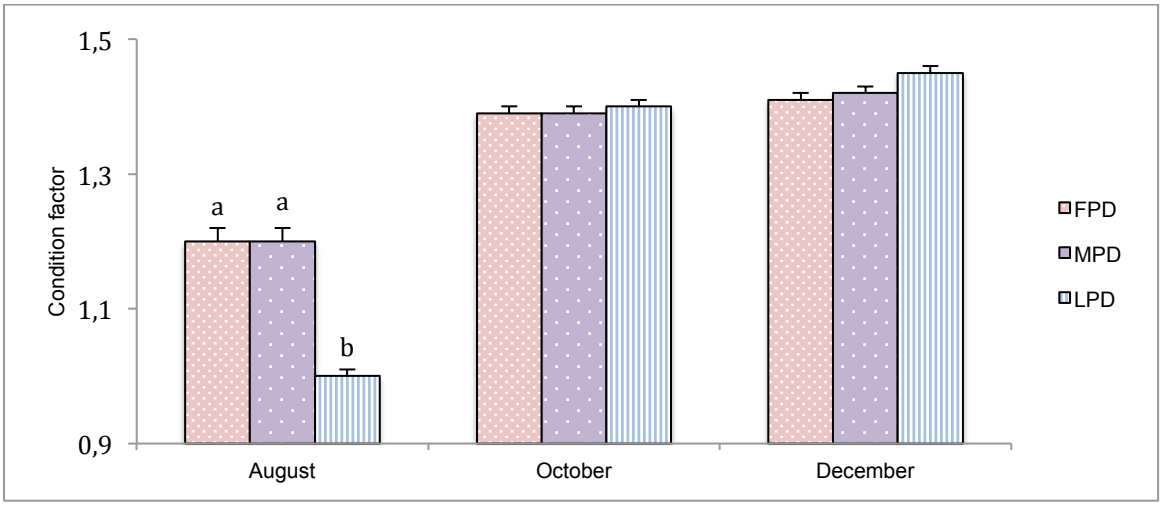
Significant differences in slaughter yield were observed between the pre-dietary fish groups in August (*P < 0.0001*) (fig. 4.1.c). The slaughter yield was lowest for the FPD group (87.9% ± 0.4%) and the highest for the LPD group (90.4% ± 0.10%). At the sampling in October, no significant difference in slaughter yield between the pre-dietary groups was observed, but there was a trend (*P = 0.1*) towards a lower slaughter yield for the FPD group (89.9% ± 0.16%) compared to the MPD group (90.4% ± 0.16%) and the LPD group (90.3% ± 0.16%). In December the FPD and MPD groups had significantly higher slaughter yield (90.1% ± 0.13% and 90.3% ± 0.13%, respectively) than the LPD group (89.7% ± 0.13%) (*P = 0.0043*).

No significant difference in slaughter yield between the MO and VO dietary groups (*P = 0.46*) was observed in October (90.1% ± 0.13% and 90.3% ± 0.13%, respectively). At the sampling in December the MO group had significantly lower slaughter yield (89.7% ± 0.10%) compared to the VO group (90.4% ± 0.10%) (*P < 0.0001*).

a)



b)



c)

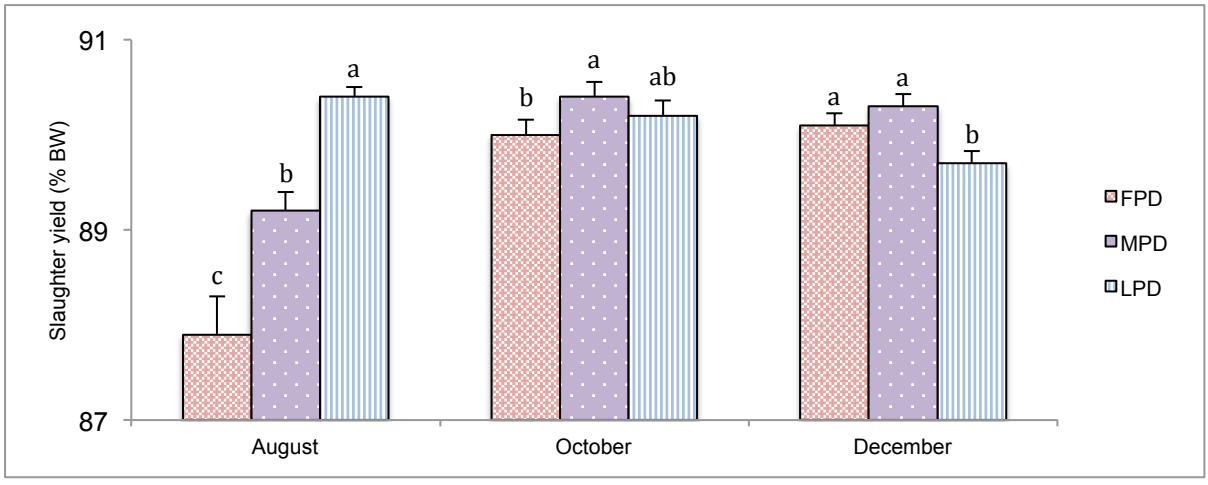


Figure 4.1 Biometric parameters: body weight (a), condition factor (b), and slaughter yield (c) of Atlantic salmon fed three pre-diets (FPD, MPD, LPD). Results are given as LSMeans ± SE. Different letters indicate significant differences ($P < 0.05$) between the pre-dietary groups within sampling time.

4.2 Fillet

Fillet yield

The fillet yield for each of the six dietary treatments measured in October and December are presented in table 4.2. Fillet yield was not recorded in August. No significant differences were observed between the dietary treatments in October. In December the fillet yield of the MPD group was significantly higher (63.4% ± 0.19%, *P = 0.0182*) compared to the FPD and LPD groups (62.8% ± 0.19% and 62.6% ± 0.19%, respectively) (fig. 4.2). No significant effects of main diet were observed for fillet yield during the experimental period.

Table 4.2 Average fillet yield (% of BW) of Atlantic salmon fed three pre-diets (FPD, MPD, LPD) from May-August and thereafter two main diets (MO, RO). Results are shown for salmon sampled in October and December (LSmeans ± SE). Different superscript letters indicates significant differences (*P < 0.05*) between groups within sampling time.

	October			December		
	FPD	MPD	LPD	FPD	MPD	LPD
MO	61.3 ± 0.4	62.2 ± 0.8	62.3 ± 0.5	62.8 ± 0.3^{b}	63.1 ± 0.2^{ab}	62.6 ± 0.3^{b}
VO	61.6 ± 0.3	62.2 ± 0.3	61.5 ± 0.3	62.8 ± 0.3^{b}	63.6 ± 0.3^{a}	62.7 ± 0.2^{b}

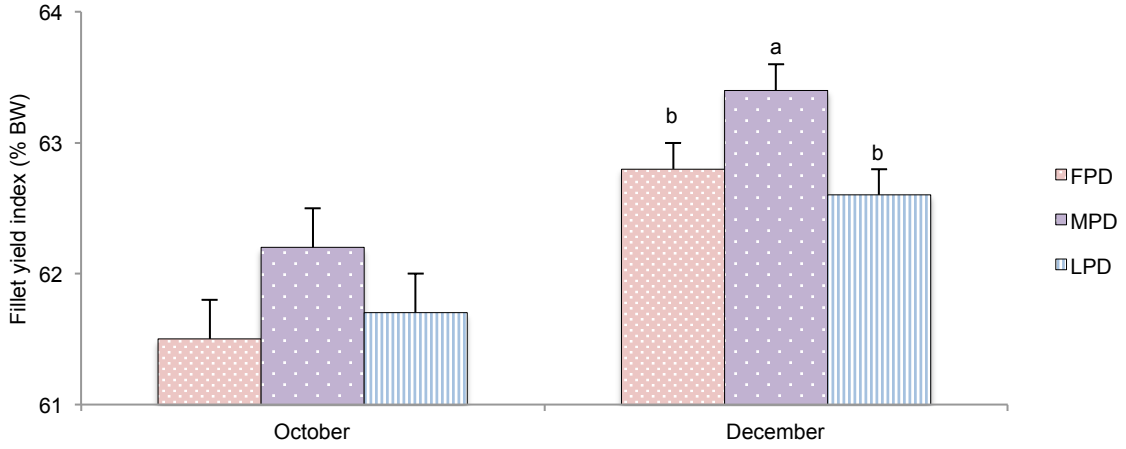


Figure 4.2 Fillet yield (% BW) of Atlantic salmon fed three pre-diets (FPD, MPD, LPD) during the period May-August. Results are presented as overall LSmeans±SE, independent of subsequent dietary treatment (VO and MO diet). Different letters denote significant differences (*P < 0.05*) between dietary treatments within one sampling time.

Lipid content

Due to the pre-dietary treatment in the period from May to August, three groups of fish with different fat content were produced (tab. 4.3).

Table 4.3 Development in lipid content (% wet weight) in skeletal muscle of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO and VO). Results are shown for salmon sampled in October and December (LSmeans ± SE). Different letters show statistical difference within the same row ($P < 0.05$).

	MO			VO		
	FPD	MPD	LPD	FPD	MPD	LPD
August	15.5	11.8	8.7	15.5	11.8	8.7
Δ Aug—Oct	1.5 ± 0.4^{c}	3.1 ± 0.4^{b}	6.2 ± 0.4^{a}	0.7 ± 0.4^{c}	2.6 ± 0.4^{bc}	5.7 ± 0.4^{a}
October	16.9 ± 0.4^{a}	14.9 ± 0.4^{b}	14.9 ± 0.4^{b}	16.1 ± 0.4^{ab}	14.4 ± 0.4^{b}	14.4 ± 0.4^{b}
Δ Oct—Dec	-2.5 ± 0.4^{b}	-0.7 ± 0.4^{a}	-1.3 ± 0.4^{ab}	-1.1 ± 0.4^{a}	-0.3 ± 0.4^{a}	-0.7 ± 0.4^{a}
December	14.3 ± 0.4^{a}	14.0 ± 0.4^{a}	13.4 ± 0.4^{b}	15.1 ± 0.4^{a}	14.4 ± 0.4^{a}	13.9 ± 0.4^{a}
Δ Aug — Dec	-1.1 ± 0.4^{c}	2.2 ± 0.4^{b}	4.7 ± 0.4^{a}	-0.4 ± 0.4^{c}	2.6 ± 0.4^{b}	5.2 ± 0.4^{a}

During the period from August to October the muscle fat content increased, but fat retention differed significantly between the three pre-dietary groups ($P < 0.05$). The highest fat accumulation intensity was in the LPD group (5.4% points increase), and the lowest in the FPD group (0.9% points increase). Hence, the variation between the pre-dietary groups decreased in December as compared with August. In the period from October to December, the fat content showed an overall reduction (fig. 4.3), but the decrease was significantly more pronounced ($P < 0.05$) in the FPD group (-1.7%) in comparison to MPD and LPD (0.0 and -0.4, respectively). During the main dietary treatment period, from August to December, the muscle lipid content declined significantly in the FPD group (-0.8%) and increased in MPD fat and LPD groups (2.4 and 5.2% respectively). The fat content in December ranged from 14.7% (FPD group) to 13.8% (LPD group).

The main diet had no significant effect on fat accumulation in skeletal muscle in the period from August to October ($R^2 = 0.09$ in comparison to $R^2 = 0.84$ for pre-diet). During the whole experiment the pre-diets were more important for development of muscle lipid content than the main diets ($R^2 = 0.90$ and $R^2 = 0.01$).

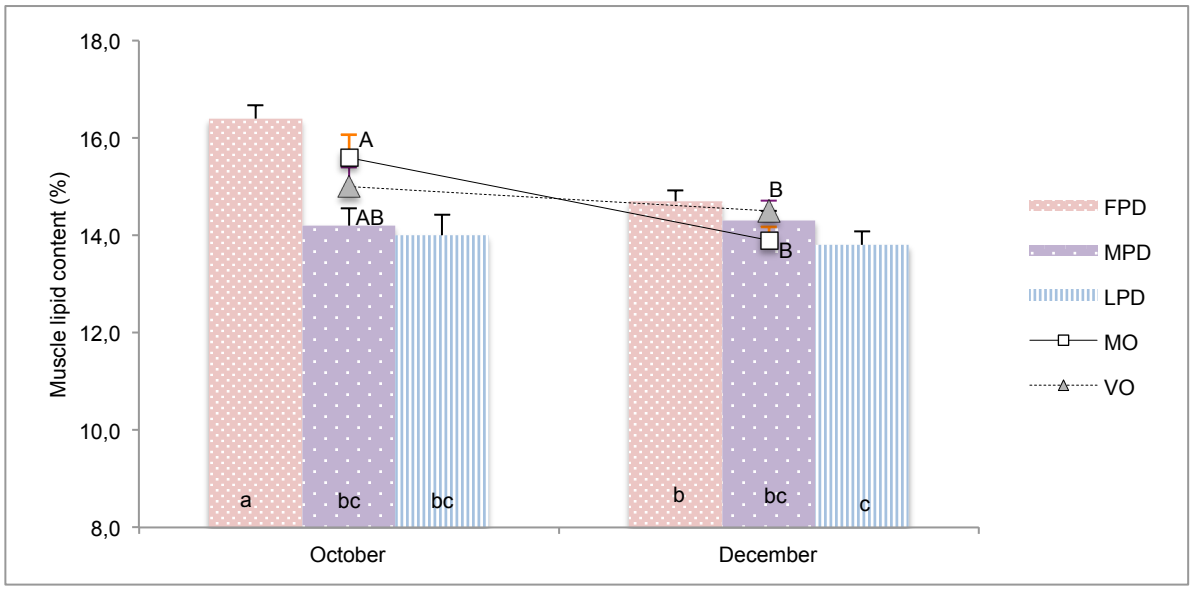


Figure 4.3 Lipid content of muscle tissue in Atlantic salmon sampled in October and December according to diets (graphic chart) and pre-diets (bar chart). Different letters shows statistical differences between treatments (capital for diets, small letterfor pre-diets) and sample times ($P < 0.05$).

Fatty acid (FA) composition

FA profile (the most representative FA) of skeletal muscle tissue of Atlantic salmon from each of the six dietary groups, sampled in October and December, are presented in table 4.4. As shown in the table, the content of each presented fatty acid varied significantly between both pre-diets and main diets within both sampling times.

As shown in table 4.5, the muscle of MO in October had a significantly increased percentage of the FAs 16:0, 20:5n-3 and 22:6n-3, and significantly decreased percentages of 18:1n-9 and 18:2n-6 compared with VO. The FA 18:2n-6 and 18:3n-3 had a tendency to be lower in the muscle of the MO.

In December, the FA composition of the skeletal muscle was not significantly different compared to October. FA 18:2n-6 and 18:3n-3 had a tendency to be higher in the VO group.

In October and December, the FPD group had significantly lower content of FA 16:0 and sum of the saturated FA (SAFA) compared to the MPD and LPD groups. The content of FA 18:3n-3 and sum n-3 FA in the MPD group was significantly lower during the experimental period (tab. 4.5).

Table 4.4 FA composition (% of total FA) of total lipid in skeletal muscle of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO and VO). Results are shown for salmon sampled in October and December (LSmeans ± SE). Different superscript letters indicate significant differences (*P < 0.05*) between groups within one sampling time.

		October			December		
FA	Diet	FPD	MPD	LPD	FPD	MPD	LPD
C 16:0	MO	11.1 ± 0.16^{c}	13.4 ± 0.08^{a}	12.8 ± 0.04^{b}	11.0 ± 0.13^{c}	12.8 ± 0.18^{a}	12.3 ± 0.06^{b}
	VO	9.7 ± 0.05^{e}	11.5 ± 0.00^{c}	10.3 ± 0.25^{d}	9.6 ± 0.05^{d}	10.7 ± 0.11^{c}	10.0 ± 0.06^{e}
C 16:1 n-7	MO	4.6 ± 0.19^{c}	5.9 ± 0.05^{a}	5.6 ± 0.02^{b}	4.5 ± 0.06^{c}	5.5 ± 0.06^{a}	5.0 ± 0.07^{b}
	VO	3.2 ± 0.00^{e}	4.1 ± 0.04^{d}	3.3 ± 0.01^{e}	3.2 ± 0.05^{e}	3.6 ± 0.08^{d}	3.2 ± 0.03^{e}
C 18:0	MO	2.7 ± 0.06^{bc}	3.0 ± 0.01^{a}	2.9 ± 0.03^{a}	2.6 ± 0.02^{c}	2.9 ± 0.07^{a}	2.9 ± 0.02^{a}
	VO	2.4 ± 0.03^{d}	2.8 ± 0.02^{b}	2.7 ± 0.07^{c}	2.4 ± 0.02^{d}	2.7 ± 0.02^{bc}	2.6 ± 0.02^{c}
18:1 n-7	MO	2.9 ± 0.05	2.8 ± 0.19	3.0 ± 0.03	3.2 ± 0.03^{a}	3.2 ± 0.03^{a}	3.2 ± 0.04^{a}
	VO	2.8 ± 0.11	3.0 ± 0.09	2.9 ± 0.15	3.1 ± 0.01^{b}	3.1 ± 0.01^{bc}	3.1 ± 0.01^{c}
18:1 n-9	MO	31.1 ± 0.27^{d}	24.0 ± 0.11^{e}	24.5 ± 0.07^{e}	31.5 ± 0.30^{d}	26.1 ± 0.37^{f}	26.9 ± 0.08^{e}
	VO	37.9 ± 0.16^{a}	33.2 ± 0.14^{c}	36.3 ± 0.12^{b}	38.6 ± 0.09^{a}	35.4 ± 0.14^{c}	37.3 ± 0.27^{b}
18:2 n-6	MO	10.5 ± 0.05^{d}	8.0 ± 0.05^{e}	8.5 ± 0.03^{f}	10.8 ± 0.11^{d}	9.0 ± 0.12^{f}	9.4 ± 0.04^{e}
	VO	12.8 ± 0.01^{a}	11.0 ± 0.14^{c}	12.4 ± 0.03^{b}	13.2 ± 0.04^{a}	12.0 ± 0.10^{c}	12.9 ± 0.09^{b}
18:3 n-3	MO	4.0 ± 0.02^{d}	2.8 ± 0.02^{f}	3.1 ± 0.01^{e}	4.1 ± 0.06^{d}	3.2 ± 0.04^{f}	3.5 ± 0.05^{e}
	VO	5.2 ± 0.00^{a}	4.2 ± 0.07^{c}	4.9 ± 0.01^{b}	5.3 ± 0.04^{a}	4.8 ± 0.06^{c}	5.2 ± 0.04^{b}
20:1 n-9	MO	3.6 ± 0.01^{b}	3.7 ± 0.07^{ab}	3.4 ± 0.02^{b}	3.4 ± 0.03^{ab}	3.4 ± 0.01^{ab}	3.1 ± 0.03^{c}
	VO	3.6 ± 0.11^{ab}	3.8 ± 0.03^{a}	3.4 ± 0.12^{b}	3.4 ± 0.04^{b}	3.5 ± 0.05^{ab}	3.1 ± 0.03^{c}
20:5 n-3	MO	5.0 ± 0.16^{b}	5.7 ± 0.81^{b}	6.6 ± 0.03^{a}	5.0 ± 0.06^{c}	6.1 ± 0.04^{b}	6.3 ± 0.05^{a}
	VO	3.2 ± 0.02^{c}	3.9 ± 0.03^{c}	3.6 ± 0.03^{c}	3.2 ± 0.03^{f}	3.7 ± 0.02^{d}	3.6 ± 0.03^{e}
22:5 n-3	MO	2.0 ± 0.08^{b}	2.5 ± 0.02^{a}	2.6 ± 0.02^{a}	2.1 ± 0.03^{b}	2.6 ± 0.04^{a}	2.6 ± 0.01^{a}
	VO	1.3 ± 0.03^{d}	1.6 ± 0.04^{c}	1.5 ± 0.03^{cd}	1.4 ± 0.01^{e}	1.6 ± 0.02^{c}	1.5 ± 0.01^{d}
22:6 n-3	MO	6.0 ± 0.06^{b}	7.7 ± 0.05^{a}	7.7 ± 0.07^{a}	6.2 ± 0.11^{b}	7.5 ± 0.08^{a}	7.5 ± 0.11^{a}
	VO	4.4 ± 0.00^{d}	5.7 ± 0.26^{b}	4.9 ± 0.08^{c}	4.3 ± 0.07^{e}	5.2 ± 0.06^{c}	4.7 ± 0.05^{d}
EPA+ DHA	MO	11.0 ± 0.22^{c}	13.4 ± 0.76^{b}	14.4 ± 0.10^{a}	11.2 ± 0.17^{b}	13.6 ± 0.12^{a}	13.8 ± 0.11^{a}
	VO	7.5 ± 0.01^{e}	9.6 ± 0.29^{d}	8.5 ± 0.10^{e}	7.4 ± 0.09^{e}	8.9 ± 0.07^{c}	8.3 ± 0.07^{d}
Sum n-3	MO	17.7 ± 0.26^{c}	19.4 ± 0.73^{b}	20.8 ± 0.14^{a}	18.3 ± 0.18^{c}	20.1 ± 0.23^{b}	20.7 ± 0.12^{a}
	VO	14.7 ± 0.01^{d}	16.1 ± 0.28^{d}	15.5 ± 0.34^{d}	15.0 ± 0.12^{e}	15.9 ± 0.06^{d}	15.6 ± 0.11^{d}
Sum n-6	MO	12.6 ± 0.06^{b}	9.9 ± 0.28^{d}	10.5 ± 0.14^{c}	12.9 ± 0.11^{d}	11.0 ± 0.15^{f}	11.5 ± 0.06^{e}
	VO	14.8 ± 0.02^{a}	12.9 ± 0.09^{b}	14.4 ± 0.03^{a}	15.2 ± 0.04^{a}	14.0 ± 0.12^{c}	14.8 ± 0.09^{b}
SAFA	MO	18.3 ± 0.04^{c}	22.4 ± 0.02^{a}	21.4 ± 0.02^{b}	18.2 ± 0.24^{c}	21.1 ± 0.26^{a}	20.3 ± 0.10^{b}
	VO	15.8 ± 0.05^{e}	18.5 ± 0.05^{c}	16.8 ± 0.28^{d}	15.6 ± 0.08^{f}	17.4 ± 0.15^{d}	16.4 ± 0.11^{e}

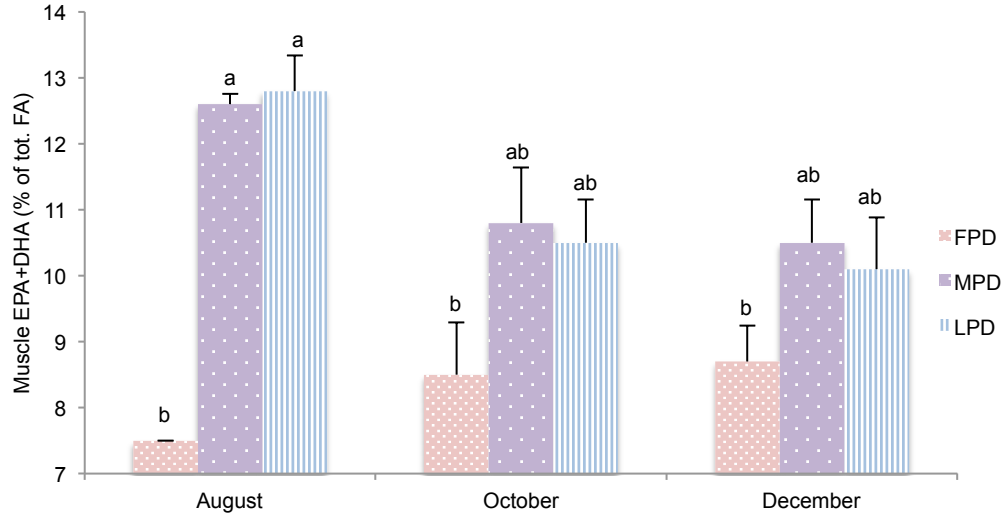
Table 4.5 Fatty acid composition (% of total FA) of total lipid in skeletal muscle of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO and VO). Results are shown for salmon sampled in August, October and December (LSmeans ± SE). Different superscript letters indicate significant differences (*P < 0.05*) between groups within one sampling time (capital letters) and between sampling times (small letters) (October and December for main diets; August, October and December for pre-diets).

		Main diet						Pre-diet		
FA		August	diet	October	diet	December		August	October	December
16:0	MO	12.2 ±0.78	12.7	12.4± 0.45^{a*}	8.5	12.0± 0.25^{a}	F	10.0 ± 0.05^{d}	^{B}10.2 ± 0.29^{c}	^{B}10.0 ± 0.20^{d}
	VO	12.2 ± 0.78	8.5	10.5 ± 0.33^{b}	8.5	10.1± 0.15^{b}	M	14.2 ± 0.15^{a}	^{A}12.1 ± 0.44^{b}	^{A}11.5 ± 0.29^{bc}
							L	12.4 ± 0.04^{ab}	^{A}11.2 ± 0.53^{bc}	^{B}10.8 ± 0.32^{c}
18:1 n-9	MO	28.8 ± 2.31	26.7	26.5 ± 1.44^{b}	41.7	28.2± 0.73^{b}	F	36.0 ± 0.20^{ab}	35.8 ± 1.49^{ab}	36.2 ± 1.00 ^{a}
	VO	28.8 ± 2.31	41.7	35.8 ± 0.88^{a}	41.7	37.1 ± 0.4^{a}	M	24.1 ± 0.17^{c}	30.0 ± 1.90^{bc}	32.2 ± 1.30 ^{b}
							L	26.3 ± 0.07^{cb}	32.4 ± 2.50^{ab}	33.8 ± 1.46
18:2 n-6	MO	9.4 ± 0.87	8.1	9.0 ± 0.4^{b}	13.8	9.7 ± 0.25^{b}	F	12.0 ± 0.05^{ab}	12.1 ± 0.50^{ab}	12.4 ± 0.34^{a}
	VO	9.4±0.87	13.8	12.1± 0.35^{ab}	13.8	12.7± 0.16^{a}	M	7.2 ± 0.08^{c}	10.0 ± 0.63^{b}	10.9 ± 0.42^{b}
							L	8.9 ± 0.01^{b}	11.1 ± 0.84^{ab}	11.7 ± 0.49^{ab}
18:3 n-3	MO	3.3 ± 0.47	3.4	3.3 ± 0.23^{b}	6.4	3.6 ± 0.12^{b}	F	4.7 ± 0.00^{ab}	^{A}4.8 ± 0.25^{ab}	^{A}4.9 ± 0.18^{a}
	VO	3.3±0.47	6.4	4.7 ± 0.19^{ab}	6.4	5.1 ± 0.08^{a}	M	2.2 ± 0.04^{c}	^{B}3.8 ± 0.30^{b}	^{B}4.2 ± 0.22^{b}
							L	2.9 ± 0.02^{c}	^{A}4.3 ± 0.39^{ab}	^{A}4.6 ± 0.24^{ab}
20:5 n-3	MO	4.1 ± 0.36	10.2	5.8 ± 0.36^{a}	4.6	5.8 ± 0.18^{a}	F	3.0 ± 0.05	3.7 ± 0.41	3.7 ± 0.26
	VO	4.1 ± 0.36	4.6	3.5 ± 0.14^{b}	4.6	3.5 ± 0.07^{b}	M	4.8 ± 0.02	4.5 ± 0.43	4.5 ± 0.33
							L	4.6 ± 0.01	4.6 ± 0.64	4.5 ± 0.39
22:6 n-3	MO	6.9 ± 0.72	7.3	7.7 ± 0.37^{a}	3.4	7.3 ± 0.19^{a}	F	4.6 ± 0.00^{c}	4.8 ± 0.39^{c}	4.9 ± 0.28^{c}
	VO	6.9 ± 0.72	3.4	5.0 ± 0.26^{b}	3.4	4.7 ± 0.12^{b}	M	7.8 ± 0.14^{a}	6.3 ± 0.44^{ab}	6.0 ± 0.32^{b}
							L	8.2 ± 0.14^{a}	5.9 ± 0.59^{bc}	5.6 ± 0.40^{bc}
Sum n-3	MO	16.6 ± 0.82	24.4	19.3 ± 0.61^{a}	15.3	19.7± 0.33^{a}	F	14.1 ± 0.10	15.5 ± 0.68	16.1 ± 0.47
	VO	16.6 ± 0.82	15.3	15.4 ± 0.28^{b}	15.3	15.5± 0.13^{b}	M	17.5 ± 0.01	17.2 ± 0.73	17.4 ± 0.59
							L	18.3 ± 0.08	17.3 ± 1.11	17.4 ± 0.70
Sum n-6	MO	11.2 ± 0.87	9.5	11.0 ± 0.53^{c}	14.6	11.8± 0.25^{b}	F	13.8 ± 0.00^{ab}	^{A}14.1 ± 0.47^{ab}	^{A}14.4 ± 0.31^{a}
	VO	11.2 ± 0.87	14.6	14.0 ± 0.37^{a}	14.6	14.7± 0.16^{a}	M	9.1 ± 0.07^{c}	^{B}11.9 ± 0.66^{b}	^{B}12.9 ± 0.41^{b}
							L	10.9 ± 0.04^{b}	^{A}13.1 ± 0.83^{ab}	^{A}13.7 ± 0.46^{ab}
SAFA	MO	19.7 ± 1.18	22.8	20.7 ± 0.77^{a}	15.2	19.9± 0.38^{a}	F	16.3 ± 0.05^{c}	^{B}16.7 ± 0.53^{c}	^{B}16.5 ± 0.38^{c}
	VO	19.7 ± 1.18	15.2	17.0 ± 0.52^{b}	15.2	16.5± 0.24^{b}	M	22.7 ± 0.08^{a}	^{A}19.8 ± 0.81^{b}	^{A}18.7 ± 0.51^{b}
							L	20.2 ± 0.06^{b}	^{A}18.4 ± 0.95^{bc}	^{B}17.7 ± 0.56^{bc}

In August the EPA+DHA muscle content was influenced by pre-dietary treatment. The lowest content was observed for the FPD group (7.5 ± 0.0) compared to the MPD (12.6 ± 0.2) and LPD (12.8 ± 0.5). In October and December, no significant differences in EPA+DHA content were observed between the pre-dietary groups, but a trend (*P = 0.1*) towards a lower content of EPA and DHA was detected for the MPD and LPD groups in December compared to August and October (fig. 4.4.a).

Main diet was shown to have a significant effect on EPA+DHA content in skeletal muscle (fig. 4.4.b), with increased level in the MO by 2%-units (from 10.9 ± 0.8 to 12.9 ± 0.6) during the period August-October and a 2.4%-unit decrease in the VO group (from 10.9 ± 0.8 to 8.5 ± 0.3) during the same period.

a)



b)

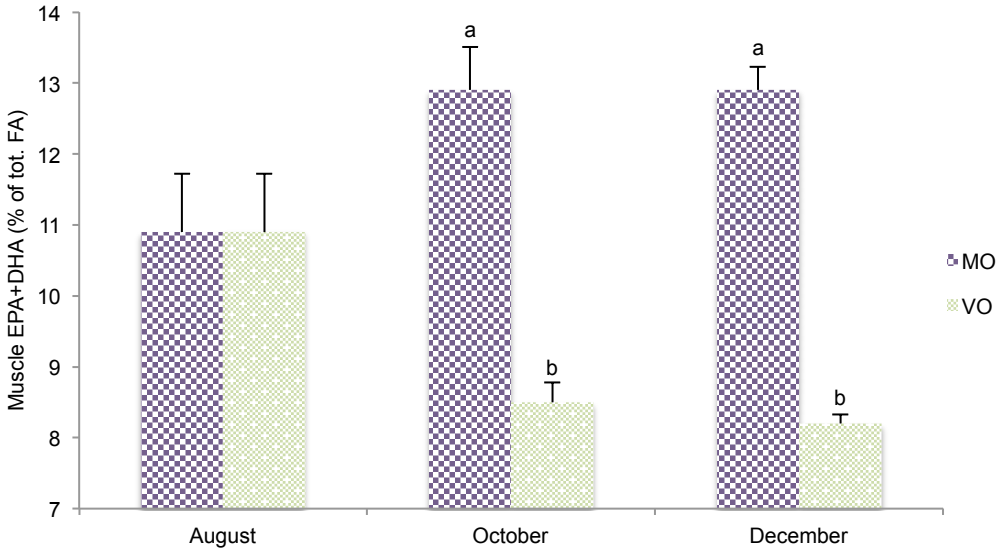


Fig. 4.4 EPA and DHA (% of total fatty acids) of total lipid in the skeletal muscle of Atlantic salmon sampled in August, October and December according to a) pre-diets (fat, FPD; medium fat, MPD; lean, LPD) and b) diets (Marine 70%, MO; Rapeseed 70%, VO). The data are given as mean ± standard error of mean. Different letters shows statistical differences ($P < 0.05$) between dietary treatments and sampling times.

Temporal changes g/100g EPA+DHA muscle content between the main dietary groups are shown in figure 4.5 within each of the pre-dietary groups FPD (a), MPD (b) and LPD (c) diets. The MO group had significantly higher level of EPA+DHA in October and December compared with the VO group, with the maximal content observed in October for the LPD group (2.15 g/100g).

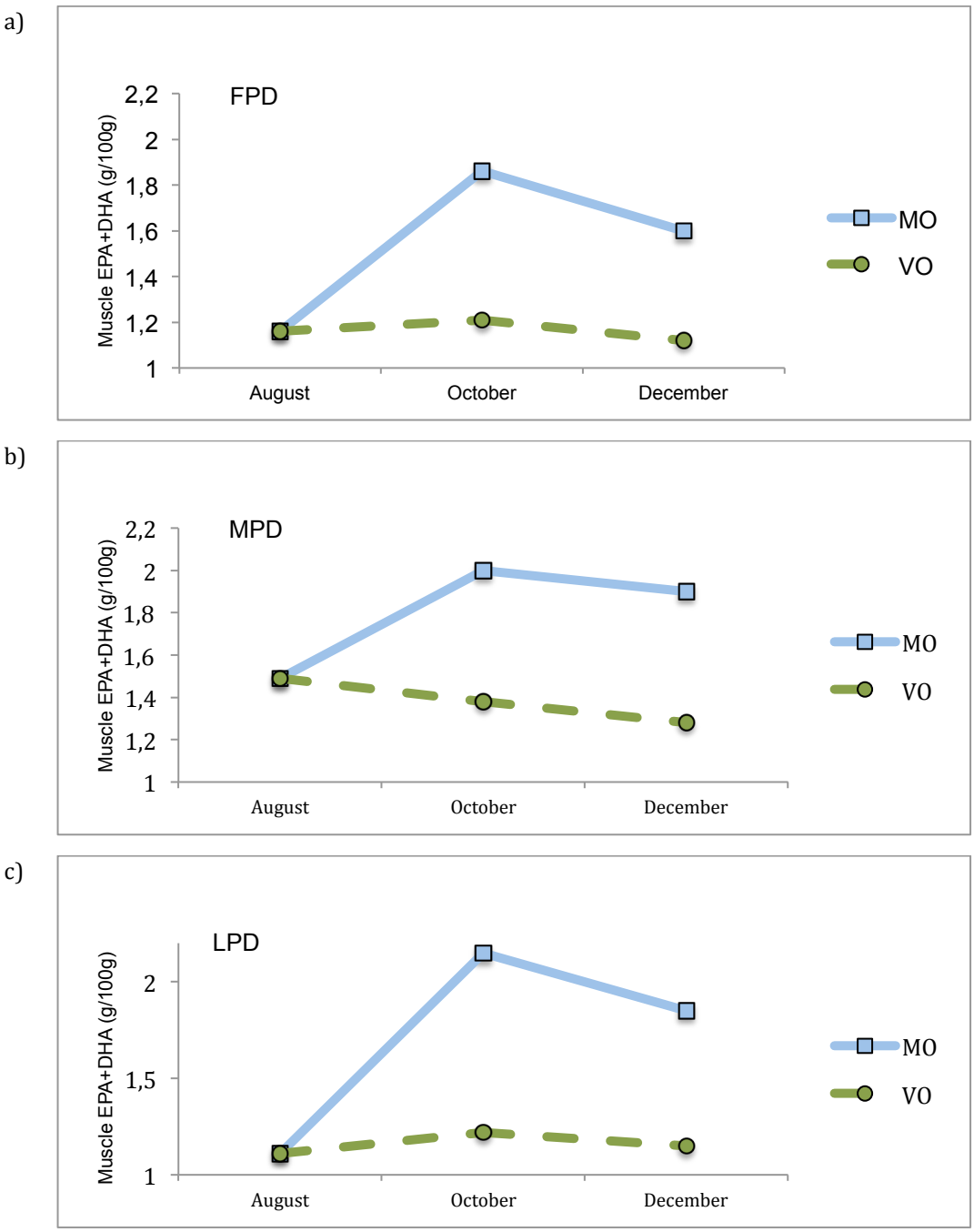


Fig. 4.5 EPA and DHA (g per 100 g of tissue) in the muscle tissue in Atlantic salmon sampled in August, October and December fed the a) FPD, b) MPD and c) LPD pre-diets, and MO and VO diets.

4.3 Viscera

Mass index

In August the LPD group had significantly lowest visceral mass indexes (*P < 0.0001*), whereas the highest index were observed for the FPD group (tab. 4.6). No significant differences in viscera mass index were observed between pre-dietary groups in October, but there was detected a trend (*P = 0.08*) towards a lower viscera mass index for the MPD group (8.5 ± 0.2) compared to the FPD (9.0 ± 0.2) and LPD (8.7 ± 0.2) groups (fig. 4.6). In December the LPD group had significant higher viscera mass index (9.2 ± 0.1) than the FPD and MPD groups (8.9 ± 0.1 and 8.7 ± 0.1, respectively) (*P = 0.0*1).

At the sampling in October no significant difference between the MO (8.8 ± 0.1) and VO (8.7 ± 0.1) was detected (*P = 0.5*). In December the MO main diet group had significantly higher (*P < 0.001*) viscera mass index compared with the VO (9.3 ± 0.1 and 8.6 ± 0.1, respectively).

Table 4.6. Average viscera mass index (% BW) of Atlantic salmon fed three pre-diets (FPD, MPD, LPD) from May-August and thereafter two main diets and (MO, RO). Results are shown for salmon sampled in August, October and December (LSmeans ± SE). Different superscript letters indicate significant differences (*P < 0.05*) between groups within sampling time.

	August			October			December		
	FPD	MPD	LPD	FPD	MPD	LPD	FPD	MPD	LPD
MO	11.3±0.4^{a}	9.6±0.2^{b}	8.5±0.1^{c}	9.1 ± 0.21	8.5 ± 0.17	8.8 ± 0.23	9.1 ± 0.19^{ab}	9.2 ± 0.17^{ab}	9.5 ± 0.15^{a}
VO				8.9 ± 0.20	8.5 ± 0.30	8.6 ± 0.22	8.6 ± 0.16^{bc}	8.1 ± 0.15^{c}	8.9 ± 0.17^{b}

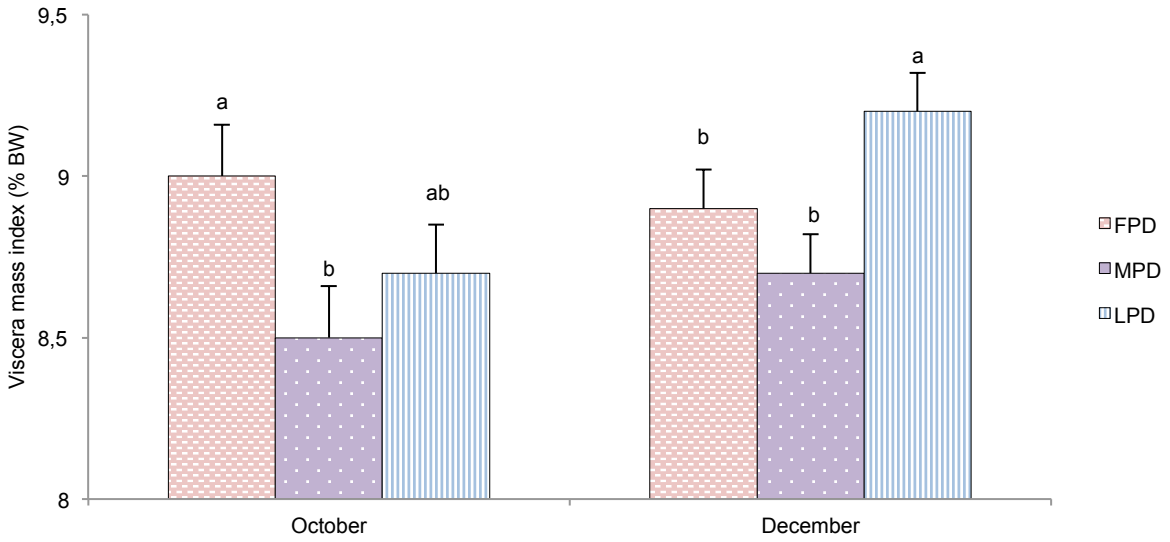


Figure 4.6 Viscera mass index (% BW) of farmed Atlantic salmon fed three pre-diets (FPD, MPD, LPD). Different letters denote significant differences (*P < 0.05*) between dietary treatments within sampling time.

Lipid content

Development of lipid content in visceral tissue according to pre-diets and main diets is presented in table 4.7.

Pre-dietary feeding treatment resulted in high difference in visceral fat content between group in August and caused different fat accumulation rate after the pre-dietary period. Thus, fish from the FPD group had the highest lipid content in viscera (39.0%) and showed significantly lowest fat increase rate (19.5%) in the period August – October, while the highest rate of fat accumulation (26.2%) was in the LPD group which had initially the lowest fat content (26.6%) in August. Although the fat accumulation in viscera was lowest for the FPD group from August to October, the fat content of this group was still significantly highest compared to the MPD and LPD groups in October. In December, no significant differences were observed between the pre-dietary treatments

Fat accumulation rate related to main dietary treatments showed no significant difference between MO and VO groups in October and averaged in 23.2% and 22.2% increase, respectively. In December the VO group had significantly higher visceral fat content (60.7%) compared to MO group (55.9%, $P < 0.05$). Hence, in the period from October to December, viscera fat content was more correlated with main dietary treatment than with pre-dietary treatment (R^2 = 0.41 vs. R^2 = 0.09), opposite to the previous period (R^2 = 0.02 vs. R^2 = 0.54).

Table 4.7 Development in lipid content (% wet weight) in viscera of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO, VO) until December. Results are shown for salmon sampled in October and December (LSmeans ± SE). Different letters shows statistical difference within the same row ($P < 0.05$).

	MO			VO		
	FPD	MPD	LPD	FPD	MPD	LPD
August	39.0	29.0	26.6	39.0	29.0	26.6
Δ Aug—Oct	18.7 ± 2.22	23.2 ± 2.22	27.9 ± 2.22	20.3 ± 2.22	21.7 ± 2.22	24.5 ± 2.22
October	57.7 ± 2.20	52.1 ± 2.20	54.5 ± 2.20	59.3 ± 2.20	50.6 ± 2.20	51.1 ± 2.20
Δ Oct—Dec	-0.0 ± 2.83	0.2 ± 2.83	0.00 ± 2.83	3.5 ± 2.83	9.15 ± 2.83	4.2 ± 2.83
December	57.4 ± 1.67^{b}	54.8 ± 1.67^{b}	55.8 ± 1.67^{b}	62.4 ± 1.67^{a}	60.9 ± 1.67^{ab}	58.7 ± 1.67^{ab}
Δ Aug — Dec	18.7 ± 1.88^{b}	23.4 ± 1.88^{b}	27.9 ± 1.88^{ab}	23.8 ± 1.88^{b}	30.8 ± 1.88^{a}	28.7 ± 1.88^{ab}

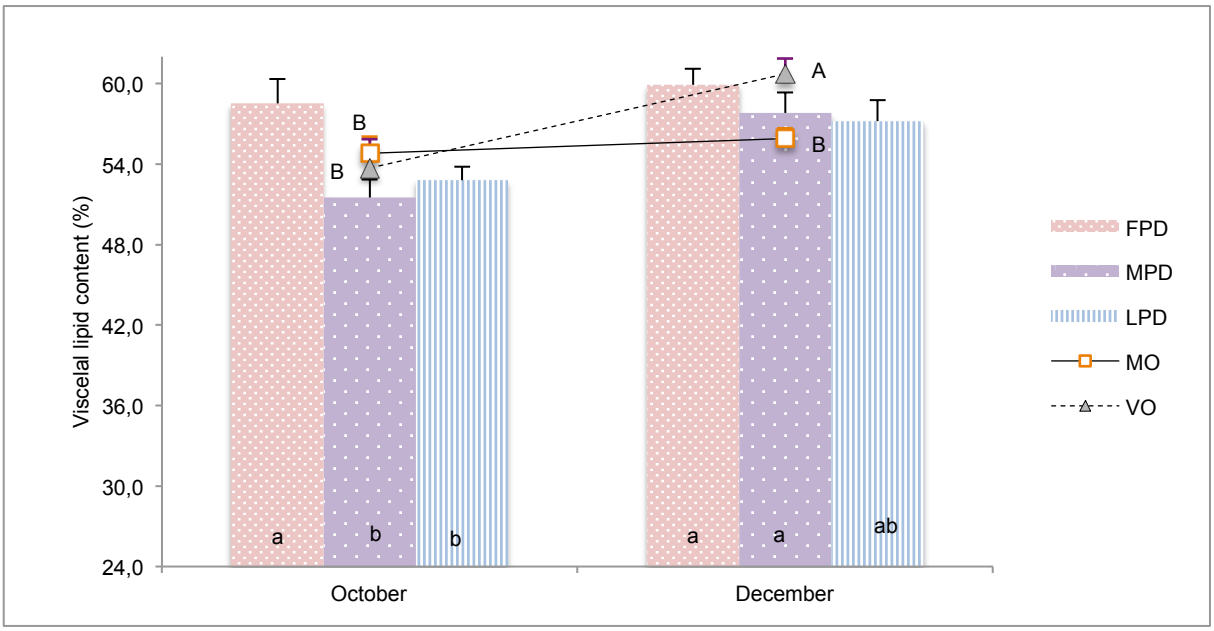


Figure 4.7 Lipid content of visceral tissue in Atlantic salmon sampled in August, October and December according to main diets (VO and MO; graphic chart) and pre-diets (FPD, MPD and LPD; bar chart). Different letters shows statistical differences between treatments (capital for diets, small for fre-diets) and sample times ($P < 0.05$).

FA composition

As shown in table 4.8, the viscera of MO in October had a significantly increased percentage of the FAs 16:0, 20:5n-3 and 22:6n-3, and significantly decreased percentages of 18:1n-9, 18:2n-6 and18:3n-3 compared with VO.

In December as shown in table 4.8, percentages of FA 16:0, 20:5n-3 and 22:6n-3 in the MO group significantly decreased, whereas percentages of 18:1n-9, 18:2n-6 and 18:3n-3 significantly increased compared to October.

Table 4.8 Fatty acid composition (% of total fatty acids ± SE) of total lipid in visceral tissue of Atlantic salmon sampled in August, October and December. Results are shown for salmon fed three pre-diets (LPD:L, MPD:M, FPD:F) from May-August and thereafter two main diets (MO, VO) until December. Different letters show statistical differences between treatments and sample times ($P < 0.05$).

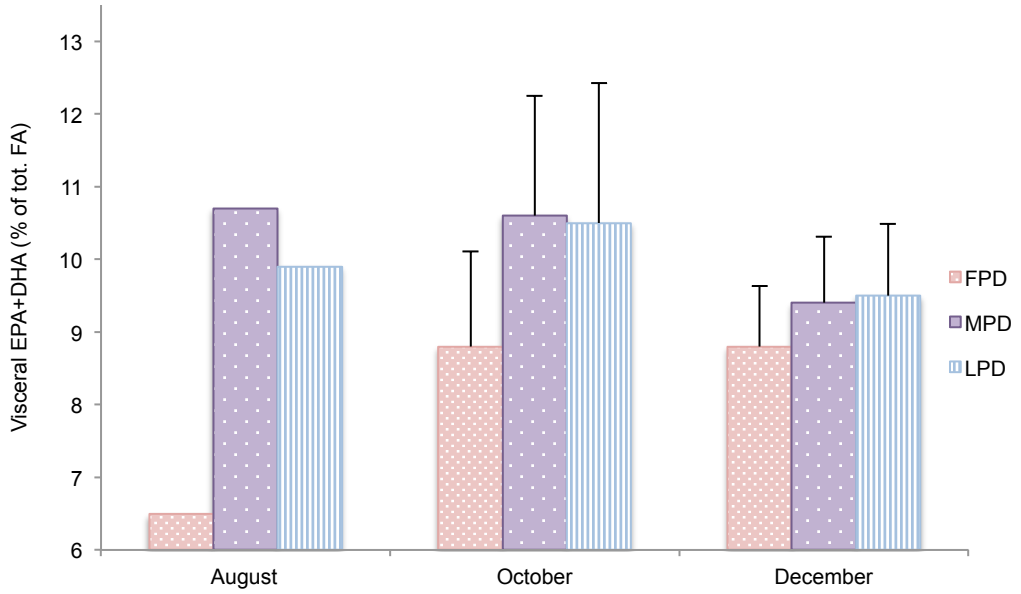
FA	Main diet	August	diet	October	diet	December	Pre-diet	August	October	December
16:0	MO	11.1 ± 1.02	12.7	12.0 ± 0.31^{a}	8.5	11.2 ± 0.12^{b}	F	9.5	10.1 ± 0.56	9.9 ± 0.37
	VO	11.1 ± 1.02	8.5	9.6 ± 0.23^{c}	8.5	9.2 ± 0.07^{c}	M	13.0	11.5 ± 0.67	10.7 ± 0.39
							L	10.8	11.0 ± 0.87	10.3 ± 0.40
18:1 n-9	MO	30.5 ± 3.15	26.7	26.6 ± 1.06^{c}	41.7	29.8 ± 0.41^{b}	F	36.5	34.4 ± 2.60	35.3 ± 1.65
	VO	30.5 ± 3.15	41.7	37.9 ± 0.50^{a}	41.7	39.3 ± 0.02^{a}	M	25.9	30.6 ± 3.34	34.0 ± 1.87
							L	29.0	31.8 ± 3.90	34.4 ± 1.99
18:2 n-6	MO	10.4 ± 1.04	8.1	9.3 ± 0.35^{d}	13.8	10.4 ± 0.11^{c}	F	12.2	11.9 ± 0.85	12.2 ± 0.57
	VO	10.4 ± 1.04	13.8	13.0 ± 0.21^{b}	13.8	13.6 ± 0.07^{a}	M	8.6	10.5 ± 1.07	11.8 ± 0.61
							L	10.4	11.1 ± 1.28	12.0 ± 0.66
18:3 n-3	MO	3.5 ± 0.59	3.4	3.4 ± 0.17^{d}	6.4	3.7 ± 0.06^{c}	F	4.6	4.6 ± 0.39	4.7 ± 0.30
	VO	3.5 ± 0.59	6.4	5.1 ± 0.12^{b}	6.4	5.4 ± 0.03^{a}	M	2.6	3.9 ± 0.48	4.5 ± 0.33
							L	3.3	4.2 ± 0.56	4.6 ± 0.34
20:5 n-3	MO	3.6 ± 0.45	10.2	6.2 ± 0.27^{a}	4.6	5.7 ± 0.12^{b}	F	2.7	4.1 ± 0.74	4.2 ± 0.43
	VO	3.6 ± 0.45	4.6	3.2 ± 0.11^{c}	4.6	3.2 ± 0.03^{c}	M	4.2	5.0 ± 0.87	4.5 ± 0.48
							L	3.8	5.0 ± 1.04	4.6 ± 0.53
22:6 n-3	MO	5.5 ± 0.84	7.3	6.6 ± 0.29^{a}	3.4	6.0 ± 0.10^{b}	F	3.8	4.6 ± 0.59	4.6 ± 0.40
	VO	5.5 ± 0.84	3.4	4.0 ± 0.13^{c}	3.4	3.7 ± 0.04^{c}	M	6.5	5.6 ± 0.77	4.9 ± 0.43
							L	6.1	5.5 ± 0.90	4.9 ± 0.47
Sum n-3	MO	14.7 ± 1.12	24.4	19.5 ± 0.52^{a}	15.3	18.3 ± 0.21^{b}	F	12.5	15.8 ± 1.21	15.9 ± 0.70
	VO	14.7 ± 1.12	15.3	14.3 ± 0.21^{c}	15.3	14.3 ± 0.07^{c}	M	18.8	17.4 ± 1.53	16.4 ± 0.77
							L	15.9	17.5 ± 1.76	16.5 ± 0.84
Sum n-6	MO	12.5 ± 1.01	9.5	11.6 ± 0.38^{c}	14.6	12.7 ± 0.11^{c}	F	14.2	14.1 ± 0.78	14.4 ± 0.55
	VO	12.5 ± 1.01	14.6	15.0 ± 0.22^{b}	14.6	15.7 ± 0.08^{a}	M	10.7	12.6 ± 1.04	14.0 ± 0.56
							L	12.7	13.3 ± 1.19	14.2 ± 0.62
SAFA	MO	18.5 ± 1.62	22.8	20.3 ± 0.55^{a}	15.2	18.8 ± 0.20^{b}	F	15.8	16.9 ± 0.98	16.5 ± 0.66
	VO	18.5 ± 1.62	15.2	16.0 ± 0.34^{c}	15.2	15.2 ± 0.12^{c}	M	21.4	19.2 ± 1.29	17.3 ± 0.71
							L	18.2	18.3 ± 1.52	17.1 ± 0.76

EPA+DHA content in viscera was lowest in the FPD group (6.5%) compared to the MPD and LPD groups (10.7% and 9.9%, respectively). No effect of pre-diet on EPA+DHA content was detected and no significant differences in EPA+DHA percentage were observed within pre-dietary groups during the experimental period (fig. 4.8.a).

EPA+DHA content in viscera (fig. 4.8.b) incriased significantly in the MO from 9.0 ± 1.3 to 12.8 ± 0.6 during the period August-October, and decleaned in the VO group to 7.1 ± 0.2. There were not found significant differences in muscle EPA+DHA content within the VO groups at the October and December samplings. EPA+DHA percentage in the MO group

decreased significantly in the period October-December (11.6 ± 0.2), but remained significantly higher then that in the VO group (6.9 ± 0.1).

a)



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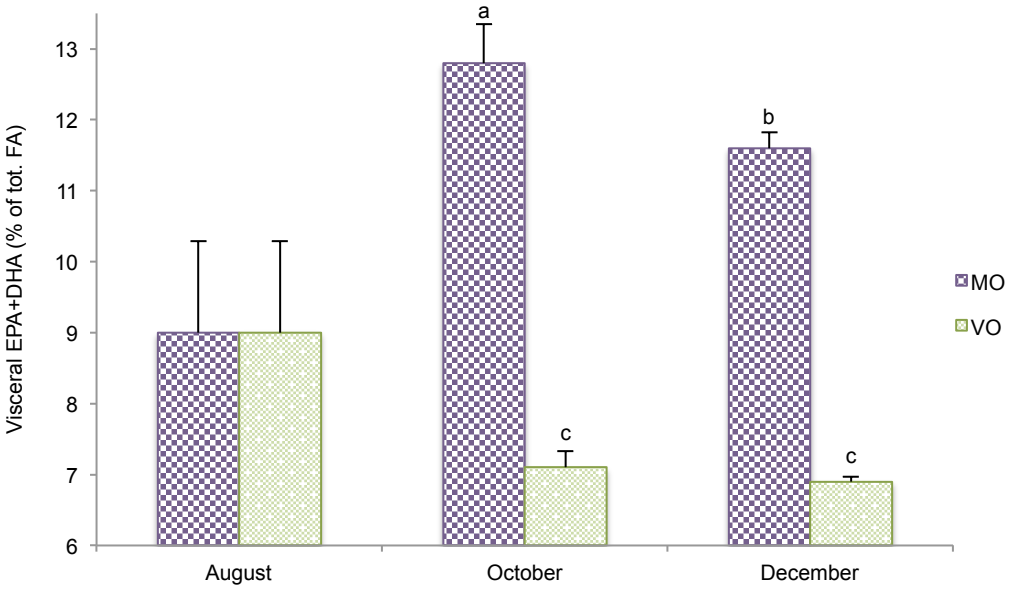


Figure 4.8 EPA and DHA (% of total fatty acids) of total lipid in visceral tissue in Atlantic salmon sampled in August, October and December according to a) pre-diets (FPD; MPD; LPD) and b) main dietary treatments (MO; VO). The data given as mean ± standard error of mean. Different letters shows statistical differences between dietary treatments and sample times ($P < 0.05$).

4.4 Liver

Mass index

The initial liver mass index (x100) in May was 101.0% ± 3.1%. In August the LPD group had significantly lowest (*P < 0.0001*) liver mass index, whereas the highest indexes were observed for the MPD group (tab. 4.9). In October there was detected a trend (*P = 0.08*) towards a lower liver mass index*100 for the FPD (98.8% ± 1.3%) and MPD (99.0% ± 1.3%) groups compared to LPD (102.6% ± 1.3%). Pre-diet was shown to have a significant effect on liver mass index in December (*P = 0.03*). The LPD group had significantly higher liver mass index*100 (100.3% ± 1.0%) compared to FPD (97.0% ± 1.0%) and MPD (96.9% ± 1.0%) groups (fig. 4.9).

No overall effect of main diets on liver mass index was detected and no significant differences in liver mass index were observed in October (*P = 0.7*) and December (*P = 0.6*)

Table 4.9. Average liver mass index (%BW*100) of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO, VO). Results are shown for salmon sampled in October and December (LSmeans ± SE). Different superscript letters indicate significant differences between pre-diet groups within sampling time (*P < 0.05*).

	August			October			December		
	FPD	MPD	LPD	FPD	MPD	LPD	FPD	MPD	LPD
MO	102.9±1.9[b]	109.0±2.2[a]	94.6±2.3[c]	98.6 ± 1.97	100.9 ± 2.45	101.6 ±1.51	96.3 ± 1.99	97.1 ± 1.22	99.9 ± 1.54
VO				99.0 ± 2.05	97.0 ± 1.73	103.7 ± 1.47	97.7 ± 1.36	96.7 ± 1.47	100.8 ± 1.23

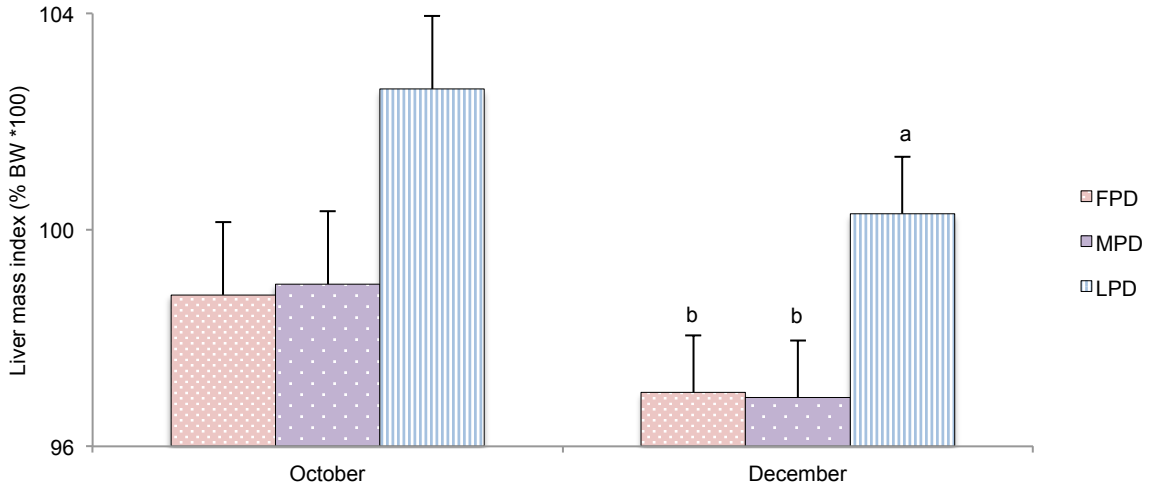


Figure 4.9 Liver mass index (% BW *100) of farmed Atlantic salmon fed three pre-diets (FPD; MPD; LPD). Results are shown as overall LSmeans ± SE for both main dietary treatments, VO and MO. Different letters denote significant differences (*P < 0.05*) between dietary treatments within one sampling.

Lipid content

Developments of the lipid content in liver according to pre-dietary and main dietary treatments are shown in table 4.10.

The fat content of liver was highest for the FPD and lowest for the LPD fish groups after the pre-diet feeding period. The fat content in the liver increased significantly for all pre-dietary groups from August-October, but the rate of fat accumulation differed significantly between the groups with the most significant accumulation being observed for the LPD group (3.5%). No significant differences were observed between the pre-dietary groups in December (fig. 4.10).

The fat content in the liver was numerically lower for the MO group compared with the VO group in October (6.3% ± 0.4% and 6.8% ± 0.28%, respectively) and December (5.8% ± 0.2% and 6.3% ± 0.3%, respectively), but the difference was not significant (fig. 4.10).

Table 4.10 Development in lipid content (% wet weight) in liver of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO, VO). Results are shown for salmon sampled in October and December (LSmeans ± SE). Different letters show statistical differences within the same row ($P < 0.05$).

	MO			VO		
	FPD	MPD	LPD	FPD	MPD	LPD
August	6.1	5.1	3.9	6.1	5.1	3.9
Δ Aug—Oct	-0.5 ± 0.71^{c}	0.2 ± 0.71^{c}	4.2 ± 0.71^{a}	1.5 ± 0.71^{bc}	1.1 ± 0.71^{bc}	2.9 ± 0.71^{ab}
October	5.6 ± 0.71^{b}	5.3 ± 0.71^{b}	8.1 ± 0.71^{a}	7.5 ± 0.71^{ab}	6.2 ± 0.71^{ab}	6.8 ± 0.71^{ab}
Δ Oct—Dec	0.3 ± 0.57^{a}	0.8 ± 0.57^{a}	-2.9 ± 0.57^{c}	-1.8 ± 0.57 ^{bc}	-0.4 ± 0.57^{ab}	0.3 ± 0.57^{a}
December	5.9 ± 0.34	6.0 ± 0.34	5.6 ± 0.34	5.7 ± 0.34	6.1 ± 0.34	7.1 ± 0.34
Δ Aug — Dec	-0.2 ± 0.34^{c}	0.9 ± 0.34^{b}	1.7 ± 0.34^{b}	-0.4 ± 0.34^{c}	0.9 ± 0.34^{b}	3.2 ± 0.34^{a}

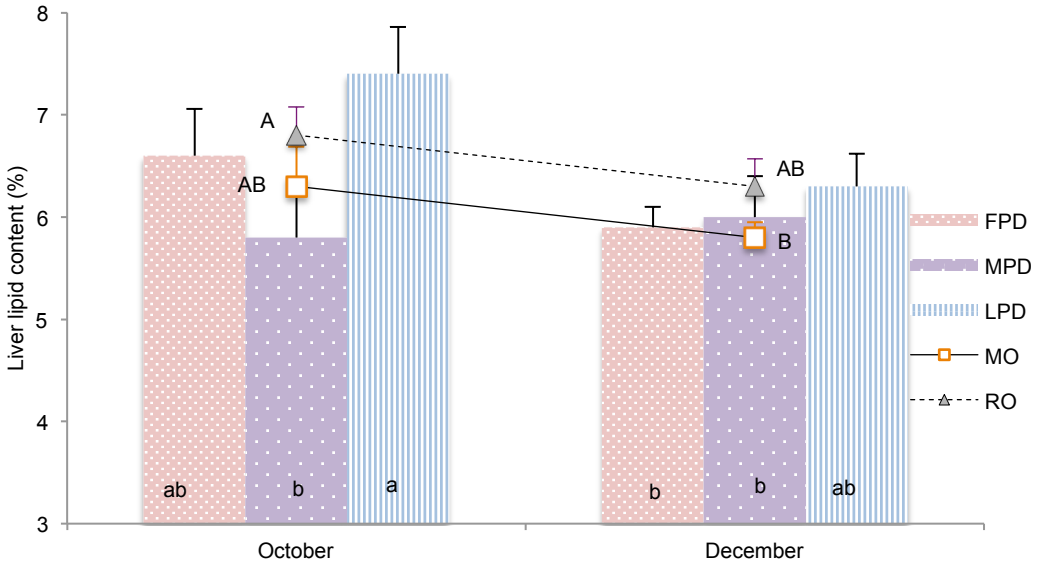


Figure 4.10 Lipid content of liver in Atlantic salmon sampled in August, October and December according to main diets (VO and MO; graphic chart) and pre-diets (FPD, MPD and LPD; bar chart). Different letters show statistical differences between treatments (capital for main diets, small for pre-diets) and sample times ($P < 0.05$).

FA composition

The FA composition of liver lipid in Atlantic salmon according to pre-dietary and main-dietary treatments are presented in tab. 4.11. FA composition of liver mirrors the FA composition of the main diets. No effect of pre-diets was detected after the pre-dietary treatment finished.

Table 4.11 Fatty acid composition (% of total fatty acids ± SE) of total liver lipids in Atlantic salmon sampled in August, October and December. The results are presented for main diets (Marine 70%, MO; Rapeseed 70%, RO) and pre-diets (FPD, F; MPD, M; LPD L). Different letters show statistical differences between treatments and sample times ($P < 0.05$).

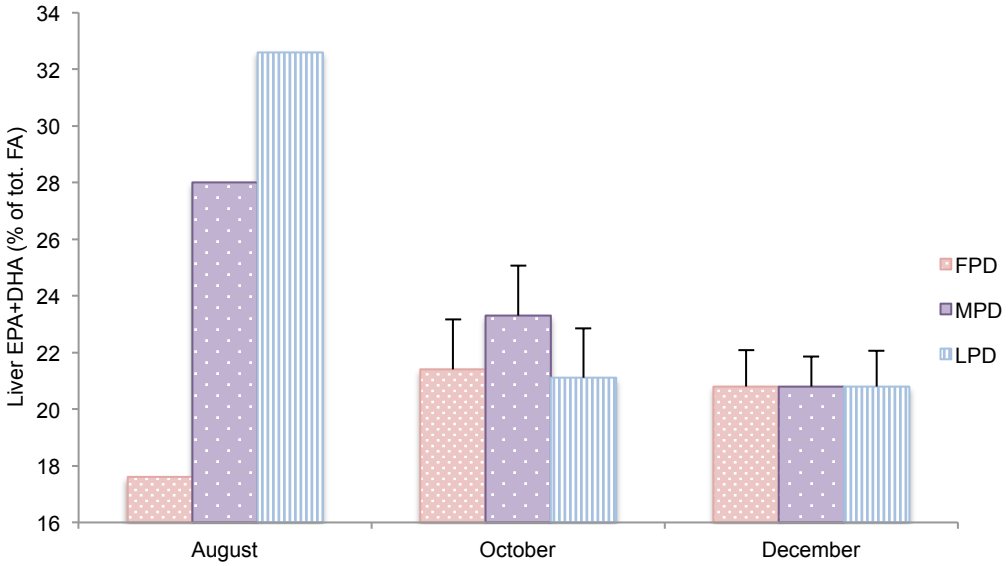
		Main diet							Pre-diet		
FA		August	diet	October	diet	December		August	October	December
16:0	MO	12.7 ± 1.88	12.7	10.6 ± 0.40 ^{a}	8.5	9.5 ± 0.15 ^{b}	F	9.0	9.6 ± 0.35	9.2 ± 0.29
	VO	12.7 ± 1.88	8.5	8.9 ± 0.32 ^{bc}	8.5	8.7 ± 0.24 ^{c}	M	14.7	10.6 ± 0.36	9.2 ± 0.36
							L	14.5	9.1 ± 0.36	8.8 ± 0.32
18:1 n-9	MO	3.0 ± 0.25	26.7	3.8 ± 0.05 ^{a}	41.7	3.5 ± 0.04 ^{b}	F	3.4	3.6 ± 0.65	3.4 ± 0.07
	VO	3.0 ± 0.25	41.7	3.3 ± 0.05 ^{c}	41.7	3.4 ± 0.04 ^{c}	M	2.8	3.5 ± 0.65	3.4 ± 0.08
							L	2.6	3.6 ± 0.65	3.5 ± 0.05
18:2 n-6	MO	0.14 ± 0.02	8.1	0.1 ± 0.02	13.8	0.1 ± 0.00	F	0.1	0.2 ± 0.02	0.1 ± 0.01
	VO	0.14 ± 0.02	13.8	0.1 ± 0.00	13.8	0.1 ± 0.01	M	0.2	0.1 ± 0.02	0.2 ± 0.01
							L	0.1	0.1 ± 0.02	0.2 ± 0.01
18:3 n-3	MO	0.1 ± 0.02	3.4	0.2 ± 0.01 ^{c}	6.4	0.2 ± 0.01 ^{a}	F	0.2	0.1 ± 0.01^{b}	0.2 ± 0.01^{a}
	VO	0.1 ± 0.02	6.4	0.2 ± 0.01 ^{b}	6.4	0.2 ± 0.01 ^{a}	M	0.1	0.1 ± 0.01^{b}	0.2 ± 0.01^{a}
							L	0.1	0.1 ± 0.01^{b}	0.2 ± 0.01^{a}
20:5 n-3	MO	8.5 ± 1.45	10.2	9.7 ± 0.30 ^{a}	4.6	7.7 ± 0.19 ^{b}	F	5.6	7.9 ± 0.61	7.6 ± 0.40
	VO	8.5 ± 1.45	4.6	6.3 ± 0.31 ^{c}	4.6	6.8 ± 0.18 ^{c}	M	9.5	8.6 ± 0.61	7.7 ± 0.36
							L	10.2	7.9 ± 0.61	7.2 ± 0.38
22:6 n-3	MO	17.6 ± 3.00	7.3	16.7 ± 0.49 ^{a}	3.4	13.3 ± 0.39 ^{b}	F	12.0	13.6 ± 1.12	12.8 ± 0.91
	VO	17.6 ± 3.00	3.4	11.1 ± 0.59 ^{c}	3.4	12.0 ± 0.38 ^{c}	M	18.4	14.8 ± 1.10	12.9 ± 0.71
							L	22.3	13.4 ± 1.10	12.3 ± 0.79
Sum n-3	MO	30.8 ± 3.83	24.4	34.9 ± 0.60 ^{a}	15.3	27.3 ± 0.57 ^{b}	F	23.7	21.4 ± 2.01	27.1 ± 1.23
	VO	30.8 ± 3.83	15.3	23.9 ± 0.88 ^{c}	15.3	25.5 ± 0.52 ^{c}	M	32.0	23.3 ± 2.01	27.1 ± 1.08
							L	36.8	21.1 ± 2.01	26.9 ± 1.10
Sum n-6	MO	9.4 ± 2.27	9.5	9.6 ± 0.16 ^{c}	14.6	12.8 ± 0.06 ^{b}	F	13.8	11.7 ± 0.12^{b}	12.9 ± 0.10^{a}
	VO	9.4 ± 2.27	14.6	13.1 ± 0.19 ^{ab}	14.6	13.7 ± 0.08 ^{a}	M	6.2	11.1 ± 0.12^{b}	12.8 ± 0.06^{a}
							L	8.3	11.3 ± 0.12^{b}	12.9 ± 0.06^{a}
SAFA	MO	20.2 ± 2.33	22.8	18.9 ± 0.43 ^{a}	15.2	16.1 ± 0.25 ^{b}	F	15.6	16.9 ± 0.74^{ab}	15.5 ± 0.54^{b}
	VO	20.2 ± 2.33	15.2	15.8 ± 0.41 ^{bc}	15.2	14.7 ± 0.35 ^{c}	M	22.4	18.4 ± 0.74^{a}	15.6 ± 0.49^{b}
							L	22.7	16.7 ± 0.74^{ab}	15.1 ± 0.50^{b}

No effect of pre-dietary treatment on EPA+DHA content in liver was observed during the period from August to December. In spite of the difference in liver EPA+DHA content between pre-dietary groups in August (fig. 4.11.a), equal level of EPA+DHA (20.8%) for the FPD, MPD and LPD groups was observed in December.

In August the initial level of EPA+DHA in liver was 26.1% ± 4.4% for the MO and VO groups. As shown in fig. 4.11.b, the level of EPA+DHA increased in the MO group (26.4% ± 0.8%) and declined in the VO group (17.5% ± 0.9%) in October. In December, after the changing the diet for MO group, EPA+DHA content significantly decreased (21.0% ± 0.6%).

Liver EPA+DHA content in the VO group increased in December (18.9% ± 0.5%), but was significantly lower then that in the MO group.

a)



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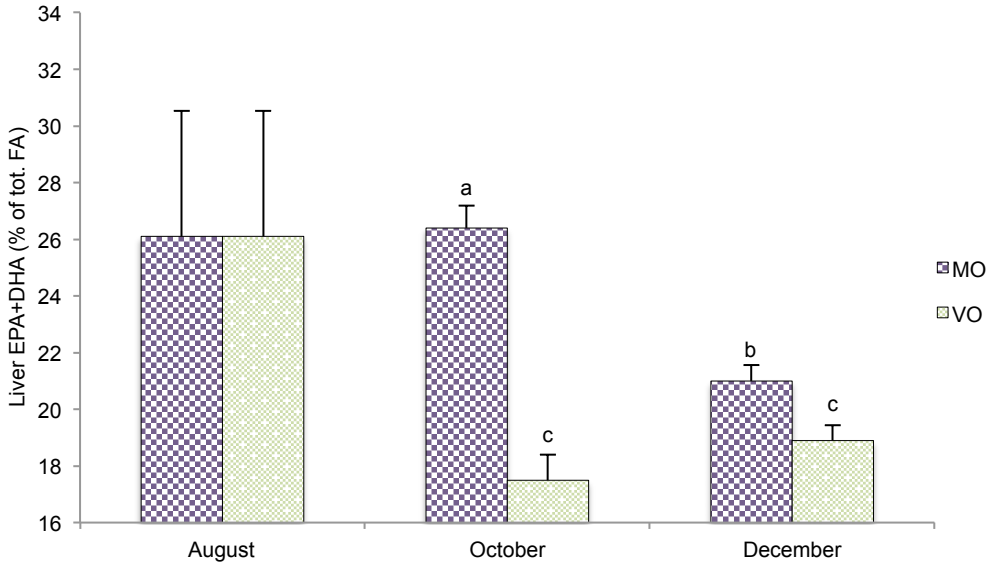

Figure 4.11 EPA and DHA (% of total fatty acids) of total lipid in liver tissue in Atlantic salmon sampled in August, October and December according to a) pre-diets (FPD, MPD, LPD) and b) main diets (MO; VO). The data given as mean ± standard error of mean. Different letters show statistical differences between dietary treatments and sample times ($P < 0.05$).

4.5 Heart

Mass index

The heart mass index showed no significant differences between the pre-dietary fish groups in August (*P = 0.87*) (tab. 4.12). Heart mass index*100 in October was significantly higher for salmon fed the LPD pre-diet (10.0 ± 0.2) compared to the FPD (9.3 ± 0.2) and MPD (9.6 ± 0.2) treatments, and in December the heart mass index of the LPD group was significantly higher (9.4 ± 0.1, *P = 0.0003*) compared with FPD and MPD treatments (8.9 ± 0.1) (Fig. 4.12).

There was no significant difference between main dietary treatments in October (*P = 0.4*), but in December fish fed the MO diet had significantly (*P = 0.005*) lower relative heart weight (8.9 ± 0.0) compared to fish fed the VO (9.2 ± 0.0).

Table 4.12 Average mass of heart (%BW*100) of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO, VO). Results are shown for salmon sampled in October and December (LSmeans ± SE). Different superscript letters indicate significant differences between groups within sampling time (*P < 0.05*).

	August			October			December		
	FPD	MPD	LPD	FPD	MPD	LPD	FPD	MPD	LPD
MO	9.9±0.2	10.0±0.2	10.0±0.2	9.20 ± 0.13	9.61 ±0.23	9.91 ± 0.24	8.71 ± 0.13^{c}	8.75 ± 0.14^{c}	9.17 ± 0.12^{b}
VO				9.44 ± 0.29	9.59 ± 0.33	10.16 ± 0.17	9.06 ± 0.12^{bc}	8.98 ± 0.14^{bc}	9.55 ± 0.115^{a}

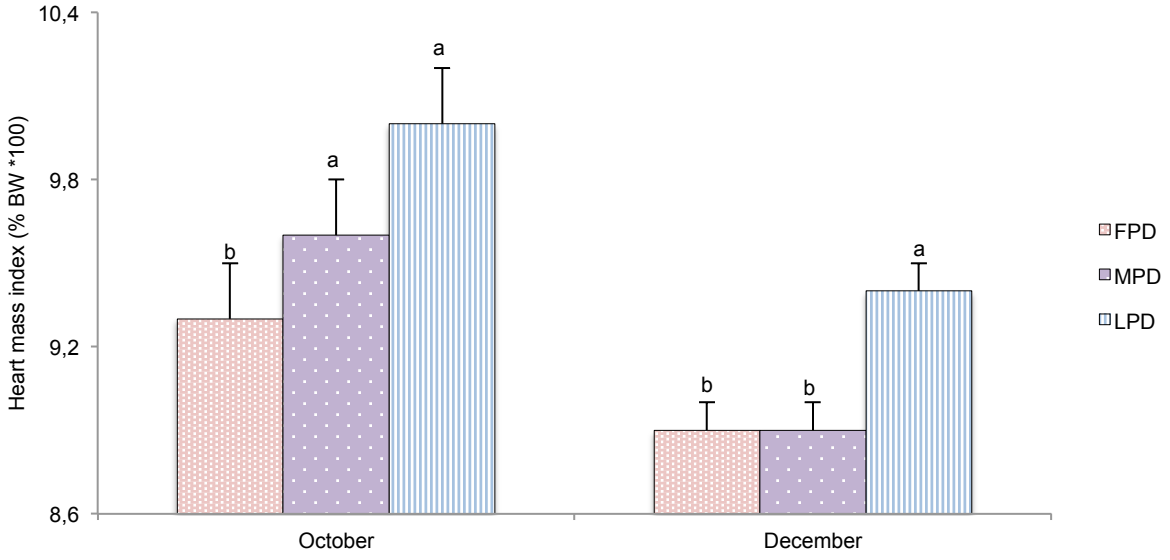

Figure 4.12 Heart mass index (% BW*100) of farmed Atlantic salmon fed three pre-diets (FPD, MPD, LPD). Different letters denote significant differences (*P < 0.05*) between dietary treatments within one sampling.

Lipid content

As shown in the table 4.13, the lipid content in heart tissue of Atlantic salmon from the MPD group in August was lower than in the FPD group (Δ 1.5%) and higher than in the LPD group (Δ 0.7%). The average percentage heart lipids increased for all pre-dietary groups in October, with the most significant accumulation being observed for the LPD group, independent of main dietary treatment. No significant differences were observed between main dietary groups (*P > 0.05*).

Table 4.13 Development in lipid content (% wet weight) in heart of Atlantic salmon fed three pre-diets (LPD, MPD, FPD) from May-August and thereafter two main diets (MO, VO). Results are shown for salmon sampled in October (LSmeans ± SE). Different letters show statistical differences within the same row (*P < 0.05*).

	MO			VO		
	FPD	MPD	LPD	FPD	MPD	LPD
August	5.1	3.6	2.9	5.1	3.6	2.9
Δ Aug—Oct	0.8 ± 0.47^{c}	2.6 ± 0.47^{ab}	3.2 ± 0.47^{a}	0.9 ± 0.47^{c}	1.6 ± 0.47^{bc}	2.0 ± 0.47^{ac}
October	5.9 ± 0.46	6.1 ± 0.46	6.1 ± 0.46	6.0 ± 0.46	5.1 ± 0.46	4.9 ± 0.46

FA composition

The fatty acid profile of the main diets was mirrored in the heart tissue (Table 4.14). The level of saturated fatty acids significantly increased in the MPD group and decreased in the VO group in the period from August to October. Additionally, the decrease in palmitic acid, the most abundant SAFA, was most pronounced in the VO group. Concentrations of 18:1n-9, 18:2n-6 and 18:3n-3 increased from August to October in both groups, but the levels of these FAs was significantly higher in the VO group (*P < 0.05*), as well as total level of n-6 fatty acids. Decrease in EPA and DHA compared to August in the MO and VO groups was most pronounced in the VO group that resulted in significantly higher level of these fatty acids in the MO group in October (*P < 0.05*). There was no statistically confirmed correlation between the fatty acid profile in the heart and pre-dietary treatments.

Table 4.14 Fatty acid composition (% of total fatty acids ± SE) of total heart lipids in Atlantic salmon sampled in August and October. Results are shown for main diets (MO, RO) and pre-diets (FPD, F; MPD, M; LPD, L). Different asterisk indicate statistical differences between diets in October (*P < 0.05*).

		Main diet			Pre-diet		
FA		August	diet	October		August	October
C 16:0	MO	16.3 ± 1.13	12.7	15.8 ± 0.18 *	F	14.2	14.2 ± 0.58
	VO	16.3 ± 1.13	8.5	14.1 ± 0.36	M	18.0	15.5 ± 0.29
					L	16.8	15.1 ± 0.62
C 18:1 n-9	MO	19.4 ± 3.46	26.7	21.4 ± 0.80	F	26.3	26.9 ± 1.89
	VO	19.4 ± 3.46	41.7	28.9 ± 0.61 *	M	15.9	23.8 ± 2.17
					L	16.0	24.7 ± 2.53
C 18:2 n-6	MO	6.3 ± 1.12	8.1	6.8 ± 0.22	F	8.6	8.7 ± 0.71
	VO	6.3 ± 1.12	13.8	9.3 ± 0.29 *	M	5.0	7.5 ± 0.61
					L	5.4	7.9 ± 0.82
C18:3 n-3	MO	2.2 ± 0.56	3.4	2.5 ± 0.10	F	3.4	3.4 ± 0.33
	VO	2.2 ± 0.56	6.4	3.6 ± 0.14 *	M	1.6	2.8 ± 0.28
					L	1.8	3.0 ± 0.37
C 20:5 n-3	MO	7.0 ± 0.86	10.2	6.9 ± 0.22 *	F	5.3	5.5 ± 0.49
	VO	7.0 ± 0.86	4.6	5.1 ± 0.18	M	8.0	6.3 ± 0.52
					L	7.7	6.3 ± 0.57
C 22:6 n-3	MO	19.5 ± 2.83	7.3	13.5 ± 0.50	F	14.1	11.8 ± 0.69
	VO	19.5 ± 2.83	3.4	11.9 ± 0.51	M	20.9	13.3 ± 0.64
					L	23.6	13.0 ± 0.73
EPA+ DHA	MO	26.5 ± 3.64	17.5	20.4 ± 0.69 *	F	19.3	17.3 ± 1.12
	VO	26.5 ± 3.64	8.0	17.1 ± 0.67	M	28.9	19.6 ± 1.05
					L	31.3	19.3 ± 1.26
Sum n-3	MO	32.0 ± 3.66	24.4	26.7 ± 0.76 *	F	24.9	23.7 ± 1.05
	VO	32.0 ± 3.66	15.3	23.6 ± 0.65	M	34.2	25.9 ± 1.10
					L	37.0	25.9 ± 1.26
Sum n-6	MO	8.2 ± 1.03	9.5	9.1 ± 0.22	F	10.3	11.0 ± 0.72
	VO	8.2 ± 1.03	14.6	11.6 ± 0.28 *	M	6.9	9.9 ± 0.63
					L	7.4	10.3 ± 0.84
SAFA	MO	23.9 ± 1.32	22.8	24.8 ± 0.27 *	F	21.4	22.2 ± 1.06
	VO	23.9 ± 1.32	15.2	21.5 ± 0.49	M	25.8	23.9 ± 0.67
					L	24.5	23.4 ± 1.18

5. DISCUSSION

The aim of pre-dietary treatment was to produce salmon with different fat content and growth potential. The fat fish with initially high body weight and muscle fat content compared to medium fat and lean fish had lowest growth and the fat accumulation rate during the experimental period. Viscera mass index significantly decreased in fat fish and increased in lean fish. The development of lipid content in viscera, liver and heart showed the same tendency as development of lipid content in skeletal muscles. During the experimental period the fat accumulation rate in viscera and liver was the lowest in fat fish and the highest in lean fish. Liver mass index increased significantly during the experimental period in lean fish that indicates the high intensity of metabolic processes in this group. Heart mass index and the rate of fat accumulation in heart were the highest in lean fish as well.

The main dietary treatment started in August has no effect on body weight. Slaughter yield, previously affected by the pre-dietary treatment, in December showed the significant difference between MO (lower) and VO (higher) groups. The main dietary treatment influenced viscera and heart mass index, and viscera and liver fat content.

The most important result of the main-dietary treatment was the variation in fatty acid composition of organs and tissues in Atlantic salmon. The special focus was on polyunsaturated fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA+DHA percentage in muscle lipids significantly increased in MO group compared to VO group at the start of the main dietary treatment. After the switched MO diet to VO diet which had the low content of EPA and DHA, the percentage of these fatty acids decreased insignificant in muscle fat, that indicates the high intensity of fatty acids retention in muscles of Atlantic salmon in autumn. The other examined tissues changed the fatty acid profile accordant with fatty acid composition of the diet.

6. CONCLUSIONS

It is suggested that improved sustainable utilization of marine fish oil may be achieved through feeding fish lower levels during periods where the fish is utilizing lipid for energy production and elevated levels in periods with high fat retention; i.e. using elevated levels of dietary fish oil during the autumn for Atlantic salmon.

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8. ATTACHMENT

Approximately 2.5 g of the tissue homogenates were weighted out and placed in Erlenmeyer flasks. Then 6 ml saltwater solution (0.9 % NaCl) and 50 ml chloroform/methanol (2:1) were added, before the samples were homogenized for 60 seconds with an Ultra-Turrax knife-homogenizer (IKA Werke GmbH & Co. KG, Germany). After 60 seconds with homogenization 6 ml of saltwater (0.9% NaCl) was added and the mixed solutions were separated into two layers. The top layer consists of mainly salt water and methanol (water soluble phase) and the bottom layer consists of mainly lipids and chloroform (lipid phase). Afterwards the solutions were filtered into a measuring cylinder for further separation over several hours. The water-soluble phases were then discarded and 20 ml of the lipid phase were transferred with a pipette into numbered beakers. The rest of the lipid phase were transferred into test tubes and corked for fatty acid analysis. The chloroform in the breakers was then evaporated, by placing the beakers on a heat plate. After the evaporation the beakers were placed in an incubator with a temperature of 100 °C for 30 minutes. Finally, the beakers cooled off in room temperature before end weighing. Parallel samples were conducted. For each series of analysis control samples were carried out, using LT-fishmeal as a reference of the performed analysis. The total lipid content was calculated using equation 4, see calculations 3.5.