# Dietary impact on texture, gaping and liquid loss in fillets of farmed Atlantic salmon (Salmo salar L.)

Kristine Misund Bringslid

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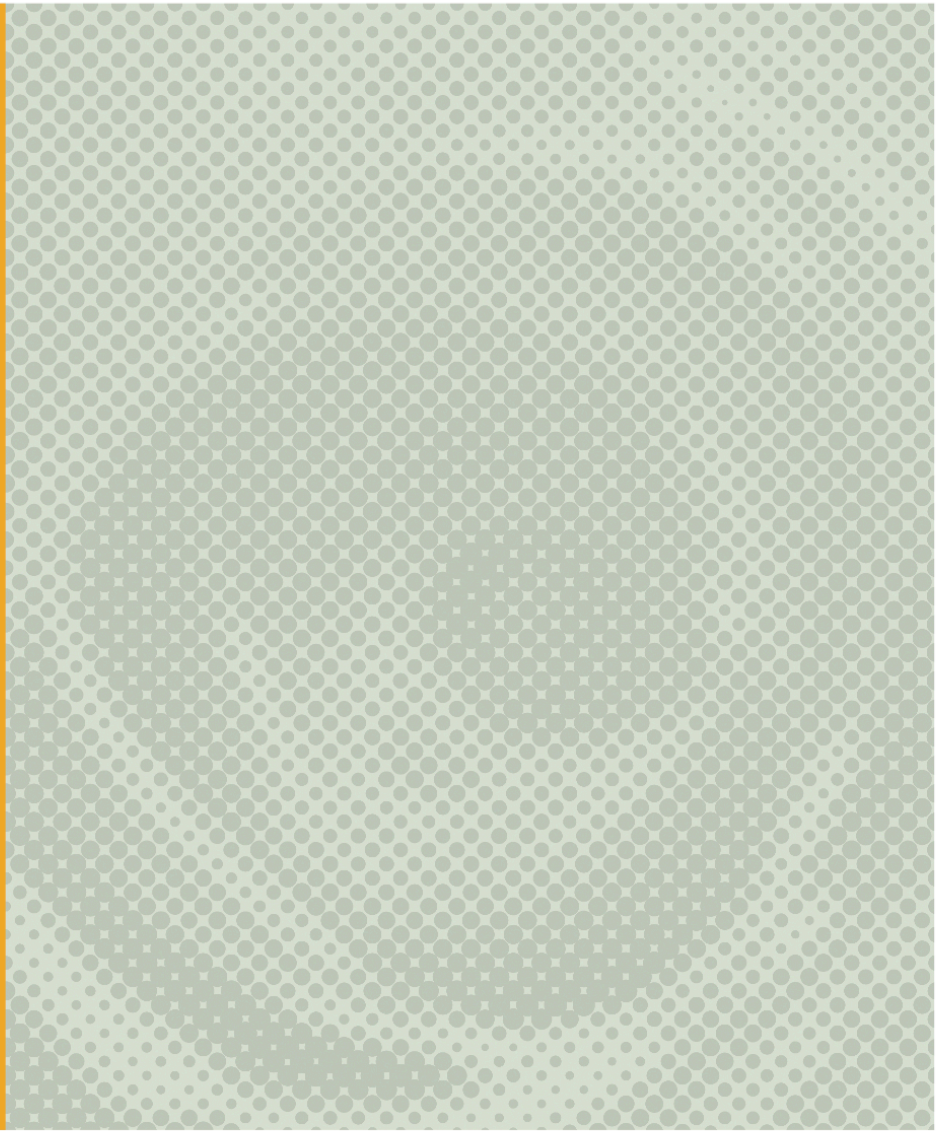

## Preface

This study was a part of the project “Fat and texture in fillet of farmed salmon”. It was performed by Nofima, under leadership of Dr. Turid Mørkøre and financed by the FHF (Fiskeri- og havbruksnæringens forskningsfond).

I wish to express my sincere gratitude to my supervisor Dr. Turid Mørkøre and my co-supervisor Prof. Kjell-Arne Rørvik for including me in this project and advising me through the thesis.

A special thanks to Målfrid T. Bjerke, who guided me through large parts of the field- and laboratory work.
I also want to thank Magnus Åsli for answering many of my questions and sharing pictures from the sampling.

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Finally, I want to thank my family for moral and economic support during my studies at the Norwegian University of Life Sciences (UMB).

## Abstract

A seven-month long feeding trial was conducted to investigate the dietary influence on texture, gaping and liquid loss in fillets of Atlantic salmon.
During the first 3 months of the experiment the salmon where fed a diet with high fat content (36%) and a diet with low fat content (18%). One group where also fed the low fat diet in half ration. As expected, this resulted in groups of salmon with significant differences in body weight and fat content. By achieving this, we could study the differences in flesh quality and fat composition among groups with different growth potential through the autumn.
These groups where then mixed in new net pens and fed different diets for the next 4 months. The new diets had equal fat content, but different main lipid source (rapeseed oil/marine oil) and protein level.
Main dietary oil source in the diet did not affect the slaughter parameters, except for the slaughter yield. The fatty acid composition of the fillets, were also strongly affected by dietary oil source. Quality parameters were not affected by oil source, except for a somewhat lower liquid loss in the group fed the marine70 diet.
The present study did not see any significant effect of adding extra amino acids to a diet in terms of slaughter parameters, fat and fatty acid composition and quality parameters.

# Table of contents

# 1. INTRODUCTION

Atlantic salmon is one of the most significant species in global aquaculture, especially in terms of value. The production has increased dramatically over the last 10 years and in 2010 the total production was over 1 460 000 tonnes (FAO, 2010). Norway was the largest producer with 944 600 tonnes, accounting for about 65 % of the total amount. Most of the Norwegian salmon was exported, reaching a value of 31,3 billion NOK. This exceeded the value of export from fisheries the same year, making salmon farming one of Norway's most important industries (FHL, 2011).
Production of Atlantic salmon is predicted to continue growing in the future to meet food demands for the increasing human population (FAO, 2010). Though there are some limitations as the situation is today.

Harvesting of wild fish stocks for fishmeal and fish oil have already reached its maximum sustainable level (FAO, 2010). Today 70% of the fishmeal and almost 90 % of the fish oil, produced globally, are used in feed for aquatic animals (Naylor et al, 2009). It has therefore been a strong focus on improving the utilization of raw materials in the fish feed industry (Bostock et al, 2010). Replacing fish oil and fishmeal with vegetable protein and oils has been one of the solutions, but this has created some new challenges. The Norwegian institute of public health recommends intake of fatty fish like salmon, due to its high content of long chained omega-3 fatty acids; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are known to be health beneficial, especially in relation to the cardiovascular system and development of the brain in humans (Frøyland et al 2011). Unfortunately, the fatty acid composition of fish flesh tends to reflect the fatty acid composition of the diet (Bell 1998). Hence, the replacement of fish oil lowers the EPA and DHA levels and thereby reduces the health effects of consuming salmon.

To maintain a good reputation and a large market share, it is of importance to the salmon farming industry to achieve high flesh quality.
Concerning the inclusion of vegetable proteins and oils in feed for salmon and its effect on flesh quality, the results from earlier studies are somewhat contradicting (Thomassen & Røsjø, 1989; Hardy et al, 1987; Johnston, 1999; Regost et al, 2004).

The effect of seasonal variations and growth rate on texture of farmed salmon fillets, are to a larger extent a more established fact (Mørkøre & Rørvik, 2001; Roth et al 2005). A desirable high growth rate, can cause softening of the fish flesh and thereby downgrading after slaughtering. A recent study has however shown that addition of amino acids in the feed can improve the texture without affecting the growth (Mørkøre et al, 2010).

The aim of this thesis is to look at dietary effects on texture, gaping and liquid loss in fillets of farmed Atlantic salmon, in relation to fat level, fat source and amount of amino acids in the feed.

# 2. THEORETICAL BACKGROUND

## 2.1 Flesh quality

Flesh quality is usually defined by taste, smell, appearance, firmness, juiciness and process characteristics. Feeding regime, diet composition and the environment are factors known to affect the condition of the muscle tissue and thereby the flesh quality of fish (Johnston, 1999).

Sustaining high flesh quality is important to the fish farming industry, to maintain a good reputation and market share. Low fish flesh quality leads to reduced consumer acceptability (Ando, 1999) and downgrading (Mitchie, 2001). Thereby lowering the value of the product resulting in an economic loss for the farmers.

The most important flesh quality traits for Atlantic salmon are colour, texture, fat content and chemical composition of the flesh.

## 2.2 Muscle composition

The main part of fish flesh is water, and in fatty fish like salmon the water content is approximately 64%. Protein levels vary from 20 – 22 %, while lipids usually constitutes about 13 -15 % (Fennema, 1996).

The fillet of an Atlantic salmon consists of three major components; muscle blocks of contractile protein (myotomes), connective tissue (myocommata) and lipids (Kiessling, 2006). The myotomes and myocommata are found in separate, parallel layers, giving the fillet a striated, W-shaped formation (Figure 2.1). Actin and myosin constitutes the main part of the contractile protein, which are organised in myofibrils inside the muscle fibres (Love, 1970). The connective tissue is made up by collagen and forms the cytoskeleton of the muscle. Lipids are located in the cell membranes as phospholipids, in the cytoplasma of the muscle fibres, as lipid droplets or in the adiposities as storage lipids (Kiessling, 2006).

Salmon has two types of muscle tissue. White muscle composed of high contracting fibres used for rapid swimming and dark muscle containing slow contracting fibres used for sustained swimming (Johnston et al, 1977).

The white muscle, which is high glycolytic and anaerobic is the dominating muscle type constituting up to 90% of the total muscle (Kiessling, 2006). In Atlantic salmon the white muscle is pigmented red, from the carotenoids in the diet.

The dark muscle is aerobic and has its colour due to rich blood supply and high concentrations of myoglobin (Johnston, 1977).
In meat from mammals, the texture is directly affected by the connective tissue. Fish muscle contains less connective tissue (Sato et al, 1986), and is not as important for the texture except for in raw and smoked products (Johnston et al, 2000). Studies have revealed that there is a correlation between muscle diameter, muscle fibre density and firmness (Hatae et al 1986, Hurling et al 1996, Johnston et al 2000). Small average diameter and high density of muscle fibres, generally gives the highest firmness.

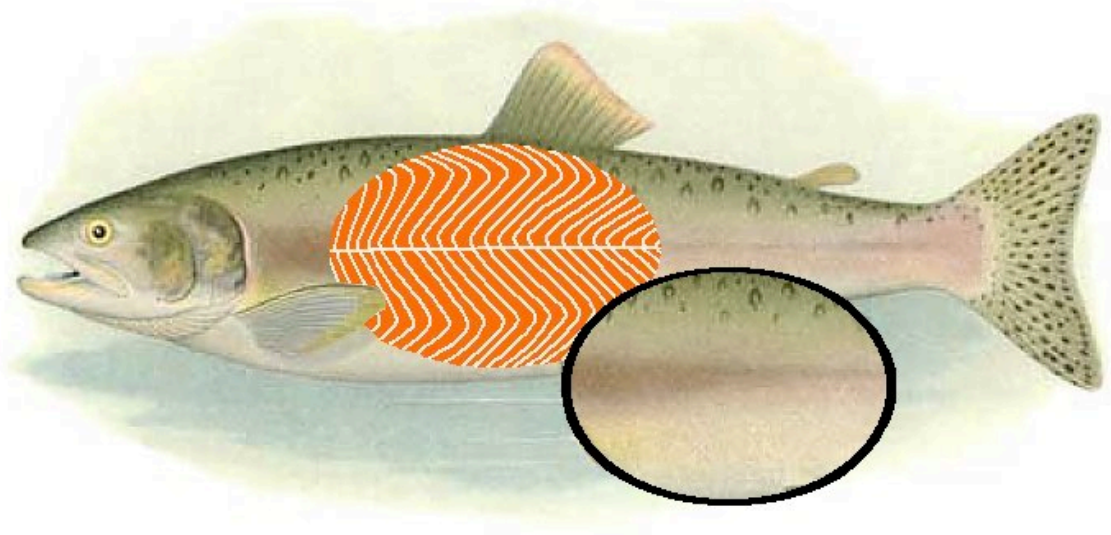

**Figure 2.1:** Picture showing the striated, W- shaped muscle of salmon, where the thick orange lines are the muscle blocks (myotome) and the thin white lines are the connective tissue (myocommata).
The illustration is adapted from http://www.earthlife.net/fish/muscles.html.

### 2.3 Lipids

Lipids are organic substances, insoluble in water, but soluble in non-polar organic solvents. They are an important source of metabolic energy (ATP), since they have the highest energy level of all nutrients, with a gross energy value of 9.5 kcal/g (Tacon, 1987). Lipids are especially important as energy source for carnivorous coldwater species like salmonids, which has a low ability to utilize carbohydrates for energy. Dietary lipids may be protein sparing, and thereby have a positive effect on feed utilisation and growth (Watanabe 1982; Hardy 1999).

Lipids can be categorized as; neutral lipids, where triacylglycerides constitutes the largest part or polar lipids, dominated by phospholipids (Tacon, 1987).
In salmonids, the triacylglycerides are the most abundant lipid group, and may constitute more than 90 % of the total fat (Aursand et al, 1994). The main function of triacylglycerides is to work as fat depots. Several studies have shown that the fatty acid composition of triacylglycerides is strongly affected by the diet (Thomassen & Røsjø, 1989; Waagbø et al, 1991).
Phospholipids constitute approximately 6% of the total fat in salmonids (Aursand et al, 1994), and generally contain high levels of n-3 fatty acids. Phospholipids functions as the major building blocks of cell membranes. Contrary to the triacylglycerides, the fatty acid profile of phospholipids is not strongly correlated to the diet (Lie & Huse, 1992).

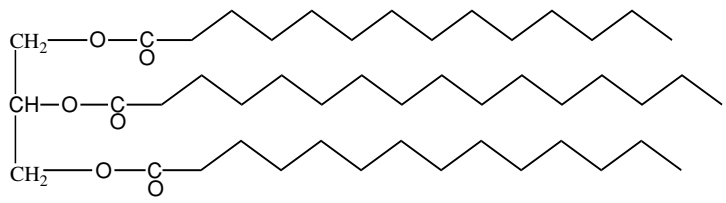

**Figure 2.2:** Triacylglycerol. (Halver, 2002)

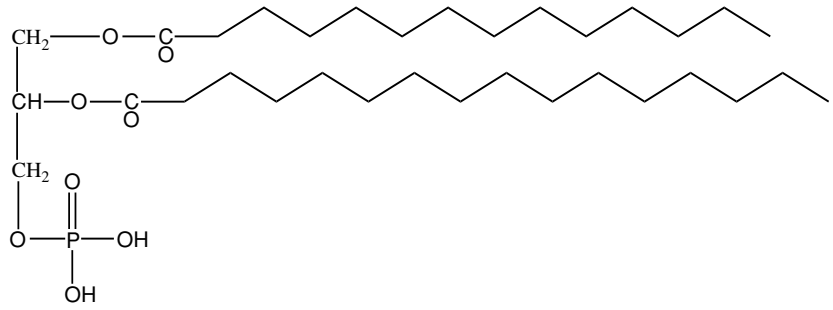

**Figure 2.3:** Phosphatic acid, the backbone of phosholipids (Halver, 2002)

### 2.3.1 Fatty acids

Fatty acids are the building blocks of most lipids, composed of carboxylic acids with long hydrocarbon chains. They are classified by chain length, number of double bonds and position of double bonds. Number of double bonds signifies degree of unsaturation. Fatty acids with no double bonds are saturated, with one double bond are monounsaturated and with more than one double bond are polyunsaturated (PUFA) (Halver, 2002).

Omega-3 fatty acids are PUFA's with the first double bond at the third position counted from the methyl end of the molecule. The body cannot synthesize omega-3 fatty acids itself, and they are therefore considered essential fatty acids – EFA (Børresen, 2008). The omega-3 fatty acids can be divided in two groups; the alpha-linolenic acid (C18:3 n-3) and the highly unsaturated fatty acids (HUFA - 20 or more carbons and 3 or more double bonds). C18:3 n-3 can be found in vegetable oil and nuts, while marine organisms are considered the main source of HUFA (Tacon, 1987)

The main omega-3 fatty acids in fish are eicosapentaenoic acid C20:5 n-3 (EPA) and docosahexaenoic acid C22:6 n-3 (DHA). Fatty fish like salmon, has a high relative oil content and is therefore rich in EPA and DHA (Halver, 2002).

| | Lard[a] | Palm[a] | Rape[a] | Soya[a] | Olive[a] | Linseed[a] | Herring[b] | Anchovy[b] |
|---|---|---|---|---|---|---|---|---|
| Global Production (tons × $10^{-6}$) in 1996[c] | 6.1 | 17.1 | 11.4 | 20.8 | 2.0 | 0.7 | 1.4[d] | 1.4[d] |
| Fatty acid | | | | | | | | |
| 16:0 | 26 | 61 | 5 | 11 | 14 | 7 | 13 | 17 |
| 16:1*n*-7 | 3 | tr[e] | tr | tr | 2 | tr | 7 | 9 |
| 18:0 | 15 | 5 | 2 | 4 | 3 | 5 | 1 | 4 |
| 18:1*n*-9 | 49 | 26 | 60 | 22 | 69 | 18 | 10 | 12 |
| 18:2*n*-6 | 9 | 7 | 21 | 54 | 12 | 17 | 1 | 1 |
| 18:3*n*-3 | tr | tr | 10 | 8 | 1 | 54 | 1 | 1 |
| 20:1*n*-9 | tr | 0 | 2 | tr | tr | 0 | 13 | 2 |
| 20:5*n*-3 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 17 |
| 22:1*n*-9 | 0 | 0 | 1 | tr | 0 | 0 | 0 | 0 |
| 22:1[f] | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 2 |
| 22:6*n*-3 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 9 |

[a] Data are mean values for the ranges quoted by Gunstone *et al.* (1994).
[b] Data from Sargent and Henderson (1995).
[c] Data from O'Mara (1998).
[d] Value for total global fish oil production.
[e] Trace.
[f] The *n*-9 isomer in the vegetable oils; the *n*-11 isomer in the fish oils.

**Figure 2.4:** Fatty acid composition of commercially available fats and oils. Table adapted from Fish nutrition: Halver, 2002.

## 2.4 Protein

Proteins are large molecules, with complex three-dimensional structures. They are made up by 20 different amino acids and are bound together by peptide bonds.

Protein is the major organic material in fish tissue, constituting between 65 and 75 % on a dry weight basis (Halver, 2002).

Consuming proteins is essential for the salmon to obtain essential amino acids. 10 amino acids are regarded as indispensable or essential for fish. This means that they cannot be synthesized in the body and have to be provided through the diet. These are; threonine, tryptophan, histidine, arginine, lysine, leucine, isoleucine, methionine, valine and phenylalaline (Tacon, 1987). Digestion of protein releases free amino acids that can be used to synthesize new protein by different tissues in the fish body. Amino acids are constantly used by the salmon, to either replace existing muscle fibres, or to build new ones, and a regular intake of protein is therefore necessary (Halver, 2002).

Insufficient intake of protein leads to reduction in growth, and can also result in weight loss due to a relocation of protein from less vital to vital tissues in the fish body.

In case of excessive intake of protein, only some of it will be used to synthesize new protein as most of it will be utilized as energy (Halver, 2002).

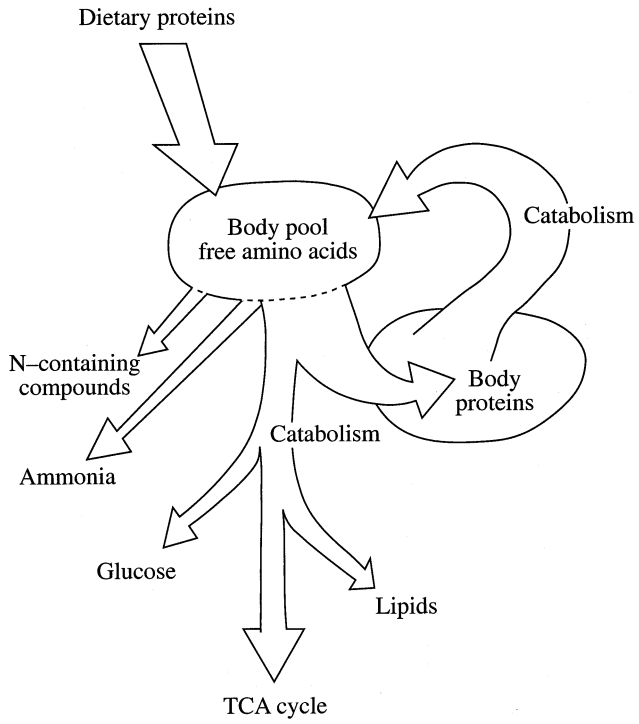


**Figure 2.5:** Main pathways of protein metabolism (Halver, 2002)

### 2.4.1 Arginine

Arginine is classified as an essential amino acid for birds, carnivores and young mammals. Organisms with a normal urea cycle produces arginine, and the amino acid is therefore only regarded as semi essential. Because fish has a limited urea cycle, it synthesizes too little arginine, and arginine is therefore essential in fish nutrition (Wilson, 1989)

Arginine serves as a precursor for nitric oxide, polyamines, proline, creatine, ornithine and agmatine. These compound plays important roles in signalling, growth regulation and cell proliferation (Wu and Morris, 1998).

Arginine can also stimulate the release of hormones, such as insulin and growth hormone (Mommsen, 2001)

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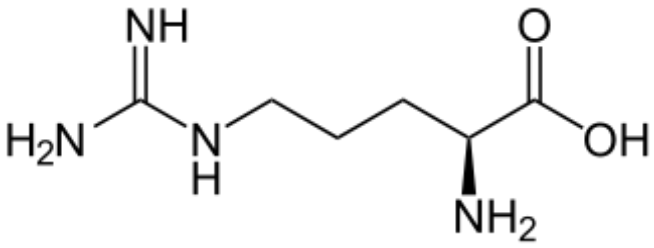



**Figure 2.6:** Showing the chemical structure of arginine.

### 2.4.2 Glutamate

Glutamate is regarded as a non- essential amino acid in fish nutrition, which mean that the body can synthesize sufficient amounts.

It plays an important role in the synthesis of protein and several pathways of the metabolism (Neu et al, 1996) Glutamate is also the precursor of other amino acids including proline, arginine, aspartate, ornithine and alanine. Proline can be of importance for muscle texture, since it plays an important part of synthesis of collagen and connective tissue (Tapiero et al, 2002).

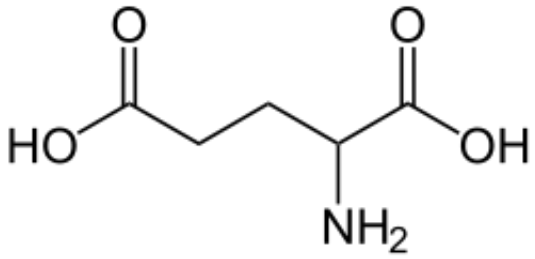



**Figure2.7:** Showing the chemical structure of glutamate.

### 2.5 Muscle pH

Under the anaerobic conditions in the fish muscle after slaughter, glycogen is converted to lactate. The level of muscle glycogen before slaughter is therefore of importance for the pH level in the muscle post rigor (Black and Love, 1988)

The pH of the muscle post-mortem can affect the texture, gaping and water holding capacity of fresh fillet (Love, 1988)

Low pH has resulted in higher frequency of gaping in Atlantic salmon (Lavety et al, 1988)

### 2.6 Liquid loss

Liquid loss describes the amount of water released from the muscle (Mørkøre, 2002). In fresh fish tissue, the water is tightly bound to the proteins, and will stay in the muscle structure even under high pressure. After a period of storage, the proteins gradually loose its ability to retain all the water and it will be lost from the fish muscle as drip, negatively affecting the flesh quality (Murray and Burt, 2001).

Liquid loss causes weight reduction and is therefore of economic importance. Exudates can also make the product less appealing to the consumer. The water holding capacity is decreasing when the pH is approaching the isoelectric point. This often occurs during rigor mortis. When the rigor stage is over, the pH will slightly increase and the water holding capacity will improve. (Lynnum, 2005)

### 2.7 Gaping

In fresh fish, the muscle blocks are firmly attached to the connective tissue.

According to Love (1970) gaping is a post-mortem phenonomen, caused by rupture of the connective tissue in the fillet, leading to flaking of the fillet.

Generally gaping occurs together with tissue softening (Bremner, 1992), although studies have shown that it may occur even when the flesh is firm (Mørkøre & Rørvik, 2001). Gaping can lead to economic losses as the fillets gets a less attractive appearance and cannot be mechanically skinned or sliced (Johnston, 1999).

### 2.8 Texture

Texture is an important quality parameter in farmed salmon, due to the consumer's low accept for soft fish flesh (Ando, 1999). Soft fish flesh can also cause problems during processing, not being able to be sliced and skinned mechanically (Michie 2001). This leads to significant economic losses for the fish industry, and it is therefore important to know the factors that affect the texture.

Growth pattern is one of the factors known to affect flesh texture. Studies have shown that rapid growth in the period before slaughter can cause soft fillet texture (Mørkøre et al, 2001). This is especially valid for salmon that has had a low growth rate, before the rapid growth period (Folkestad, 2008).

Contrary to most animals, fish grow both by increasing existing muscle fibres (hypertrophy) and by addition of new muscle fibres (hyperplasia) (Kiessling, 2006). Some studies indicate that salmon with small muscle fibres have a firmer flesh and less gaping than salmon with larger muscle fibres (Johnston et al, 2000). Amount of connective tissue can also affect the texture. Fillets with high content of connective tissue generally have a firmer texture than fillets with low content (Kiessling, 2006). High fat levels (> 18%) in the flesh may have unfavourable effects on texture and processing characteristics (Gjedrem, 1997).

It is important to consider the timing of the texture measurements, as the texture varies between pre-rigor, rigor and post-rigor states. (Børresen, 2008).

### 2.9 Seasonal variations in flesh quality

The physiology and metabolism of salmon farmed during the seawater phase is affected by the changes in environmental conditions throughout the seasons (Oheme, 2010). Temperature and photoperiod are parameters that vary greatly and are known to affect growth, feed conversion and quality of farmed fish.

When sexual maturation occurs protein and lipids from the fish muscle are moved to the gonads. The fillet quality decreases because of a loss of protein and lipid, replaced by increasing water content (Aksnes et al,1986).

### 2.10 Dietary effect on muscle composition

The fatty acid composition of fish flesh usually reflects the fatty acid composition of the diet, therefore the fatty acid profile of the fish flesh can be altered by changing the feed oil composition (Bell, 1998). Intake of all dietary energy over the maintenance requirements will result in lipid storage (Shearer, 1994). This means that both the level of energy in the diet and ration can affect the level of fat in the fish flesh.
Lipid and water content are inversely related in fatty fish like salmon (Shearer, 1994). Meaning that if the fat content increases the water content decreases and vice versa.

Protein levels in fish are regulated by endogenous factors like fish size and life cycle stage (Shearer, 1994). Dietary levels of protein will therefore not influence the protein level in the fish to a large extent. When the protein intake exceeds the ability of the fish to synthesize additional protein it will be deaminated and stored as fat.
According to Smith et al (1988) who fed diets containing plant or animal protein to rainbow trout, the source of protein has little effect on the protein content of the fish. The amino acid profile of the fish flesh neither seems to be affected by the diet formulation, although the free amino acid pool can be influenced (Schwarz and Kirchgessner, 1988).

# 3. MATERIALS AND METHOD

## 3.1 Experimental setup

The study was performed in 2011 at Nofima's research station on Averøy, Norway. In July the year before, 15 800 Atlantic salmon (*Salmo salar L.*) were put to sea (1+), with an average weight of 62 g. The fish originally came from "Urke fiskeoppdrett" and had been PD vaccinated. At start up of the experiment in May 2011, 1950 fish weighing approximately 1 kg were distributed evenly in 3 (7x7x7 m) net pens. The weight and length were recorded and pit tags were inserted to be able to follow each individual's performance. 30 fish were also taken out for analysis.

From May to August the fish were fed 3 different pre – diets; high fat, low fat and low fat in half ration. In August the fish were evenly redistributed in 12 (5x5x5 m) net pens, and fed 3 different main diets; 70% marine oil, 70% rapeseed oil and 70% rapeseed oil coated with extra protein, until the end of the experiment in December.

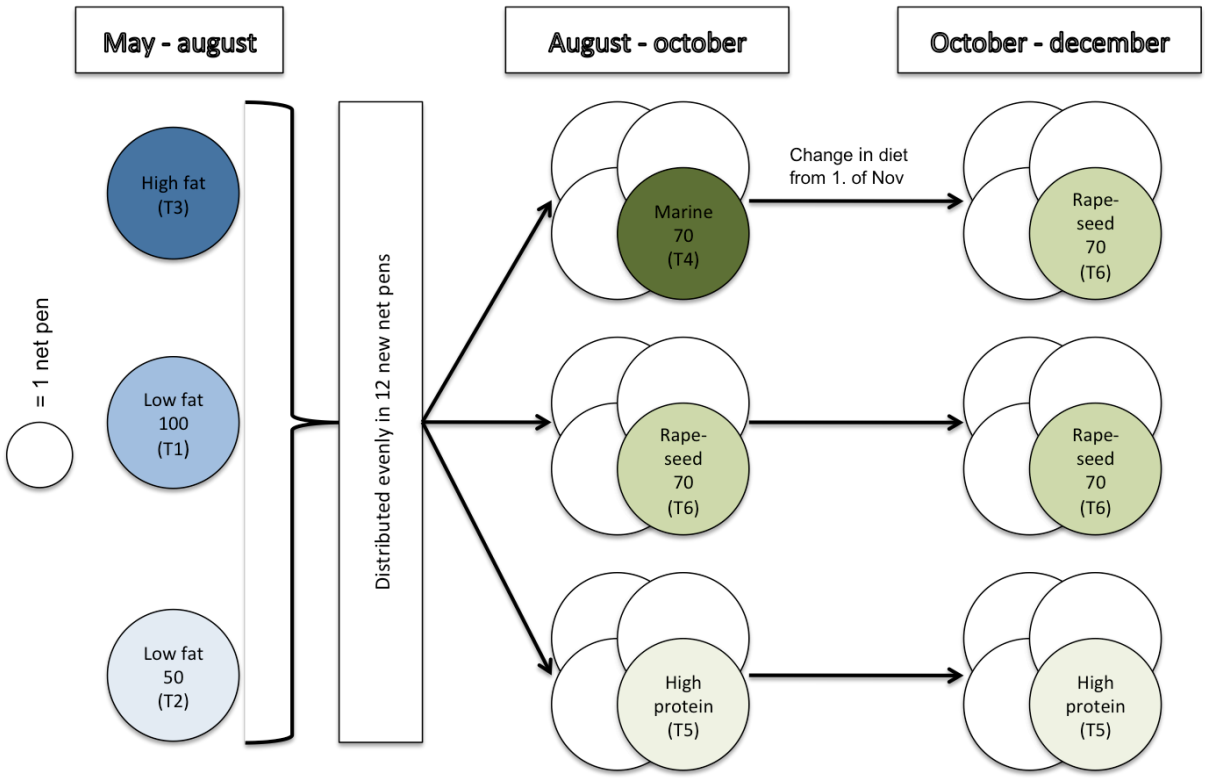



**Figure 3.1:** Overview of the project, showing the experimental setup.

The net pens in the last part of the experiment (August – December) had different number of fish, due to sampling from only the 6 first net pens in October.
Net pen 1-6 had 150 fish in each, 50 from each pre-diet, while net pen 7-12 had 120 fish in each, 40 from each pre-diet. The fish were also marked with different coloured floy tags on the back fin, so that one could easily see which pre-diet group they belonged to in the mixed net pens.

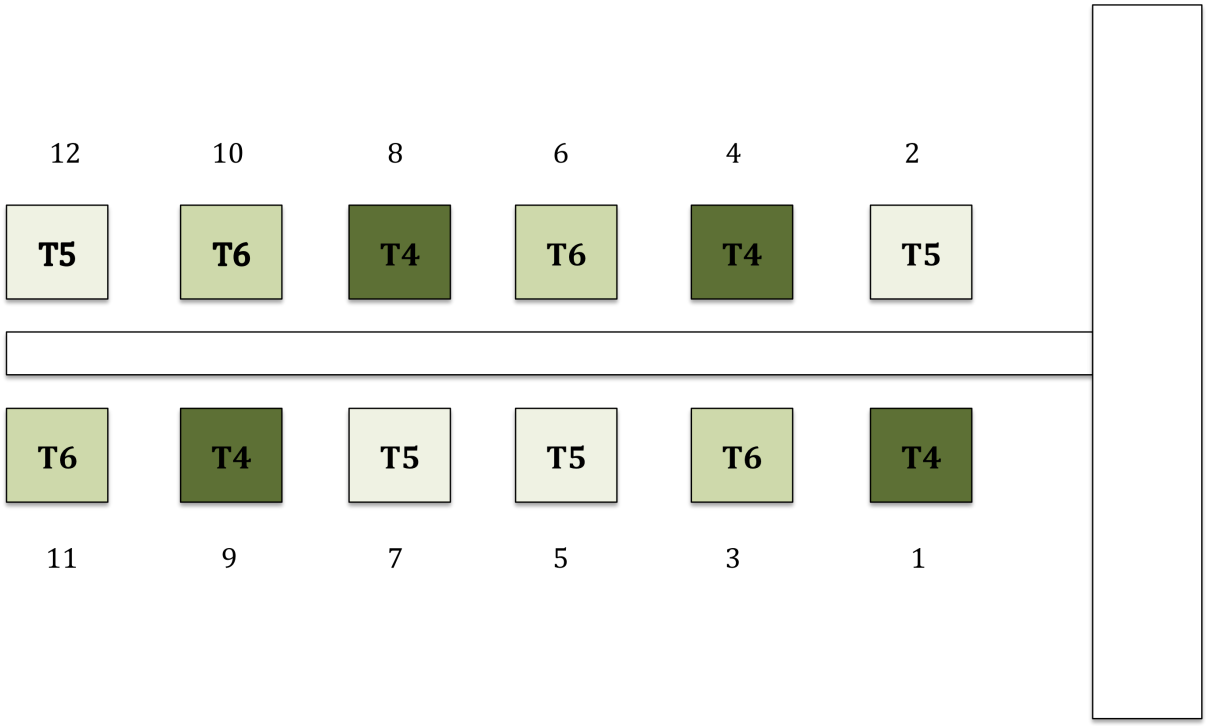



**Figure 3.2:** Overview of the net pens, where 1-12 is the number of the net pen and T4-T6 represents the different diets.

### 3.2 Diets and feeding

The fish were fed 3 - 4 times a day to satiation during the whole experimental period, except the group fed 50 % ration and in periods of starvation prior to handling.
Excess feed were collected on the bottom of the net pens, to get an accurate feed intake (method described by Einen et al 1999).

*3.2.1 Pre-diets (May - August)*

This part of the experiment was performed to create fish groups with different fat levels entering autumn, reflecting fish farmed at different degrees of longitude in Norway.
The diets consisted of:
Low fat100 (LF100) T1 - a commercial cod feed with a fat level of 18%, coated with astaxanthin. Given in 100% ration.
Low fat50 (LF50) T2- a commercial cod feed with a fat level of 18%, coated with astaxanthin. This group were given ½ ration of what the group on diet T1 ate the day before and only fed 2 times a day to ensure even distribution of the fed in the net pen.
High fat (HF100) T3 - a commercial salmon feed with a fat level of 36%.
Diet T1 was given to create a group with low fat level, diet T2 low fat level and a high potential for growth and diet T3 high fat level.
Amount of marine oils were equal in the salmon and the cod feed.

*3.2.2 Main diets (August - December)*

In this period the fish were given 3 diets with different oil source and protein level.
The diets consisted of:
Rapeseed70 (Rape70) T6 – control diet, a standard commercial salmon feed with 35% protein and 35% fat. Rapeseed oil constituting 70% of the fat.
High protein (HiPro) T5 – similar fat content and oil source as the control diet. Thus, added gelatine (coated on) to raise the protein level.
Marine70 (Mar70) T4 – similar protein/ fat ratio as the control diet, but with 70% marine oils.

From 1 of November to the termination of the experiment the group that had been given the diet with 70% marine oils, were switched to the control diet with 70% rapeseed oil.

*3.2.3 Chemical analysis of the diets*

The pre-diets were analysed for macronutrients only, while the main diets were analysed for amino acid and fatty acid profile as well. Amino acid content of the coating for the high protein diet (T5) is based on information from the producer.

| **Macronutrients in the pre-diets** | **Low fat100 (T1) & Low fat50 (T2)** | **High fat (T3)** |
|---|---|---|
| Crude protein Kjeldahl N*6,25 (%) | 49.9 | 33.5 |
| Ash (%) | 7.2 | 4.6 |
| Lipid (%) | 17.5 | 34.1 |
| Starch (%) | 6.2 | 9.3 |
| Total dry matter (%) | 91.7 | 93.4 |
| Free astaxanthin (mg/kg) | <1 | 46 |

**Table 3.1:** The macronutrients in the pre-diets. The feed was analysed by Nofima BioLab in Bergen. Vitamins and minerals were not analysed, but included in the total dry matter. Analysis was done prior to astaxanthin coating of diet T1 and T2.

| **Macronutrients in the main diets** | **Marine70 (T4)** | **High protein (T5)** | **Rapeseed70 (T6)** |
|---|---|---|---|
| Crude protein Kjeldahl N*6,25 (%) | 41.7 | 41.4 | 41.8 |
| Ash (%) | 4.9 | 4.9 | 4.9 |
| Lipid (%) | 31 | 32.3 | 31.1 |
| Starch (%) | 6.8 | 6.5 | 6.4 |
| Total dry matter (%) | 93,8 | 94,3 | 94 |
| Water (%) | 6.2 | 5.7 | 6 |
| Free astaxanthin (mg/kg) | 52 | 48 | 51 |

**Table 3.2:** The macronutrients in the main diets. The feed were analysed by Nofima BioLab in Bergen. Vitamins and minerals were not analysed, but included in the total dry matter. The analysis was performed prior to coating of the high protein diet.

| Amino acids in main diet (%) | | | |
|---|---|---|---|
| *Essential* | | Non essential | |
| Histidine | 0.91 | Alanine | 1.69 |
| Leucine | 2.87 | Aspartic acid | 3.39 |
| Isoleucine | 1.71 | Cysteine | 0.55 |
| Lysine | 2.42 | Glutamic acid | 8.88 |
| Methionine | 0.86 | Glycine | 1.73 |
| Phenylalaline | 1.86 | Hydroxylysine | 0.06 |
| Threonine | 1.35 | Proline | 2.46 |
| Tryptophan | 0.30 | Serine | 1.85 |
| Arginine | 2.36 | Tyrosine | 1.15 |
| Valine | 1.83 | Hydroxyproline | 0.0 |

**Table 3.3:** The amino acid content (%) of the main diets. The analysis was done prior to coating of the high protein (T5) diet, and was the same for all the 3 diets.

| Amino acids in coating (g/100 g protein) | | | |
|---|---|---|---|
| *Essential* | | Non essential | |
| Histidine | 1.1 | Alanine | 9.7 |
| Leucine | 2.5 | Aspartic acid | 5.9 |
| Isoleucine | 1.1 | Cysteine | 0 |
| Lysine | 3.8 | Glutamic acid | 12.0 |
| Methionine | 0.9 | Glycine | 19.6 |
| Phenylalaline | 1.7 | Hydroxylysine | 0.5 |
| Threonine | 2.6 | Proline | 13.2 |
| Tryptophan | 0 | Serine | 3.2 |
| Arginine | 10.0 | Tyrosine | 0.2 |
| Valine | 2.0 | Hydroxyproline | 9.0 |

**Table 3.4:** The amino acid content (%) of the coating applied on the high protein diet (T5).

| Fat level and fatty acid profile of the main diets | | |
|---|---|---|
| **Main diets** | **Rapeseed70 / High protein** | **Marine70** |
| **Fat level (%)** | 31.7 | 32.4 |
| **Fatty acids (%)** | | |
| C 14:0 | 2.4 | 4.9 |
| C 15:0 | 0.3 | 0.3 |
| C 16:0 | 8.5 | 12.7 |
| C 17:0 | 0.4 | 0.9 |
| C 18:0 | 2.7 | 3.3 |
| C 22:0 | 0.9 | 0.8 |
| C 14:1 n-5 | 0.2 | 0.2 |
| C 15:1 | 0.2 | 0.4 |
| C 16:1 n-5 | 0.2 | 0.1 |
| C 16:1 n-7 | 2.9 | 6.0 |
| C 16:1 n-9 | 0.2 | 0.4 |
| C 17:1 n-7 | 0.2 | 0.2 |
| C 18:1 n-7 | 0.2 | 0.1 |
| C 18:1 n-9 | 41.7 | 26.6 |
| C 20:1 n-7 | 0.0 | 0.2 |
| C 20:1 n-9 | 1.5 | 1.4 |
| C 20:1 n-11 | 0.8 | 0.5 |
| C 22:1 n-9 | 0.5 | 0.3 |
| C 22:1 n-11 | 0.9 | 1.4 |
| C 24:1 n-9 | 0.3 | 0.3 |
| C 16:2 n-3 | 0.1 | 0.1 |
| C 16:3 n-4 | 0.5 | 1.0 |
| C 18:2 n-6 | 13.8 | 8.1 |
| C 18:3 n-3 | 6.4 | 3.4 |
| C 18:3 n-6 | 0.1 | 0.3 |
| C 18:4 n-3 | 0.2 | 0.2 |
| C 20:2 n-6 | 0.2 | 0.1 |
| C 20:3 n-6 | 0.0 | 0.2 |
| C 20:4 n-3 | 0.0 | 1.8 |
| C 20:4 n-6 | 0.4 | 0.8 |
| C 20:5 n-3 | 4.6 | 10.2 |
| C 22:5 n-3 | 0.6 | 1.3 |
| C 22:6 n-3 | 3.4 | 7.3 |
| **Total EPA/DHA** | 8.0 | 17.5 |
| **Total n-0** | 15.1 | 22.8 |
| **Total n-3** | 15.3 | 24.4 |
| **Total n-6** | 14.5 | 9.4 |

**Table 3.5:** The fat level (%) and fatty acid profile of the main diets (T4 – T6).

### 3.3 Sampling

Samples of the fish were taken 4 times during the experiment. At starting point in May, entering autumn in August, during autumn in October and at termination in December.

*Procedure*

The fish were taken up from the net pen in batches, and then anesthetized in seawater with metacaine (MS 222 0.1 g/L, Alpharma, Animal Health Ltd, Hampshire, UK). Weight and length were measured, before the fish were returned to the net pen or taken out for analysis.

Fish that had lost weight from one sampling to the next were removed from the experiment, as well as sexually mature fish and fish that did not recover from the anaesthetization.

The fish taken out for analysis were killed by a strike to the head, cut through the gills and bled in a tank of seawater for 20 minutes.

After exsanguination the weight was recorded. The fish where then gutted and weight was recorded again. This was followed by manual filleting and recording of filet weight. On the right filet of 5 fish from each pre-diet the pH and temperature was measured using a pH meter (Wissenchaftlich-Techniche Werkstatten GmbH WTW, Weilheim, Germany) with an electrode (BlueLine 21, Schott Instruments Electrode, SI Analytics GmbH, Mainz, Germany) and a temperature compensator (TFK 325, WTW).

The NQC (Norwegian quality cut) area from all the right filets were packed and frozen for subsequent analysis. The left filets were packed whole after recording of gaping.

| **Sampling** | May | August | October | December |
|---|---|---|---|---|
| Dates | 10 – 13 | 9 - 11 | 18 - 19 | 6 - 9 |
| Net pens sampled | 1-3 | 1-3 | 1-6 | 1-12 |
| Individuals recorded | 1950 | 1620 | 900 | 1620 |
| Individuals for analysis | 30 | 60 | 180 | 360 |

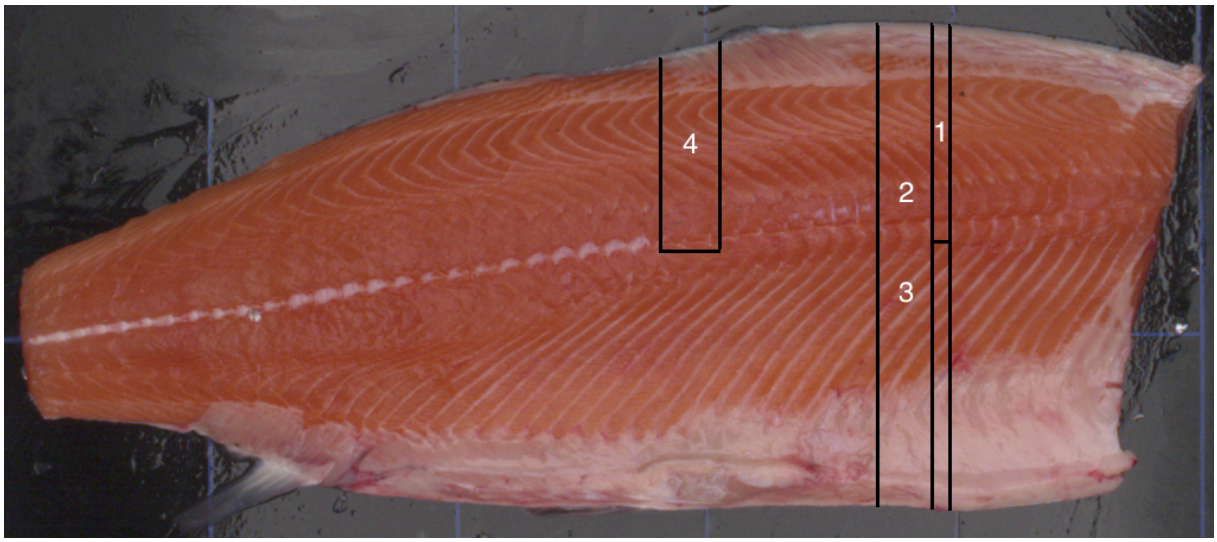
**Table 3.6 :** Overview of the sampling dates, witch net pens were sampled, number of individuals weighed and measured and number of individuals taken out for further analysis.

### 3.4 Seawater temperature

The temperature was registered at 3 meters depth, every day through the whole experimental period. In the period May – August the average temperature was 10.7 °C, while in August – December it was 10.9 °C. The coldest month was December with an average temperature of 6.9 °C and September was the warmest month with an average temperature of 14.1 °C.


**Figure 3.3:** Seawater temperature from May – December measured in °C.

### 3.5 Analysis




**Picture 3.1:** A left fillet side from the sampling in December. Illustrates which part of the fillet that was utilized in the different analysis:

1 – Liquid loss

2 – Texture

3 – pH

4 – Fat % and fatty acid composition (chemical analysis)

#### 3.5.1 Texture

The texture was measured 6 days after slaughter, using a Texture Analyser TA-XT2 (Stable Micro System Ltd., Surrey, UK). It was equipped with a 5 kg load cell and a flat-ended cylinder of 12.5 mm diameter, type P/0.5. The travelling speed was 1mm $s^{-1}$, and the trigger force was 0.2 N. A 25 mm thick cutlet, from the anterior part of the fillet was utilized for this analysis, and the probe were pressed down to 90% of the cutlet height (22.5 mm).

#### 3.5.2 Gaping

Gaping was recorded on the left fillet-side directly after slaughtering at Averøy. No scale was used, but it was noted whether the fillet had gaping or not.

Degree of gaping was measured in the laboratory in Ås 6 days after slaughtering. Up to then the fillets had then been stored at -20 °C.

The filets were scored on a scale ranging from 0 - 5, were 0 is no gaping and 5 is extreme gaping (Andersen et al 1994).

| **Gaping score** | **Explanation** |
|---|---|
| 0 | No gaping |
| 1 | < 5 small gapings |
| 2 | < 10 small gapings |
| 3 | > 10 small and possibly some large gapings |
| 4 | Many large gapings |
| 5 | Extreme gaping |

**Table 3.7:** The gaping scale utilized during analysis (Anderesen et al 1994). Small gapings < 2 cm, large gapings > 2 cm.

### 3.5.3 Liquid loss

The liquid loss analysis were performed 6 days after slaughter, and until then the samples had been stored at - 20°C.

An untrimmed muscle sample of approximately 12 g was placed on a thin-bedded honeycomb multiple sheeted pad made from cellulose. A perforated nylon sheet was placed between the muscle and the pad to avoid it sticking together.

The samples were stored at 4 °C for 4 days. To measure liquid leakage the muscle tissue was removed and the pad was weighed. At the end the pad was dried, and the fat leakage was calculated.

Liquid leakage was calculated as

100 * (weight of the pad after 4 days of storage – initial weight of the pad) / initial weight of muscle sample.

Fat leakage was calculated as

100 * (weight of the pad after drying – initial weight of the pad) / initial weight of muscle sample.

### 3.5.4 Analysis of fat % and fatty acid composition of the muscle

The analysis of the fat % and fatty acid composition of the muscle were performed by the principles of lipid extraction described by Folch et al (1957).

*Preparation*

During preparations the NQC (Norwegian quality cut) was cut in half and the skin was removed. Only the upper part was utilized for the analysis.

Then, tissue samples from 10 fish, belonging to the same net pen and pre- diet group, were grinded together to a homogenous mass. This was stored in plastic bags at – 20 °C until the analyses were performed.

Approximately 2.5 grams of the sample was weighed out in an Erlenmeyer flask, then added 6 ml of 0.9% NaCl and 50 ml chloroform:methanol (2:1). This was homogenized for 60 seconds with an Ultra Turrax homogenizator. Another 6 ml of 0.9% NaCl was added and the sample was homogenized again for 5 seconds.

The solution then separated into two phases. The upper phase containing water soluble components with a chloroform:methanol:water ratio of 86:14:1, and the lower phase containing lipids with a chloroform:methanol:water ratio of 3:48:47.

Next, the homogenate was filtered through a piece of cotton using a funnel and a graded cylinder. The cylinder with the solution was then stored in a fume cupboard for further separation. The upper water/methanol phase and protein were removed and the lower chloroform phase was transferred to two new reservoirs using a pipette. One part in a beaker for fat % calculation and one part in a test tube for analysis of fatty acid composition.

*Calculation of fat%*

The beaker with the sample was put on a heat plate in a fume cupboard until the next day, for evaporation of remaining water phase. Then the sample was weighed before the fat % was calculated as follows:

$$\% \text{ fat} = (\text{g fat} * 100) / ((I * U)/TV)$$

g fat = evaporated sample in beaker
100 = %
I = weight of the sample in g
U = Pipetted chloroform extract in ml
TV = Total volume of the solvent

*Fatty acid composition*

The sample was first evaporated at 60 °C with nitrogen overflow. 2 ml of benzene, 2 ml of metanolic HCl and 0.2 ml dimetoxypropane was then added and the sample was methylated over night in room temperature.

Following, 2 ml of hexane was added as well as 3 ml 6 % $NaHCO_3$ for neutralization. After mixing, the sample divided into two phases, and the upper phase was removed. The remaining sample consisting of methylated lipids, hexane and benzene was again evaporated at 60 °C with nitrogen overflow and dissolved in hexane.

Fatty acid composition of the sample was determined using a Hewlett Packard 6890 Gas Chromatograph equipped with a split injector SGE capillary column 60 m * 0.25 mm (length * diameter). Helium was the carrier gas and the temperature program was as follows:

- Starting temperature of the column: 50°C
- Temperature increased 4°C/min up to 170°C
- Temperature increased 0.5°C/min up to 200°C
- Temperature increased 10°C/min up to 300°C

HP GC ChemStation software (Agilent Technologies) was used to analyze the results and concentrations of individual fatty acids were expressed as percentage of total fatty acids.

### 3.6 Statistics

Data from the trial were statistically analyzed by analysis of variance (ANOVA) using the general linear model (GLM) statement of the Statistical Analysis System (Version 9.2; SAS Institute Inc., Cary, USA).

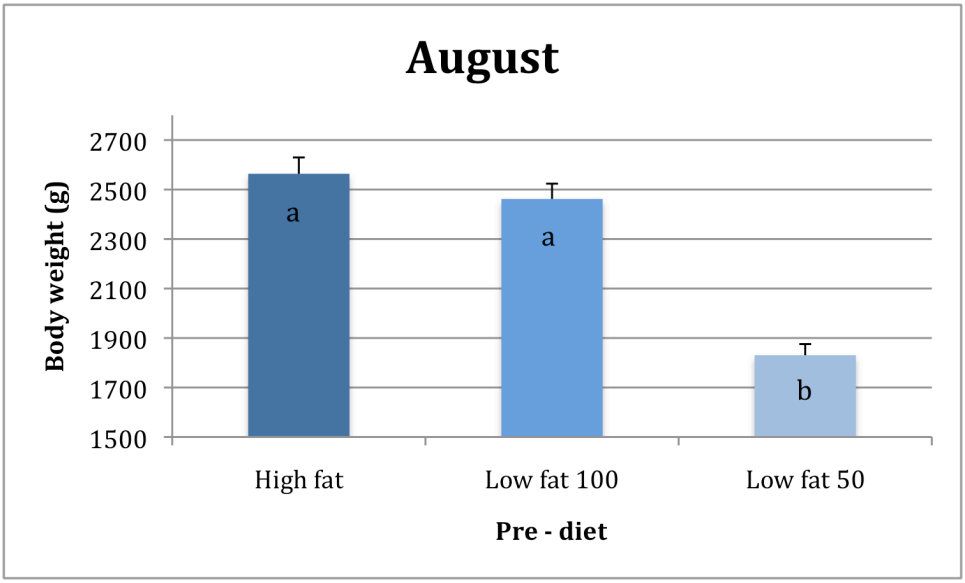
The level of significance was indicated at $P \leq 0.05$ and $P \leq 0.1$ was considered as a trend.

# 4. RESULTS

## 4.1 Body weight

In August, the group fed the LF50 pre-diet had a significantly lower body weight than the groups fed the HF100 and LF100 pre–diets (P<0.0001).
There where no significant differences in body weight among the groups fed the different diets in October (P<0.0001). Numerically, the groups fed the LF50 pre-diet still had the lowest body weight.
In December the groups fed the LF100 pre–diet followed by the HiPro or Mar70 diet had a significantly higher body weight compared to the group fed the LF50 pre-diet followed by the Mar70 diet (P<0.0001). The groups fed the LF50 pre–diet still had the lowest body weight numerically.

a)



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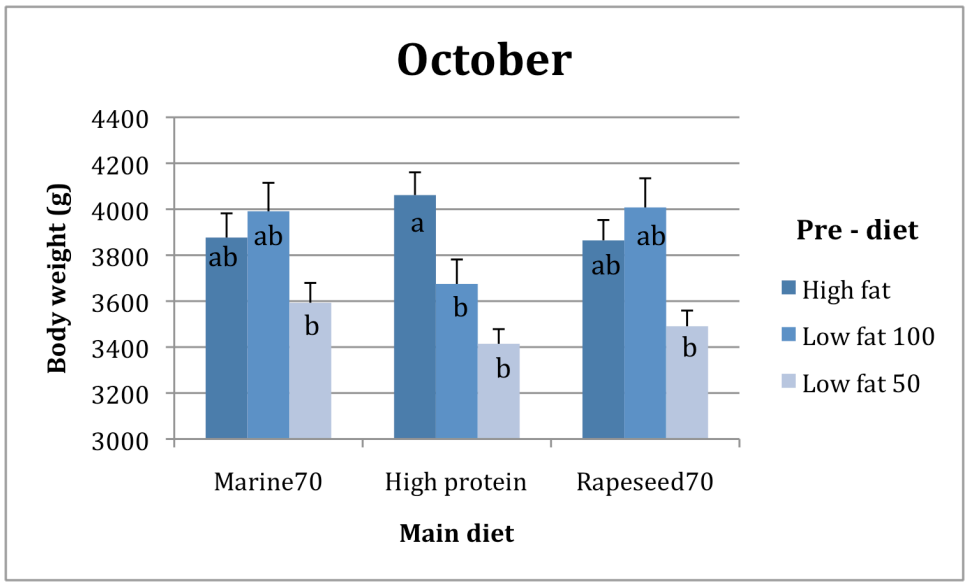



c)

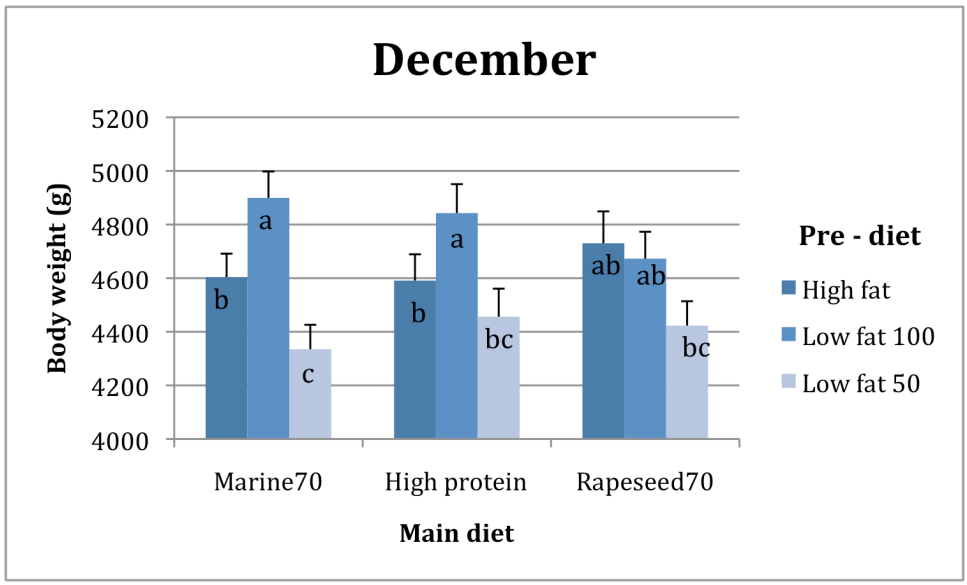



**Figure 4.1 a - c:** The body weight (g) of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

### 4.2 Thermal growth coefficient (TGC)

Pre-diets

In the period August to October the TGC differed significantly among all the pre–diet groups (P<0.01). The group fed the LF50 pre–diet had the highest TGC (4.34) and the group fed the HF100 pre-diet had the lowest TGC (2.94).

From October to December the group fed the LF50 pre–diet had a significantly higher TGC compared to the groups fed the HF100 and LF100 pre–diets (P<0.01).

Main diets

In the period August to October the group fed the Mar70 diet had a significantly higher TGC compared to the group fed the HiPro diet (P=0.02). From October to December there were no significant differences in TGC between the groups fed the different dietary treatments (P=0.56). Numerically, the group fed the Rape70 diet had the highest TGC (2.49) and the group fed the Mar70 had the lowest TGC (2.18).

**Thermal growth coefficient**

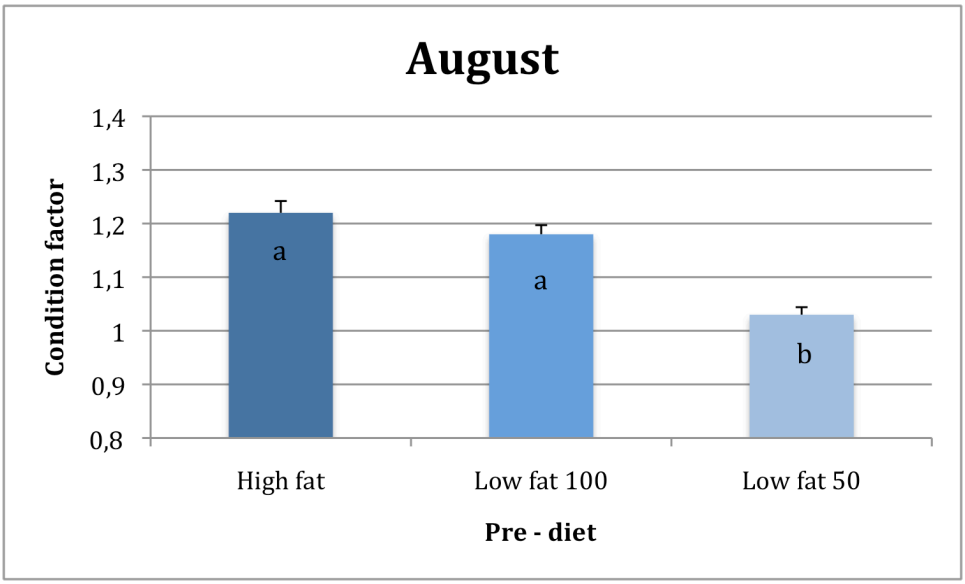
| **Dietary treatment** | | **Period** | |
|---|---|---|---|
| | | *August – October* | *October - December* |
| **Pre - diet** | High fat | 2.94±0.02$^{c}$ | 2.12±0.12$^{b}$ |
| | Low fat 100 | 3.38±0.07$^{b}$ | 2.18±0.11$^{b}$ |
| | Low fat 50 | 4.34±0.05$^{a}$ | 2.77±0.17$^{a}$ |
| **Main diet** | Marine70 | 3.61±0.01$^{a}$ | 2.18±0.17 |
| | High protein | 3.42±0.04$^{b}$ | 2.39±0.28 |
| | Rapeseed70 | 3.53±0.02$^{ab}$ | 2.49±0.22 |

**Table 4.2:** The thermal growth coefficient of the pre–diet groups and the main diet groups in the periods August - October and October – December ± SE. Letters indicates significant differences (P<0.05) within diet type (pre–diet/main diet) and period.

### 4.3 Condition factor

In August the group fed the LF50 pre–diet had a significantly lower condition factor compared to the groups fed the HF100 and the LF100 pre–diets (P<0.0001).
No significant differences in condition factor were observed among the groups fed different dietary combinations in October and December (P=0.0006)
In December the groups fed the LF50 pre-diet had the highest condition factor numerically.

a)



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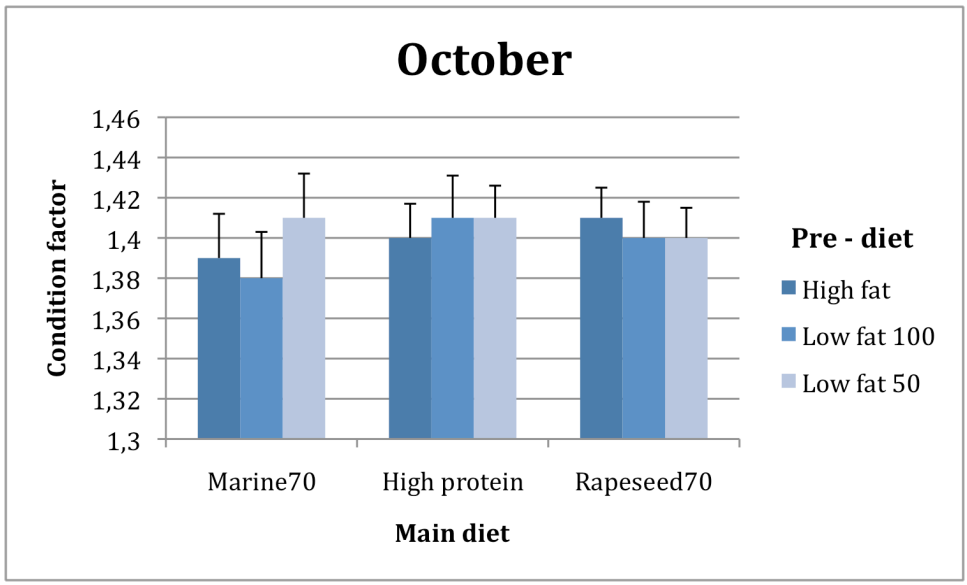


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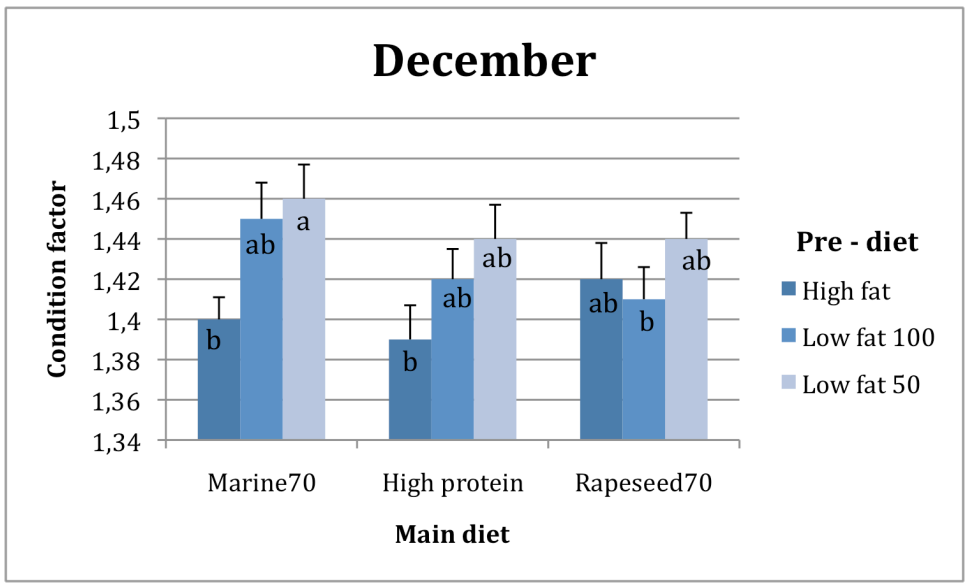



**Figure 4.3 a-c:** The condition factor (bodyweight/length$^3$) of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oils, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

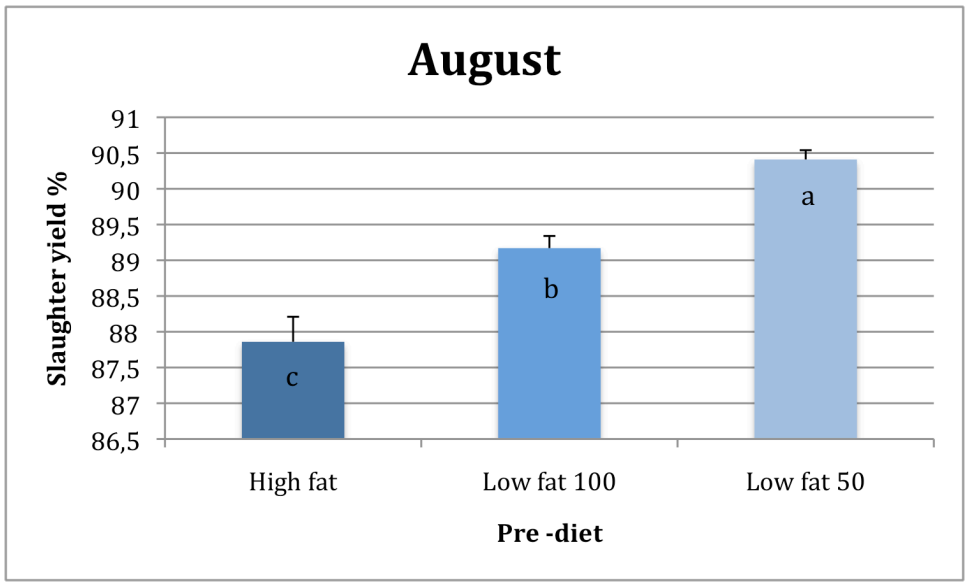
### 4.4 Slaughter yield

In August there were significant differences in slaughter yield among all the pre–diet groups (P<0.0001). The group fed the LF50 pre–diet had the highest slaughter yield (90.41±0.13) and the group fed the HF100 pre–diet had the lowest slaughter yield (87.86±0.35).

No significant differences were observed in slaughter yield among the groups fed different dietary combinations in October (P=0.66).

In December the group fed the LF100 pre–diet followed by the Rape70 diet had a significantly higher slaughter yield than the groups fed the LF100 or LF50 pre-diet followed by the Mar70 diet (P<0.0001). Numerically, the groups fed the LF50 pre-diet had the lowest slaughter yield.

a)



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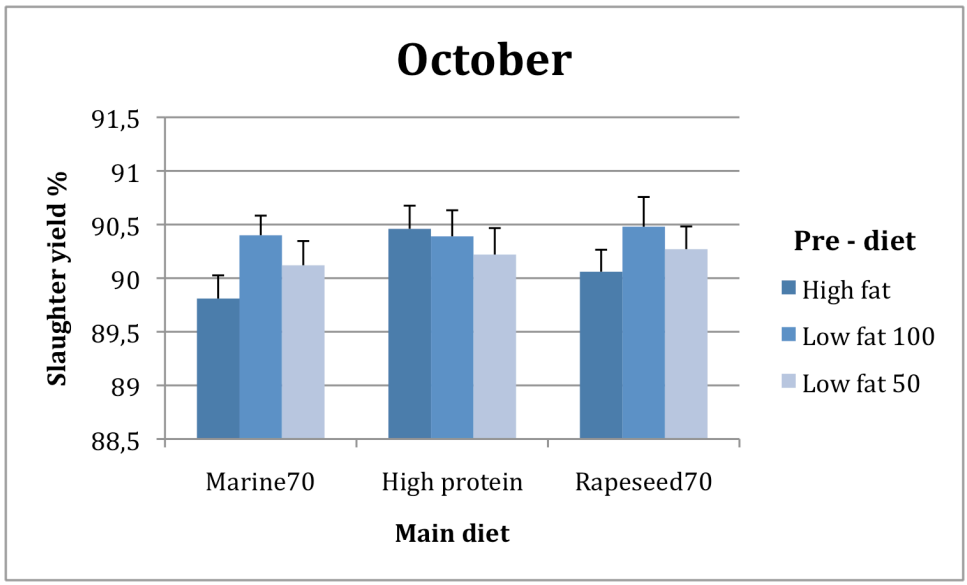


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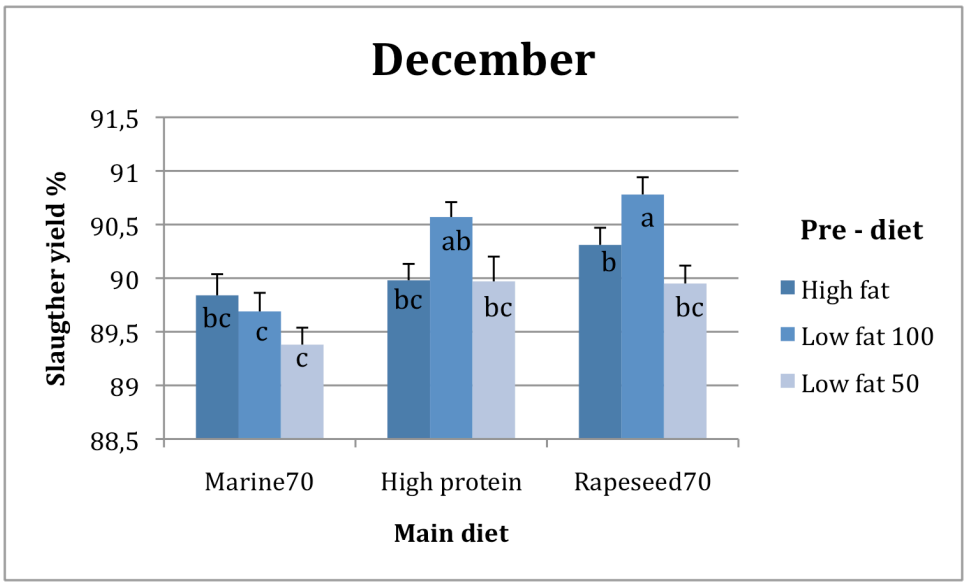



**Figure 4.4 a-c:** The slaughter yield (body weight – guts) in % of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

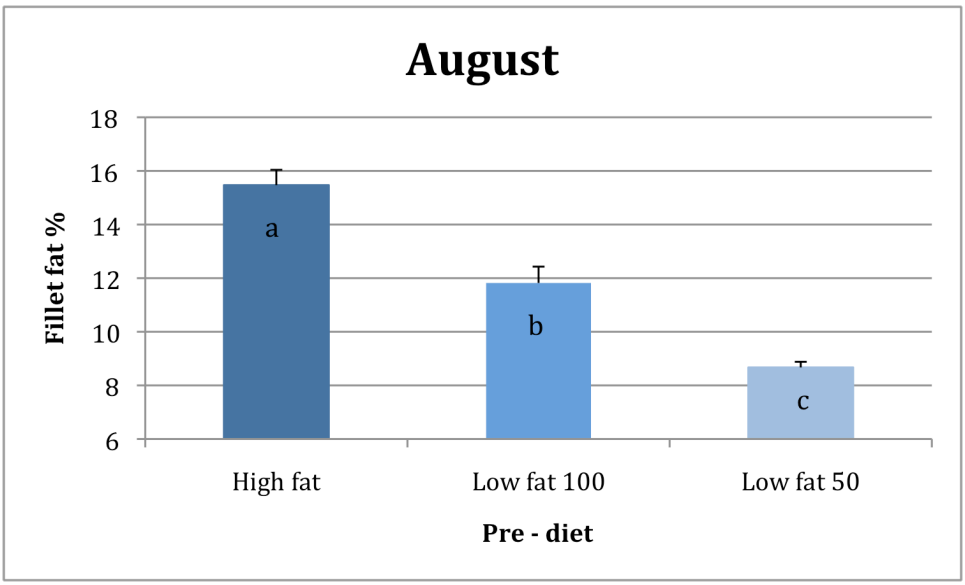
### 4.5 Fillet fat content

The fillet fat content differed significantly among all the pre–diet groups in August (P=0.0056). The group fed the HF100 pre–diet had the highest fillet fat content (15.47±0.57) and the group fed the LF50 pre–diet had the lowest fillet fat content (8.67±0.21).

In October the groups fed the HF100 pre-diet still had the highest fillet fat content numerically. The group fed the HF100 pre-diet followed by the Mar70 diet had significantly higher fillet fat content than the groups fed the LF100 and LF50 pre-diet followed by the HiPro diet (P=0.0011).

In December there were no significant differences among the groups fed the different dietary combinations (P=0.3529). Numerically, the groups fed the HF100 pre-diet had the highest, and the groups fed the LF50 pre-diet had the lowest fillet fat content.

a)



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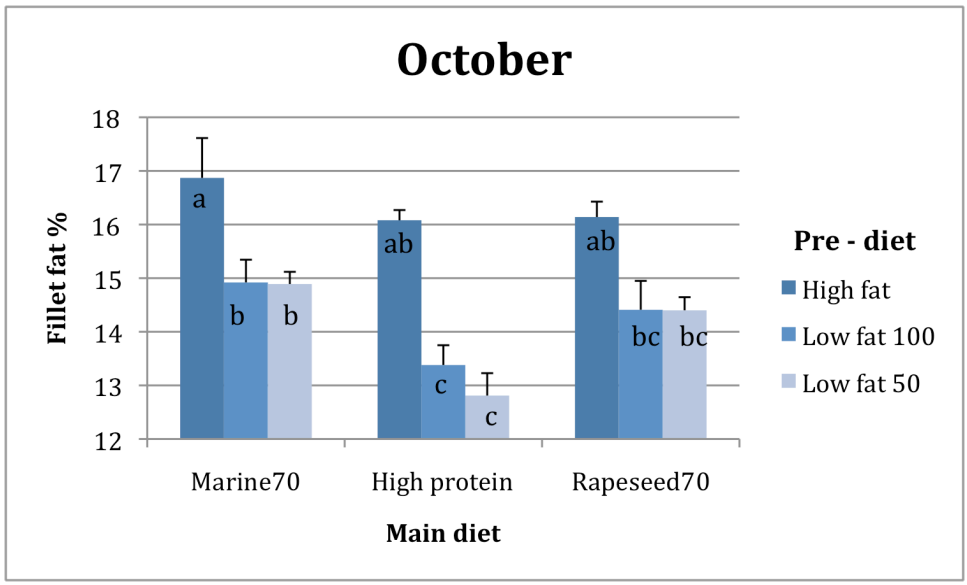


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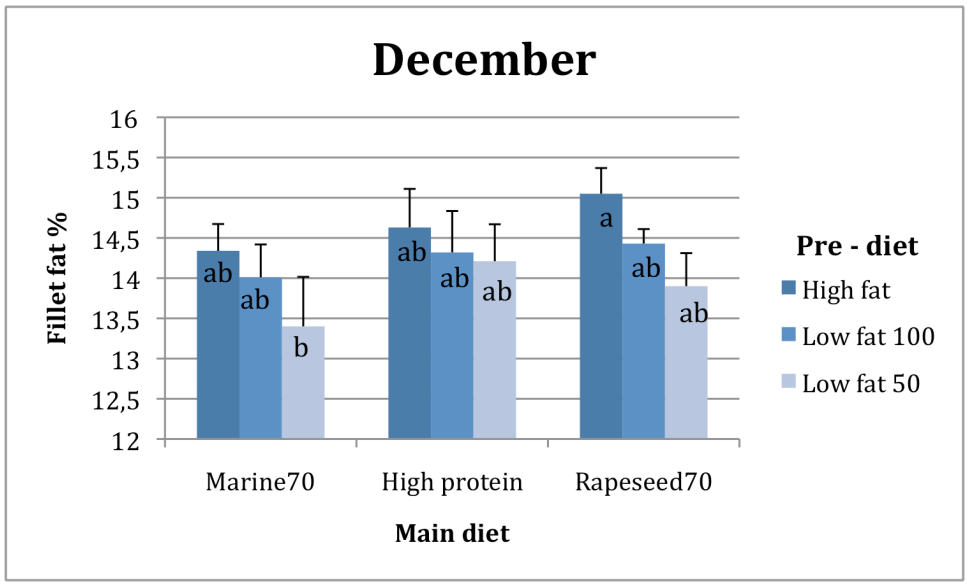


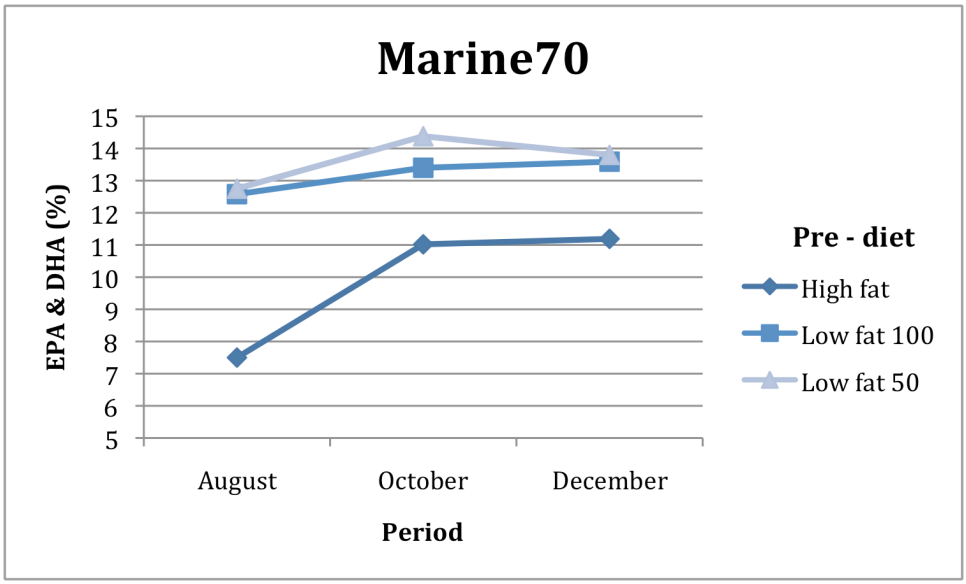

**Figure 4.5 a-c:** The fillet fat content in % of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

### 4. 6 EPA & DHA content

The EPA & DHA content were significantly lower in the group fed the HF100 pre-diet compared to the groups fed the LF100 and LF50 pre–diets in the period May – August (P=0.0001).

In October and December the EPA & DHA content were significantly higher in the groups fed the LF100 and LF50 pre-diets followed by the Mar70 diet, than in the groups fed other dietary combinations (P<0.0001). The group fed the HF100 pre–diet followed by the HiPro or Rape70 diet had a significantly lower EPA & DHA content, compared to the groups fed other dietary combinations.

a)



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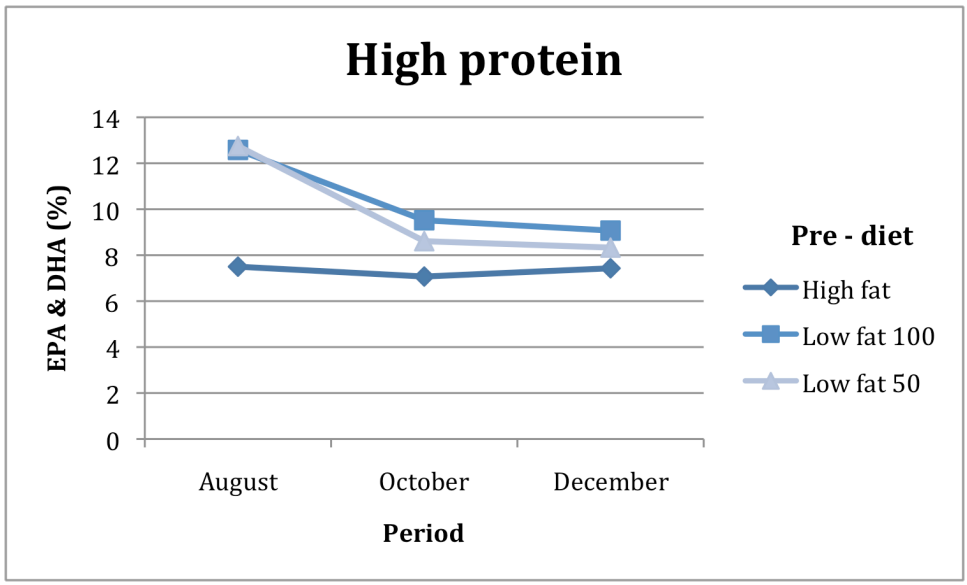


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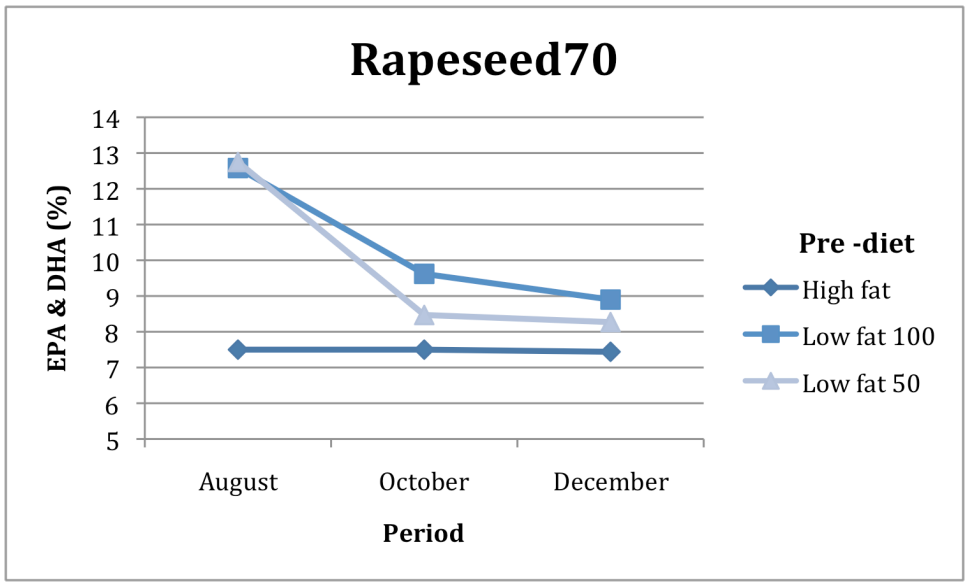



**Figure 4.6 a-c:** The EPA & DHA content (%) in fillets of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are shown as mean values of the different groups.

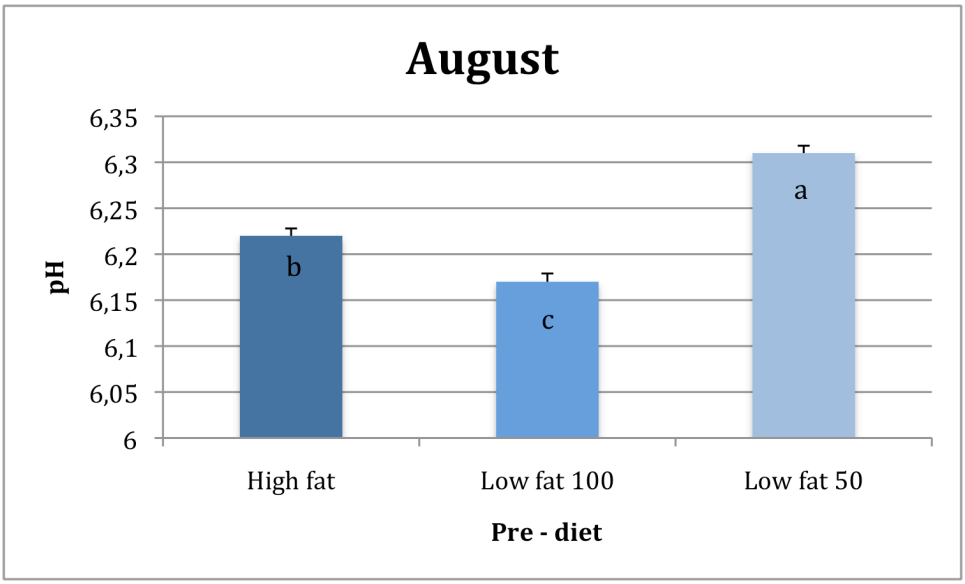
### 4.7 pH

In August the pH differed significantly among all the pre-diet groups (P<0.0001). The group fed the LF50 pre-diet had the highest pH score (6.32±0.008) and the group fed the LF100 pre-diet had the lowest pH score (6.17±0.009).

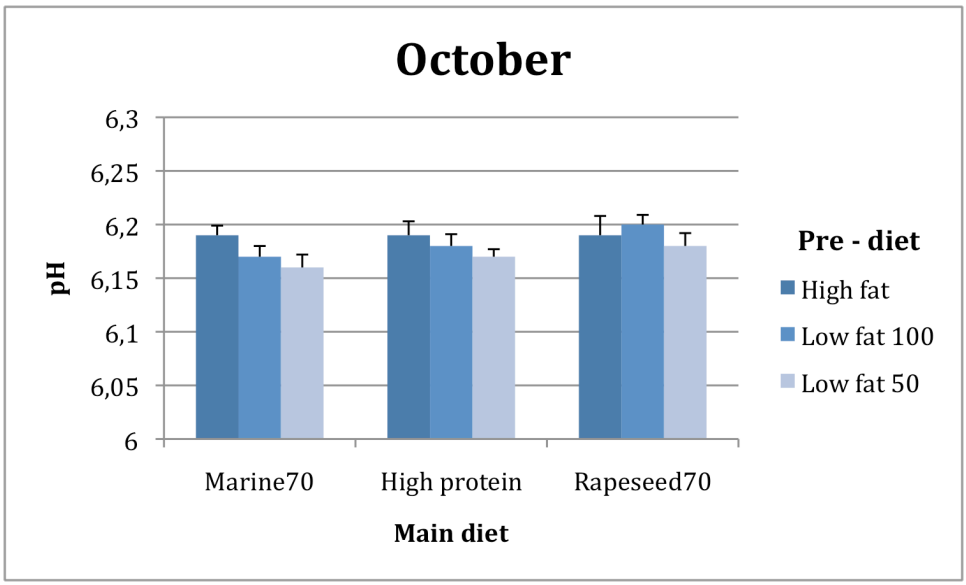
There were no significant differences in pH among the groups fed the different dietary combinations in October (P = 0.26). Numerically, the groups fed the LF50 pre-diet had the lowest pH score.

In December there were no significant differences in pH among the groups fed different dietary combinations (P=0.06).

a)



b)

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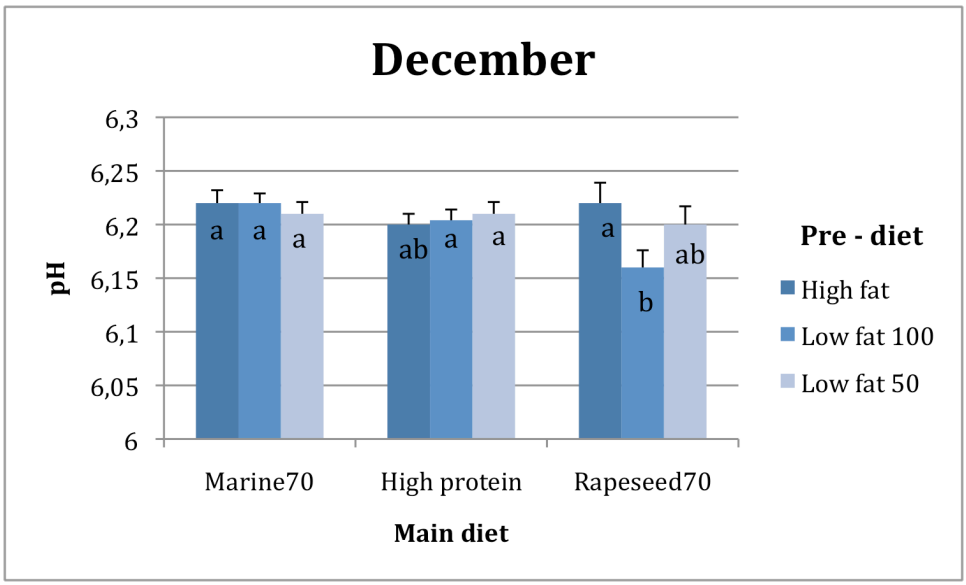
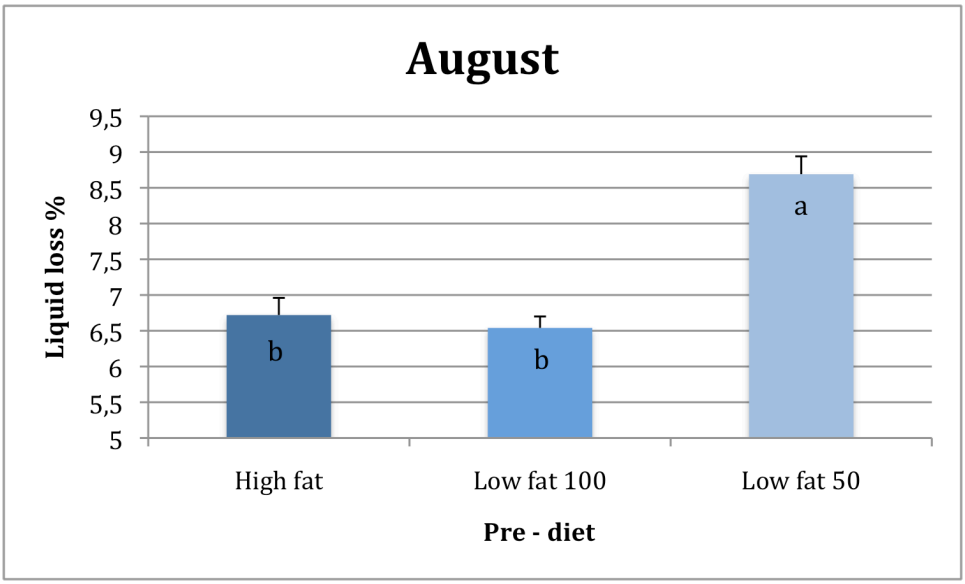



**Figure 4.7 a-c:** The fillet pH level (6 days after slaughtering) of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

### 4.8 Liquid loss

In August the group fed the LF50 pre-diet had a significantly higher liquid loss compared to the groups fed the HF100 or LF100 pre-diets ($P < 0.0001$).
The liquid loss results from October showed no significant differences among the groups fed different dietary combinations ($P = 0.22$)
In December the group fed the HF100 pre-diet followed by the HiPro diet had a significantly higher liquid loss compared to the group fed the LF50 pre-diet followed by the Mar70 diet ($P = 0.0002$). Numerically, the groups fed the Mar70 diet had the lowest liquid loss.

a)



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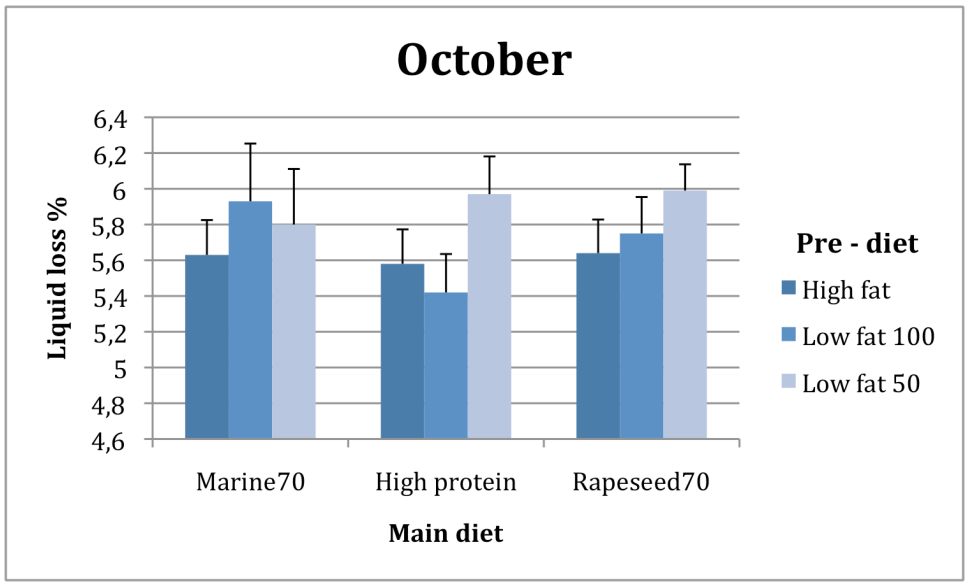



c)

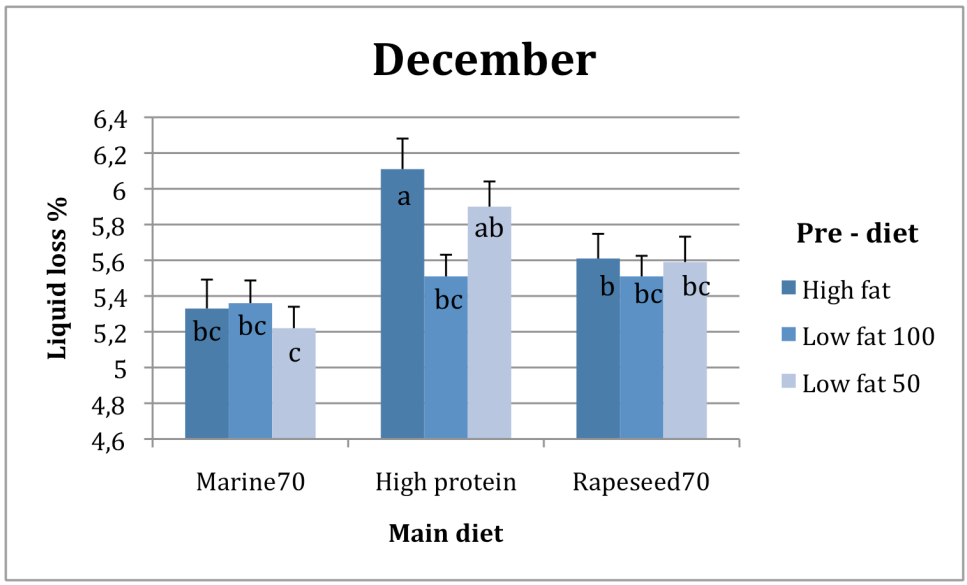
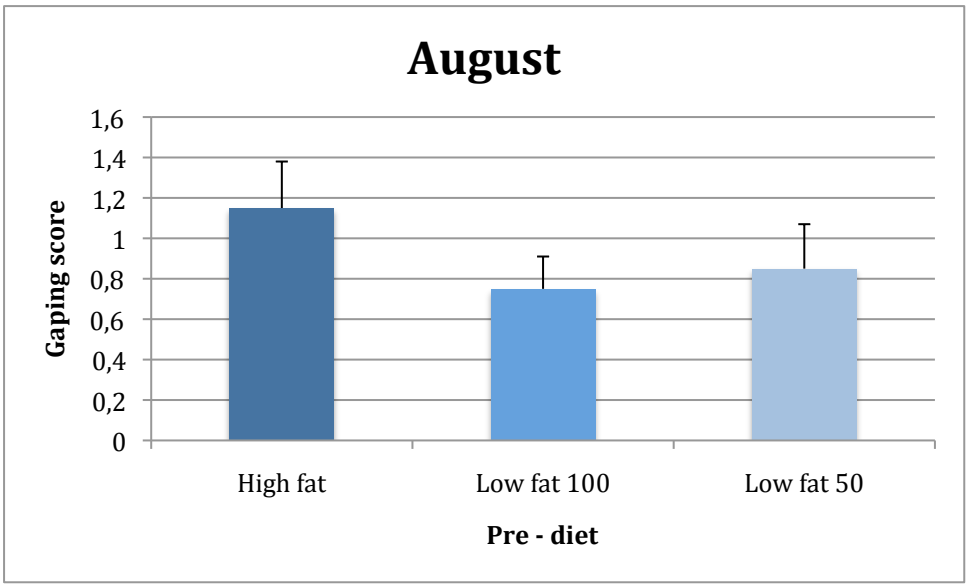



**Figure 4.8 a-c:** The liquid loss % from fillets of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

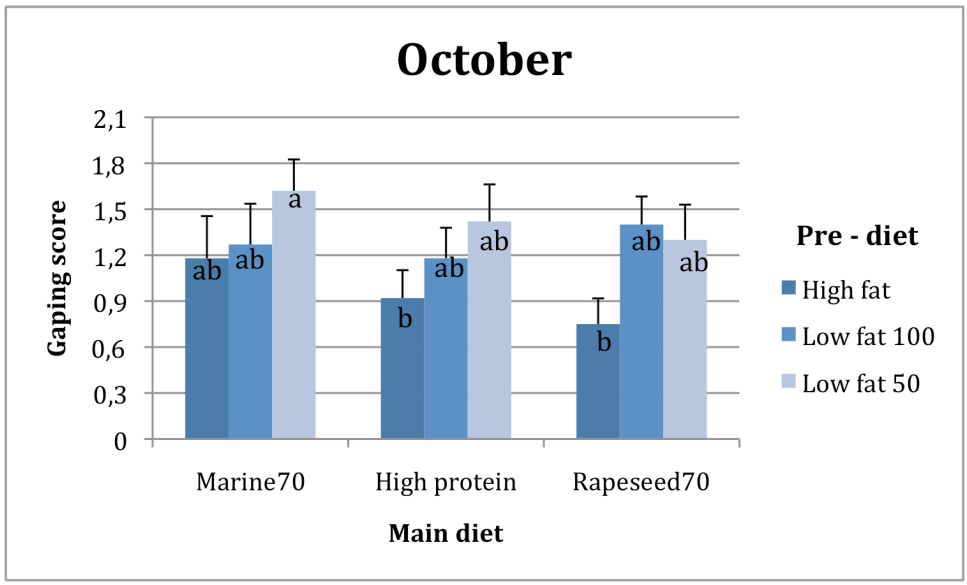
### 4.9 Gaping

There were no significant differences in gaping score among the groups fed different pre–diets in August (P= 0.37). Numerically, the group fed the HF100 pre-diet had the highest average gaping score (1.15±0.23), and the group fed the LF100 pre-diet had the lowest average gaping score (0.75±0.16).
In October there were no significant differences in gaping score among the groups fed the different dietary combinations. The group fed the HF100 pre-diet had a numerically lower gaping score than the groups fed other pre-diets (P=0.17).
There were no significant differences in gaping score among the groups fed different dietary combinations in December (P=0.54).

a)



b)




c)

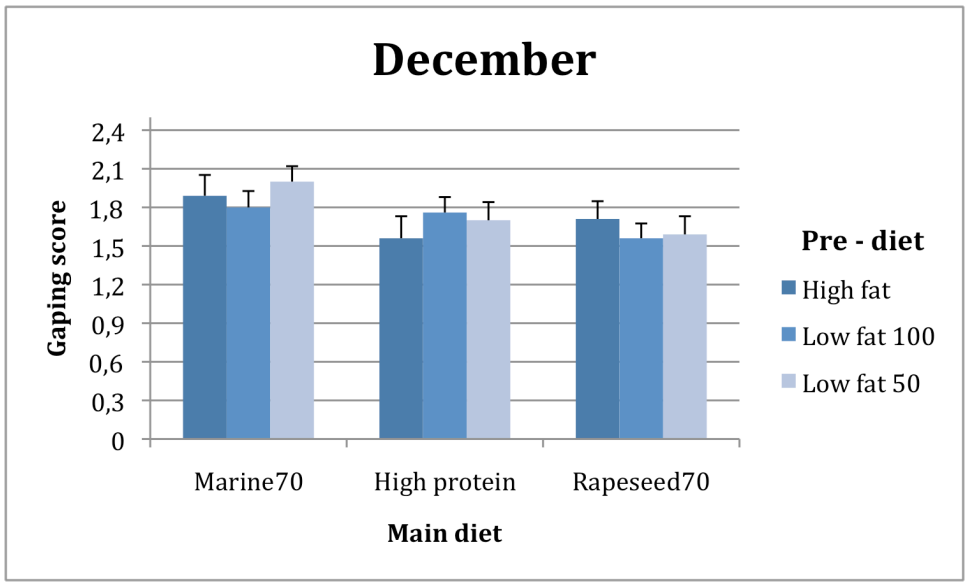



**Figure 4.9 a-c:** The fillet gaping score (6 days after slaughtering) of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

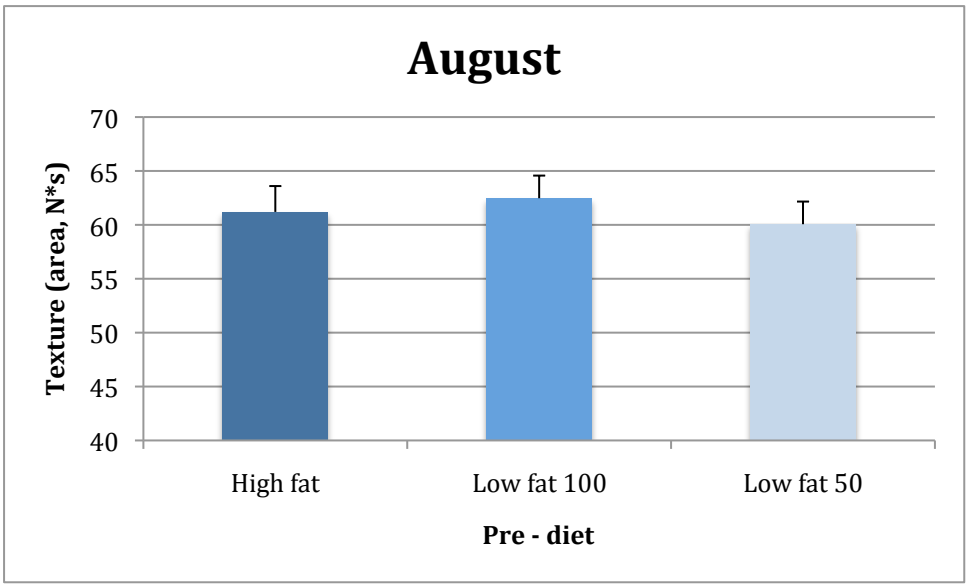
### 4.10 Texture

In August there were no significant differences in texture among the groups fed the different dietary treatments (P=0.04). Numerically, the group fed the LF100 pre-diet had the highest texture score.

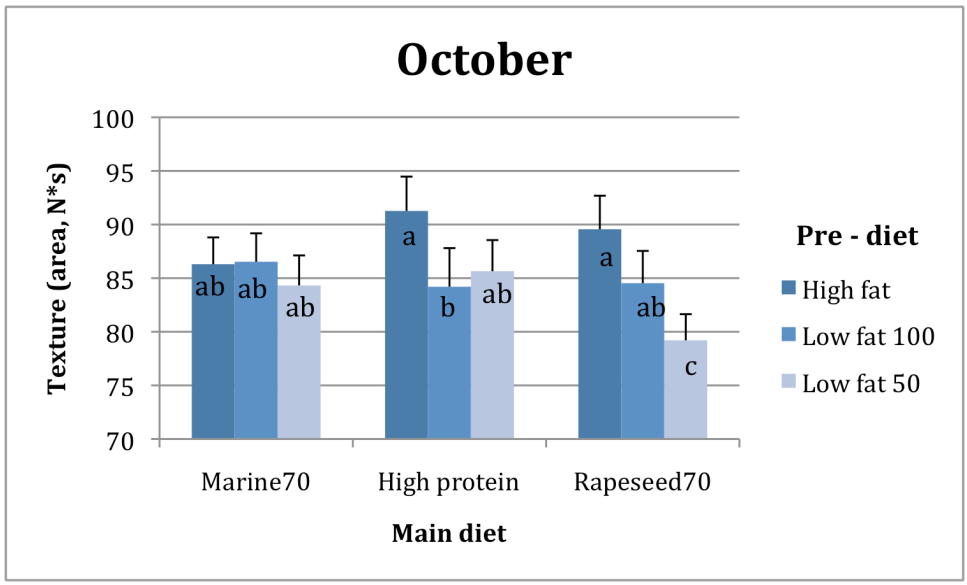
The samples from October showed that the groups fed the HF100 pre-diet followed by the HiPro or Rape70 diet had a significantly higher texture score compared to the group fed the LF50 pre-diet followed by the Rape70 diet.

In December there were no significant differences in texture score among the groups fed the different dietary combinations.

a)



b)




c)

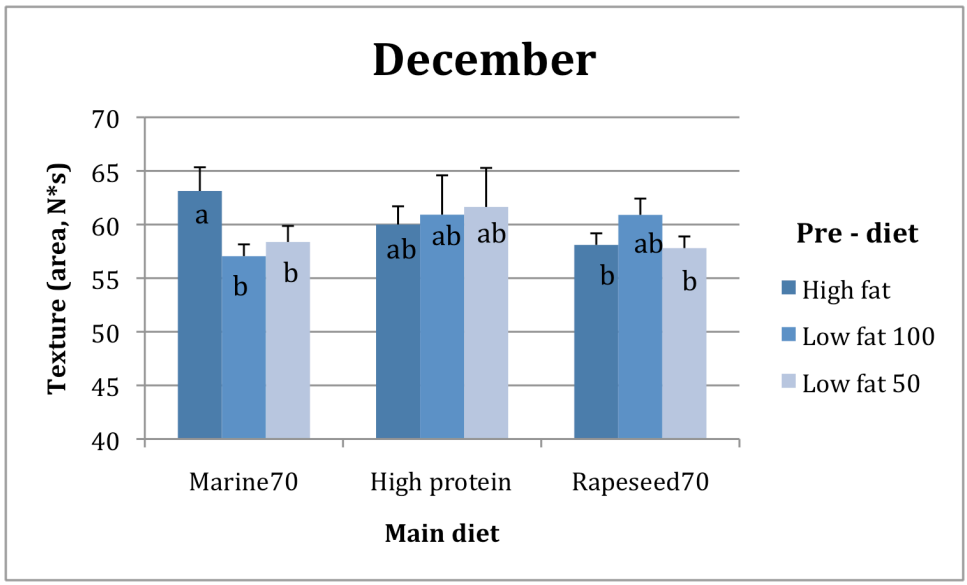



**Figure 4.10 a-c:** The fillet texture of Atlantic salmon fed pre-diets with high fat level, low fat level or low fat level in half ration (May – Aug), and main diets with 70% of the fat from marine oil, 70% of the fat from rapeseed oil or 70% of the fat from rapeseed oil added extra protein (Aug – Dec). The results are mean values of the different groups ± SE and the letters indicates significant differences ($P<0.05$) between the groups within the sampling periods.

# 5. DISCUSSION

## 5.1 Body weight and growth

The present study showed that feeding a diet with high fat content (35%) versus a diet with low fat content (18%) did not result in significant differences in body weight. The body weight was only significantly lower in the group fed half ration. This is in agreement with previous studies by Azevedo et al (2004) and Solberg (2004) showing that the growth rate was not affected by feeding high protein/low fat diet versus low protein/high fat diet to Atlantic salmon.
The group fed half ration in the first period of the trial, had as expected an explosive growth when fed full ration, while the group fed a diet with high fat in full ration had a more slow but steady growth through the autumn.

Feeding a diet with 70% rapeseed oil versus a diet with 70% marine oil did not affect the growth (TGC) significantly. This coincides with results reported by Bell et all (2001), who concluded that rapeseed oil is an effective substitute for fish oil in feed for Atlantic salmon in terms of growth rates.
In the beginning of the autumn, the group fed the high protein diet had the lowest growth in terms of TGC. A possible explanation to this can be that the transfer to this feed was larger due to the protein gel coating, creating a harder surface on the pellet.

## 5.2 Condition factor and slaughter yield

Feeding diets with different fat content did not significantly affect the condition factor, when given in full ration. The condition factor refers to the body shape of the fish, and is correlated to the body weight. Only in the group where the ration was reduced to 50%, the condition factor was significantly lower. Due to rapid growth and fat retention through the autumn this group surpassed the groups fed full ration in the first period, and ended up with the numerically highest condition factor.
In line with a study by Rosenlund et al (2001), use of rapeseed oil versus marine oil in the diet did not significantly affect the condition factor.

The slaughter yield differed significantly among all the groups 3 months into the trial; whereas the group fed the low fat diet in half ration had the highest slaughter yield and

the group fed the high fat diet had the lowest slaughter yield. This is in agreement with a study by Hillestad et al (1994), showing that slaughter yield increased with increasing protein level and decreased with increasing fat level.
Our results also showed some small but significant differences in slaughter yield between the groups fed the rapeseed70 diet and the group fed the marine70 diet at the end of the trial.

### 5.3 Fillet fat content and fatty acid composition

Salmon fed a diet with high fat content (35%) had as expected a higher fillet fat % than salmon fed a diet with low fat content (18%). The lowest fillet fat content where found in the group fed half ration. This is in line with Solberg 2004, who showed that feed with higher dietary oil content (36%), resulted in a significantly higher fat content in the fillets, compared to feed with lower dietary oil content (26%)

The groups fed low fat diets (18%) had significantly higher amount of EPA and DHA in the fillet compared to the group fed a high fat diet (35%).
Salmon fed the diet with the largest portion of marine oils had as expected the highest amount of EPA and DHA in the fillet. From the 1 November to the end of the experiment in December the group originally fed the marine70 diet were fed the rapeseed70 diet. Nevertheless, the results showed an increase in EPA/DHA level in the groups fed the high fat and the low fat100 pre-diet from sampling in October to sampling in December. The low fat50 pre-diet had a minor decline in EPA/DHA content from October (14.38%) to December (13.8%). These results are in agreement with Bell (2001) finding that inclusion of rapeseed oils in excess of 50 % of supplementary lipid in Atlantic salmon result in significant decline in EPA and DHA concentrations in the flesh, such that the nutritional benefits of the fish to the consumer would be considerably reduced.

### 5.4 Quality parameters

The pH of the salmon fillets where highest in the group fed half ration in the first period of the trial. The lowest pH where found in the fillets of the salmon fed the low fat diet in full ration. To the contrary, Solberg 2004 found no significant differences in pH due to fat level in the diet, only variations during season.
Feeding salmon diets with different fat sources and protein levels did not result in

differences in the pH of the fillets.
A correlation between the pH and liquid loss is well documented by Ofstad et al (1995). The results from this study where in line with this as the group with the highest pH also had the highest liquid loss.
Gaping score of fillet did not differ significantly among any of the diets during the whole experimental period. Generally gaping occurs together with tissue softening (Bremner, 1992), although studies have shown that it may occur even when the flesh is firm (Mørkøre & Rørvik, 2001).
The results from the texture analysis in august did not show significant differences among the groups fed diets with different fat levels or diets given in different rations. A previous study showed to the contrary that high fat levels (> 18%) in the flesh may have unfavourable effects on texture and processing characteristics (Gjedrem, 1997). Einen et al (1999), showed that feeding reduced feed ration prior to slaughtering increased the firmness of the flesh. There might be differences in texture along the fillet, and the contradicting findings can be a result of measuring on different parts of the flesh.

# 6. CONCLUSION

The level of fat in the diet did not affect the slaughter parameters, except for the slaughter yield, which was significantly lower in the group fed a diet with high fat content. It also affected the fat content and the amount of EPA and DHA in the salmon fillet. Regarding the quality, the level of fat provoked significant differences in pH and liquid loss, but not in gaping and texture.

Main dietary oil source in the diet did not affect the slaughter parameters, except for the slaughter yield. The fatty acid composition of the fillets, were also strongly affected by dietary oil source. Quality parameters were not affected by oil source, except for a somewhat lower liquid loss in the group fed the marine70 diet.

The present study did not see any significant effect of adding extra amino acids to diets for Atlantic salmon, in terms of slaughter parameters, fat and fatty acid composition and quality parameters.

# 7. REFERENCES

Norge - verdensledende på laks. (FHL) Available: http://laksefakta.no/nokkelinfo.html [Accessed 19.02.2012 2012].

AKSNES, A. G., B; ROALD, S.O 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, Salmo salar. Aquaculture, 53, 7-20.

ALNE, H., OEHME, M., THOMASSEN, M., TERJESEN, B. & RORVIK, K. A. 2011. Reduced growth, condition factor and body energy levels in Atlantic salmon Salmo salar L. during their first spring in the sea. Aquaculture Research, 42, 248-259.

ANDERSEN, U. B., STRØMSNES, A. N., STEINSHOLT, K. & THOMASSEN, M. S. 1994. Fillet gaping in farmed Atlantic salmon (Salmo salar). Norwegian Journal of Agricultural Sciences, 8, 165-179.

ANDO, M. 1999. Correspondence of collagen to the softening of meat during refrigeration. Extracellular matrix of fish and shellfish India.

AURSAND, M. B., B; RAINUZZO, J. R; JORGENSEN, L; MOHR, V 1994. Lipid distribution and composition of commercially farmed Atlantic salmon. Journal of the Science of Food and Agriculture, 64, 85-104.

AZEVEDO, P. A. L., S; CHO, C. Y; BUREAU, D. P 2004. Growth and feed utilization of large size rainbow trout and Atlantic salmon reared in freshwater: diet and species effect, and responses over time. Aquaculture Nutrition, 10, 401-411.

BELL, J. G. 1998. Current aspects of lipid nutrition in fish farming. In: BLACK, K. D. & PICKERING, A. D. (eds.) Biology of farmed fish. Sheffield, UK: Sheffield Academic Press.

BELL, J. G. M., J; TOCHER, DR; MCGHEE, F; CAMPBELL, PJ; SARGENT, JR 2001. Replacement of Fish Oil in Diets of Atlantic salmon Affects Tissue Lipid Compositions and Hepatocyte Fatty Acid Metabolism. Journal of Nutrition, 1535-1543.

BØRRESEN, T. 2008. Improving seafood products for the consumer, Boca Raton, Fla., CRC Press.

BOSTOCK, J., MCANDREW, B., RICHARDS, R., JAUNCEY, K., TELFER, T., LORENZEN, K., LITTLE, D., ROSS, L., HANDISYDE, N., GATWARD, I. & CORNER, R. 2010. Aquaculture: global status and trends. Philosophical Transactions of the Royal Society B-Biological Sciences, 365, 2897-2912.

BREMNER, H. A. 1992. Fish flesh structure and the role of collagen - its post mortem aspects and implications for fish processing. In: HUSS, H. J., M; LISTON, J (ed.) Quality Assurance in the Fish Industry. Amsterdam: Elsevier Science Publishers.

BLACK, D., LOVE., R.M 1988. Estimating the carbohydrate reserves in fish. J. Fish Biol. , 32, 335-340.

EINEN, O., MORKORE, T., RORA, A. M. B. & THOMASSEN, M. S. 1999. Feed ration prior to slaughter - a potential tool for managing product quality of Atlantic salmon (Salmo salar). Aquaculture, 178, 149-169.

FAO 2010. The state of world fisheries and aquaculture. Rome.

FENNEMA, O. R. 1996. Food Chemistry, New York.

FHL. 2011. Nøkkeltall for havbruk [Online]. Available: http://www.fhl.no/sjoematfakta/noekkeltall-for-havbruk-article174-43.html.

FOLCH, J., LEES, M. & STANLEY, G. H. S. 1957. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. Journal of Biological Chemistry, 226, 497-509.

FOLKESTAD, A., RORVIK, K. A., KOLSTAD, K. & MORKORE, T. 2008. Growth rates of individual farmed Atlantic salmon Salmo salar L. influence the texture of raw and smoked fillets. Aquaculture Research, 39, 329-332.

FRØYLAND, L. B., H; GRAFF, I; MYHRSTAD, M; PAULSEN, J; RETTERSTØL, K; ULVEN, S. 2011. Evaluation of negative and positive health effects of n-3 fatty acid as constituents of food supplements and fortified foods.

GJEDREM, T. 1997. Flesh quality improvement in fish through breeding. Aquaculture International, 5, 197-206.

HALVER, J. E. & HARDY, R. W. 2002. Fish nutrition, Amsterdam, Academic Press.

HARDY, R. W. 1999. Problems and opportunities in fish feed formulation. Aquaculture Magazine, 25, 56-60.

HARDY, R. W., SCOTT, T. M. & HARRELL, L. W. 1987. Replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens. Aquaculture, 65, 267-277.

HATAE, K. T., A; TAKEYAMA, M; MATSUMOTTO, J. J 1986. Contribution of the connective tissues on texture differences of various fish species. Bulletin of the Japanese Society for the Science of Fish, 52, 2001-2008.

HILLESTAD, M. J., FREDDY 1994. High-energy/low-protein diets for Atlantic salmon: effects on growth, nutrient retention and slaughter quality. Aquaculture, 124, 109-116.

HURLING, R. R., J. B; HUNT, H. D 1996. Fibre diameter and fish texture. Journal of Texture Studies, 27, 679-685.

JOHNSTON, I. A. 1999. Muscle development and growth: potential implications for flesh quality in fish. Aquaculture, 177, 99-115.

JOHNSTON, I. A., LI, X. J., VIEIRA, V. L. A., NICKELL, D., DINGWALL, A., ALDERSON, R., CAMPBELL, P. & BICKERDIKE, R. 2006. Muscle and flesh quality traits in wild and fanned Atlantic salmon. Aquaculture, 256, 323-336.

JOHNSTON, I. A. A., R; SANDHAM, C; DINGWALL, A; MITCHELL, D; SELKIRK, C; NICKEL, D; BAKER, R; ROBERTSON, B; WHYTE, D; SPRINGATE, J 2000. Muscle fibre density in relation to the colour and texture of smoked Atlantic salmon Aquaculture, 189, 335-349.

JOHNSTON, I. A. D., W; GOLDSPINK, G 1977. Energy metabolism of carp swimming muscles. J. comp. Physiol, 114, 203-216.

KESTIN, S. C. & WARRISS, P. D. 2001. Farmed fish quality, Malden, Mass., Fishing News Books.

KIESSLING, A., ESPE, M., RUOHONEN, K. & MORKORE, T. 2004. Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO2 anaesthesia. Aquaculture, 236, 645-657.

KIESSLING, A. R., K; BJORNEVIK, M 2006. Muscle fibre growth and quality in fish Archives Animal Breeding, 49, 137-146.

LAVETY, J. A., O. A; LOVE, R. M 1988. The connective tissues of fish. J. Food Sci. Tech, 23, 23-30.

LIE, O. H., I 1992. The effect of starvation on the composition of Atlantic salmon. Fisk.Dir. Skr., Ser. Ernaering,, 5, 11-16.

LOVE, R. M. 1970. The chemical biology of fishes, London, Academic Press.

LOVE, R. M. 1988. The FOOD FISHES their intrinsic variation and practical implication, London, Farrand Press.

LYNNUM, L. 2005. Fisk som råstoff, Gyldendal.

MACCALLU.WA, JAFFRAY, J. I., CHURCHIL.DN & IDLER, D. R. 1968. CONDITION OF NEWFOUNDLAND TRAP-CAUGHT COD AND ITS INFLUENCE ON QUALITY AFTER SINGLE AND DOUBLE FREEZING. Journal of the Fisheries Research Board of Canada, 25, 733-&.

MITCHIE, I. 2001. Causes of downgrading in the salmon farming industry. In: KESTIN, S. C. W. P. (ed.) Farmed fish quality. Oxford.

MOMMSEN, T. P. M., TW; PLISETSKAYA, E.M 2001. Effects of arginine on pancreatic hormones and hepatic metabolism in rainbow trout. Physiological and Biochemical Zoology, 74, 668-678.

MØRKØRE, T. 2002. Texture, fat content and product yield of salmonids. 2002:07, UMB.

MORKORE, T. & AUSTRENG, E. 2004. Temporal changes in texture, gaping, composition and copper status of Atlantic salmon (Salmo salar, L) fed moist feed or extruded dry feed. Aquaculture, 230, 425-437.

MORKORE, T. & EINEN, O. 2003. Relating sensory and instrumental texture analyses of Atlantic salmon. Journal of Food Science, 68, 1492-1497.

MORKORE, T., HANSEN, A. A., UNANDER, E. & EINEN, O. 2002. Composition, liquid leakage, and mechanical properties of farmed rainbow trout: Variation between fillet sections and the impact of ice and frozen storage. Journal of Food Science, 67, 1933-1938.

MORKORE, T. & RORVIK, K. A. 2001. Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (Salmo salar) transferred to seawater as 0+smolts or 1+smolts. Aquaculture, 199, 145-157.

MØRKØRE, T. K., E; ESPE, M; LARSSON, T; VEISETH-KENT, E; TERJESEN, B; STANDAL, I; RØRVIK, K 2010. Optimalt fôr som gir fast filet. Nofima.

MORRIS, P. C., BEATTIE, C., ELDER, B., FINLAY, J., GALLIMORE, P., JEWISON, W., LEE, D., MACKENZIE, K., MCKINNEY, R., SINNOTT, R., SMART, A. & WEIR, M. 2003. Effects of the timing of the introduction of feeds containing different protein and lipid levels on the performance and quality of Atlantic salmon, Salmo salar, over the entire seawater phase of growth. Aquaculture, 225, 41-65.

MURRAY, J. & BURT, J. R. 2001. The composition of fish, Torry Research Station.

NAYLOR, R. L., HARDY, R. W., BUREAU, D. P., CHIU, A., ELLIOTT, M., FARRELL, A. P., FORSTER, I., GATLIN, D. M., GOLDBURG, R. J., HUA, K. & NICHOLS, P. D. 2009. Feeding aquaculture in an era of finite resources (vol 106, 15103, 2009). Proceedings of the National Academy of Sciences of the United States of America, 106, 18040-18040.

NEU, J. S., V; CHAKRABARTI, R 1996. Glutamine nutrition and metabolism: where do we go from here? The FASEB Journal, 10, 829-837.

OEHME, M., GRAMMES, F., TAKLE, H., ZAMBONINO-INFANTE, J. L., REFSTIE, S., THOMASSEN, M. S., RORVIK, K. A. & TERJESEN, B. F. 2010. Dietary supplementation of glutamate and arginine to Atlantic salmon (Salmo salar L.) increases growth during the first autumn in sea. Aquaculture, 310, 156-163.

OFSTAD, R., KIDMAN, S., MYKLEBUST, R., OLSEN, R. L. & HERMANSSON, A.-M. 1995. Liquid-holding capacity and structural changes in comminuted salmon (Salmo salar) muscle as influenced by pH, salt and temperature. LWT - Food Science and Technology, 28, 329-339.

REGOST, C., JAKOBSEN, J. V. & RØRÅ, A. M. B. 2004. Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. Food Research International, 37, 259-271.

ROSENLUND, G. O., A; SANDBERG, M; STANDAL, H; TVEIT, K 2001. Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon. Aquaculture, 32, 323-328.

ROTH, B., JOHANSEN, S. J. S., SUONTAMA, J., KIESSLING, A., LEKNES, O., GULDBERG, B. & HANDELAND, S. 2005. Seasonal variation in flesh quality, comparison between large and small Atlantic salmon (Salmo salar) transferred into seawater as 0+ or 1+ smolts. Aquaculture, 250, 830-840.

SATO, K. Y., R; SATO, M; SHIMIZU, Y 1986. Collagen content in the muscle of fishes in association with their swimming movement and meat texture. Bulletin of the Japanese Society for the Science of Fish, 52, 1595-1600.

SCHWARZ, F. K., M 1988. Amino acid composition of carp ŽCyprinus carpio L.. with varying protein and energy supplies. Aquaculture, 72, 307-317.

SHEARER, K. D. 1994. FACTORS AFFECTING THE PROXIMATE COMPOSITION OF CULTURED FISHES WITH EMPHASIS ON SALMONIDS. Aquaculture, 119, 63-88.

SMITH, R. K., H; REGENSTEIN, J; RUMSEY, G 1988. Growth, carcass composition, and taste of rainbow trout of different strains fed diets containing primarily plant or animal protein. Aquaculture, 70, 309-321.

SOLBERG, C. 2004. Influence of dietary oil content on the growth and chemical composition of Atlantic salmon. Aquaculture Nutrition, 10, 31-37.

TACON, G. J. A. 1987. The nutrition and feeding of farmed fish and shrimp - a training manual, Brazil, FAO.

TAPIERO, H. M., G; COUVREUR, P; TEW, K.D 2002. Free amino acids in human health and pathologies. Biomedicine & Pharmacotherapy, 56, 446-457.

THOMASSEN, M. S., GUDDING, R., NORBERG, B. & JØRGENSEN, L. 2006. Havbruksforskning: Fra merd til mat, Oslo, Norsk forskningsråd.

THOMMASSEN, M. R., C 1989. Different fats in feed for salmon: influence on sensory parameters, growth rate and fatty acids in muscle and heart. Aquaculture, 79, 129-135.

THORPE, J. E., ADAMS, C. E., MILES, M. S. & KEAY, D. S. 1989. SOME INFLUENCES OF PHOTOPERIOD AND TEMPERATURE ON OPPORTUNITY FOR GROWTH IN JUVENILE ATLANTIC SALMON, SALMO-SALAR L. Aquaculture, 82, 119-126.

TOCHER, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science, 11, 107-184.

WAAGBØ, R. S., K; SANDVIN, A; LIE, Ø 1991b. Feeding three levels of n-3 polyunsaturated fatty acids at two levels of vitamin E to Atlantic salmon. Fisk.Dir. Skr., Ser. Ernaering,, 4, 51-63.

WATANABE, T. 1982. Lipid nutrition in fish. Comparative Biochemistry and Physiology 73, 3-15.

WILSON, R. P. 1989. Amino Acids and Proteins. In: HALVER, J. E. (ed.) Fish Nutrition. New York: Academic Press.

WU, G. Y. M., S.M 1998. Arginine metabolism: nitric oxide and beyond. Biochemical Journal, 336, 1-17.