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Feeding Strategy and Effect on Fillet Quality of Atlantic Salmon (*Salmo salar*)

Jakob Berntzen

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Supervisor: Turid Rustad, IBT

Norwegian University of Science and Technology
Department of Biotechnology

Preface

I hereby declare that the work is performed independently and according to the regulations of NTNU.
Lab work performed partly at Department of Biotechnology in Trondheim and SalMar's processing facility, Innovamar at Frøya. Fish were collected from Lerang Research station and a Salmar farm by the coast of Nord-Trøndelag.
The thesis is a result of two distinct projects related to feeding strategy and the effect on fillet quality of Atlantic salmon (*Salmo salar*).

Seven years ago I decided to leave the comfort of a steady pay check and a promising career as an electrician. Little did I know that seven years later I was going to submit a master thesis in biotechnology at NTNU. I have always been curious and I think I always will be. I am sure it will be beneficial when entering a new role and a new career. This thesis originated through approached contact with key personnel in the Norwegian aquaculture industry.
First of all I want to thank my supervisor Turid Rustad, for being flexible in designing this master thesis and for supporting me with her expertise and enthusiasm in the field of food chemistry. I am grateful towards lab engineers Siri, Kåre and Trude, for helping me with the analyses.
Thanks to SalMar and Skretting for cooperation and for increasing the industry relevance of my thesis. Especially Merete Sandberg and the SalMar biology research group, including Head of Biology and Nutrition, Bård Skjelstad and Feed Technical Manager Eldar Bendiksen. Special thanks also to the SalMar quality division led by Eva Haugen, Quality Manager Hanne Tobiassen and lab scientist Miroslaw Lucas for samples, training and guidance in analyses. Thanks to Skretting, Project Manager Gunvor Baardsen and Manager of Lerang research station, Mads Martinsen for sending me samples and for inviting me to the facilities in Stavanger.

At last I want to thank my family for support and my beloved fiancé for putting up with me during this hectic period.

Trondheim May 15, 2016

Abstract

This thesis seeks to explore how changes in feeding strategy can affect the fillet quality of farmed Atlantic salmon (*Salmo salar*). By changing the number of daily feedings or the degree of marine raw materials it will be reasonable to expect an effect from some of the main quality parameters in fish fillet. The thesis consists of two separate experiments. In the feed frequency experiment protein solubility, colouration, dry matter, lipid content and lysosomal protease activity was compared for fish fed one and three times daily. The results indicate that the group fed three times per day had wider spread in colouration, lower lipid content, higher content of sarcoplasmic proteins and lower cathepsin B+L activity compared to fish fed one time daily. No significant difference was detected in mean colouration, fillet hardness, breaking strength or water contents from changes in feeding rate.

In the diet experiment, five groups of Atlantic salmon were reared in full-scale net pens and fed with marine based feed (MBF) at different durations under the growth phase (Group A: 41 weeks, B: 40 weeks, C: 27 weeks, D: 25 weeks and E: 0 weeks). A conventional industrial feed (CF) was otherwise used. Pigmentation, lipid content, fatty acid profile, amino acid profile and water content was compared between the groups. Changes of lipid content and colouration was compared in fresh fish and after freeze storage using visual analysis, NIR and UV spectrometry. Amino acids, fatty acids and water content was examined after freeze storage. Growth, weight spread, downgrading content, feed factors and visceral deposition was compared from slaughter data for the different feeding groups. Results indicates that increased dietary marine feed can lead to higher growth rates, lower feed factor, stronger red pigmentation, higher lipid contents, changes in amino acid profile and visceral deposition. No significant differences was detected in protein content, water content or superior quality grading. *UltraPerformance Convergence Chromatography*^{TM} (UPC^2) may be a possible method for screening of fatty acid profile of unesterified fatty acids of fish fillets, however further method development are needed to clarify its potential.

These experiments show that chosen feeding strategy can have an impact on several growth and quality parameters in Atlantic salmon. Further research is recommended in order to describe the mechanisms underlying these observations. The development into more sustainable feed raw materials still results in high quality products.

Sammendrag

Formålet med masteroppgaven var å undersøke hvordan endring i fôringsstrategi kan ha en innvirkning på kvalitet på oppdrettslaks. Ved å variere fôringsfrekvensen eller andelen marine råvarer i fôret, vil det være mulig å forvente en påvirkning på enkelte kvalitetsparametere i muskel hos Atlantisk laks (*Salmo salar*). Masteroppgaven består av to separate forsøk. I forsøket på fôringsfrekvens ble proteinløselighet, farge, tørrstoff, mengde fett, i tillegg til aktiviteten av den lysosomale proteasen cathepsin B+L sammenlignet i prøver av fisk fôret én og tre ganger per døgn. Resultatene indikerer at utvalget som var fôret 3 ganger i døgnet hadde større spredning i farge, høyere konsentrasjon av vannløselige proteiner, lavere fettinnhold og lavere aktivitet i cathepsin B+L enn utvalget som var fôret én gang i døgnet. Ingen signifikant forskjell ble målt i gjennomsnittlig farge, hardhet, bruddstyrke eller vanninnhold som følge av endring i fôringsfrekvens.
I diettforsøket ble fem grupper av Atlantisk laks ble holdt i fullskala oppdrettsnøter og fôret med marint basert fôr (MBF) i ulikt antall uker under vekstfasen (Gruppe A: 41 uker, B: 40 uker, C: 27 uker, D: 25 uker, E: 0 uker). Et konvensjonelt industrfôr (CF) ble ellers brukt. Pigmentering, lipidinnhold, fettsyre- og aminosyreprofiler og vanninnhold ble sammenlignet mellom gruppene. Endring i fett og farge ble sammenlignet i fersk fisk og etter fryselagring ved bruk av visuell analyse, NIR og UV-spektrometri. Aminosyrer, fettsyrer og vanninnhold ble analysert etter fryselagring. Tilvekst, vektspredning, nedgraderingsprosent, fôrfaktor og innvolledeponering ble sammenlignet i slaktedata fra de ulike gruppene. Resultatene indikerer at økt marint basert fôr kan føre til høyere vekstrater, lavere fôrfaktorer, sterkere rød pigmentering, høyere fettinnhold, endringer i aminosyreprofil og større innvolleandel. Ingen signifikante forskjeller ble oppdaget i proteininnhold, vanninnhold eller andel superior kvalitetsgradering. *UltraPerformance Convergence Chromatography*^{TM} (UPC^2) kan være en mulig metode for screening av fettsyreprofil av uestrifiserte fettsyrer fra fiskefilet, men videre metodeutvikling behøves for å avdekke dette potensialet.

Disse eksperimentene viser at den valgte fôringsstrategien kan ha en innvirkning på flere vekst- og kvalitetsparametere i laksefilet. Ytterligere forskning anbefales for å forklare mekanismene som ligger til grunn for disse observasjonene. Utviklingen mot mer bærekraftige fôrråvarer resulterer fortsatt i høykvalitets produkter.

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List of Abbreviations

AGD ..... Amoebic gill disease

ALA ..... α-linolenic acid

ANOVA ..... Analysis of variance

AQS ..... American Society for Quality

ARA ..... Arachidonic acid

CMS ..... Cardiomyopathy syndrom

CV ..... Conventional feed

CVD ..... Cardiovascular disease

DHA ..... Docosahexaenoic acid

EPA ..... Eicosapentaenoic acid

FCR ..... Feed Conversion Ratio

FO ..... Fish oil

GC ..... Gas Chromatography

HPLC ..... High-Performance Liquid Chromatography

HSMI ..... Heart and skeletal muscle inflammation

HUFA ..... Highly unsaturated fatty acids

IPN ..... Infectious Pancreas Necrosis

ISA ..... Infectious Salmon Anemia

LPL ..... Lipoprotein lipase

MBF ..... Marine based feed

NQC ..... Norwegian Quality Cut

OS ..... Oxidative stress

PCA ..... Principal Component Analysis

PD ..... Pancreas Disease

PRV ..... Piscine orthoreovirus

SFA ..... Saturated fatty acids

SPC ..... Soy Protein Concentrate

UPC^2 ..... UltraPerformance Convergence Chromatography^{TM}

VO ..... Vegetable oil

WHC ..... Water Holding Capacity

1 Introduction

1.1 Background and Motivation

The global population is growing. Resulting in increasing demand for marine protein. Due to this, the Norwegian aquaculture industry is estimated to increase its production to 5 million tonnes Atlantic salmon (*Salmo salar*) in 2050 (Olafsen et al., 2012). This raise in production will increase the competition for feed ingredients like oils and proteins. It may also increase the pressure on water supply and land area for feed production and on-shore production sites.

Salmon sea-farm aquaculture as we know it today has evolved through three decades of continuous transformation. An ongoing struggle for increased productivity, as well as strictly imposed requirements for sustainable farming, has been driving forces for technological developments in the industry since the seventies. Through increased and targeted investments in research, technology and operations, it is now visible that the Norwegian aquaculture industry is moving from an experimental to a knowledge-based business. This implies documentation regarding parts of the production in need for improvements, which will secure a sustainable development in the sector.
One such area with potential for improvements are within feed and feeding technologies. The goal for every sea farmer should be to produce high quality, healthy, nutritious fish in an economically, environmentally and socially sustainable supply chain (Carter & Rogers, 2008). The development shows that an increasing amount of the feed ingredients comes from plant based sources. This is recognized as more sustainable than processing fish into fish meal for aquaculture purposes, as energy are lost when moving up each trophic level of the food chain (Naylor et al., 2000; Ellingsen et al., 2009).
In order to secure the leading position in the worlds salmon aquaculture, Norway has to be leading in the development of knowledge in production technology and feeding systems for Atlantic salmon.
New raw materials, optimised diets and relevant research in how to develop feeding strategies without compromising fish health, quality, nutritional value, or the environment will be the key in this context.

In this master thesis, challenges regarding today's dominating feeding strategies will be addressed. Feeding strategy in this context implies feeding frequency, or feedings per day, in addition to feed chemical composition.
The differences and similarities in physical and chemical quality parameters of salmon fillets from two separate experiments with changes in feeding strategy have been investigated.

This thesis will examine the feeding aspect in a production perspective. More precisely, look into how feeding frequency and diet composition affect the fillet quality through parameters as proximate composition, pigments, texture, protease activity as well as fatty acid and amino acid profiles.
The parameters were mostly analysed with well-recognised methods, but also some method development efforts.

In the first experiment, post mortem quality analyses were completed on two groups given one or three daily feedings, without changing the daily feed intake. Fish was held in closed land based tanks. This was done to investigate if the quality could be affected by such basic changes in the feeding regime.
The second experiment involved larger changes. It took place at an operative sea farm and involved changes in feed recipe. The objective of the experiment was measuring the effect on fillet quality and yield from an artificial feed pellet based on marine protein and lipid sources. These samples was compared to fillets from a conventional feed program, where a considerable fraction of the marine, fish-based ingredients are substituted with terrestrial, plant based options. This was done to investigate how the development to more plant based resources impacts the sensory and nutritional characteristics of the product.
Slaughter data from the fish groups was provided by SalMar (hereafter "the company") to illustrate the effect on yield and classification from specified dietary changes.
Five groups of fish given different amount of the marine feed was investigated in order to uncover dose/response impacts.
Here, some methods for quality measurements was performed on the fillet at different stages of the supply chain and compared. This was done to explain how quality characteristics are changing with time and handling, as well as trying to challenge today's accepted methods of quality assurance.
Inclusion of more fish ingredients in the feed increases both the environmental footprint through energy use and emission of greenhouse gasses and the economic pressure for the producer through increased feed costs (Naylor et al., 2000; Hernández et al., 2007; Ellingsen et al., 2009). Thus, it could be of interest to evaluate if the potential benefits in growth and quality could be substantial enough to defend such costs. However, a detailed economic or environmental evaluation is not in the scope of this thesis.
To sort out which of the various quality variables to compare and present from the second part of the assignment, a statistical Principal Component Analysis (PCA) was conducted to investigate essential correlations and independences. All measured numeric response variables in this thesis are arranged into statistical models and investigated with computational tools.

1.2 Quality

Quality can be defined as "the degree to which a set of inherent characteristics fulfils a set of requirements" (Standard-Norway, 2015). American Society for Quality (AQS, 2016), describes quality as "A subjective term for which each person or sector has its own definition. In technical usage, quality can have two meanings. It can mean the characteristics of a product or service that bear on its ability to satisfy stated or implied needs. It can also mean a product or service free of deficiencies".
In other words, quality can be interpreted as the manner which a product or service fulfils the demands of a customer. These demands and expectations vary between different markets and can be based on religion, tradition and use (Kiessling et al., 2007; Bahuaud et al., 2009b).

In the food industry, the fundamental parameter of quality is that the food should be safe to eat.
The primary quality of food is controlled by hygiene, nutrition, sensory, parameters during storage and processing, as well as ethical conditions like sustainable production and animal welfare. In terms of animal products, biological aspects like species, size, season and health, as well as factors regarding livestock like feeding regime, handling and production would also affect the primary quality.
Total, or secondary quality involves the experienced quality and the market quality, such as if the product is delivered according to specifications, price, size, packaging, service, information and distribution (Bahuaud et al., 2009b; Nortvedt et al., 2007).

The perception of the term fish quality will also vary between participants in the supply chain, but freshness and shelf life are central factors that demands correct handling and control of temperature in processing, storage and distribution. The fish appearance is the first feature a customer will evaluate. The consumer would expect that unharmed fillets with smooth colouration implies higher sensory quality, firmer texture and low degree of bacterial growth. It would probably also be better suited for secondary processing than fillets with visible damage. In the nineties, external characteristics was regarded as sufficient in order to determine the fish quality, while internal factors like muscle integrity, composition and texture was given less attention, with the exception of fat content and pigmentation (Rasmussen, 2001).
Quality can be manipulated through numerous measures, both before and after harvest. Targeted breeding, nutritional feed content, feeding regime, feed frequency, handling, stress managing, environmental concerns, temperature control and packaging are examples of parameters with critical influence on the product quality and these therefore need to be controlled (Kiessling et al., 2007; Bahuaud et al., 2009b; Nortvedt et al., 2007).

It is essential to raise the knowledge of fish quality in order to satisfy the growing demand for seafood from customers with increasing quality conciousness.

1.3 Feeding Strategy

Feeding strategy implies changes in dietary compositions, as well as how and when feeding occurs. (Waagbø et al., 2001)
Quality and proximate composition (Figure 1) of the fish fillet is a result of series of endogenous and exogenous factors including genetics, environment and diet. The chosen feeding strategy has therefore an influence on fish metabolism and the value of the product (Shearer, 1994).

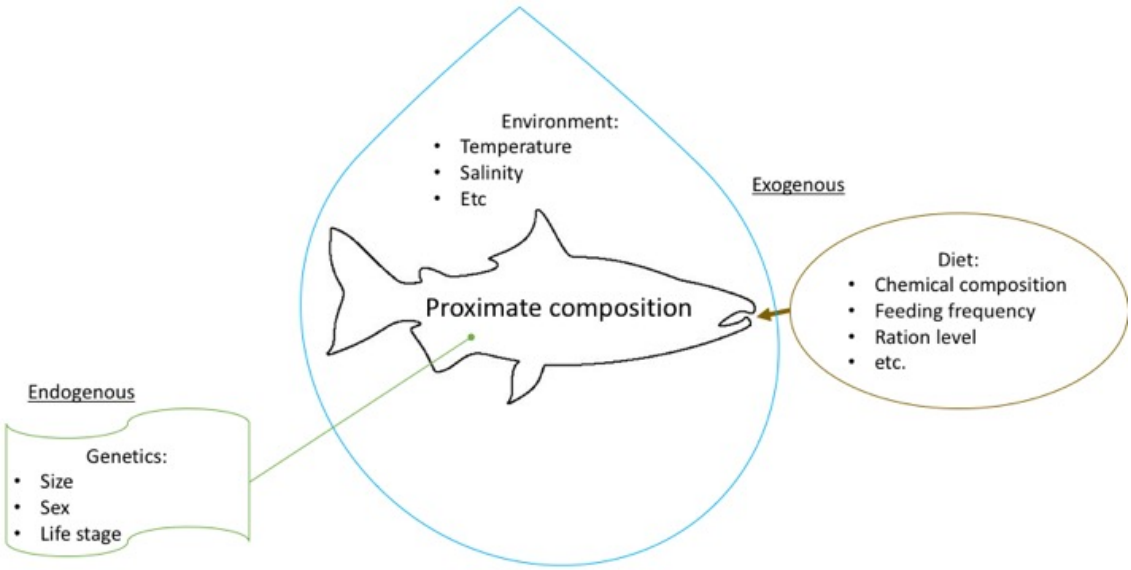

Figure 1: Proximate composition in Atlantic salmon is influenced by endogenous and exogenous factors (Shearer, 1994)

In a intensive cultivating system, the fish farmer has a unique ability to structure the architecture of the food product through the feeding regime. By controlled modification of the dietary chemical composition, feeding frequency or ration at different life stages, the quality and profile of the product can potentially be tailored in great detail (Norges-Forskningsråd & Thomassen, 2007). Table 1 illustrates the plasticity of the Atlantic salmon as a marine livestock.

Table 1: Proximate composition of farmed Atlantic Salmon (*Salmo Salar*). Plasticity of fish content during a life cycle. Values are presented as percent of wet weight (Shearer et al., 1994).

Component	Content [g/g fish]
Dry matter	$\sim 0.15 - 0.40$
Protein	$\sim 0.11 - 0.30$
Lipid	$\sim 0.02 - 0.20$
Ash	$\sim 0.01 - 0.03$

The lipid and protein contents will normally be higher in fast growing larger fish than in smaller fish (Shearer et al., 1994; Einen et al., 2006).

1.3.1 Diet

The nutritional content in the diet naturally has great impact on the fish composition. It is important to provide appropriate levels of energy, macronutrients, vitamins and minerals to ensure both fish well being, rapid growth as well as high product quality. Lack or excess of nutrients can result in a wide spectre of fish health and development issues, as reviewed in Section 1.4. Pigments, proteins, lipids and carbohydrates are featured in Sections 1.8, 1.6, 1.5 and 1.7, respectively.

1.3.2 Feeding Frequency

Rate of feeding during the growth phase is usually selected based on the size of the salmon, the day length and the water temperature. Normally the fish are fed after appetite to optimize the feed intake and the growth rate. Endogenous rhythms are affecting the activity and preferred hours of feeding. The appetite are usually highest at after sunrise and right before sunset.

The absolute limitation for feed intake is the stomach capacity, including volume and rate of stomach depletion. By varying the number of feedings per day, the farmer can limit the fish ability to eat maximally. The daily feed intake usually drops when fish are moved from a appetite regulated feeding regime to a interval controlled system, but the fish will adapt after some weeks, the stomach capacity stretches and the intake will rise again (Waagbø et al., 2001; Halver & Hardy, 2002).

There are examples on restrictive feeding with longer periods of starvation interrupted by intervals of feeding. This leads to increased competition and physical aggression between the fish and can alter the quality through wider spread in size, leaner fish and physical injuries.

Feeding rate will affect metabolic activity related to feed uptake, growth, energy storage and feed utilization. The complete effect on fish quality are not completely identified (Waagbø et al., 2001). Experiments to examine the effect of feeding rate variations needs to be performed in controlled environments in order to ensure that the daily feed intake remains constant.

1.3.3 Feed Timing

The nutritional requirements change during the salmon life cycle, prior to smolting, during smolting, after sea stocking, or sea-transfer and in the growth phase. The size and nutritional content in the fillet are affected by how the chemical composition of feeds meet the metabolic capacity at different stages of the production. If high levels of a desired nutrient are fed late in the growth phase, it is more likely to give high levels also in the fillet, than the same composition right after the sea stocking (Shearer et al., 1994; Einen et al., 2006).

The proximate composition and growth potential are naturally fluctuating with seasons and life stage, affected by alteration in water temperature and photo periods. The salmon is usually leaner in the spring time and grows rapidly towards the autumn. Elongation of photo periods and rise of water temperature results in increased activity, appetite, muscle growth and gain of fat. This can lead to variation in quality if not compensated by the feeding regime. Elevating the dietary lipid levels in the spring time, and corresponding lowering over the summer can ensure a more constant product year-round (Einen et al., 2006).

The feed industry has gradually developed a wide range of adapted feed products in order to face the different fish health and quality challenges throughout the fish life cycle. Seawater transfer is a critical stage of the production. The smolts are then usually fed with specific high energy transition feeds rich in nutrients to support growth, increase the immunocompetence and prevent development complications (Waagbø et al., 2001; Halver & Hardy, 2002).

The post slaughter quality and shelf life can also be affected by the feed used late in the growth phase. Specific slaughter feeds are sometimes used as an adjustment to optimise the result for specific characteristics. Rørå et al. (1995) claimed that lean diets prior to slaughter resulted in lower visceral and fillet fat deposition and stronger post slaughter red pigmentation than fish fed high lipid diets. and A wash-out period with high levels of n-3 fatty acids prior to slaughter have been shown to increase the flesh levels of these fatty acids. To counteract the following risk of post slaughter fatty acid oxidation and generation of free radicals, increased levels of antioxidants are supplied to the pellet (Einen et al., 2006).

1.3.4 Starvation

In the wild, Atlantic salmon can go several weeks without feeding. This ability is usually utilized in intensive aquaculture of salmon at the end of the growth phase. A starvation period prior to slaughter results in a decrease in condition factor ((weight/length^3)x100), but is a powerful tool in order to improve the yield, as well as improve quality (Wathne et al., 1995b; Waagbø et al., 2001; Einen et al., 1999).

Under starvation, the growth decreases and the fish utilizes the endogenous energy reserves in muscle, liver and abdominal tissue to cover the metabolic needs (Einen et al., 1999).

Starvation prior to slaughter can alter the proximate composition, give a leaner shape, a higher water binding capacity and will alter the fillet yield of the salmon (Fennema, 1996; Gómez-Guillen et al., 2000). However, studies by Einen et al. (1998) have shown that the rate of lost body mass decreases with increasing starvation time and only marginal variations in proximate composition was detected up to 56 days of starvation. Both protein and fat from muscle, abdominal tissue and liver are used as energy sources under long-term starvation. This results in more of a shrinkage of total body mass, than a distinct change in intrinsic balance. However, a shorter-term starvation period at 30 days prior to slaughter, resulted in increased slaughter yields, due to reduction in the visceral lipid content. Quality analyses have revealed rise in pH, reduced gaping and fat content, slight improvement of colour intensity and textural improvements in raw and smoked fillets (Einen et al., 1999).

1.4 Fish Health

Proper nutrition is critical in order to ensure healthy fast growing fish. Artificial diets serves as a tool not only for promoting normal growth through essential nutrients, but also in providing compounds with possible health improving effects if applied in appropriate concentrations. Nutritional imbalance is a source of health issues for fish, which again affects growth rate and fish quality (Halver & Hardy, 2002). Some frequently occurring symptoms of nutritional imbalance are listed in Table 2.

Table 2: Symptoms in relation to malnutrition in cultured fish (Tacon, 1992; Waagbø et al., 2001).

Symptom	State	Nutrient
Scoliosis/Lordosis (spinal deformities)	Deficiency	Tryptophan, magnesium, phosphorous, vitC
	Poisoning	Lead, cadmium, vitA, oxidized fish oil, leucine
Cataract (eye disorder)	Deficiency	Methionine, tryptophan, zinc, magnesium, copper, selenium, manganese, vitA, riboflavin, pyridoxine
	Poisoning	Choline, oxidized fish oil
Fin rot	Deficiency	Lysine, tryptophan, zinc riboflavin, niacin, inositol, vitC
	Poisoning	Lead, vitA
Fatty liver	Deficiency	Cholin, essential fatty acids
	Poisoning	Oxidized fish oil
Exophthalmia (bulging eyes)	Deficiency	Pantothenic acid (PANT), niacin, folat, vitA, vitE
	Poisoning	Oxidized fish oil
Fin and skin bleeding	Deficiency	Riboflavin, niacin, PANT, thiamine, inositol, vitA, vitC and vitK
	Poisoning	Oxidized fish oil
Anaemia (low haemoglobin)	Deficiency	Iron, selenium, vitC, vitD, vitE, vitK
	Poisoning	Lead, oxidised fish oil
Scoliosis/Lordosis (spinal deformities)	Deficiency	Tryptophan, magnesium, phosphorous, Vit C
	Poisoning	Lead, cadmium, VitA, oxidized fish oil, leucine
Gill damage	Deficiency	essential fatty acids, vitC, vitE, magnesium, PANT, biotin
	Poisoning	Unknown

Stress, parasites, bacterial and viral diseases will impact the yield and quality of the

product. Wounds, ulcers, deformities, pigment disorders, lost growth and mortality is quality related indicators of fish health issues like pancreas disease (PD), infectious pancreas necrosis (IPN), infectious salmon anaemia (ISA), furunculosis, and exposure to sea lice (Hjeltnes et al., 2016). A detailed review of these issues is outside the scope of this thesis. However, some of the disease related issues can be counteracted through dietary actions.

High energy feed and nucleotide-supplementation can reduce IPN related mortality and improve osmoregulation capacity. Intrinsic fatty acid composition can affect the phagocytotic capacity of macrophages and production of eicosanoid signal molecules, especially in cold water ($< 5\,^{\circ}\mathrm{C}$). Cataract have shown to be affected by the nutritional profile of the feed. Lack of methionine and tryptophan, as well as alterations in fatty acid composition can induce the disease (Halver & Hardy, 2002; Einen et al., 2006). Dietary additives are also proposed as a remedy against sea lice, through stimulation of epidermal mucus production (Provan et al., 2013).

It is unclear if the fish experiences life style diseases like humans (e.g. cardiovascular disease, atherosclerosis), but lipoprotein secretion is higher when feeding high levels of vegetable oils and can thereby be a useful side effect of fish oil substitution (Einen et al., 2006).

A variety of reports have shown that there is an ability to replace relative large levels of marine ingredients with vegetable alternatives, without inducing notable effects on growth or feed utilization. It is important to notice that some vegetable ingredients involves compounds with anti-nutritional effects. One example is phytic acid (Figure 2) from carbohydrate sources, which can inhibit digestive anionic and cationic trypsin proteases (Einen et al., 2006).

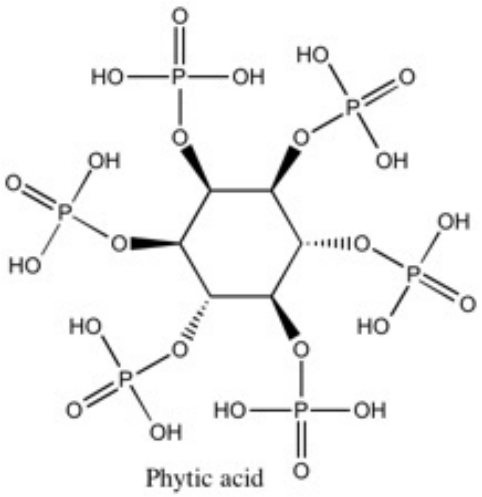

Figure 2: Phytic acid structure

The n-3/n-6 balance in feed pellets affect the fish health condition, through its impact on structural fatty acids in membranes. Studies have claimed that low n-3/n-6 balance, combined with stress can induce muscle necrosis, ventricular wall thinning and mortal membrane lesion in Atlantic salmon heart (Sargent, 1991). However, more research needs to be done to better understand the relationship between fish health and duration of feeding vegetable ingredients.

1.5 Lipids and Fatty Acids

Lipids are a wide group of essential chemical compounds and a macronutrient in foods. They are the evolutionary preferred and far most effective energy source (Table 3) for many marine fish and constitutes together with proteins and carbohydrates the macro nutrients for fish and mammals.

Table 3: Gross Energy of Macro nutrients (FAO et al., 1985).

Nutrient	Energy [kJ/g]
Fat	39
Protein	23
Carbohydrate	17

Lipids and fatty acids have numerous biological functions, but the most important ones includes providing structure to membranes, source of energy and energy storage in various tissues. The structure and function of membranes are highly affected by the degree of unsaturation in dietary fatty acids (Waagbø et al., 2001).

Fish, as well as humans holds the ability to biosynthesize the majority of needed lipids for growth and development. Most lipids in eukaryotes derives from acetyl-CoA through lipogenesis and can be divided into three classes: linear fatty acids; specialized, cyclic or branched fatty acids; and isoprenoids like carotenoids, sterols and hormones (Leaver et al., 2008). The fatty acids are mainly stored as triglycerides in muscle, liver and adipose visceral tissue, but also as phospholipids in membranes (Zhol et al., 1995; Jobling & Johansen, 2003; Nanton et al., 2007).

1.5.1 Lipid Storage

Liver, heart and red muscles has the most active cells for the catabolic pathway of fatty acids (Henderson, 1996). However, due to the large volume of tissue, white muscle are recognized as the most important contributor (Frøyland et al., 2000; Nanton et al., 2003;

Stubhaug et al., 2005).

Lipid transportation from storage cells to energy demanding cells are facilitated by several enzymes. Lipoprotein lipase (LPL) is a crucial control point. LPL activity is hormonally reciprocally regulated in by e.g. insulin and catecholamines, stimulating the balance between release of energy in muscle or storage of energy in adipose tissue, respectively (Mead et al., 2002).
It is believed that seasonal changes affect lipid energy distribution in salmon, but the detailed mechanisms are not fully known. In a study by Saera-Vila et al. (2005) it was shown that LPL-encoding genes in gilthead sea bream (*Sparus aurata L.*) are up-regulated in spring corresponding with a high body fat content. The annual peak of LPL mRNA expression was measured in summertime, coinciding with high requirements for energy in response with increased muscle growth.

1.5.2 Biosynthesis of Highly Unsaturated Fatty Acids

Salmon is capable of transforming a slight amount of α -linolenic acid (ALA; 18:3n-3) to eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid(DHA; 22:6n-3). However, the requirements for both fish and human nutrition depend upon the bottom of the food web to provide these essential highly unsaturated fatty acids (HUFAs). Due to these limited enzymatic capacities of ALA transformation, the essential fatty acids EPA, DHA and arachidonic acid (ARA; 20:4n-6) are therefore supplied in the artificial diets for aquaculture (Sargent et al., 2002).
Polyunsaturated fatty acids (PUFA) like 18:2n-6 and 18:3n-3 are precursors for essential HUFAs like EPA, DHA and ARA. Vertebrates lack the enzymatic ability to desaturate $\delta 12$ and $\delta 15$ double bonds to make n-3 and n-6 PUFAs from mono unsaturated fatty acids. These features are present in marine plants and plankton species and makes these PUFAs into essential nutrients for fish and human diets.

The biochemical pathways to synthesize HUFAs are through sequential desaturation and elongation of 18:2n-6 and 18:3n-3 PUFAs obtained from the diet. The biosynthetic pathways of EPA, DHA and ARA are illustrated in Figure 3.

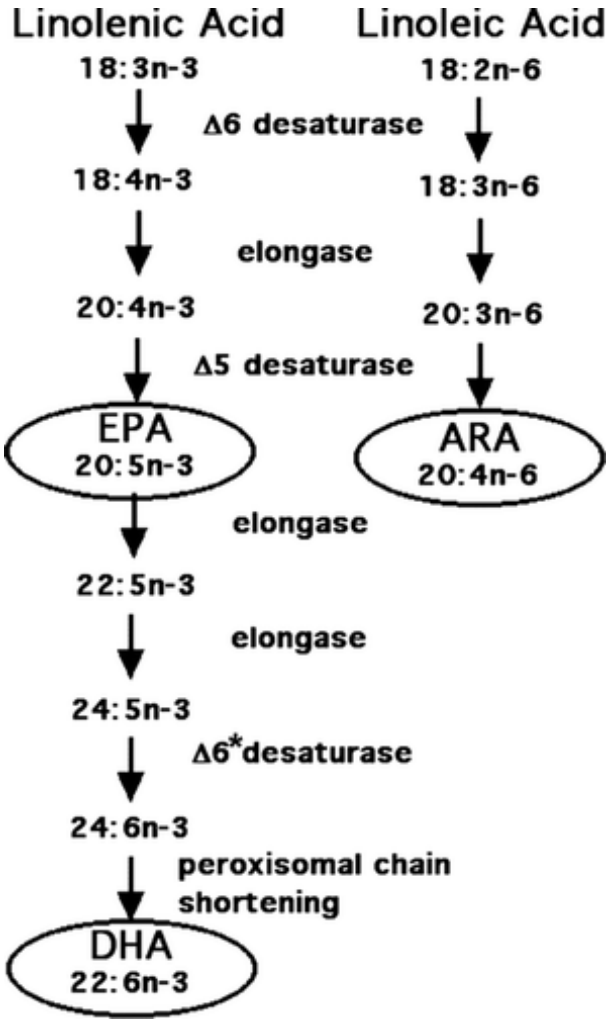

Figure 3: Biosynthetic pathway of EPA and DHA (Leaver et al., 2008)

Inclusion of vegetable oils (VO) in Atlantic salmon sea phase diets are related to a generally higher genetic expression of $\delta 5$ and $\delta 6$ desaturase compared to fishoil-based feeds (Zheng et al., 2005). A study conducted by Jordal et al. (2005), illustrated a significant up-regulation of the $\delta 5$ desaturase genes for Atlantic salmon fed 75% rapeseed oil, compared to the FO-fed group. He concluded that fresh water species feeding of terrestrial resources, rich in C18 fatty acids, are generally more adapted to scarcity of these fatty acids and possess up-scaled capacities of transformation into long-chained (LC) HUFAs.

1.5.3 Fish Oil versus Vegetable Oils

As a component in commercial feeds for intensive production of salmon, increased level of lipids is shown to give better digestibility for protein and a higher feed conversion rate (FCR). When more energy is provided from fats, less protein is metabolised for energy and stays available for muscle growth (Hemre & Sandnes, 1999). As feedstuffs like fish meal and fish oil (FO) from wild fisheries are highly limited, addition of VO can be good

resource economy. However, this requires that the requirements for essential fatty acids are met (Bell et al., 2002; Regost et al., 2004).
Farmed salmon are generally higher in crude fat, than wild fish. Even though the n-3 PUFA content is higher in wild fish, the total amount of n-3 PUFA tend to be similar, due to the higher amount of total lipid.
An issue that aquaculture companies has to address is that high levels of lipids in the feed which provides high growth rates, also leaves a higher volume of fats left with the viscera (Cowey & Young Cho, 1993). Larger industrial processing plants are therefore equipped with facilities for utilisation of the by-products to produce lipids and protein, resulting in up to 100% reuse of by-products (Solsletten, 2006; Bekkevold & Olafsen, 2007).

In a controlled system like intensive aquaculture, the main focus should always be to produce healthy fish, with high growth rates nutritional and sensory quality for the end consumer (Sargent et al., 2002; Tocher, 2003; Leaver et al., 2008). EPA and DHA content of farmed and wild fish species are shown in Table 4.

Table 4: Content of EPA+DHA in wild and cultured fish fillets (Haard, 1992).

Species	Wild (mg/100 g)	Farmed (mg/100 g)	Wild/farmed (%)
Channel catfish	170	100	170%
Red drum	160	210	76%
Carp	150	190	78%
Rainbow trout	560	1120	50%
Coho salmon	1490	1330	112%
Atlantic salmon	1450	980	148%

High concentrations of n-3 PUFAs in the feed have been claimed to be correlated to an increased risk for oxidative stress (OS) in salmon liver (Kjær et al., 2008; Østbye et al., 2011) and adipose tissue (Todorčević et al., 2009). Some of these effects are proposed to be reduced by addition of dietary antioxidants (Li et al., 2006)

Experiments shows that substitution of FO with VO in fish diets may change the β-oxidizing capacity of salmon (Torstensen & Stubhaug, 2004; Stubhaug et al., 2006, 2007). High EPA and DHA levels may lead to increases in FA β-oxidation through up-scaled genetic expression (Yamazaki et al., 1987; Willumsen et al., 1993, 1996; Brown et al., 1997; Berge et al., 1999; Østbye et al., 2011). However, the complete mechanisms underlying these effects are not fully revealed.
Lipogenesis activity is affected by HUFAs like EPA and DHA through modification of hepatic levels of the lipogenic enzymes glucose-6-phosphate desaturase (G6PD) and malic enzyme (ME) (Leaver et al., 2008; Menoyo et al., 2003; Alvarez et al., 2000). However,

no drastic variations in lipogenesis are detected switching from FO to VO, probably due to high levels of lipids in aquaculture feeds (Kiessling et al., 2007).
The effect on quality of VO substitution has been extensively researched. Some scientists have claimed that water binding capacity have declined, something that later has been rejected. However, the colouration and the lipid content are changed. It was claimed that quality indicators like texture, gaping and consumer experience was not directly affected (Kiessling et al., 2007).

1.6 Proteins and Amino Acids

Proteins are classified as macronutrients and represents a wide range of biological functions in living organisms. Fish, humans and all higher eukaryotes are depending on protein through the diet in maintaining essential enzyme activities and muscle growth. Digested dietary proteins are absorbed from the intestinal tract as amino acids, which serves as building blocks for functional and structural proteins encoded by the salmon genome. Amino acids are also transformed into signal molecules like hormones, neurotransmitters and nucleotides (Figure 4) or oxidized further into nitrogen and carbon compounds and energy (Nelson et al., 2008).

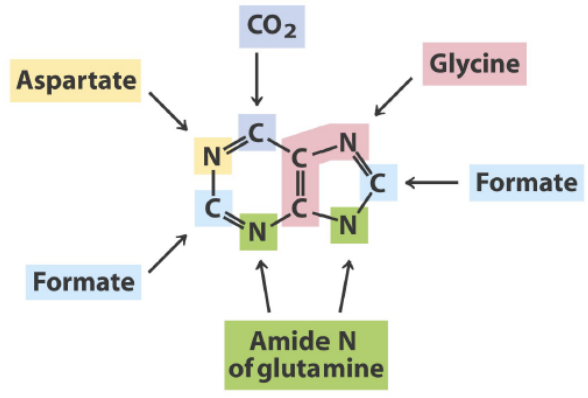

Figure 4: Origin of purine ring atoms (Studyblue, 2016)

The diet has to provide an adequate level of essential amino acids to ensure rapid growth and avoiding unwanted proteins catabolism (Wilson, 2002). If the dietary protein exceed the demands for amino acids, the remainder will be transformed into demanded metabolites or stored as energy.

The source of protein for feed pellets in salmon aquaculture has until recently relied on fish meal from species like capelin, herring, blue whiting and anchovy. These species have low consumer value for humans. However they consists of nutrients that are evolutionary preferred and valuable for the farmed salmon as feed ingredients, which also gives natural character and content in the end product. Fish meals are nutritious and

highly digestible as component in fish pellets because it simulates the natural diet for wild Atlantic salmon (Waagbø et al., 2001).
These fish stocks have until recently been abundant and highly accessible as feed raw materials. Now they are reaching their limits of sustainable harvest. This leads the industry over to alternative protein sources, ensuring the intended growth in the industry (Olafsen et al., 2012). The far most common substitutes are soy beans, an energy efficient protein source available in large volumes. However, the compositions of fish meal and soy protein are different, due to their origin and natural properties. This implies challenges in balancing the diet to ensure an equivalent recipe (Cowey & Young Cho, 1993; Waagbø et al., 2001; Halver & Hardy, 2002).
Fish meal ensures high digestibility due to low carbohydrate levels and strong nutritional value, through applicable amino acid and fatty acid profiles, as well as vitamins and minerals useful for the immune system (Waagbø et al., 2001).
Soy bean meal contains indigestible carbohydrates which are correlated to intestinal enteritis in farmed salmon. This matter is avoided by processing soy bean meal into extracted soy bean protein concentrate (SPC). Still, the amino acid profile in SPC differs from that in fish meal. Something that could lead to reduced digestibility, growth and unwanted metabolic impacts if proper dietary balance is not achieved. As humans, fish lack the ability to synthesize all needed amino acids, these essential amino acids has to be supplied through the diet (Table 5). Amino acid deficiency can be detected through measurement of free amino acids in plasma samples or muscle amino acid profile (Waagbø et al., 2001; Espe et al., 2014).

Table 5: Essential and non-essential amino acids for fish (Halver & Hardy, 2002).

Essential	Non-essential
Arginine	Asparagine
Histidine	Aspartic acid
Isoleucine	Cysteine
Leucine	Glutamic acid
Lysine	Glutamine
Methionine	Glycine
Phenylalanine	Histidine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

SPC and other plant based feeds usually have a lower content of in lysine and deficient in the sulphur containing methionine. As the protein synthesis is genetically regulated through transcription and translation of the fish genetic material, it is essential to have the mRNA-corresponding free amino acids available for peptide elongation and muscle

growth (Dersjant-Li, 2002). If free indispensable amino acids are deficit, generally the transcription of peptides will stop, resulting in reduced muscle growth, however other health related symptoms may appear. Methionine and tryptophan are substrates for compounds like cysteine and taurine, that have shown to be crucial in ensuring the hepatic regulation of the free amino acid pool. Methionine deficiency are correlated to symptoms like stressed or increased liver and cataract, which turns the eye lens opaque, impairs the light transmission and weakens its vision (Barash et al., 1982; Council, 1993). Arginine, isoleucine, leucine, phenylalanine and valine levels are generally higher than in fish meal (Miles & Chapman, 2006). Essential amino acid composition in fish meal, SPC and soy bean meal is presented in Table 6 (Dersjant-Li, 2002).

Table 6: Approximate protein composition of common protein ingredients and quantitative requirements of essential amino acids for Atlantic salmon (*Salmo salar*) (Scott, 1998; Sveier et al., 2001; Dersjant-Li, 2002; Halver & Hardy, 2002).

	Fish Meal	Soy Protein Concentrate	Soy Bean Meal	Atlantic salmon Requirements
Dry matter [%]	92	93	89	-
Crude protein [%]	62.9	65	44	-
Essential Amino Acids [%]	28	30.6	19.8	-
Arginine [%*]	5.8	7.6	7.3	4-5
Histidine [%*]	2.8	2.8	2.7	1.8
Isoleucine [%*]	4.1	4.9	4.5	3.2
Leucine [%*]	7.2	8.0	7.8	5.2
Lysine [%*]	7.6	6.5	6.4	3-6
Methionine [%*]	2.8	1.4	1.4	2.3-3.1
Phenylalanine [%*]	4.0	5.3	5.0	5.8
Threonine [%*]	4.2	4.2	3.9	3.2
Tryptophan [%*]	1.0	1.2	1.4	?
Valine [%*]	4.8	5.2	4.7	3.9
Sum [%*]	44.5	47.1	45.1	-

[%]: Percentage of wet weight, [%*]: Percentage of crude protein

Generally the requirement is considered covered when the amino acid supports optimal growth. Amino acid requirements for fish are estimated with the use of various methods and have different considerations for when the need is covered. This makes it complicated to determine exact limits. Some amino acids can be transformed into others and decreases the specific need. This is the case for phenylalanine and tyrosine, as well as

methionine and cysteine (Halver & Hardy, 2002).
The requirements for methionine are reported to range between 2.24 and 3.1 g/16 g N (% of protein), based on the values in Table 6 (Scott, 1998; Sveier et al., 2001; Halver & Hardy, 2002). This implies that fish meal are providing sufficient levels of methionine, but that SPC leaves a shortage, which needs to be balanced with added crystalline methionine or other corresponding ingredients.
It is important to note that if one of the essential amino acids are deficient, then growth will slow down (Halver & Hardy, 2002). Reports state that dietary protein has little if any effect on the whole body amino acid profile of fish, as long as the need for indispensable amino acids are covered (Shearer, 1994).

Protein accounts for 35-50% of the growth phase salmon feed, and the utilization of protein is crucial. The growth of the fish is primarily due to the intake of protein, so insufficient protein equals less growth. However, too much protein in the diet will lead to increasing levels in the faeces and higher nitrogen pollution. There should be a balance between energy/protein ratio in the feed. A high E/P-ratio gives higher fat accumulation, and a low E/P-ratio results in more energy spent on burning fat. (Wilson, 2002). Protein demand is higher in small fast growing stages, than for larger slower growing fish. By optimizing the amino acid compositions in the feed, the need for protein will decrease (Dersjant-Li, 2002; Wilson, 2002).

Muscles consists mainly of water soluble, sarcoplasmic protein (mostly enzymes and salt soluble myofibrillar (mostly structural) proteins, as well as stroma proteins (mostly collagen) (Shahidi, 1994). The distribution varies a little between different animals and between fish species (Table 7).

Table 7: Protein distribution in meat sources (Suzuki, 1981; Haard, 1992).

Source	Sarcoplasmic protein (%)	Myofibril protein (%)	Stroma Protein (%)
Fish, general	10-25	70-90	3-10
Sardine	22.5-34.7	59.2-66.1	1.3-2.5
Carp	24	∼71	∼5
Flounder	∼21	∼76	∼3
Cod	∼21	∼76	∼3
Beef	16-28	39-68	16-28

Pelagic and actively swimming species like mackerel and sardines (Table 7) are generally higher in sarcoplasmic proteins than the pelagic and fish staying in a more limited areas (Suzuki, 1981). This can imply a higher content of sarcoplasmic proteins in salmon.

1.7 Carbohydrates

The nutritional value of carbohydrates for Atlantic salmon is limited, due to evolutionary adaptations as carnivores, where biological needs for structural and storage carbohydrates (glycogen) are met by amino acid catabolism (Cowey & Walton, 1989). However, the compounds are recognized as inexpensive sources of energy which provides structure as well as technical and physical properties to the pellets and are included into balanced commercial feeds (Halver & Hardy, 2002). Salmon has a relatively short intestine (Figure 5) with relative low level of specific enzymes, the ability to digest starch is limited. Pre-treating of the carbohydrates, like heating or boiling is therefore common to increase the digestibility (Aksnes, 1995).

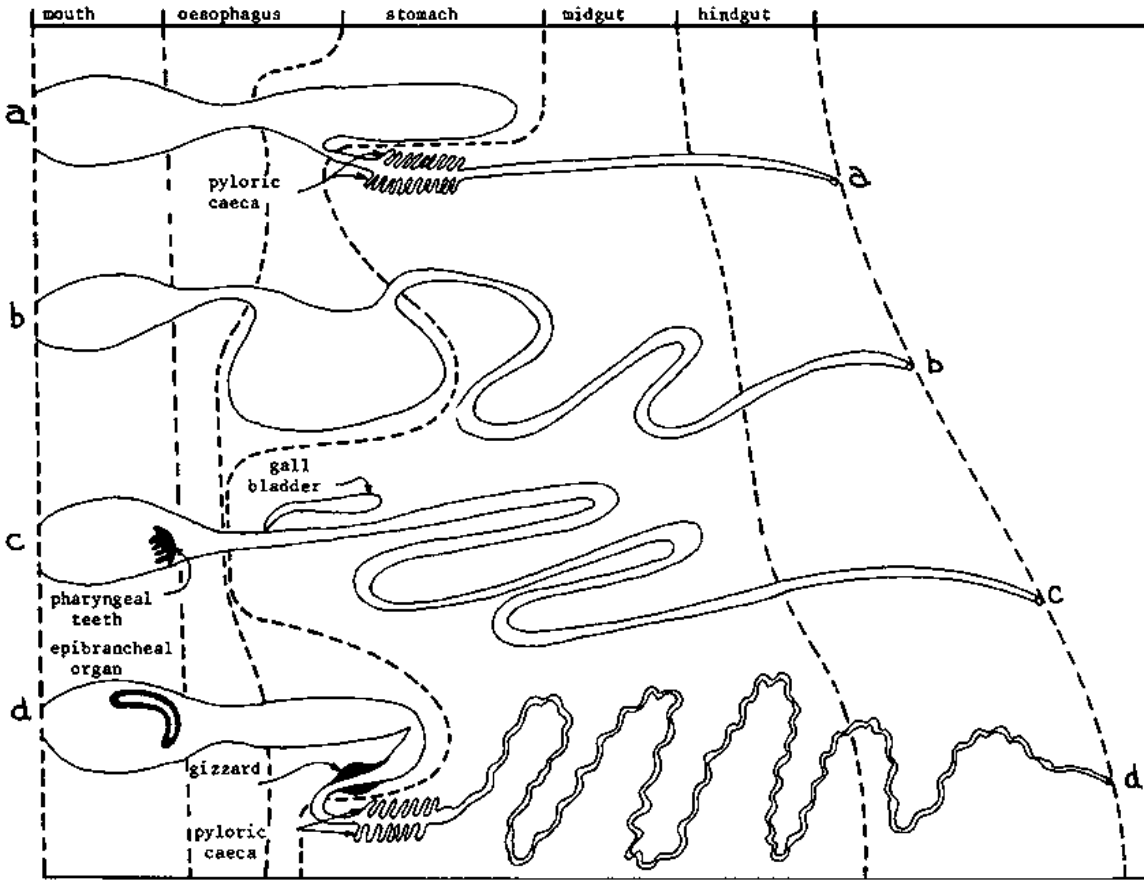

Figure 5: Digestive configurations of teleost fish. a: carnivores including Atlantic Salmon (*Salmo Salar*), b, c and represents omnivores and planktivores with various digestive traits (Halver & Hardy, 2002)

The need for carbohydrates is not crucial for salmon as it is for other macronutrients, because the liver can synthesize glucose to feed the muscle cells from fats and proteins. However salmon grows faster with carbohydrates in the feed than without.
If the level of carbohydrates are too high ($> 20\%$), the growth of the salmon is reduced (Aksnes, 1995).

1.8 Pigments

Colouration is a key parameter in evaluation of the primary quality of food. Carotenoids accounts for yellow and orange colours in a wide range of foods, from fruit and vegetables to some seafood. The subgroup of xanthophylls, more specifically astaxanthin ($3,3-dihydroxy-\beta,\beta-carotene-4,4-dione$) (Figure 6) provides most of the recognizable pink pigmentation in Atlantic salmon (Bjerkeng et al., 1997a). Due to the high cost of Astaxanthin as feed ingredient, an efficiency improvement of pigment deposition and visual colouration could be of great interest for the industry (Torrissen & Christiansen, 1995; Bjerkeng et al., 1997a).

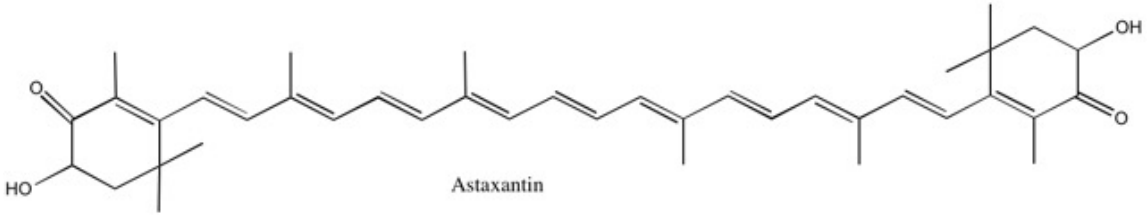

Figure 6: Astaxanthin structure

Astaxanthin consists of two terminal rings linked by a long conjugated system of trans double bonds that is essential for its light absorbency properties and colour intensity. The molecule can be transformed into less colour intense cis isomers if stored under sub-optimal conditions (Bjerkeng & Johnsen, 1995; Bjerkeng et al., 1997a; Coultate, 2009).
Nutritionally, the compound is recognized as a potent antioxidant and a preventer of numerous human diseases (Guerin et al., 2003; Hussein et al., 2006; Krinsky & Johnson, 2005).
It is claimed that high levels of astaxanthin in feed rations prior to slaughter can have a preventive effect on post slaughter lipid oxidation from high concentrations of n-3 PUFAs (Nordgarden et al., 2003). As the biosynthetic pathway of astaxanthin is missing in fish, the compound is obtained through the diet. In fish feed the molecule are most commonly chemically synthesized, but it is also retrieved from natural sources or biosynthesized in yeast or bacteria (Boussiba et al., 1998; Johnson & An, 2008; Scaife et al., 2009).

The levels and unsaturation of dietary fatty acids n-3 PUFAs are claimed to affect red carotenoid concentrations and redness in salmon fillet. It is discussed that it can be a correlation between concentration of saturated fatty acids and increased catabolism of astaxanthin (Bjerkeng et al., 1999). Astaxanthin is a non-polar pigment and freely soluble in the fish oil. Oil composition are claimed to make impact on the light absorbency properties of astaxanthin (Coultate, 2009). Scientists disagree upon how dietary oil source and amount can have a notable effect on the light absorption and visible colouration of

the fillet (Torrissen et al., 1989; Bjerkeng et al., 1999; Bell et al., 2001, 2002).

The quantification of colour can be done through chemical extraction, visually or instrumentally. The instruments can define color through several models, like red, green, blue (RGB) or cyan, magenta, yellow and key (CMYK). However a recognized system used for fish colouration is the three dimentional L*a*b* system described by Hunter (1948). The system defines color in three dimensions: L* - lightness; a* - red/green; and b* - yellow/blue. L* ranges from 0 (Deep Black) to 100 (Bright white), a* and b* ranges from -128 to 128. These parameters can be used to calculate intensity (Hue, H*, 360°) and clarity (Chroma, C*, 0-100) of the colour as shown in Equation 1.

$$\begin{aligned} C^* &= \sqrt{(a^* * a^* + b^* * b^*)} \\ H^* &= arctan(\frac{b^*}{a^*}) \end{aligned} \tag{1}$$

Figure 7 illustrates this relationship.

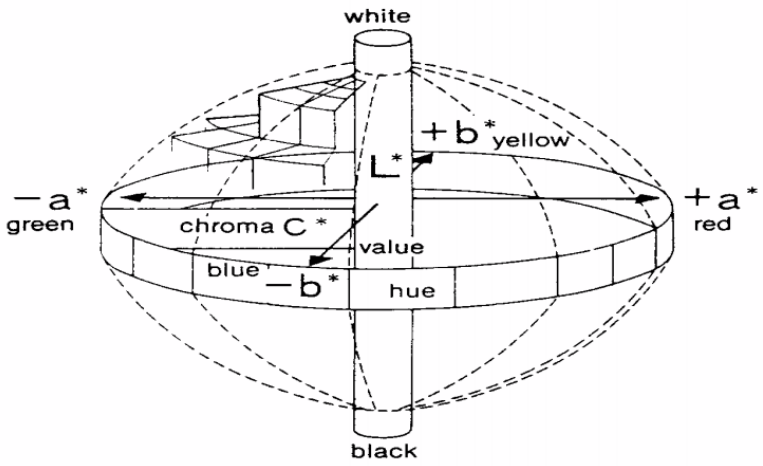

Figure 7: L*a*b* Colour wheel (Anderson, 2001)

A fast and effective tool in characterization of flesh colour utilized by the industry is the *DSM SalmoFan*^{TM} (Figure 8).

Figure 8: *DSM SalmoFan^{TM} (DSM, 2016)*

This is a internationally recognized scale from 20 to 34, normally used under constant light conditions.

1.9 Texture

One of the main factors causing changes in muscle texture is the degree of myofibre-myofibre and myocommata-myofibre decoupling (Taylor et al., 2002). High dietary EPA levels are claimed to have a possible damaging effect on these junctions (Bahuaud et al., 2009a). Histological examinations by executed by Erdal et al. (1991) revealed skeletal and cardiac degenerative muscle lesions as response to high dietary EPA and DHA, which could have a softening effect on fillet texture.

Instrumental texture analyses showed that Glu supplementation resulted in significantly ($P \leq 0.05$) firmer fillets after ice storage (10.1 vs. 9.1 N) and after frozen storage (8.7 vs. 6.3 N) (Larsson et al., 2014). Water binding and thereby myofibril protein properties are important because water content have been shown positively correlated to firmer texture (Section 1.11) (Hultmann & Rustad, 2004).

1.10 Cathepsins

Cathepsins are a group of lysosomal proteolytic enzymes that contributes to degradation of fish muscle texture (Ashie et al., 1996; Kolodziejska & Sikorski, 1996; Hultmann & Rustad, 2002). High cathepsin D, H, B and L activities are detected in muscle of spawning chum salmon. That is why the evolutionary developed function are thought to be energy provision from muscle tissue during long periods of starvation (Yamashita & Konagaya, 1990a; Hultmann & Rustad, 2002).
The various cathepsins are adapted for hydrolysis of specific sites of the peptides. Cathep-

sin B and L are both cysteine proteases and are recognized as the main contributors to muscle degradation (Kolodziejska & Sikorski, 1996; Aoki et al., 2000; Yamashita & Konagaya, 1991; Hultmann & Rustad, 2002).
Proteolytic cathepsin activity is affected by type of tissue, stress, temperature (optimum: 40-50 °C), pH (pH optimum: 3-4, some 6-6.5) and activation by reducing agents and maturation hormones (Aoki et al., 2000; Hultmann & Rustad, 2002).
When fish are slaughtered during periods of high growth, the fillet quality may decrease due to high enzymatic activity. Specified slaughter feeds used prior to slaughter with high levels of antioxidants can be used to lower the effect (Nordgarden et al., 2003).
It is claimed that if lysosomes are ruptured, like during water crystallization, the cathepsins will leak out and lead to softing of fish muscle and reduced shelf life (Yamashita & Konagaya, 1990b; Hultmann & Rustad, 2002). This is one reason why much emphasis are put on optimizing freezing and processing technologies.

1.11 Water

Water is the most abundant compound is fish muscle ($\sim 60-65\%$) and has crucial influence on physiological and chemical characteristics through its nature as solvent of polar molecules (Waagbø et al., 2001).
As a substantial contributor to the fillet content, water can affect the primary quality in many ways. Freezing, salting and drying of fish will lower the water activity and increase the hygienic quality and limits microbial growth (Coultate, 2009). Dissolved minerals, vitamins and other compounds will affect the nutritional quality (Halver & Hardy, 2002), while the water holding capacity (WHC) will influence the sensory and technical quality (Fennema, 1996). Water in fish is found mainly as physically entrapped. The amount of water in the product is controlled by the WHC, which affect tissue performance and other quality properties like juiciness, texture, storability and suitability for secondary processing (Fennema, 1996). Fillet texture is affected by the fish WHC, which is connected to pH, ionic strength and the structure and concentration of salt soluble myofibil proteins because they retains most of the water in muscle tissue ($\sim$70%). Hultmann and Rustad (2004) have investigated these properties and showed that breaking strength can be positively correlated with water content.
How water is bound and water activity are important factors to control for storage and processing of salmon.
The WHC of fish are claimed to be affected by the nutritional status of the fish and increased WHC is detected after periods of starvation prior to slaughter (Fennema, 1996; Gómez-Guillen et al., 2000; Olsson et al., 2007).

1.12 Human Benefits of Marine Fatty Acids

It has been shown that a higher intake of n-3 LC-PUFA can help prevent against cardiovascular disease (CVD) in humans. Several hypotheses on this subject derives from early discoveries presented by the Aarhus scientists Dyerberg and Bang in 1982, when they linked the high fatty fish consumption in Inuit communities with their significantly low CVD rate.

There is much that indicates that the n-3 LC-PUFA DHA and EPA have the ability to reduce inflammation and thereby dissolve, and stabilize atherosclerotic plaque. DHA is thought to have the ability to decrease the risk of atherosclerosis, by reducing the level of the inflammatory marker C-reactive protein (CRP) in the blood stream.

Multiple studies states that a higher level of n-3 LC-PUFA are related to a notable reduction in blood pressure, removal of triglycerides, factors that together will decrease the chance of death from heart disease (Bergé & Barnathan, 2005).

Dietary fish oils are associated with a lowered risk of developing prostate cancer (Norrish et al., 1999), as well as adenocarcinomas, an epithelial form of cancer. It showed that consumption of n-3 LC-PUFA could have a positive effect against extreme weight loss for cancer patient (Barber et al., 1999; Bergé & Barnathan, 2005).

Fish oil is recommended as a supplement for patients with the skin condition psoriasis. The disease can be recognized much because of the ARA-rich plaque on the skin and the patients lowered ability to metabolize eicosanoids like prostaglandins and leukotrienes that are thought to decrease inflammation (Bergé & Barnathan, 2005).

Rheumatic patients are thought to get an effect in relief of pain and stiffness in joints because of the n-3 LC-PUFA ability to lower production of protein interleukin-1β (Kremer, 2000; Bergé & Barnathan, 2005).

Several science reports states that dietary n-3 LC-PUFA can prevent asthma in children, and benefit subjects with lung diseases like asthma, cystic fibrosis and emphysema, because the LC-PUFA leads eicosanoids away from the ARA-pathway that is forming bronchoconstrictive leukotrienes (Schwartz, 2000). Attention-deficit hyperactivity disorder (ADHD) is a very common disorder, most common among children in school age (4-20%). The symptoms are poor coordination, inattention, hyperactivity, impulsivity, and learning disorders. ADHD, dyslexia and dyspraxia have been linked to deficiency of certain LC-PUFA, especially ARA, EPA and DHA (Stevens et al., 1995). One possible hypothesis is that children who do not get breast milk gets less DHA (very abundant in breast milk), and therefore are more exposed to ADHD, dyslexia and dyspraxia, and therefore should consider to eat fish rich in n-3 LC-FA (Stordy, 2000; Bergé & Barnathan, 2005).

Epidemiological studies show that low intake of EPA and DHA compared with a high intake of linoleic acid (LA) could lead to increased risk of developing dementia and cog-

nitive reduction. A diet rich in EPA and DHA tend to help the brain cell membrane stay as fluid as possible, unlike n-6 LC-FA and saturated fatty acids (SFA) that tend to make the membranes rougher. Due to this and the anti-inflammatory properties, supplementation of EPA and DHA could lead to avoiding retardation of the brain function. Reports suggests that intake of n-3 LC-PUFA could prevent depression, schizophrenia, Alzheimer's disease and other mental illnesses (Stoll et al., 1999; Kyle et al., 1999; Bergé & Barnathan, 2005).
There are differences between saturated FAs effect on the human health. Stearic acid is a LC-SFA that appears to be less affecting on the serum cholesterol than palmitic acid, and is thereby more appropriate for cholesterol-lowering diets (Grundy, 1994). However, dietary LC-SFA are thought to enhance the risk for gallstone among men (Tsai et al., 2008).

1.12.1 What Is Appropriate Intake?

The healthiness of LC-FAs are linked to the amount ingested and the relative ratio to other nutrients and fatty acids, but some types of LC-FAs are considered to be healthier than others. Trans-FA is an example of a group of fatty acids that most scientists think should be avoided completely, and LC-SFA from animal fats should be moderately used. The balance between n-3 and n-6 PUFAs are considered a main reason for many human diseases like CVD, cancer, inflammatory and autoimmune diseases.
The western diet have a n-3/n-6-ratio between 1:15 and 1:20. Optimal ratios are believed to be between 1:1 and 1:4, and cutting the diet to 1:4 could lead to a 70% decreased in mortality for patients with CVD. Studies have shown that patients with rectal cancer could slow down the growth of rectal cells when they decreased their ratio to 1:2.5, the 1:4-ratio gave no effect on growth (Simopoulos, 2002).
The optimal balance is showed to vary with different health conditions, but the main consensus is that many people could gain health benefits with a higher intake of n-3 LC-PUFA (Bergé & Barnathan, 2005).

2 Materials and Method

2.1 Feeding Frequency

2.1.1 Sampling

Atlantic salmon (*Salmo salar*) was delivered from Lerang research station, outside Stavanger, Norway.

6 fish with similar weight and length (∼3000 grams, ∼57 cm) was picked out from each of two groups under different feeding frequency: one and three feedings per 24 hours. The fish was anaesthetized, euthanized, bled out and transported on ice, by plane to Værnes airport the same day. After arrival to NTNU, Trondheim, the styrofoam boxes was emptied of meltwater, supplied with ice and stored in cold storage 4 °C before the fresh fish were analysed the next morning.
The fish was filleted, kept on ice and used in subsequent experiments.

Each right-side fillet was used for chemical analysis. 4 grams of the back loin was cut out for water- and salt soluble protein extraction, 4 grams were used for dry matter analysis and 10 grams were frozen −18 °C and stored for lipid extraction (Figure 9).

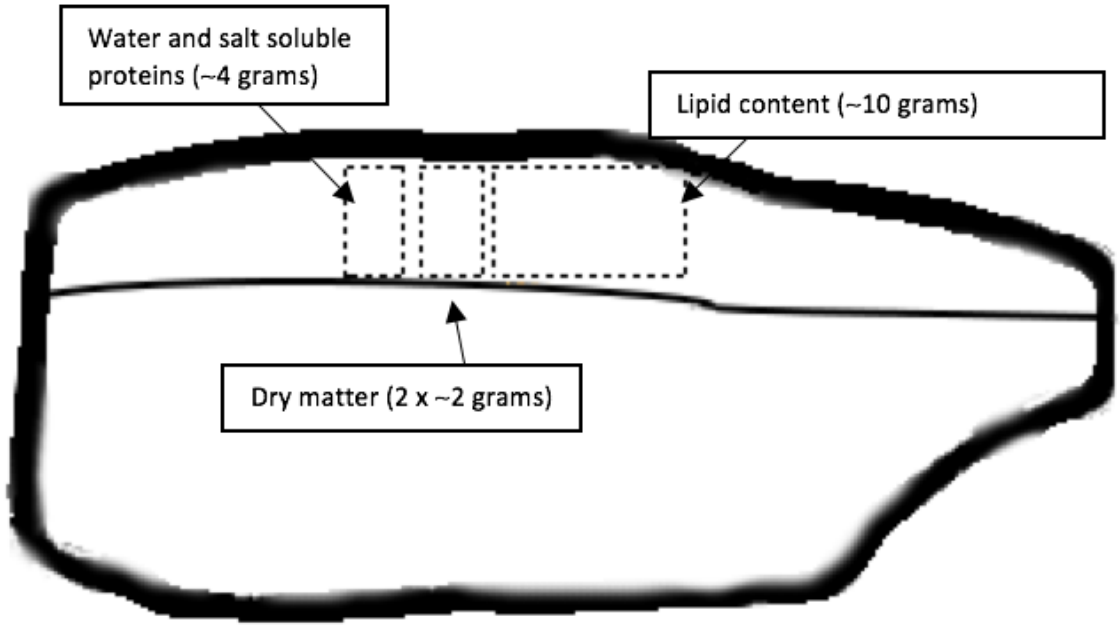

Figure 9: Sample extraction, feed frequency experiment. The figure illustrates the right fillet of Atlantic Salmon, *Salmo salar*, which was used for quality analyses.

2.1.2 Texture

The texture analysis was performed on the left fillet from each fish. The instrument TA-XT plus Texture Analysator was used (*SMS Stable Micro Systems, Surrey, England*), according to the method described by Einen & Thomassen (1998) and modified by Hultmann & Rustad (2002).

A load cell (5 kg) were linked to a flat-ended cylindrical plunger (12 mm diameter). Resistance force (N) was recorded as the plunger was pressed downwards into the fillets at a constant speed of 1 mm/s until it reached 60% of the sample height, with a holding time of 5 seconds between 2 repetitive compressions. The recorded data were used to calculate the breaking strength and hardness of the fillets (Figure 10), as described by Bourne (Bourne, 1978).

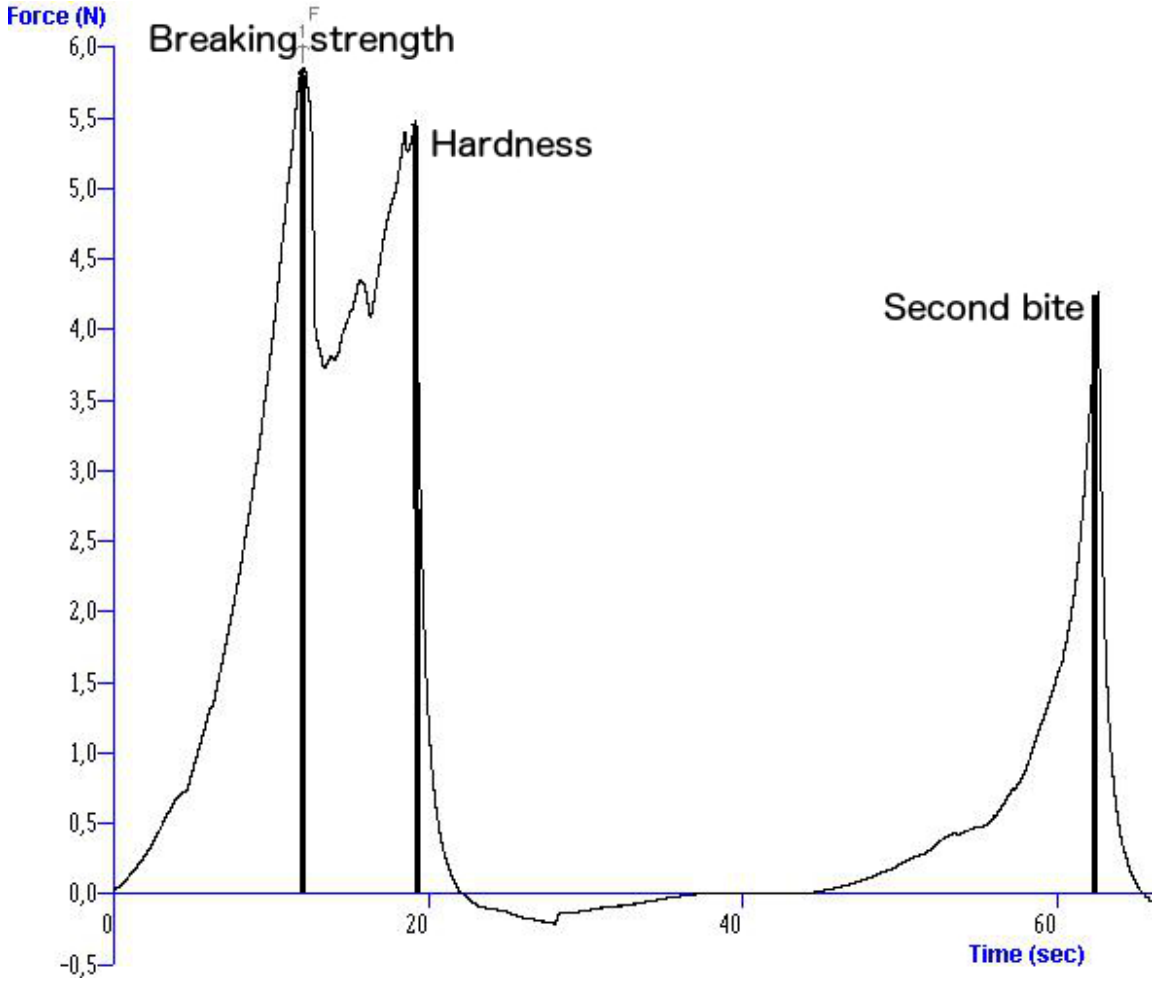

Figure 10: Curve from Texture Analysis of Atlantic Salmon (*Salmo salar*).

Texture measurement was repeated at 5 spots above the lateral line on each fillet, from posterior to exterior end, with an interval of 5 centimetres. The following figure

(Figure 11) illustrates the setup.

Figure 11: Texture analysis. The photo shows an Atlantic Salmon (*Salmo salar*) fillet being analysed with a Stable Micro Systems Texture Analysator, TA-XT plus.

2.1.3 Pigmentation

The pigment analysis was carried out on the left fillet of each fish.
The fillets was photographed with a single-lens reflex camera, under constant lighting conditions. The colouration of the pictures was examined by use of a colour recognition feature (*Digital Colour Meter* v5.10, Apple Inc, California, USA). In each photo, three fixed spots (11*11 pixels) was focused (lateral posterior, centre of backloin, lateral anterior), which provided a comparable Lightness, red/green, yellow/blue (L*a*b*) code that indicates the variation in pigmentation between the samples. Average L*a*b* values for each fillet was used in further calculations.

2.1.4 Water and Salt Soluble Proteins

This analysis was performed on a sample of the right fillet of each fish, and examined after the method of Anderson & Ravesi (1968) and Licciardello et al. (1982) modified by Hultmann and Rustad (2002).
A sample of white muscle ($\sim$ 4 grams) was weighed in. The sample was added 0.05 M phosphate buffer (pH 7, 80.0 mL) and homogenized with ultra turrax (14,000 rpm, 15 seconds). The mixture was centrifuged (8000 g, 20 minutes, 4 °C). The supernatant was filtrated and phosphate buffer was added to a total volume of 100 mL. This constitutes the water soluble fraction. The remaining sample from the centrifugation was homogenized with 0.05 M phosphate buffer with 0.6 M KCl. The mixture was centrifuged (8000 g, 20 minutes, 4 °C). The supernatant was filtrated and phosphate buffer with KCl was added to a total volume of 100 mL. This constitutes the salt soluble fraction.
The protein content of the fractions was analysed with the Biorad method, described by Bradford (1976).

Water soluble protein extracts was frozen and stored at −20 °C, to be used for the protease activity analysis (Section 2.1.7).

2.1.5 Lipid Content

The analysis of total amount of fat was conducted with a sample from the same area of the right fillet from each fish (Figure 9), and examined with the method described by Bligh and Dyer (1959).

A sample of white muscle from the fish fillet (∼ 10 grams) was weighed out and kept on ice together with the rest of the materials used. Distilled water (10 mL), chloroform (20 mL) and methanol (40 mL) was added and the sample was homogenized (2 minutes). Chloroform (20 mL) was added, then the sample was homogenized (30 seconds), before addition of distilled water (20 mL) and repetition of homogenization (30 seconds). The mixture was centrifuged (9000 g, 20 minutes, 4 °C) before a sample of the chloroform phase (1.00 mL, one parallel) was transferred to a pre-weighed test tube and evaporated on heating block (4 °C) and weighed to determine the lipid content.

2.1.6 Dry Matter

The dry matter analysis was conducted on the same area on the right fillet from each fish.
Two parallels of a sample of white muscle (∼ 2 grams) was weighed. The sample was dried in heating cabinet (105 °C, 24 hours). The dry matter was cooled and weighed, before the initial water content was calculated.

2.1.7 Protease Activity Cathepsin B+L

The analysis was performed on the water soluble extracts (Section 2.1.4), as described by (Barrett & Kirschke, 1981).

Four different solutions was prepared for this experiment:

- A substrate solution of 3 mM benzyloxycarbonyl-phenylalanylarginine-4-methylcoumaryl-7-amide (300 μL) was diluted with distilled water (1:32).
- A stop solution (150 mL) of 1% SDS and 50 mM EDTA, adjusted to pH 7.0.
- An analysis buffer (100 mL) of 150 mM bis-Tris, 30 mM EDTA, 6 mM DTT, adjusted to pH 6.0.
- The protein extracts was thawed and diluted with water (1:10)

Analysis buffer (100 μL) was added to a test tube, three parallels for each fish, in addition to one blank. Suitably diluted protein extract (100 μL) was added, to the samples. To the blank water was added instead of protein extract. The test tubes was incubated in water bath (30 °C, 15 minutes).
0.09375mM substrate solution (100 μL) was added to each tube, before the incubation was repeated. After incubation, the stop solution (3.0 mL) was added and the tubes were put on ice.

Fluorescence values was measured with UV-spectrometer (excitation: 360 nm, emission: 460 nm).

2.2 Dietary Composition

2.2.1 Feed Composition

Recipes for the conventional feed (CF) and the marine based feed (MBF) used in this experiment are shown in Table 8.

Table 8: Feed recipes, diet experiment.

Ingredients	Conventional feed	Marine feed
Fish meal	8.8 %	40 %
Soy Protein Concentrate	23.0 %	2.5 %
Sun flower meal	8.1 %	
Wheat gluten	4.0 %	10.5 %
Wheat meal	9.8 %	15.5 %
Bean meal	3.0 %	
Fish oil	9.3 %	15.2 %
Rape seed oil	23.1 %	15.2 %
Astaxanthin	50 mg/kg	50 mg/kg
Recycled shattered feed	8.4 %	
Other	2.7 %	1.1 %
Protein	32.9 %	38.8 %
Starch	7.2 %	9.8 %
Fibre	3.5 %	0.5 %
Fat	35.5 %	35.9 %
EPA+DHA	6.0%	8.6%
Saturated fats	16.5%	15.3%

2.2.2 Sampling

The salmon samples all originated from the same operative sea farm in Mid-Norway. The samples was extracted from 5 groups (A-E) fed different amounts of fMBF, where group E fed only CF. A timeline presenting the feeding regime are presented in Figure 12.
When the fish grew into a size of approximately 4 to 6 kilograms, they were transferred with well boat to a nearby slaughterhouse where the samples were collected directly from the production line, heads-on gutted. Hereafter the fish was measured and weighed, before the Norwegian Quality Cut (NQC) were taken out, and used in further analyses. Sampling of groups A, B and C were done according to the slaughter plan between 21th of july to 21th of october. Groups which where not yet planned to harvest (group D and E), were sampled on the farm and shipped as NQC on ice by express mail over night. Groups A, B and C went through post-harvest starvation, while groups D and E were collected from the netpens unstarved.

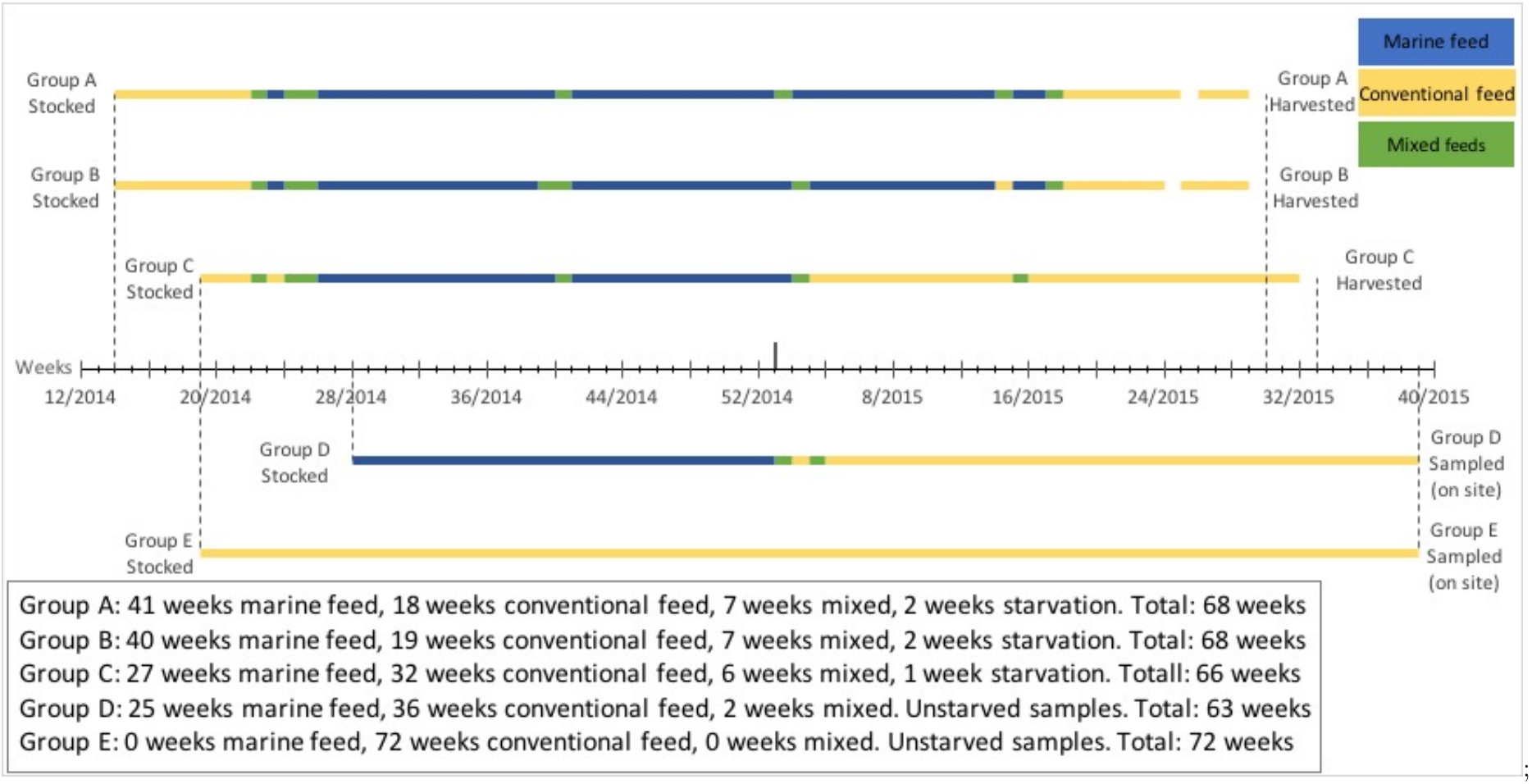

Figure 12: Timeline, diet experiment. A feed based mainly on marine raw materials is provided in varying amounts to growth phase Atlantic salmon (*Salmo salar*) at the same sea farm. Weeks feeding fish with marine based feed are marked in blue, weeks with conventional feeds are marked in yellow and weeks with both marine based and conventional feeds are marked as green on the time line. Breaks in the lines illustrates weeks of pre-slaughter starvation. Samples from groups A, B and C (n=15) were collected during slaughter, while groups D and E (n=10) were sampled on the sea farm and shipped on ice with express mail over night.

2.2.3 Growth and Fish Health

Data considering growth rate, slaughter weight and fraction of superior quality fish, as well as on-site quality measurements from each group was provided by the company and examined in statistical analyses.

2.2.4 Fresh Fish Pigment Analysis

The pre-rigor NQC sections were stored for two days in refrigerator. The right fillet of the post-rigor section was then cut from the sample and visually evaluated for colouration with *SalmoFan*^{TM} cards (Hoffmann-La Roche Basel, Switzerland). The NQC fillet was homogenized, then pigmentation and fat was estimated with Near Infrared (NIR) spectroscopy from an industrially recognized calibration.

2.2.5 Storage and Transportation

The remaining NQC sections was individually packed, labeled and stored in a freezer ($-18\,^{\circ}$C) at the slaughterhouse lab at Frøya. The samples was packed on ice in closed Styrofoam boxes, and transported by refrigerated truck to a cold storage at Trondheim pier and transported by car to a freezer room at NTNU ($-18\,^{\circ}$C).
The samples was examined on arrival to make sure they were still frozen.

2.2.6 Homogenization

The NQC samples was thawed on ice, filleted, skinned and homogenized with a food processor before it was divided into separate containers for subsequent experiments.

2.2.7 Dry Matter

2 grams of homogenized sample was weighed (2 parallels) and dried at $105\,^{\circ}$C for 24 hours before the dry weight was registered.

2.2.8 Lipid Analyses

The lipid was extracted and total lipid content was determined after the method described in Section 2.1.5 with following modifications.

A homogenized sample ($\sim$ 10.00 gr) was weighed. Distilled water (16 ml), chloroform (20 ml) and methanol (40 ml) were added, before homogenizing with ultra turrax (14,000 rpm, 2 minutes). Chloroform (20 ml) was added and homogenized (30 seconds). Distilled water (40 ml) was added and homogenized (30 seconds).
The sample was centrifuged (5000 g, $4\,^{\circ}$C, 15 minutes), before lipid fraction was determined.

The remaining chloroform phase was transferred to centrifuge tubes and stored in freezer (−18 °C) and were hold for analysis of fatty acid composition and content of carotenoids.

Fatty Acid Composition

A sample of the chloroform phase (10 ml) was evaporated on heating block (60 °C) with supply of nitrogen gas. 2M NaOH (2 ml) was added, and the sample was put on heating block (min. 2 hours). The lipid phase was washed with ion-free water and dissolved in CH_2Cl_2 (1.5 ml), whirl mixed and stored in freezer (−18 °C).
A filtrated sample of the solution, with 10 μM Palmitic-d_{31} acid was analysed with UltraPerformance Convergence Chromatography™.
As the washing can result in loss of sample, a threshold value was set.
Before statistical analyses, samples with total peak area below 100,000 was ignored, in order to prevent disturbance from negligible fluctations.

2.2.9 Carotenoids

The carotenoid analysis was performed after the method of (Tolasa et al., 2005). A sample of the chloroform phase was evaporated. Oil (0.69 g) was dissolved in n-hexane (1.5mL), and the absorbency at 472 nm was read with UV-spectrometer.

2.2.10 Amino Acid Analysis

A sample of the homogenized fillet was freeze dried.
The total amino acid composition was determined after the method described by Blackburn et al. (1968).
A sample of dry matter was weighed and hydrolysed in 6M HCl for 22 hours at 105 °C and cooled. The pH was adjusted to pH 7, before filtration and addition of doubly distilled water to a total volume of 10 ml. The amino acid profile was analysed in reverse phase HPLC as explained by Hultmann (2004), by precolumn fluorescence derivatization with *o*-phthaldialdehyde (GP 50 gradient pump, RF 2000 fluorescence detector, ultimate 3000 autosampler, and ultimate 3000 column compartment, all parts Thermo Scientific Dionex, Sunnyvale, CA, USA), was performed using a NovaPak C18 cartridge (Waters, MA, USA).
As a measure of total protein, dry matter fraction of amino acid was summed. The highest and the lowest values from each feed group was removed before data processing, in order to easier reveal potential trends.

2.2.11 Statistical Analyses

To address the statistical significance related to both the feed frequency and chemical composition experiments, several statistical models were fitted in the statistical modelling software RStudio (v.0.99.887, RStudio Inc, Boston, USA). One-way and two-way analyses of variance (ANOVA) as well as coupled T-tests, all at the $p < 0.05$ level, was used to reveal significant differentiations between any response and explanatory variables of interest.
Due to the large volume of data, a Principal Component Analysis (PCA) was first applied for the chemical composition experiment, to uncover possibly correlated and uncorrelated variables.
Scripts used for statistical modelling are found in Appendix A.

3 Results and Discussion

In this section the results from the two experiments are presented and discussed under mutual headlines concerning the various quality topics. This is done to prevent repetitive discussions and to be able to evaluate the results from different feeding strategies in a production perspective.

3.1 Principal Component Analysis, Diet Experiment Data

The fish analysed in the varied diets experiment was taken out of a full-scale operative salmon production site, with feeding regimes not optimized for a dose response analysis (Figure 12). However, this gives the possibility to examine a more authentic setting than in the feed frequency experiment.

It is important to note that the feeds used in the diet experiment (Table 8) are based on recipes that is different in many ways, which have led to a complex analysis. There are variations in protein source (fish meal, SPC, wheat gluten and bean meal), oil source (fish oil and rape seed oil) as well as in macro-nutrient balance (protein, carbohydrates and fat). The MBF has a total content of protein and fat at 74.7%, while the CF has 68.4%, something that implies higher access for energy for group A and B than the other groups.

This means that it is hard to point out one single factor affecting each of the results from the quality analyses, but interesting correlations may appear.

In order to decide how to approach such a dataset, there are several statistic tools that can be used. Principal component analysis were chosen to reveal possible correlations and independences. The result from the PCA of the varied diets experiment is visualized in Figure 13. R-script are found in Appendix A.

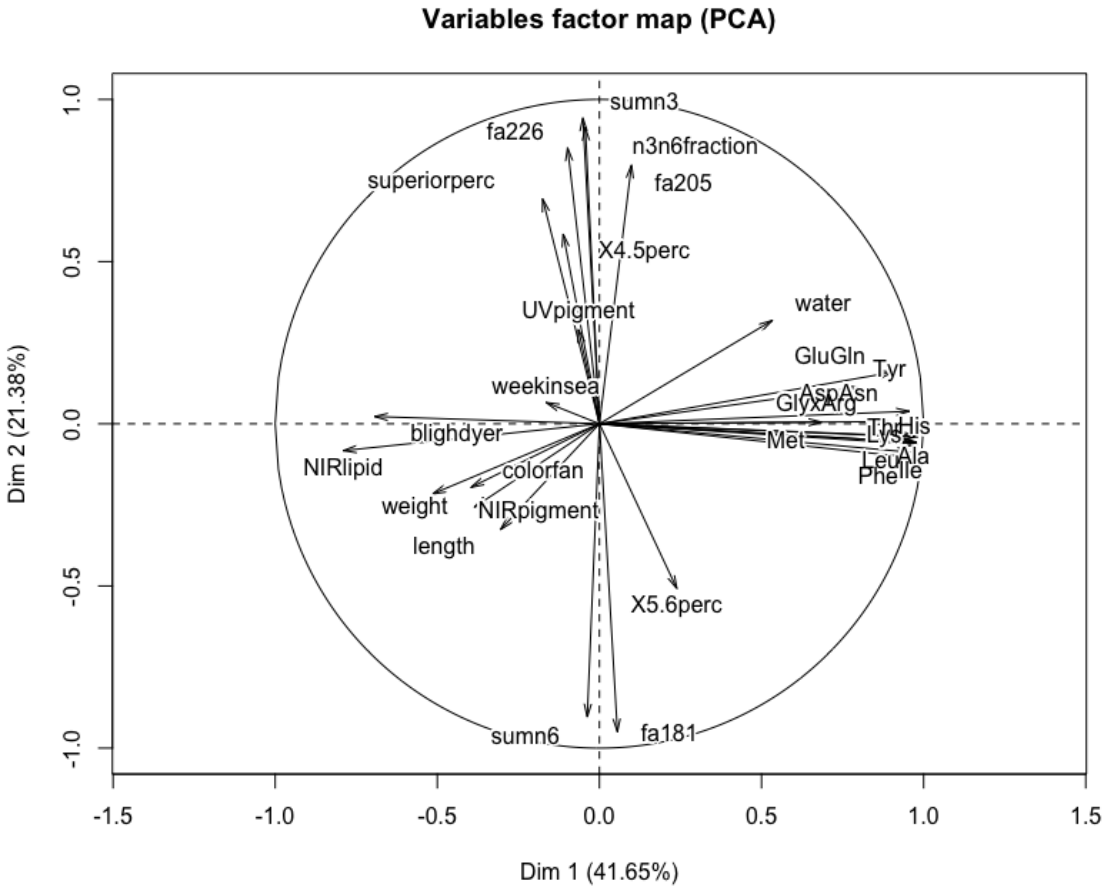

Figure 13: PCA, diet experiment data. The parameters represents Atlantic salmon (*Salmo salar*) fed different amounts of marine raw materials.

Correlations are visible, but not perfect between the results from Bligh and Dyer lipid extraction and NIR lipid analysis, which indicates a value of studying the correlation between them.

There seems to be a possible correlation between superior quality fish and n-3 fatty acid fraction, which may be an indication that fish health are linked to fatty acid composition. A clear inverse correlation is detected between amino acid and lipid fractions, but these factors are not correlated with fatty acid composition or superior slaughter quality.

The perpendicular relationship between the fresh fish colour analysis and the carotenoids identified with UV after frozen storage may be an indication that the measured carotenoids deviates from the fresh fish colouration. Water content is inversely correlated to colouration. Something that proposes that water content has a lightening effect on salmon fillet. These results will be taken to consideration when discussing the various topics in following sections.

3.2 Pigment Analysis

When a customer evaluates a food product, colouration is naturally one of the first considered characteristics. Strong and even colouration is an indicator of good fish health and is something the consumer associates with a high quality product. This is why the salmon industry emphasize research on astaxanthin pigmentation and evaluates the pigmentation through the everyday quality assurance systems, to assure consistency of strongly coloured products.

3.2.1 Photometric Analysis, Feed Frequency

Average L*a*b*-dimensions and H* and C* values from the digital photography analysis after varied feeding frequency, described in Section 2.1.3 and calculated with Equations 1 are presented in Figure 14. Raw data are presented in Appendix B.

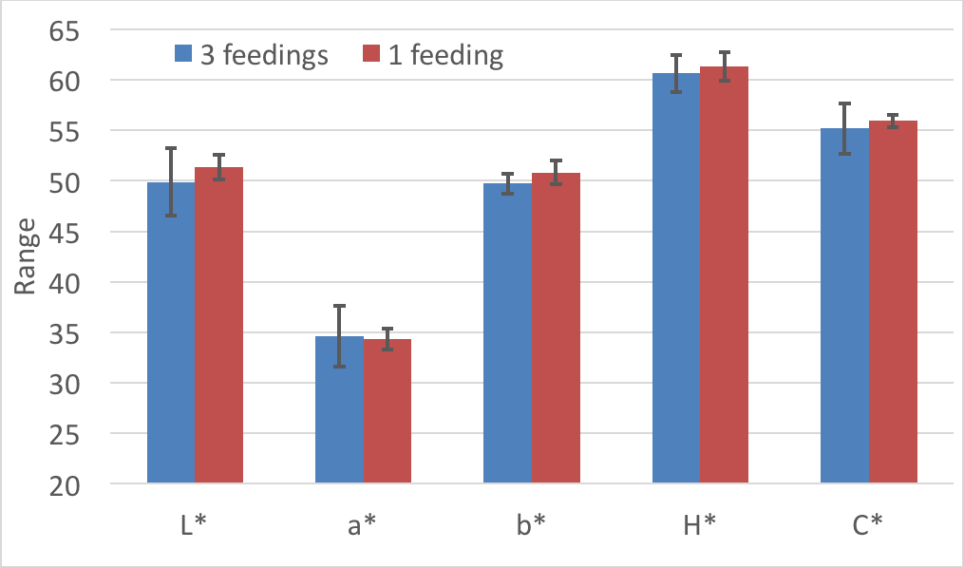

Figure 14: L*, a*, b*, H* and C* values, feed frequency experiment. Photometric analysis of Atlantic Salmon (*Salmo salar*) colouration. The figure illustrates average of 3 sample spots of left fillet (lateral anterior, center of back loin and lateral posterior) from fish fed one versus three times daily (n=6). Standard deviations are indicated on each column.

No significant difference was found between the fish groups, but there are some matters to consider.

There is a marginal trend that fish fed three times a day are darker, more red and less yellow than fish fed once a day. Fish fed 3 times shows generally higher standard deviations than fish fed 1 times per day, which indicates that this group showed less consistency in appearance and consisted of both the most coloured and the palest fillets.

It is shown that the chosen feeding strategy affects the muscle colouration, through the choice of diet. There is not found proof in literature to assume a direct effect of feeding rate on colouration, as long as the same amount of total daily feed is provided and star-

vation intervals are short enough to prevent increased hierarchy in the fish stock.

Average L*a*b*, intensity (H^*) and satiation (C^*) dimension scores (coupled t-test, $p \leq 0.05$), calculated with Equation 1, are listed in Table 9. Raw data are found in Appendix B.

Table 9: L*, a*, b*, H* and C* values, feed frequency experiment. Photometric analysis of Atlantic Salmon (*Salmo salar*) colouration. Average values of 3 sample spots of left fillet (lateral anterior, center of back loin and lateral posterior) from fish fed one versus three times daily (n=6), presented with standard deviation. (coupled t-test, $p \leq 0.05$).

	Mean 3 feedings	Mean 1 feeding	p-value
L*, Lightness	50 ± 3	51 ± 1	0.344
a*, Red/green	35 ± 3	34 ± 1	0.822
b*, Yellow/blue	50 ± 1	51 ± 1	0.115
H*, Hue	$61^\circ \pm 2$	$61^\circ \pm 1$	0.483
C*, Chroma	55 ± 2	56 ± 1	0.493

No significant difference in colouration was detected through photometric analysis of salmon fillet from variation in feed frequency. However, a marginally significant difference at 10% level was detected in the red/green dimension (p_{b^*}=0.115), indicating that increased daily feedings might have correlation to red colouration.
To speculate, fish fed once a day might possess higher enzymatic lipid turnover and transportation activity, through the less continuous access of energy, which may affect the lipid soluble pigment (Torrissen et al., 1989; Bjerkeng et al., 1999; Bell et al., 2001, 2002).
To clarify if these variations are significant, a more accurate method of photometric analysis are suggested.
Folkestad et al. (2008) describes a *Rapid and non-invasive* method in digital photometric analysis of fat and pigmentation in Atlantic salmon muscle. The method requires a specifically designed apparatus including a light proof aluminium box with standardized illumination and a perpendicularly mounted camera which is controlled through a computer system. Unlike the method applied in this experiment, Folkestad´s design also includes an optimalized algorithm for calibration of lightness and white balance in order to transcribe the data into fat and pigment concentrations or *SalmoFan^{TM} values*.
In an industrial setting, this makes a powerful tool in utilizing the concept of digital photography in evaluation of the primary sensory quality, directly from the production line.

However, in this experiment the dietary composition, consumed energy, genetics environment and post slaughter treatment was unchanged. The experiment was conducted

in closed land based tanks under controlled environments, which implies minimal risk for external stresses like parasites and infections. All in all, other factors would probably make a stronger influence on the fillet colouration than variation in feed frequency.

3.2.2 NIR Spectrometry, Diet

The fresh fish pigment analysis through NIR spectrometry of the groups with varied diets, after the methods described in Section 2.2.4 are presented in Table 10 and Table 11, respectively. Raw data are found in Appendix C.

Table 10: NIR, red pigment content, diet experiment. Pigmentation in NQC from Atlantic salmon (*Salmo salar*) fed with decreasing amounts of marine raw materials (Group A>E, n=10-15), presented with standard deviations. Instrument is calibrated to HPLC. P-value indicates significant difference from group A (anova, $p \leq 0.05$).

Fish group	Red pigments [mg/kg]	p-value
A	7.4 ± 0.7	
B	7.2 ± 0.7	0.6200
C	7.1 ± 0.7	0.4441
D	6.6 ± 1.3	0.0318
E	6.9 ± 0.6	0.2031

Group A, which had the diet consisting of most MF (41 weeks), appears to have a higher mean concentration of red pigments (7.35 mg/kg) than than the other groups, and significantly higher than group D (6.63 mg/kg, p=0.0318). All groups showed great consistency in the colouration based on the similar standard deviations (stdev=0.6-0.7) except group D, which had twice the variation (stdev=1.3). The analysis also indicates a decreasing trend of groups B, C, E and D with decreasing levels of red pigments. This corresponds well with the expectations based on findings by Bjerkeng et al. (1999) and coincides with the decreasing amount of MBF (Figure 12). Some differentiation was expected due to the variation in feed and oil composition (CF: 23.1% rape seed oil, 9.3% fish oil, MF: 15.2% rape seed oil, 15.2% fish oil) and because the degree of EPA and DHA was higher in the high-marine feed (6.0 vs. 8.6%) (Figure 8). However, it is notable that group D showed lower red pigmentation than group E, fish fed entirely on the conventional feeds. It could be assumed that the conventional feed is highly developed and might be balanced for pigment deposition effects, or poor smolt quality for group D. This is something that could be detectable through genetics or growth rate analysis (Gjerde & Gjedrem, 1984; Rye & Gjerde, 1996; Quinton et al., 2005).

3.2.3 Visual Colour Analysis, Diet

Results from the SalmoFan^{TM} colouration evaluation of fresh fish are presented in Table 11. Raw data are found in Appendix C.

Table 11: SalmoFan^{TM} results, diet experiment. Pigmentation in NQC from Atlantic salmon (*Salmo salar*) fed with decreasing amounts of marine raw materials (Group A>E, n=10-15), presented with standard deviations. P-value indicates significant difference from group A (anova, $p \leq 0.05$)

Fish group	SalmoFan^{TM}-value	p-value
A	27.4 ± 0.7	
B	26.9 ± 0.6	0.191
C	27.0 ± 0.7	0.904
D	27.2 ± 1.2	0.162
E	26.6 ± 0.5	0.591

As from the NIR results in Table 10, group A appears to have a higher mean pigmentation (SalmoFan ^{TM}-score = 27.4) than than the other groups, but no significant difference was detected between the groups. The analysis indicates a decreasing trend in red colouration, correlating to the amount of dietary MBF (Group A, B, C, E and D, respectively). However, regarding that the SalmoFan^{TM} spans from 20 to 34, relatively modest differences are detected between as response to the increased MBF. The results from visual analysis corresponds well, but not perfectly to the variations detected through NIR analysis (Figure 15), both in score and in standard deviations (Tables 10 and 11). Raw data are found in Appendix C.

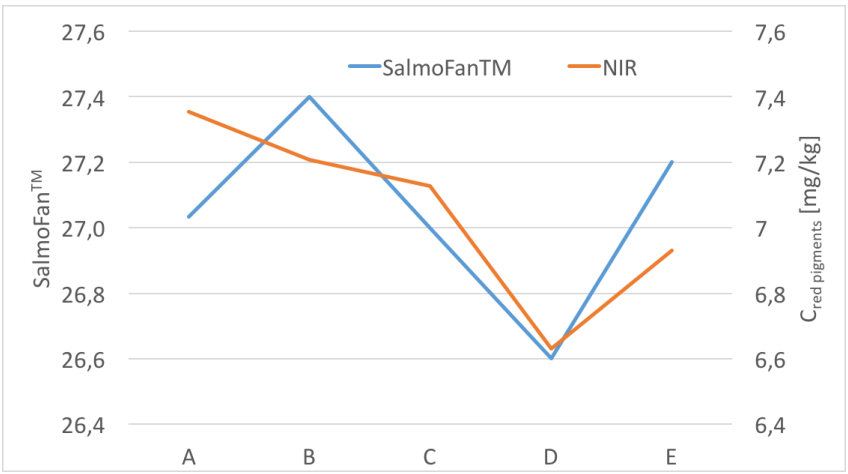

Figure 15: NIR vs. SalmoFan^{TM} results, diet experiment. Colouration in NQC from Atlantic salmon (*Salmo salar*) fed with decreasing amounts of marine raw materials (Group A>E, n=10-15)

The figure indicates that visual colouration to some degree can be affected by other

factors than detected by NIR. It could be assumable that the calibration of the NIR instrument is based mainly on fish fed on conventional feeds, which can affect the light absorbence characteristics and the ability to detect smaller changes (Liu et al., 2013). The dietary oil source has been shown to influence the light absorbance characteristics of the carotenoid, something that also is widely discussed in relation to salmon colouration (Torrissen et al., 1989; Bjerkeng et al., 1999; Bell et al., 2001, 2002). In salmon fillet, astaxanthin is mainly bound to proteins in muscle tissue and not the fat tissue. Matthews et al. (2006) claims that the protein α-actinin is the main binding cite for astaxanthin. In shellfish like lobster and shrimp, the protein bound to astaxanthin has major influence on the light absorbency and apparent colouration. However, this is not directly transferable principles.

The colouration of salmon can change as a response to freezing, storage and thawing, especially under sub optimal conditions (Bjerkeng & Johnsen, 1995; Jensen et al., 1998; Coultate, 2009).

3.2.4 UV Spectrometry of Carotenoids, Diet

The results from the analysis of carotenoids in salmon oil, conducted after freeze transport ($-18\,^{\circ}\mathrm{C}$), are presented in Table 12. Raw data and calculation example are found in Appendix D.

Table 12: UV-spectrometry, carotenoids in oil, diet experiment. Extracted from NQC of freeze stored Atlantic salmon (*Salmo salar*) fed with decreasing amounts of marine raw materials (Group A>E, n=10-15), presented with standard deviations. P-value indicates significant difference from group A (anova, $p \leq 0.05$).

Fish group	Absorbance (472 nm)	Astaxanthin [mg/kg]	p-value
A	0.358 ± 0.072	3.7 ± 0.8	
B	0.385 ± 0.045	4.0 ± 0.5	0.459
C	0.297 ± 0.064	3.1 ± 0.7	0.009
D	0.335 ± 0.062	3.5 ± 0.6	0.309
E	0.312 ± 0.081	3.2 ± 0.8	0.066

The oil from group B appears to have the highest concentration of carotenoids compared after the process of freeze storing, thawing, homogenizing and Bligh and Dyer lipid extraction. The concentration was marginally significantly higher in group B than in Group D (coupled t-test; p=0.07), significantly higher than group E (coupled t-test; p=0.03) and group C (coupled t-test; p=0.0003).
First of all, this extraction method resulted in very low amounts of carotenoids, compared to the NIR fresh fish analysis. It is notable that the sample preparation nor the execution

was optimized for carotenoid analysis as explained by Britton (1995), in terms of storage time, light protective environments during preparation and execution or accuracy like HPLC (Schiedt & Liaaen-Jensen, 1995; Lerfall et al., 2016). However, all the samples was given the same treatments, so the differences between the groups are of interest.
In comparison to the trend from fresh fish NIR analysis (Table 10), these results indicate that groups A, C and E have lost more pigmentation than the other groups during freeze storage and processing. The extractabilty and detectability of astaxanthin depends on the chosen solvent and type of oil and complete recovery is a complicated process (Ambati et al., 2014). Stability of Astaxanthin is varying for different oils and is affected by such factors as freezing, illumination and heating (Rao et al., 2007). It is expected that part of the astaxanthin would transform into metabolites like idoxanthin or less colour intense cis-isomers of astaxanthin (Aas et al., 1997). The applied method detects total carotenoids which would include carotenoid metabolites, but this could result in decreased light absorbance characteristics (Coultate, 2009).
A complete recovery of astaxanthin was therefore not expected in this analysis, but the pigmentation seems to have changed despite equal treatments. In contrast to earlier founds by Lerfall et al. (2016), these results may indicate difference in carotenoid stability between the groups, since group A, C and E weakens more rapidly and is more affected by storage and handling than the other groups. However, it is not possible to conclude a correlation between dietary regime and carotenoid stability. Minor differences in handling could have had an impact on the carotenoid recovery (Schiedt & Liaaen-Jensen, 1995). Further examination of this matter with accurate quantification through high pressure liquid chromatography is needed in order to verify the findings and better understand the underlying mechanisms.

Feed strategy can affect colouration in growth phase Atlantic salmon fillet.
Photometric analysis with single lens reflex camera, constant light source and standard software seems useful as a fast and easy method in detection of variations in color characteristics in food. Slight trends, including less consistency in pigmentations was detected in fish fed three times per day compared to fish fed once a day, however no significant difference was detected in colouration due to variations in feed frequency.
Visual colour evaluation with Salmofan^{TM} seems to be affected by factors important for light absorption which are not detected through NIR spectrometry. The colouration evaluated visually can possibly give a more realistic image of how the customer will assess the product.
Marine raw materials can have a positive effect on pigmentation in fresh raw Atlantic salmon fillet. A significantly increased level of astaxantin was detected in fish fed a diet based on marine raw materials for 41 weeks, compared to those fed the same diet 25 weeks.

Significant difference in carotenoid pigmentation was detected in fish fed different intervals of marine based feeds, however, no dose/response relationship was identified. Validation experiments are needed to justify these findings.

3.3 Lipid Content

Lipids, especially fat composition and degree of oxidization is of great influence for consumers and producers of Atlantic salmon. It describes properties related to appearance, freshness, odour, palatability, texture, storage stability, fish health, human nutrition and yields (Sheehan et al., 1996; Bjerkeng et al., 1997b).
High dietary and proximate lipid content is desired in a producer perspective sustaining optimal growth, since it have shown to prevent muscle protein degradation into energy (Hemre & Sandnes, 1999). Thus, total lipid content can be of great interest when optimising a feed strategy.

3.3.1 Lipid Content, Feed Frequency

Total lipid content from the feed frequency experiment are presented in Figure 16. Raw data is presented in Appendix E.

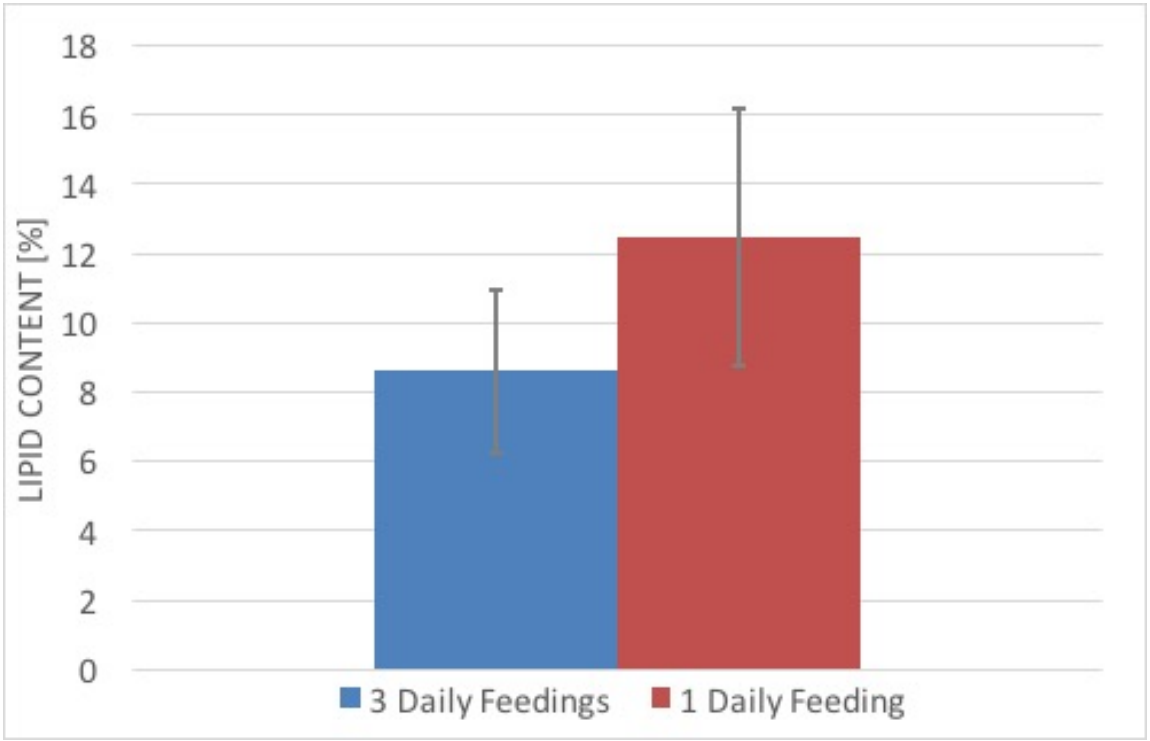

Figure 16: Extractable lipid content, feed frequency experiment. Sample of Atlantic salmon (*Salmo salar*) back loin from fish fed one versus three times daily (n=6). Results are presented as percent of wet weight. Standard deviations are illustrated on each column.

The results claims a marginally significantly (coupled t-test; p=0.0628) higher lipid

content in fish fed one time per day (12.5%) than fish fed three times per day (8.6%). This indicates that variation in feed frequency leads to differences in lipid deposition in Atlantic salmon muscle.
It could be assumed that fish fed once a day could have ability to store more energy as fat than fish fed three times per day, due to needed up-regulation of lipid transport capacity to support a continuous supply of energy to the tissues (Halver & Hardy, 2002).

The lipid values from this analysis are both relatively low compared to the literature values in Table 1 ($\geq 20\%$) (Shearer et al., 1994). However, the samples used in this analysis was cut from back loin, something that would lead to lower expected values than for homogenized NQC or whole fillets. The fish was harvested at approximately 3000 grams, a smaller size than desired for an industrial production and the fat content are known to be higher in larger fish. EPA and DHA levels are known to decrease in fish feed, it could therefore be beneficial to keep a sensibly high and steady fat content in the fillets, to ensure the nutritional requirements for human diets. Fat content are of great interest for some consumers due to the high gross energy compared to proteins and carbohydrates.

Further research with larger selections and larger fish is necessary in order to investigate the effect of feed frequency on lipid storage patterns in Atlantic salmon muscle fibers and myosepta (Zhol et al., 1995; Zhou et al., 1996).

Even if feed frequency might have some effect on lipid deposition in fillet, it seems to be a general consensus in literature that dietary lipid profile has direct impact on the lipid content in Atlantic salmon fillet (Waagbø et al., 2001; Halver & Hardy, 2002; Kiessling et al., 2007).

3.3.2 NIR Spectrometry of Fat Content, Diet

Results from the fresh fish NIR analysis of fish fed with varied amount of marine raw materials is presented in Table 13. Raw data are found in Appendix C.

Table 13: NIR, lipid content, diet experiment. NQC from Atlantic salmon (*Salmo salar*) fed decreasing amounts of marine raw materials (Group A>E, n=10-15), presented as percent of wet weight, with standard deviations. P-value indicates significant difference from group A (anova, $p \leq 0.05$).

Fish group	Mean [%]	p-value
A	17.1 ± 1.7	
B	16.6 ± 1.9	0.4847
C	16.2 ± 1.2	0.1677
D	15.2 ± 2.7	0.0136
E	15.4 ± 2.0	0.0316

Group A appears to have a higher concentration of fat than the other groups, and significantly higher than groups E (anova, p=0.03) and D (anova, p=0.01. The trend also indicates that samples from groups A, B, C, E and D consisted of decreasing levels of fat, something that coinsides with the dose of marine based feed provided.
Least difference was measured between group D and E, where group D has the lowest fat content. Even though group D was fed with MBF for 25 weeks (Figure 12), this group were fed with CF the last 35 weeks. The effect of the marine based feed is then likely to drop (Einen et al., 2006).
The feed composition in Table 1 shows that the marine based feed contains 6.3% more of the energy-rich compounds (Table 3) fat and protein combined than the conventional feed. There is evidence that high energy and fatty diets will lead to a higher muscle lipid deposition (Wathne et al., 1995a; Gjedrem, 1997; Bjerkeng et al., 1997b; Einen & Skrede, 1998).
On the other hand, it is not possible possible to point to only one factor, since digestibility, genetics, ingredient composition and micronutrients may have influenced the result.
The low fat level and large standard deviation of group D could explain the low degree of colouration measured. These results could be another sign on bad genetics for the group (Rye & Gjerde, 1996). It is not expected that the lack of starvation period in samples from group D and E have influenced the relative fat content of white muscle, since the starvation in the other groups (1 week) was relatively modest (Einen & Thomassen, 1998).

3.3.3 Lipid Extraction, Diet

Results from Bligh and Dyer lipid extraction after frozen storage is presented in Table 14. Raw data and examples of calculations are found in Appendix E and F.

Table 14: Extractable lipid content, diet experiment. NQC from Atlantic salmon (*Salmo salar*) fed decreasing amounts of marine raw materials (Group A>E, n=10-15), presented as percent of wet weight, with standard deviations. P-value indicates significant difference from group A (anova, $p \leq 0.05$).

Fish group	Mean [%]	p-value
A	19.6 ± 1.2	
B	18.9 ± 2.0	0.3452
C	18.5 ± 1.1	0.1523
D	18.0 ± 2.8	0.0606
E	17.9 ± 1.8	0.0456

Group A appears to have a higher lipid content than than the other groups. Marginally significantly higher than group D (anova, p=0.06) and significantly higher than group E (anova, p=0.046). The trend indicates that group A, B and C are having the highest fat contents followed by group D and E, respectively. Unlike the NIR-results, the Bligh and Dyer extraction shows group E as the leanest. However, there is no significant difference between the groups (group D=18.0, group E=17.9, standard error=0.85).
It would not be expected that total lipid content are changed during freeze storage, since this is standard procedure for such analyses (Bligh & Dyer, 1959).
The lipid extraction detected a higher lipid content than the NIR analysis for all groups. By including the values in a common plot, the relationship between the data becomes more clear (Figure 17).

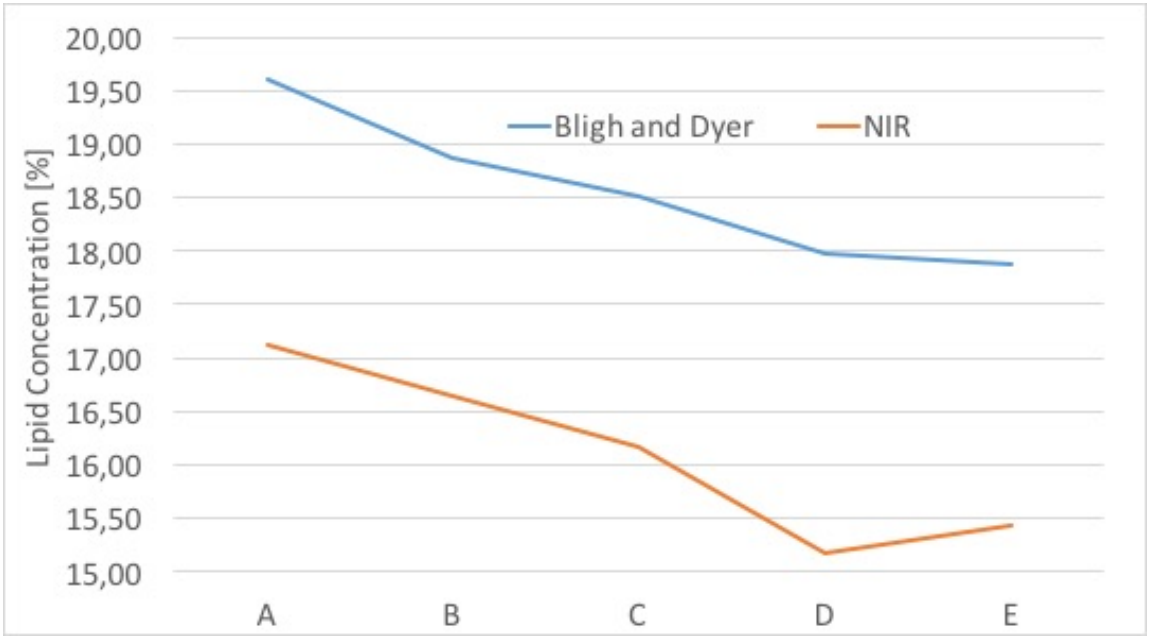

Figure 17: NIR vs. Bligh & Dyer lipid extraction results, diet experiment. Lipid content in NQC from Atlantic salmon (*Salmo salar*) fed with decreasing amounts of marine raw materials (Group A>E, n=10-15). Results are presented as percent of wet weight.

The figure illustrates that NIR analysis gives approximately 2.5 % lower lipid contents than the Bligh and Dyer extraction. One possible explanation for this, may be that some other chemical analysis are used in calibration of the NIR instrument. An other possibility is that all the samples have had a drip loss corresponding to the relative raise of 2.5% lipid content. However, this is less likely due to the high attention given to minimize this source of error. Recalibration of the NIR instrument to Bligh and Dyer extraction methods may be suggested in order to minimize this difference.

3.3.4 Fatty Acid Analysis, Diet

In screening of fatty acid profile in the diet experiment, a new method was tested. The results from UPC^2 fatty acid analysis of salmon oil is presented in Table 15. Raw data are found in Appendix G.

Table 15: Relative distribution of selected fatty acids in oil, diet experiment. Extracted from NQC of freeze stored Atlantic salmon (*Salmo salar*) fed with decreasing amounts of marine raw materials (Group A>E, n=10-15). Samples were analysed with UltraPerformance Convergence Chromatography^{TM}. Values are given as percent of selected fatty acids, presented with standard deviations. "Inseparable" includes fatty acids with both n-3 and n-6 PUFA isomers that the method was unable to separate over the column due to mutual molecular weights.

	Group A [%]	Group B [%]	Group C [%]	Group D [%]	Group E [%]
n-3 PUFAs:					
18:4	1.6 ± 0.3	1.4 ± 0.6	0.9 ± 0.4	0.9 ± 0.3	0.8 ± 0.3
20:5	14.7 ± 2.8	17.9 ± 6.4	11.7 ± 5.5	17.1 ± 5.6	13.7 ± 4.2
22:6	42.3 ± 7.6	39.3 ± 15.0	38.5 ± 10.9	40.3 ± 9.9	42.2 ± 8.4
n-6 PUFAs:					
18:2	6.5 ± 1.8	6.3 ± 2.0	6.7 ± 1.6	6.2 ± 1.8	6.5 ± 1.3
20:2	0.9 ± 0.4	0.5 ± 0.3	0.9 ± 0.3	0.8 ± 0.5	0.8 ± 0.4
Inseparable:					
18:3	3.6 ± 0.9	3.5 ± 1.1	3.4 ± 0.6	3.5 ± 0.6	3.3 ± 0.6
20:3	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
20:4	2.7 ± 0.4	2.8 ± 0.8	2.0 ± 0.8	2.4 ± 0.5	2.0 ± 0.5
22:5	4.6 ± 0.8	3.6 ± 0.6	3.6 ± 1.2	4.7 ± 1.1	3.9 ± 0.8
n-9 FA:					
18:1	22.6 ± 5.4	24.3 ± 10.6	31.9 ± 14.0	23.6 ± 14.0	26.5 ± 10.0
sum n-3	58.6 ± 9.0	58.5 ± 13.8	51.0 ± 15.0	58.3 ± 15.2	56.7 ± 11.0
sum n-6	7.4 ± 2.2	4.8 ± 3.7	7.6 ± 1.9	7.1 ± 2.2	7.3 ± 1.6
n-3/n-6	8.8 ± 3.3	10.2 ± 6.3	7.6 ± 4.3	9.4 ± 5.0	8.4 ± 3.4

Group A and B was identified with the highest mean contents of total n-3 fatty acids, EPA, DHA and Stearic acid. Group D, E and C follows with respective decreasing levels. However no significant difference was found between the feeding groups in relation to the difference in feeding regimes.

More visible differences was expected, because of findings from similar experiments by Lerfall et al. (2016). They claimed that high dietary marine raw materials resulted in significantly higher amounts of C18:4n-3, C20:4n-6, C20:5n-3, C22:5n-3 and C22:6n-3 fatty acids and a significantly higher n-3/n-6 ratio. It is from the table (Table 15) a visible trend in C18:4n-3, showing decreasing amounts (A>B>C>D>E). Group A has significantly higher fractions than groups C, D and E (anova, p>0.03). No significant correlation is visible for the other amino acids mentioned, nor the n-3/n-6 ratio from this experiment.

Under preparation of the samples, the saponified fatty acids was washed with deionized water and centrifuged. This led to decomposition of some of the pellets, which resulted in loss of sample. Direct quantification of the fatty acids was therefore not possible. This loss of sample was deflected in low peaks for some compounds, which probably caused errors in determined fatty acid ratios and high standard deviations. A threshold value (Total area $\geq 100,000 mAU * sec$) was chosen to ignore peaks in confusion with noise, something that resulted in a less representative selection of samples.

From the feed recipes in Table 1, it is clear that the level of fish oil and fish meal leads to a higher concentration of EPA and DHA PUFAs in the marine based feed. It was expected that the fatty acid profile would reflect the lipid composition of the feed (Waagbø et al., 2001; Halver & Hardy, 2002; Kiessling et al., 2007), showing a trend in EPA and DHA fillet concentrations in response to the dietary content (A>B>C>D>E) (Figure 12).
It has been claimed that the fatty acid composition provided through diet prior to slaughter can affect the post slaughter profile in muscle. It is therefore reasonable to assume that Group D would show a similar profile to Group E with an optimized screening method, due to 35 weeks with conventional feeds.

Assuming that the results are representative, first of all the n-3 PUFA levels are remarkably high considering that Haard (1992) suggested an EPA+DHA concentration at approximately 1% for farmed Atlantic salmon (Table 4).
Regarding the lack of detected difference in n-3 PUFAs, Bjerkeng et al. (1997b), Østbye et al. (2011) and Nanton et al. (2007) suggests that high dietary n-3 HUFAs can cause the FA to be stored in other tissues than white muscle. An alternative hypothesis is that the fatty acids might be reduced due to lipid oxidation in the feed or in the fillets after slaughter (Wiseman, 1996; Hong et al., 2002). From Table 1, it is clear that the astaxanthin levels are similar (50 mg/kg) for the two feeds. Astaxanthin is effective in early stage lipid oxidation, but α-tocopherol is thought to be of more importance as an antioxidant at further stages (Jensen et al., 1998).
The feeds could have been examined to measure if low antioxidant concentrations or high

degree of oxidation reduced the effect of changing between the two feeds.

No significant difference in n-3/n-6 PUFA content was detected between between group A and the other groups.
Gas Chromatography (GC) is the standard method for fatty analysis and is suggested in verification of the results in optimizing this method. A difference in the sample preparation of GC in contrast to UPC^2 is that fatty acids needs to be transformed into methyl esters, while the samples in this experiment needed to be saponified. This could give access to new applications.
UPC^2 may be a possible method for examination of fatty acid profile in fish oils, but more effort is needed in optimizing the methods both for sample preparation and for execution of the chromatographic measurements.
Inclusion of deuterated fatty acids of interest in known concentrations to each sample are recommended, in order to conduct a direct quantification with this method, eliminating the risk of inseparability due to similar molecular weight. It is also necessary to develop methods to separate the n-3 and n-6 isomers, to get a better picture of the n-3/n-6 ratio.

However, this is a new utilization of this method and requires careful adjustments, numerous runs of parallel samples and could be a separate master project. However, these results have shown method as a promising alternative to the standard methods of today. More development are needed to unveil its potential.

It is suggested that the total dietary ratio of n-3/n-6 PUFAs should go from 1:15 to be closer to 1:1. For human nutritional purposes, it is therefore important to keep the ratio in farmed salmon as high as possible. Since the human diet consists of more than foods with high n-3 PUFA content, the n-3/n-6 ratio in fish needs to be high if a ratio close to 1:1 should be reached without omega 3 supplements.
Norwegian researchers works hard for sustainable development in the aquaculture industries and new feed resources are under research and development (Patil et al., 2005; Mühlroth et al., 2013; Sørensen et al., 2016). However, a detailed review of this topic is not in the scope of this thesis.
The access to marine n-3 PUFAs are declining relative to the demand for animal protein, which increases the pressure for new sources of n-3 PUFAs (Olafsen et al., 2012). In a resource economic point of view, it could therefore be reasonable to produce fish so that not only a few, but as many people as possible can get their daily requirement for marine n-3 PUFAs. At least in anticipation of new sources or a public acceptance for EPA and DHA from genetically engineered plants and microalgae.

3.4 Protein Content

Protein is crucial for fish and human muscle growth and metabolism. Salmon is a high protein species, so total protein content is an important characteristic for consumers. Protein content in salmon is considered to be around 20 % of wet weight. If muscle protein content is low, this is an indicator for sub-optimal growth.

3.4.1 Salt and Water Soluble Proteins, Feed Frequency

The wet weight contents of extractable water and salt soluble proteins from the feed frequency experiment are presented in Figure 18. Raw data and calculation examples are presented with the standard curve in Appendix H.

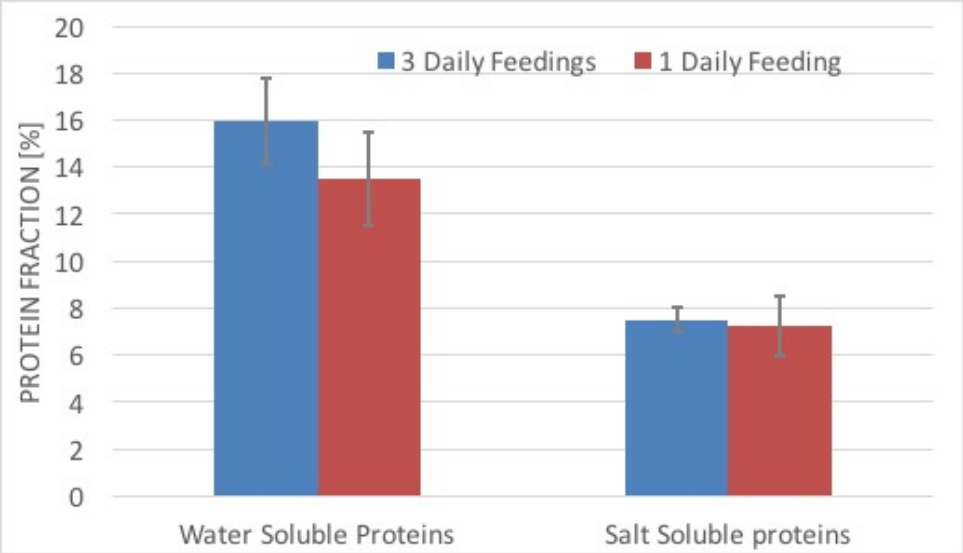

Figure 18: Extractable salt and water soluble protein, feed frequency experiment. Sample of Atlantic salmon (*Salmo salar*) back loin from fish fed one versus three times daily (n=6, 3 parallels). Results are presented as percent of wet weight. Standard deviations are illustrated on each column.

A marginally significant difference was found in water soluble proteins (coupled T-test; p=0.051) due to variation in feeding frequency, indicating a higher concentration in fish fed three times per day. The wet weight of fish fed 3 times per day contained 16% water soluble and 7.5% salt soluble protein. Fish fed 1 time per day had 13.5 % water soluble and 7.2% salt soluble protein.

No significant differentiation was detected in salt soluble proteins due to variation in feed frequency. Trends in Figure 18 indicates that fish fed 3 times per day had a higher concentration of water soluble protein than fish fed 1 time per day.

These results imply increased protein activity in metabolic turnover in fish as a response to increased feed frequency, but no effect was detected in myofibrillar protein concentrations. This was a relatively small-scaled experiment and 6 samples is probably not a large enough

selection, in order to conclude a direct correlation from these findings. For verification of the data, larger sample groups is recommended to clarify the correlation between feeding frequency and concentration of water soluble proteins in Atlantic salmon.

3.4.2 Total Amino Acid, Diet

The total amino acid content in the fish from the diet experiment is presented in Figure 19. Raw data and calculation examples are found in Appendix I.

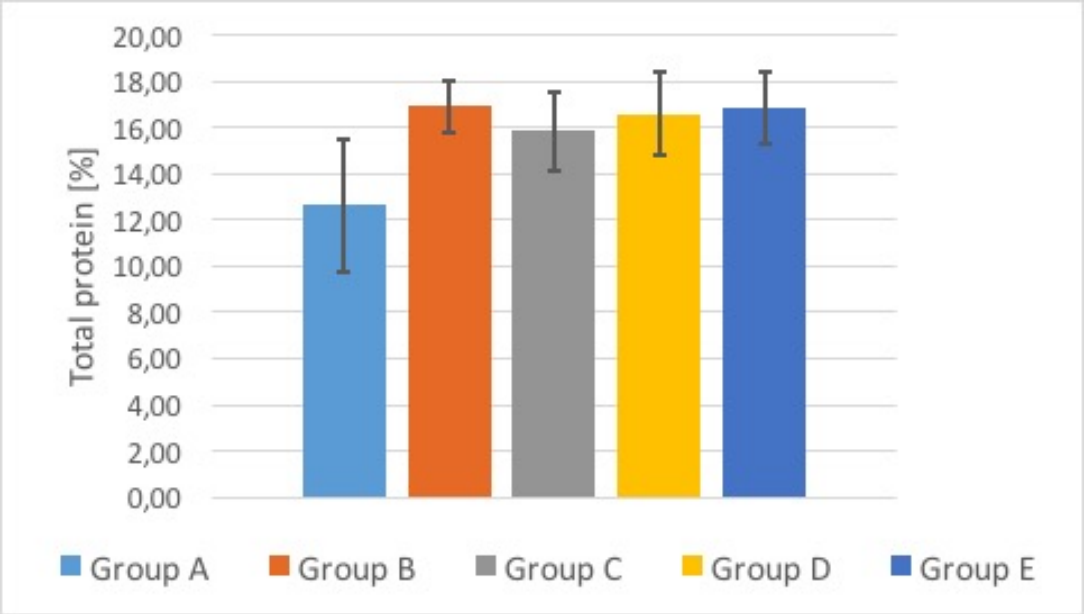

Figure 19: Total amino acid results, diet experiment. NQC from Atlantic salmon (*Salmo salar* fed with decreasing amounts of marine raw materials (Group A>E, n=10-15). Results are presented as percent of wet weight. Standard deviations are illustrated on each column.

There is a small but non-significant trend showing higher amino acid content in Group B than in the other groups. No trend is visible in relation to the changes in dietary regimes (Figure 12). On the other hand, the results shows a significantly lower content of amino acids in group A relative to group B (coupled T-test; p=0.0001). The standard deviation of group A (st.dev=2.86) is visibly larger than the other groups (B:1.1, C:1.7, D:1.82, E:1.59) which implies a larger spread in the sample protein contents.
The timeline illustrates only slight differences in feeding regimes between group A and B, would make it reasonable to expect little difference between these groups in response to the diet. It is already described that fish early in the summer can be higher in fat, while it gets leaner and higher in protein late in the summer season when the fish grows larger. There are signs of inversely correlations between amino acids and fatty acids from the PCA plot (Figure 13), however it is not said that this explains this exact case. The results from the lipid extraction (Table 14), shows that group A, larger mean lipid content than group B (19.6 vs 18.88%). As lipids are under 20% of the total weight and the differences

are only 0.7%, it is more likely that other factors could explain the difference in protein content. However, a protein extraction like presented in Figure 18, could have revealed if the protein difference was due to changes in sarcoplasmic proteins or myofibrils. Part of the proteins in group A might have been lost due to post mortem factors, like a higher drip loss than the other groups, leading to a lower content of water soluble proteins. Otherwise high temperature during storage between slaughter and analysis or during the preparation of the samples may have affected the result (Sigholt et al., 1997).
Endogenous proteases may have initiated hydrolysis of part of the protein, something that can lead to larger standard deviations and lower outputs from the HPLC (Cepeda et al., 1990). On the other hand, a high degree of degradation are required to explain this difference, since denatured proteins normally still would be detected as amino acids.

A slight but non-significant trend suggests that protein content are higher in fish fed with the marine based diet compared to the conventional feed. However, no significant correlations was detected through the analysis of protein contents of the other groups. Assuming that the total amino acid content from this analysis (total protein $= \sim 16-17\%$) is representative for the total protein content, this is lower than the results from the other experiment $\sim 21-23\%$) and low regarding the literature values (Table 1). However, some amino acids, like tryptophan will disappear during acid hydrolysis (Williams et al., 1982), something that would affect the output of this analysis. Protein extraction like in the feed frequency experiment, could have been done to control the accuracy of this method of protein content quantification.
In verification of the data, increased attention should be put into sample storage and preparation, to ensure an optimal detection of amino acids.

Fish farmers aims to achieve good fish growth through their every day work. The protein content of the proximate composition is closely related to the fish size, and life stage. The diet composition affect the fish health and muscle growth through energy content, digestibility, as well as ratio and configuration of various macro and micro nutrients. It was therefore expected to detect some differentiation in resulted protein composition in the diet experiment. However, it is notable that the conventional diet in this experiment is a commercial feed, which according to the producer are thoroughly adjusted (Sødal, 2014). Most of the potentially negative impacts of terrestrial raw material are counteracted through a balanced recipe.

3.4.3 Amino Acid Profiles, Diet

A relative quantification was chosen in illustrating the amino acid profiles (Figure 20) to compare the compositions despite different amounts of total amino acid. Even though tryptophan is considered as an essential amino acid for fish (Halver & Hardy, 2002), tryptophan and tyrosine are degraded during acid hydrolysis and are not detected in this analysis (Williams et al., 1982). Peaks of glycine and arginine merged and is therefore analysed together, like asparagine, aspartic acid and glycine, glutamic acid. Raw data are found in Appendix I.

The most visible trend in relation to the feeding regimes (Figure 12) was found for histidine. Group A shows the lowest protein histidine content (anova, $p < 0.0003$). A positive trend (A<B<C<D<E) indicates a correlation to the dietary regime.
The result claims that increased use of feed based on marine raw materials decreases the amount of histidine in muscle.
Soy bean concentrate has a slightly higher literature value concentration of histidine than fishmeal (1.82 vs. 1.78 % of protein) (Dersjant-Li, 2002), which coincides with the positive trend. High levels of histidine are claimed to have a decelerating effect on cataract development (Breck et al., 2005).

The results also indicates trends and significant difference between group A and E for serine (anova, $p_{AE} < 0.0002$), alanine and glycine+arginine, however with large deviations.
Serine and alanine are abundant non-essential amino acids with physiological functions in relation to fat and carbohydrate energy metabolism (Nelson et al., 2008). Deficiency is rare and not associated with risk of mortality (Halver et al., 1957). However, the results proposes a inversely correlation between increased dietary MBF and content of serine and alanine in muscle.
Group C, fed medium amounts of marine based and conventional feeds shows a highly significant elevation in serine content, with a corresponding low values for glycine+arginine. However, no explanation are found in literature to support these findings. Verification are recommended in order to clarify the matter.
Arginine has many important metabolic functions and positive effects on the immune system. Dietary lysine have shown to increase plasma arginine levels, so slight elevated arginine could be expected for group A from this experiment, due to the relatively higher contents in fish meal (Table 8) (Halver & Hardy, 2002).
Glycine has the least complicated amino acid structure. The main function is muscle growth, but is also important in cell signalling and as substrates for other compounds (e.g. purine nucleotide bases) (Nelson et al., 2008). Glycine deficiency is not considered likely, due to high abundance and ability for synthesis from other amino acids (Halver

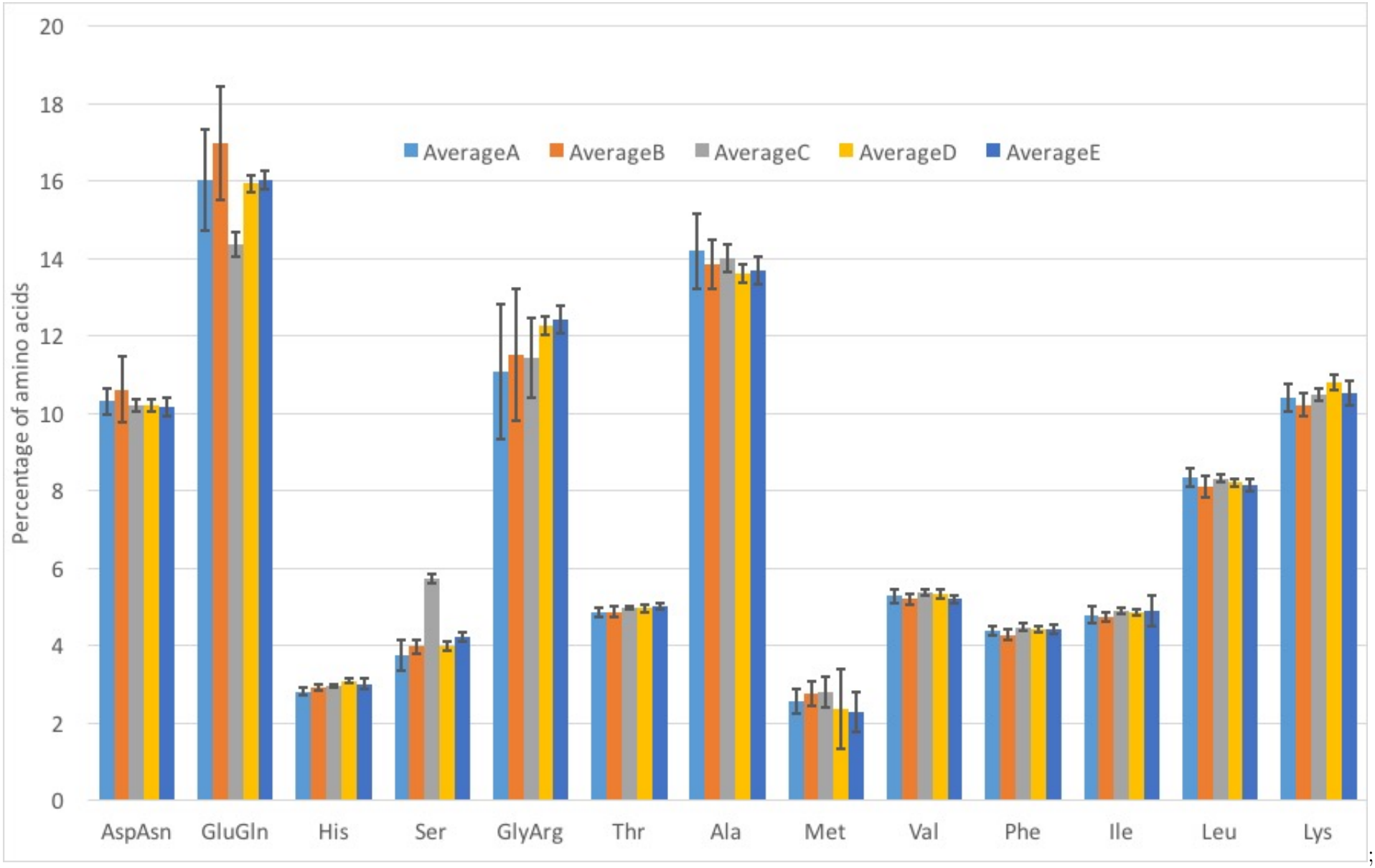

Figure 20: Amino acid profiles diet experiment. NQC from Atlantic salmon (*Salmo salar* fed with decreasing amounts of marine raw materials (Group A>E, n=10-15). Results are presented as percent of selected amino acids (sum=100). Standard deviations are illustrated on each column.

et al., 1957).
It should be noted that group D and E, with from the most intensive conventional feed regimes had the lowest mean contents of methionine. However, no significant differences was detected in relation to the changes in dietary regimes, as for the rest of the amino acids (aspartate + asparagine, glutamine + glutamic acid, threonine, valine, phenylalanine, isoleucine, leucine and lysine).

There are no results reported in literature, showing that dietary composition affects the amino acid profile of muscle, as long as the requirements for essential amino acids are met (Shearer, 1994). All proteins are encoded by the salmon DNA, and translation requires access to specific amino acids from the amino acid pool in order to elongate the peptides (Nelson et al., 2008). Lack of such amino acids may however cause decreased muscle growth and undesired health symptoms. It is therefore expected that potential variations in amino acid profiles would result from changes in sarcoplasmic proteins and free amino acids, more than myofibrillar differentiation.
It is commonly known that proteins as feed ingredients are more expensive (Goettl, 2003) and less expedient as energy sources than lipids (Table 3), so research and development is crucial when balancing a diet in a health beneficial and resource economic manner. Much research has been done in determining amino acid requirements for salmon (Halver & Hardy, 2002). This is natural, since it is closely related to fish growth. However, the available levels are inconsistent and based on different perceptions of when a need is covered. Accurate need levels would be valuable information for feed producers in limiting costs.
In order to decide accurate requirements for each of the 10 essential amino acids (Table 5), dose/response experiments. Alternatively, through a factorial approach by changing specific dietary amino acid concentrations and analyse whole body content or feed conversion rates after a lifespan. Such methods demands specialized feeds where amino acid contents is changed at high accuracy, while other factors are kept constant. Generally, the needs are recognized as covered when all amino acids are provided in surplus (Halver & Hardy, 2002; Cowey & Young Cho, 1993). In this experiment, it is likely that the amino acid profile in the MBF will fulfil the amino acid requirements due to high fish meal contents (Table 6 and 8). However only one limiting amino acid would decrease the growth rate (Cowey & Young Cho, 1993). In a resource economic point of view, it might be beneficial to include protein ingredients with amino acid contents just under the requirements and adjust the last part with pure amino acids. This would cut the costs for the producer and help rationing resources for continuous growth in the industry. However, a perfectly balanced feed in amino acids, fatty acids, antioxidants, minerals and vitamins would imply carefully adjusted recipes, which probably would impact the feed price. In that case, the consumer will decide.

3.5 Water

3.5.1 Water Content, Feed Frequency

Average water content of muscle from the feed frequency experiment is presented in Figure 21. Raw data from the analysis is presented in appendix E.

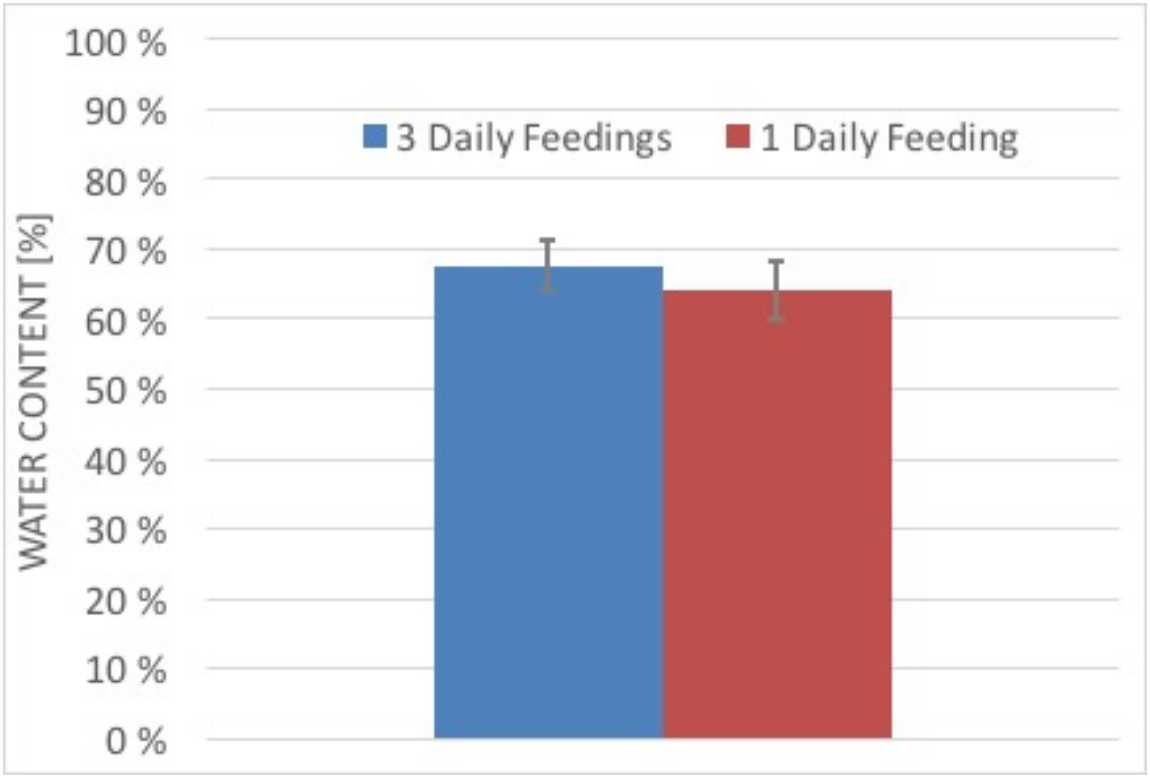

Figure 21: Water content, feed frequency experiment. Sample of Atlantic salmon (*Salmo salar*) back loin from fish fed one versus three times daily (n=6, 2 parallels). Results are presented as percent of wet weight. Standard deviations are illustrated on each column.

No significant difference was detected in water content in the groups of different feeding frequencies. However, there is a trend (Figure 21) that indicates a higher mean water content in samples from fish fed 3 times per day (68%), than the group fed 1 time per day (64%). This lack of difference could be expected, because of the similar contents of salt soluble proteins which not either shows any difference, knowing that 70 % of the water in fish muscle are held in the myofibrils (Hultmann & Rustad, 2004).

3.5.2 Water Content, Diet

Water content recorded from the dry matter analysis of the diet experiment are presented in Table 16.

Table 16: Water content, diet experiment. NQC from freeze stored Atlantic salmon (*Salmo salar*) fed decreasing amounts of marine raw materials (Group A>E, n=10-15), presented as percent of wet weight, with standard deviations. P-value indicates significant difference from group A (anova, $p \leq 0.05$).

Fish group	Mean [%]	p-value
A	63.0 ± 1.4	-
B	62.5 ± 1.1	0.459
C	62.5 ± 1.2	0.460
D	62.9 ± 2.7	0.928
E	61.8 ± 2.2	0.095

Group A appears to have a higher, but non-significant difference in water concentration than the other groups. There is no clear trend correlating to the dietary regime (Table 12).

Similar experiments have reported that higher water holding capacity could be expected for fish fed with high marine ingredients (Lerfall et al., 2016). It is visible that group E, fed the least marine ingredients contains lower water content than the group fed most MBF (group A). This difference is significant at 10% level (anova, p=0.0945).

Rørå et al. (2003) claimed that dietary substitution of VO and FO had no significant affection on the WHC.

Water affects nutritional quality as solvent for minerals and vitamins: (Thiamine, Riboflavin, Pyridoxine, Pantothenic acid, Niacin, Biotin, Folic acid, Vit B12, Ascorbic acid, Inositol, Choline, p-aminobenzoic acid, lipoic acid).

NQC samples from groups D and E were collected on site unstarved, while the other groups were collected according to the slaughter plan including one week of starvation. A correlation are claimed between starvation prior to slaughter and increased WHC. However, there is no significant difference between group A and group D, meaning that if there is a notable effect of one week of starvation, then some other factors have influenced the result of group D, since starvation have shown to increase water holding capacity (Fennema, 1996; Olsson et al., 2007) with a following firmness in texture.

Water content and WHC is lowered with decreasing pH (Fennema, 1996). It would therefore have of interest to control the pH during verification of the data.

3.6 Texture

Texture of fish is a quality parameter of great importance for the consumers experience. Soft fish and gaping are examples of quality challenges in the industry works to solve. Average hardness and breaking strength from the texture profile analysis of 5 measurements per fillet, as described in Section 2.1.2 are presented in Figure 22. Raw data from the texture profile analysis are presented in Appendix L.

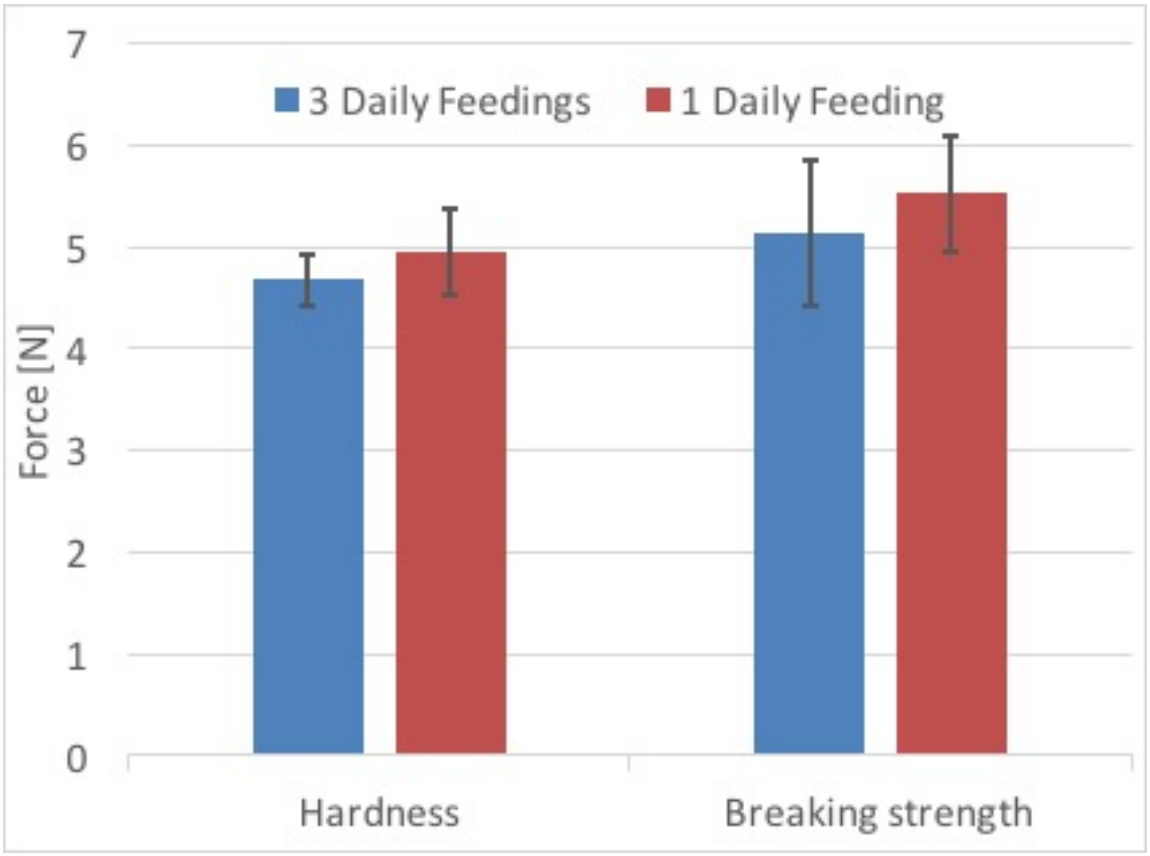

Figure 22: Hardness and breaking strength, feed frequency experiment. Average force measured from five measurements along the back loin of Atlantic Salmon (*Salmo salar*) fed one versus three times daily (n=6). Standard deviations are illustrated on each column.

A slight trend is visible indicating that fish fed one time per day has a firmer texture and a higher average breaking strength than fish fed three times per day. However no significant differences were detected between the groups.
The fish arrived with heads on, gutted and the filleting was executed by an inexperienced hand. In order to erase this source of error, the relative force per millimetre is presented in Figure 23. Raw data are found in Appendix L.

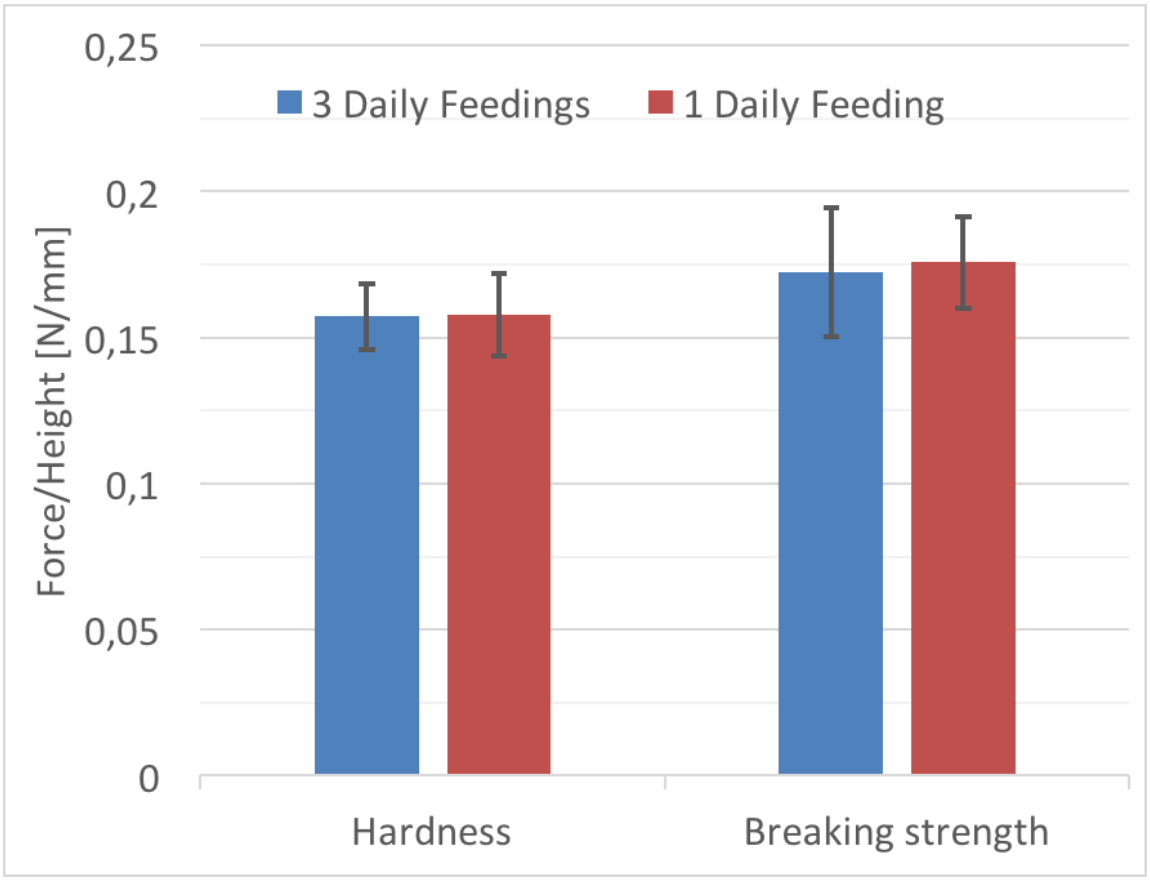

Figure 23: Height specific hardness and breaking strength, feed frequency experiment. Average force per millimetre (fillet height) measured from five measurements along the back loin of Atlantic Salmon (*Salmo salar*) fed one versus three times daily (n=6). Standard deviations are illustrated on each column.

It appears from the measurements that there was no significant difference in hardness nor breaking strength between fish fed one or three times per day. The lack of trends in relative force per height (Figure 23), compared to force in Figure 22 illustrates a difference in height between the sample groups. This height has an impact on the texture of the fish. The group fed three times per day had an average height of 29.8 mm, while the group fed one time per day was 31.5 mm high. This shows that increased fillet height leads to firmer texture and higher breaking strength. The number of muscle cells and potential myofibre-myofibre junctions between the piston and cuttingboard are varying, which makes the comparison more complicated.

It could have been reasonable to assume that higher feeding ratio might increase the intracellular metabolic activity with a following drop in pH due to anaerobic metabolism in the muscle cells. Ang & Haard (1985) demonstrated that Atlantic cod showed increased drip loss, softening of fillets and lower pH in response to intensive feeding during the influx of capelin, than the rest of the season. However, in this experiment the dietary energy is kept constant and no significant differentiation are detected.

A larger selection of samples, combined with more practice in filleting, is needed in order

to claim any correlation between feeding rate and fillet texture.

3.7 Cathepsin B+L Activity

Average values from the fluorescence analysis of cathepsin B+L is illustrated in Figure 24. Raw data is found in Appendix M.

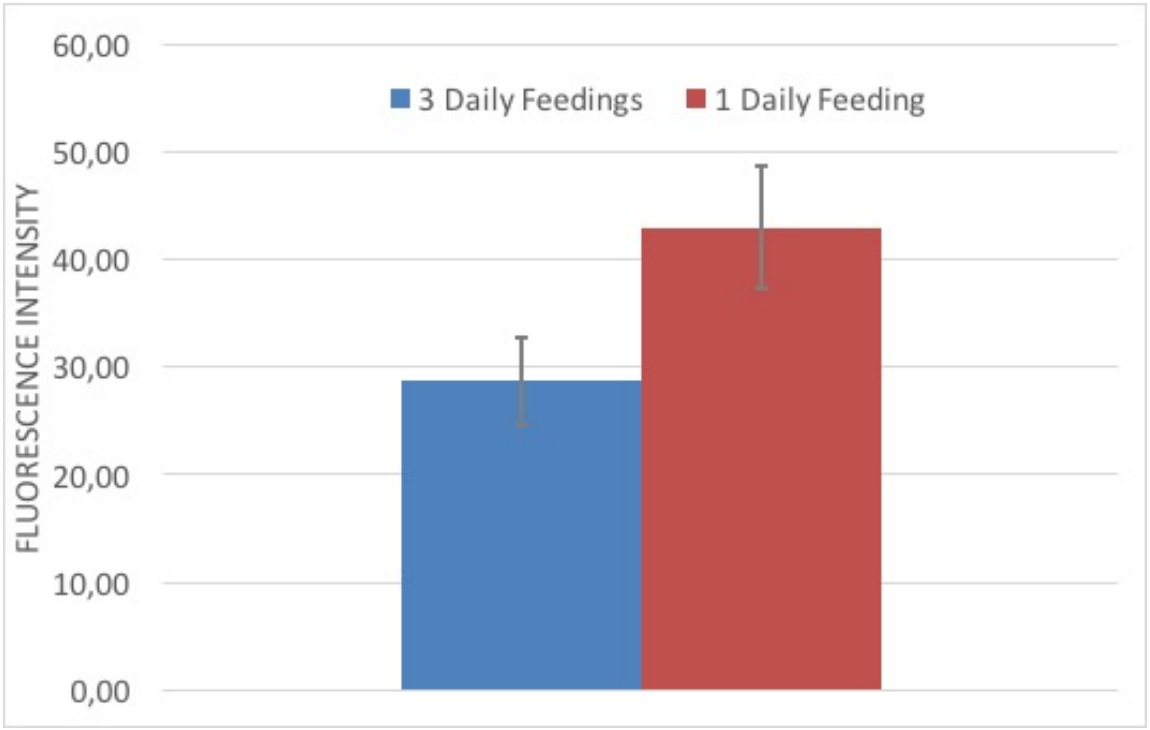

Figure 24: Cathepsin B+L activity, feed frequency. Samples from back loin of Atlantic salmon (*Salmo salar*) fed one versus three times daily (n=6, 3 parallels). The figure illustrates fluorescence absorbency from protease hydrolysed methylcoumaryl-7-amide (AMC) substrate (472 nm). The standard deviations are indicated on each column.

The results indicates a that fish fed three times per day has significantly (coupled T-test; p=0.0007) higher activity in cathepsin B+L activity in muscle than fish fed three times per day. The high activity of protease in the group fed once per day did apparently not affect the texture. However, as the texture analysis was pursued on fresh post-rigor fillets, an effect on texture after increased storage time would be expected.
From the protein extraction analysis results 18 it was detected significantly higher fractions of water soluble proteins in muscle from fish fed 3 times per day than in fish fed 1 time per day. It would therefore be more comfortable to defend an elevated cathepsin B+L concentration in the group fed three times daily.
However, higher enzyme activity is not automatically a response of concentration, other sarcoplasmic proteins than cathepsins may cause the elevated water soluble fraction shown in Figure 18.
In contrast to these founds, it is believed that high feeding rates and rapid growth can

lead to increased metabolic activity, with a following drop of pH post mortem (Cowey & Young Cho, 1993; Waagbø et al., 2001). In that case, it would be more expected to detect an increase in proteolytic activity for the group fed 3 times daily.
It is reported that cathepsins in salmon can be activated during long periods of starvation during spawning migration and that cathepsin activity are affected by endocrine signalling, thiol compounds, pH and stress (Yamashita & Konagaya, 1990a; Aoki et al., 2000; Hultmann & Rustad, 2002). Increased cathepsin activity is reported in fish where lysosomes are fractured, during rough handling before or after slaughter, or sub-optimal freezing/thawing processes (Yamashita & Konagaya, 1990b; Hultmann & Rustad, 2002). This may have been a possible explanation to these results and something that could have been verified through a drop in pH post mortem.
It is claimed that increased hierarchy may occur in populations in response to restrictive feeding which can lead to increased stress(Waagbø et al., 2001).
The fish analysed in this experiment was around 3 kg and not sexually mature, so an effect from maturation hormones is not likely.
It has been reported incidents of increased stress as a response to irregular feeding hours (Halver & Hardy, 2002).
Preferred feeding hours for Salmon are believed to be in the morning or before sunset, when the activity is high, so the feeding timing and consistency could potentially be of relevance.
However, no visible stress related physical damage was observed on any of the fish. Feeding regimes with longer starvation periods less than one meal per day are quite normal (Waagbø et al., 2001; Halver & Hardy, 2002).

In any case, verification of the results are recommended for these results due to a small selection. A combination of pH measurements, multiple runs and larger sample groups is recommended in order to investigate this correlation between feeding rate and proteolytic enzyme activity.

3.8 Growth, Yield, Fish Health

In this section, the recorded production and slaughter data provided by the company regarding yield, growth and fish health from the full-scale production from the diet experiment will be presented and discussed.

Average growth rates during the first 57 weeks in sea as response to changes in diet is illustrated in Figure 25. Raw data are found in Appendix app:growth

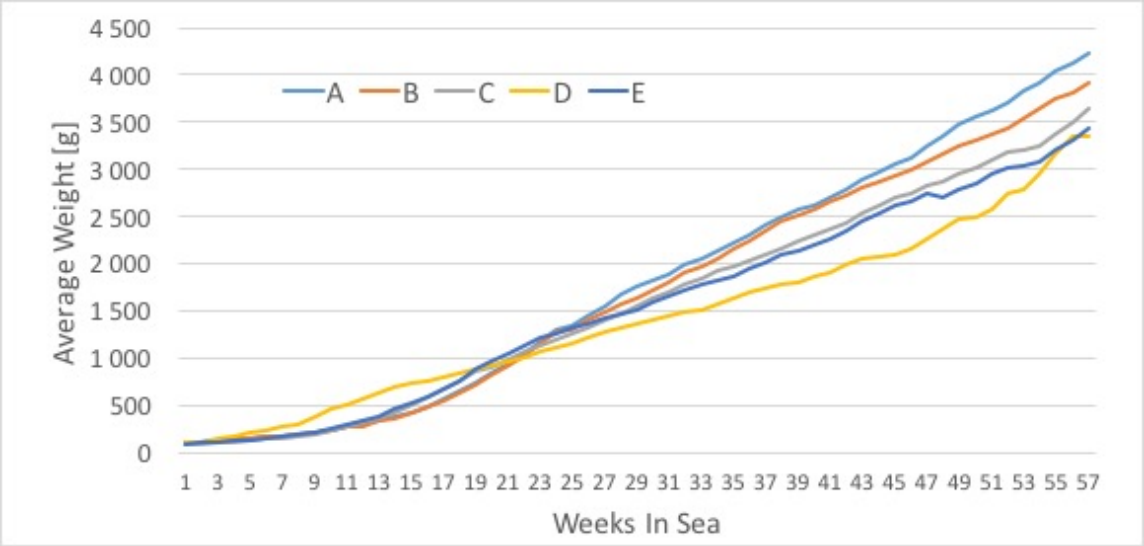

Figure 25: Growth rates, diet experiment. The figure illustrates growth the first 57 weeks in sea for Atlantic salmon (*Salmo salar*) fed with decreasing amounts of marine raw materials (Group A>E, n=10-15). Fish are stocked in sea at different dates (Group A and B: week 14/2014; Group C and E: week 19/2014; Group D: week 28/2014).

Group A had the highest mean weight after 57 weeks in sea (4233 g), higher than group B (3906 g), C (3651 g), E (3435 g) and D, (3353 g), respectively. This corresponds well to the provided amount of MBF (Figure 12).
It is notable that group D showed an irregular growth response compared to the other. The group had a rapid weight elevation between week 4 and 13, followed by period of reduced growth up to week 46. However, group D had the highest growth rate after week 46. Group D are stocked 9 weeks later than groups C and E, 14 weeks later than groups A and B. Environmental conditions like temperature, currents and day length are known to influence the appetite and growth rate of fish (Einen et al., 2006). When shifting the line of group D (Table 25) 9 positions (weeks) to the right, it is visible that weeks 11 to 21 was a period of good growth conditions for all groups. However, group D had lower growth than the other groups. Limited growth may support that group D had low fat, colouration and increased water contents (Rye & Gjerde, 1996), which may have disturbed the MBF dose response trends from these quality analyses (Table 10) and 13, 16).

Proportion of the marine based feed seems to have a positive effect on growth rate, compared to conventional feed. This can be expected because the MBF also had higher fat and energy content and than the conventional feed (Table 8), which have shown to increase growth (Wathne et al., 1995a).

Smolt quality or rough handling can be explanatory factors for reduced growth (Rye & Gjerde, 1996), however the general motivation for feeding can differ for Atlantic salmon. For wild fish, the juveniles who eats most their first autumn, grows fastest and is more likely to migrate to sea than the slower growing group, which will stay in the river for one more year. It is claimed that this feeding motivation is an decisive factor, regardless of food supply, water temperature and competition (Metcalfe et al., 1986). It is assumable that some of this behaviour also can have an effect in cultured fish as one of the explanatory factors for reduced growth and weight dispersion.

Provided data from slaughter are presented in Table 17. Raw data, formulas and plots of slaughter weight dispersion are presented in Appendix O.

Table 17: Slaughter data, diet experiment. Atlantic salmon (*Salmo salar*) fed decreasing amounts of marine raw materials (Group A>E, n=10-15).

	Group A	Group B	Group C	Group D	Group E
Smolt weight [g]	100	99	77	79	84
RGI [%]	105	103	103	101	100
Total loss [%]	3.7	3.8	3.6	7.6	3.3
BFCR	1.15	1.16	1.27	1.26	1.3
EFCR	1.16	1.17	1.28	1.28	1.32
CV	20.0	20.0	18.7	23.2	20.3
Days in sea	436	444	461	479	525
Slaughter weight [g]	5 362	5 220	5 937	5 454	6 273
Gutted weight [g]	4 557	4 489	5 076	4 647	5 336
Viscera [%]	15	14	14.5	14.8	14.9
Superior fish [%]	98.2	98.1	97.6	97.8	98.4

RGI: Relative Growth Index, BFCR/EFCR: Biologic/Economic Feed Conversion Ratio, CV: Coefficient of Variation (weight dispersion).

Group E had the highest fraction of superior quality whole fish (98.4 %), group C had the lowest (97.6%) and there seems to be no direct correspondence to the diet regime (Figure 12). This was also the case for weight dispersion, where group C was least spread

(CV=18.7), group D was reported most dispersed (CV=23.2). Seasonal changes may be a relevant factor for variation in CV, since the fish grow at different rates. Higher CV could therefore be expected for fish harvested early in the summer versus late in the autumn. However, disease or poor genetics may also be an important factor (Waagbø et al., 2001; Norges-Forskningsråd & Thomassen, 2007). Group A had the highest visceral weight fraction. Which can be expected as a response to higher dietary lipids (Cowey & Young Cho, 1993). However, there is no trend for the other groups which confirms this statement (A>E>D>C>B), thus other factors than diet composition seems to be influencing.
Group D stands out from the other groups in terms of total loss (7.6% vs. 3.3-3.8) which means higher mortality and down grading during the production. This again confirms that this group may have had poor smolt quality or rough treatment in front of or during the growth phase, resulting in low growth and lower quality parameters with higher standard deviations in several parameters. It is therefore recommended for the company to search in reported data regarding genetics and treatment for this smolt group, to reveal possible underlying explanations.
Group E, fed most conventional feed are reported with the highest economical and biological feed conversion ratios. There is are visible trends indicating that feed conversion ratios decrease with increasing dietary MBF. Group A shows lowest biological and economical feed conversion ratios (formulas in Appendix O) and higher growth than the other groups. From the quality analyses, the group also shows higher colouration and fat deposition. Knowing that MBF is more expensive than CF it is up to the producer to evaluate if the increased price will pay off.

Reports from the sea farm (Appendix O) states that the fish was in generally good health and low degrees of mortality was detected, but there was reported pancreas disease, amoebic gill disease (AGD) and heart and skeletal muscle inflammation (HSMI) in the fish stocks.

These analyses shows that there are several correlations between amount of MBF given, and quality parameters in Atlantic salmon. However, only minor differences was measured between the groups. The fish fed exclusively on terrestrial feed (Group E) also had the highest fraction of superior graded fish (98.4 %). This implies that the development of moving towards more sustainable feed sources, not necessarily lead to markable decreases in quality.

However, a cost benefit analysis in combination with a life cycle assessment of fish products from fish fed MBF is recommended. in order to evaluate the values of increased fat, colour, growth rates and decreased feed factor as well as visualizing the effects on energy

use and greenhouse emissions. The quality effects could potentially raise of price of the fish product due to improved appearance and thereby make the MBF economically viable in some markets, despite a higher feed price.
In addition to the economical aspect, it is important to evaluate the environmental impact of an increase in marine raw materials in feed for aquaculture production.
Salmon farming is an important industry for Norway and worlds salmon supplies, so if changes are done, they have to be thoroughly evaluated. Choice of feed resources should underpin fish welfare, as well as environmental, economical and social sustainable development.

The Norwegian salmon farming industry struggles with series of fish health and quality challenges, including sea lice, dark melanin spots, pigmentation, soft texture, gaping muscle filaments and infections like PD, AGD, ISA, HSMI and cardiomyopathy syndrome (CMS) (Mørkøre, 2012; Hjeltnes et al., 2016).
Some of todays quality issues, will probably be defeated in near future through research and technology, but new challenges may appear. Mørkøre et al. (2015) have claimed that dark melanin spots in fillet could be related to *piscine orthoreovirus* (PRV), stressing farming conditions, vaccination methods, fish health and mechanical injury. The reports have stated that the dark spots may be decreased with dietary adjustments.
When selling whole gutted fish, it can be a difficult to grade the quality from other parameters than external appearance. However, new methods for whole fish analysis of quality is under development, utilizing different technology for spectrometry of whole fish recording colouration, lipid, melanin spots, deformities and ulcers (Heia et al., 2016).
Ensuring high and consistent quality is one of the reasons why Norwegian farmed salmon is world leading and will be one of the challenges to address in order to keep this position in the future (Olafsen et al., 2012).

The marine fraction of ingredients in Norwegian aquaculture has declined the last 30 years. In 1990, the feed consisted of 90% marine based ingredients, which have declined to 29.2% in 2013 and the trend shows no sign of levelling (Ytrestøyl et al., 2014).
FAO has reported 2011 that feed production needs to be 70% higher in 2050 . In this case, increased efficiency, new raw materials and rationalization of resources is necessary. It is believed that new feed resources for protein, lipids, vitamins and minerals will be implemented with higher utilization of the ocean food web, algae, insects, microbiology and genetic engineering (Olafsen et al., 2012; van der Meeren et al., 2008; Sørensen et al., 2016). This could result in a more self-sufficient aquaculture production for Norway, depending less on terrestrial agriculture from other countries.

3.9 Further Work

In future studies the focus should be on extending the knowledge of underlying mechanisms for changes in quality from these changes in feeding strategy.
In further investigation of feed frequency, larger sample groups are recommended to investigate a more representative selection for an operative production. pH measurements should be included to the verification of this quality analysis to exclude possible effects on protease activity, water holding capacity and loss of water soluble proteins. Methods based on the description by Britton (1995) in combination with HPLC is proposed in examining the underlying mechanisms of the reported pigment dispersion from increased feed frequency (Table 9).

In further investigation of dose response effects from the marine based feed, it is recommended to design the feeding regime so that more distinct doses are provided to each sample group. A proposed design could be, MBF: Group A: 100%, B: 75%, C: 50%, D: 25%, E: 0%. Improved results could have been measured if all MBF rations are provided up to slaughter (Rørå et al., 1995; Einen et al., 2006). Something that could have been controlled by including a group F: 50 % MBF fed from sea transfer.
Extended fresh fish analysis from the diet experiment would be of great interest, since fresh fish is a substantial part of the salmon market. This could include sensory analysis, texture analysis, cathepsin B+L activity, salt/water soluble protein extraction and HPLC astaxanthin analysis. Gas chromatography is recommended as analysis instrument for investigation of the relationship between changes in fatty acid profile from changes in diet. Further analysis of MBF and effect on amino acid compositions, especially histidine, serine, glycine and arginine could be interesting for explaining the mechanisms behind the trends presented in Figure 20. Cost benefit analysis based on the doses provided 12 and the slaughter data (Table 17) would be an interesting extension of this project. It could also be of great interest to evaluate the energy use and emissions related to harvest, production, transport and use of the different feeds through life cycle assessments.
It is suggested that the NIR instrument used for fresh fish analysis should be calibrated with Bligh and Dyer lipid extraction and with a wider selection of fish fed MBF, if expanded use of such feeds are planned. Offering a master project within further method development of UltraPerformance Convergence Chromatography^{TM} for analyses in food chemistry would be a valuable addition to the selection of methods available at the department.

4 Conclusion

Fillet quality characteristics are affected by the chosen feeding strategy. Increased feeding frequency can lead to less colour consistency, lower fat and higher content of water soluble proteins in Atlantic salmon muscle. Feed frequency may have an impact on the lysosomal protease activity. Changing daily feeding from three to one time daily, showed no significant effect on average colouration (L*,a*,b*,H*,C*), salt soluble proteins, water content or texture. However, small sample selections (n=6) implies the need for verification. Results show that increased content of marine raw materials in the feed can lead to increased carotenoid pigmentation. Fish given marine based feed for the longest period also show higher fat content, reduced FCR, increased growth and a possibly higher water content. These studies showed a possible correlation between amino acid compositions (histidine, serine, glycine, arginine) and changes in diet. No clear relationship was found in total protein from variation in amount of marine dietary raw materials. UltraPerformance Convergence Chromatography™ have shown to be promising for fatty acid analysis of fish lipids. Experiments confirms that changes in feeding strategy, including daily feedings and variation in marine dietary raw materials can affect several quality parameters in Atlantic salmon fillets. Further analyses with larger selection groups are necessary to fully unveil the underlying mechanisms. Fish fed exclusively on conventional feed where a substantial part of marine ingredients are substituted and balanced with terrestrial alternatives showed the highest percentage of superior quality whole fish (98.4%). This implies that the development towards increased use of more sustainable feed resources still results in high quality products.

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Bekkevold, S., & Olafsen, T. (2007). Råvarer med muligheter. *Rubin*, *Inclusion of more fish ingredients in the feed increases both the environmental and the economic pressure for the producer (Naylor et al., 2000; Hernández et al., 2007). Thus, it will be of interest to evaluate if the potential benefits in growth and quality could be substantial enough to defend such costs. However, a detailed economic or environmental evaluation is not in the scope of this thesis.*.

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Appendix A Rstudio, Statistical Modelling Scripts

Attaching dataset:

$data <- read.csv(" \sim /location/dataset.csv", header = TRUE)$
$attach(data)$

ANOVA, linear model:

$result <- lm(water \sim group)$
$summary(result)$

ANOVA, generalised linear model (not normally distributed data):

$pigment <- glm(colorfan \sim group, family = Gamma)$
$summary(pigment)$

Coupled t-test:

$t.test(water[meals == "3"], water[meals == "1"])$

Principal Component Analysis:

$library(FactoMineR)$
$library(missMDA)$
$nb <- estim\_ncpPCA(data, ncp.min = 0, ncp.max = 5, method.cv = "Kfold", nbsim = 50)$
$imputed <- imputePCA(data, ncp = nb\$ncp)$
$res.pca <- PCA(imputed\$completeObs)$

Appendix B L*a*b* Data: Feed Frequency

Following are the raw data from photometric analysis of fish fed one or three times per day. Sample 3977-3983 were fed three times per day, while 3984-3989 were fed one time per day. Hue (H*) and Chroma (C*) are calculated with Equation 1.

	LATERAL POSTERIOR					CENTER OF BACKLOIN					LATERAL ANTERIOR					AVERAGE				
Sample ID	L	a	b	H*	C*	L	a	b	H*	C*	L	a	b	H*	C*	L*	a*	b*	H*	C*
3977	45.61	34.74	47.59	58.92	53.87	57.75	35.28	59.10	68.83	59.16	40.88	37.79	46.12	59.62	50.67	48.08	35.94	50.94	62.34	54.80
3978	45.21	35.87	45.70	58.10	51.87	57.74	35.86	59.92	69.83	59.10	40.83	36.55	45.31	58.21	51.11	47.93	36.09	50.31	61.92	54.34
3979	45.47	35.84	43.67	56.49	50.62	58.26	36.47	57.20	67.84	57.48	42.19	35.49	43.84	56.40	51.01	48.64	35.93	48.24	60.15	53.32
3980	46.23	36.05	47.63	59.73	52.88	58.59	36.65	58.85	69.33	58.09	39.23	38.07	43.46	57.78	48.78	48.02	36.92	49.98	62.14	53.54
3981	47.47	33.25	44.23	55.33	53.07	58.97	34.45	57.16	66.74	58.92	43.85	34.70	45.04	56.86	52.39	50.10	34.13	48.81	59.56	55.03
3983	54.02	29.52	47.85	56.22	58.33	66.82	25.17	54.70	60.21	65.29	48.89	31.71	47.59	57.19	56.32	56.58	28.80	50.05	57.74	60.08
3984	48.24	32.29	46.61	56.70	55.29	60.00	34.12	57.95	67.25	59.51	45.51	35.51	46.77	58.72	52.79	51.25	33.97	50.44	60.82	56.04
3985	49.82	34.00	48.23	59.01	54.82	59.42	38.11	62.71	73.38	58.71	44.31	36.63	47.58	60.05	52.41	51.18	36.25	52.84	64.08	55.55
3986	48.46	33.33	43.46	54.77	52.51	59.56	33.37	57.28	66.29	59.78	43.44	36.79	47.18	59.83	52.05	50.49	34.50	49.31	60.18	55.02
3987	54.23	33.54	50.12	60.31	56.21	61.54	31.70	57.03	65.25	60.93	45.35	35.81	46.65	58.81	52.49	53.71	33.68	51.27	61.34	56.69
3988	48.59	32.05	46.90	56.81	55.65	60.04	32.86	58.35	66.97	60.61	42.26	35.39	46.30	58.28	52.61	50.30	33.43	50.52	60.58	56.50
3989	48.24	34.22	47.47	58.52	54.21	62.00	32.59	57.99	66.52	60.66	43.99	35.68	46.01	58.22	52.21	51.41	34.16	50.49	60.96	55.92

Appendix C NIR Data: Diet

Following are the raw data from near infrared spectrometry of fat and pigmentation of fresh fish in the diet experiment.

Group:Fish	A:1	A:2	A:3	A:4	A:5	A:6	A:7	A:8	A:9	A:10	A:11	A:12	A:13	A:14	A:15	Average	St.dev
Gutted weight (kg)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	**0,0**	0,0
Length (cm)	74,00	73,50	77,00	74,00	77,00	77,00	77,50	73,50	71,00	75,00	75,00	75,00	74,50	75,00	76,00	**75,0**	1,7
Salmofan	26,0	27,0	26,0	27,0	28,0	26,5	28,0	26,5	27,0	26,5	27,0	28,0	27,0	27,5	27,5	**26,9**	0,7
Red pigments NIR/HPLC (mg/kg)	6,6	7,4	7,3	7,1	8,0	6,5	8,4	6,3	7,3	7,2	8,5	8,2	6,7	8,0	6,8	**7,3**	0,7
Fat NIR (%)	16,1	15,5	21,5	18,0	18,8	17,4	16,9	15,7	16,8	17,0	17,2	19,9	16,8	14,1	15,1	**17,1**	1,9
Group:Fish	**B:1**	**B:2**	**B:3**	**B:4**	**B:5**	**B:6**	**B:7**	**B:8**	**B:9**	**B:10**	**B:11**	**B:12**	**B:13**	**B:14**	**B:15**	**Average**	**St.dev**
Gutted weight (kg)	4,80	4,15	4,95	4,20	4,25	5,15	4,10	4,35	4,60	4,20	4,55	4,50	4,25	4,35	4,65	**4,5**	0,3
Length (cm)	75,00	75,00	76,00	75,00	75,00	75,00	72,00	74,50	76,00	74,00	76,00	74,50	72,50	71,50	76,50	**74,6**	1,5
Salmofan	28,5	27,5	27,5	28,0	27,0	26,5	27,5	27,0	26,5	28,0	27,0	27,0	27,0	28,0	28,0	**27,4**	0,6
Red pigments NIR/HPLC (mg/kg)	8,6	7,6	7,3	8,3	6,3	6,5	7,7	7,3	6,5	7,3	6,7	6,2	7,1	7,7	7,0	**7,2**	0,7
Fat NIR (%)	18,3	17,6	20,0	13,8	13,5	16,5	16,7	17,7	16,3	16,0	15,9	14,8	17,1	17,7	17,7	**16,6**	1,7
Group:Fish	**C:1**	**C:2**	**C:3**	**C:4**	**C:5**	**C:6**	**C:7**	**C:8**	**C:9**	**C:10**	**C:11**	**C:12**	**C:13**	**C:14**	**C:15**	**Average**	**St.dev**
Gutted weight (kg)	4,00	5,00	4,75	4,20	4,25	4,60	4,35	4,70	4,85	4,25	4,50	4,75	4,90	4,60	4,20	**4,5**	0,3
Length (cm)	73,00	79,00	76,50	77,50	77,50	77,00	74,50	73,00	76,00	73,00	75,50	76,00	78,50	79,00	75,00	**76,1**	2,1
Salmofan	26,0	28,0	27,5	27,5	26,0	27,5	27,0	27,0	27,5	26,0	26,0	28,0	27,0	27,0	27,0	**27,0**	0,7
Red pigments NIR/HPLC (mg/kg)	5,8	7,9	7,8	7,0	6,1	7,7	6,9	7,4	7,4	6,4	6,1	8,1	7,6	7,4	7,3	**7,1**	0,7
Fat NIR (%)	16,5	15,3	16,0	14,9	14,0	15,5	18,6	17,2	16,8	17,4	16,8	17,0	15,8	15,4	15,3	**16,2**	1,2
Group:Fish	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**	**D:6**	**D:7**	**D:8**	**D:9**	**D:10**						**Average**	**St.dev**
Gutted weight (kg)	6,66	3,18	5,22	5,38	4,68	4,18	4,44	5,44	2,17	3,08						**4,4**	1,3
Length (cm)	83,00	67,00	77,00	79,00	77,00	70,00	74,00	80,00	63,00	67,00						**73,7**	6,6
Salmofan	27,5	27,5	27,5	27,0	26,5	26,5	27,5	27,0	24,0	25,0						**26,6**	1,2
Red pigments NIR/HPLC (mg/kg)	7,7	7,8	7,7	7,4	6,0	6,9	7,2	6,7	4,3	4,6						**6,6**	1,3
Fat NIR (%)	18,7	14,5	15,8	17,3	15,0	16,7	16,1	15,8	9,0	12,9						**15,2**	2,7
Group:Fish	**E:1**	**E:2**	**E:3**	**E:4**	**E:5**	**E:6**	**E:7**	**E:8**	**E:9**	**E:10**						**Average**	**St.dev**
Gutted weight (kg)	6,52	5,62	4,38	3,96	7,96	5,76	5,50	5,58	5,06	4,74						**5,5**	1,1
Length (cm)	85,00	80,00	74,00	72,00	90,00	81,00	82,00	82,00	82,00	85,00						**81,3**	5,2
Salmofan	27,5	28,0	26,5	27,0	27,5	27,0	26,5	27,0	27,0	28,0						**27,2**	0,5
Red pigments NIR/HPLC (mg/kg)	7,4	7,7	6,2	6,7	7,2	7,1	6,0	6,8	6,6	7,6						**6,9**	0,6
Fat NIR (%)	14,2	18,0	14,4	12,1	18,6	15,6	16,7	16,2	14,1	14,5						**15,4**	2,0

Appendix D Carotenoid Analysis, UV: Diet

Astaxanthin concentration

$$Astaxanthin[mg/kg] = \frac{abs(472nm)}{E(g/100mL} * \frac{10000}{C(g/mL)} = \frac{abs(472nm)}{2100} * \frac{10000}{0.69g/1.5mL}$$

Where, abs = measured absorbance, E = std. abs of cuvette (1cm) with 1% astaxanthin, 10000 = adjusting to mg/kg, C = concentration

Following are the raw data from UV spectrometry of the extracted oil from the feed frequency experiment.

Oil sample Group:fish	A:1	A:2	A:3	A:4	A:5	A:6	A:7	A:8	A:9	A:10	A:11	A:12	A:13	A:14	A:15	Average A	stdev
Absorbance 472 nm	0.282	0.342	0.337	0.379	0.414	0.284	0.493	0.311	0.363	0.299	0.492	0.324	0.302	0.449	0.303	0.36	0.07
Carotenoid content in sample [ug/g]	2.92	3.54	3.49	3.92	4.29	2.94	5.10	3.22	3.76	3.10	5.09	3.35	3.13	4.65	3.14	3.71	0.75
Oil sample Group:fish	B:1	B:2	B:3	B:4	B:5	B:6	B:7	B:8	B:9	B:10	B:11	B:12	B:13	B:14	B:15	Average B	stdev
Absorbance 472 nm	0.416	0.393	0.388	0.450	0.360	0.322	0.425	0.394	0.300	0.415	0.330	0.363	0.368	0.450	0.400	0.38	0.04
Carotenoid content in sample [ug/g]	4.31	4.07	4.02	4.66	3.73	3.33	4.40	4.08	3.11	4.30	3.42	3.76	3.81	4.66	4.14	3.98	0.46
Oil sample Group:fish	C:1	C:2	C:3	C:4	C:5	C:6	C:7	C:8	C:9	C:10	C:11	C:12	C:13	C:14	C:15	Average C	stdev
Absorbance 472 nm	0.263	0.200	0.371	0.253	0.323	0.272	0.264	0.234	0.383	0.230	0.280	0.377	0.340	0.258	0.403	0.30	0.06
Carotenoid content in sample [ug/g]	2.72	2.07	3.84	2.62	3.34	2.82	2.73	2.42	3.96	2.38	2.90	3.90	3.52	2.67	4.17	3.07	0.66
Oil sample Group:fish	D:1	D:2	D:3	D:4	D:5	D:6	D:7	D:8	D:9	D:10						Average D	stdev
Absorbance 472 nm	0.347	0.471	0.386	0.342	0.277	0.323	0.348	0.266	0.315	0.271						0.33	0.06
Carotenoid content in sample [ug/g]	3.59	4.88	4.00	3.54	2.87	3.34	3.60	2.75	3.26	2.81						3.46	0.64
Oil sample Group:fish	E:1	E:2	E:3	E:4	E:5	E:6	E:7	E:8	E:9	E:10						Average E	stdev
Absorbance 472 nm	0.421	0.246	0.322	0.140	0.287	0.312	0.310	0.302	0.387	0.390						0.31	0.08
Carotenoid content in sample [ug/g]	4.36	2.55	3.33	1.45	2.97	3.23	3.21	3.13	4.01	4.04						3.23	0.83

Appendix E Lipid Extraction: Feed Frequency

Lipid Content

$$Lipid[\%] = \frac{a * b}{c * v} * 100$$

a = vaporized oil [g], b = chloroform added [ml], c = vaporized chloroform [ml], v = sample weight [g].

Following are the raw data from lipid extraction from the feed frequency experiment.

	Added $CHCl_3$ [ml]				40.0			
	Sample evaporated $CHCl_3$ [ml]				2.0			
Daily feedings:fish	1:1	1:2	1:3	1:4	1:5	1:6	Average 1 feeding	stdev
Weighted wet sample [g]	10.00	10.00	10.00	10.06	10.00	10.00	10.01	0.025
Evaporated sample [g]	0.042	0.043	0.044	0.044	0.045	0.046	0.04	0.001
Daily feedings:fish	3:1	3:2	3:3	3:4	3:5	3:6	Average 3 feedings	stdev
Weighted wet sample [g]	10.00	9.98	10.00	10.03	9.97	10.08	10.01	0.039
Evaporated sample [g]	0.083	0.083	0.083	0.083	0.083	0.083	0.08	0.000

Appendix F Lipid Extraction: Diet

Following are the raw data from lipid extraxtion from the diet experiment. Formulas are found in Appendix E.

	Added $CHCl_3$ [ml]				40.0																	
	Sample evaporated $CHCl_3$ [ml]				3.0																	
Group:fish	A:1	A:2	A:3	A:4	A:5	A:6	A:7	A:8	A:9	A:10	A:11	A:12	A:13	A:14	A:15	Average A	stdev					
Weighted wet sample [g]	10.05	10.06	10.01	10.05	10.10	10.03	10.00	10.05	10.05	10.03	10.06	10.07	10.03	10.09	10.09	10.05	0.029					
Evaporated sample [g]	0.142	0.155	0.186	0.161	0.157	0.173	0.179	0.119	0.159	0.128	0.131	0.151	0.138	0.116	0.121	0.15	0.022					
Group:fish	B:1	B:2	B:3	B:4	B:5	B:6	B:7	B:8	B:9	B:10	B:11	B:12	B:13	B:14	B:15	Average B	stdev					
Weighted wet sample [g]	10.00	10.04	10.07	10.00	10.10	10.12	10.08	9.99	10.10	10.11	10.09	10.09	10.10	10.10	10.03	10.07	0.044					
Evaporated sample [g]	0.136	0.132	0.165	0.137	0.139	0.156	0.137	0.131	0.146	0.142	0.143	0.132	0.146	0.152	0.145	0.14	0.01					
Group:fish	C:1	C:2	C:3	C:4	C:5	C:6	C:7	C:8	C:9	C:10	C:11	C:12	C:13	C:14	C:15	Average C	stdev					
Weighted wet sample [g]	10.03	10.05	10.01	10.02	10.02	10.06	10.06	10.08	10.04	10.01	10.05	10.02	10.01	10.00	10.01	10.03	0.024					
Evaporated sample [g]	0.118	0.125	0.120	0.105	0.101	0.106	0.117	0.116	0.145	0.144	0.178	0.198	0.165	0.196	0.144	0.14	0.033					
Group:fish	D:1	D:2	D:3	D:4	D:5	D:6	D:7	D:8	D:9	D:10						Average D	stdev					
Weighted wet sample [g]	10.01	10.01	10.06	10.03	10.07	10.03	10.00	10.00	10.03	10.04						10.03	0.024					
Evaporated sample [g]	0.155	0.127	0.142	0.142	0.142	0.147	0.147	0.146	0.081	0.123						0.14	0.021					
Group:fish	E:1	E:2	E:3	E:4	E:5	E:6	E:7	E:8	E:9	E:10						Average E	stdev					
Weighted wet sample [g]	10.08	10.03	10.06	10.07	10.06	10.03	10.08	10.05	10.06	10.07						10.06	0.018					
Evaporated sample [g]	0.131	0.136	0.119	0.118	0.154	0.156	0.128	0.139	0.123	0.143						0.13	0.013					

Appendix G Fatty Acid Analysis: Diet

Table G1: Raw data, peak areas from UPC^2, used to calculate relative fatty acid concentrations from the diet experiment (Table G2).

Group/Fish	16:0-d31	18:3	n6-18:2	20:4	20:3	n6-20:2	22:5	n6-22:4	n6-22:2	n3-18:4	n3-20:5	n3-22:6	n9-18:1	Sum
A/1	1283	19684	32772	20369	2863	3000	35787	1360	3	10887	136137	366165	82477	711504
A/2	10610	80207	157599	47628	12817	28027	94369	4075	876	18407	214293	499101	480417	1637816
A/3	10400	1534	3311	893	219	537	1463	82	13	480	3833	15749	16390	44504
A/4	2814	24991	43376	22709	3365	4960	36192	1472	188	14396	136817	394125	145964	828555
A/5	3648	45208	81070	32689	6785	12291	53530	2331	10	15684	148721	377949	315510	1091778
A/6	8289	108011	186064	69311	15186	31506	122175	5432	1014	29617	273686	618217	521593	1981812
A/7	1848	28401	48071	33012	4405	5132	45922	1760	190	21701	209816	521854	144707	1064971
A/8	3979	13322	25058	9599	1632	2785	17168	686	140	6141	59119	217242	98730	451622
A/9	2209	54174	95117	43429	7004	10906	82842	3153	392	28696	269024	643108	306932	1544777
A/10	3868	15299	28868	12434	2502	4404	20727	828	175	7056	61635	234912	130728	519568
A/11	1449	21172	35140	20253	2794	2997	32041	1267	123	10405	133916	368394	94986	723488
A/12	4151	16290	29425	13236	2184	3913	22676	896	125	8098	81789	281286	103425	563343
A/14	4149	30570	51157	29890	4210	6537	44863	1797	233	20247	168026	507792	185136	1050458
A/15	7355	12379	23210	7841	1387	2584	14079	560	124	5323	46332	202227	94419	410465
B/2	1391	20037	32515	20435	2897	2993	31507	1296	142	10837	132023	359576	88004	702262
B/7	12869	18966	35625	18386	2917	1087	15420	883	165	8741	137321	130469	122074	492054
B/8	6524	58506	101221	44349	6910	4514	48855	2834	384	13104	288387	358747	307615	1235426
B/9	6853	14913	26206	8609	1341	1490	13403	600	136	3096	50281	67703	112959	300737
B/10	11837	6004	11989	3294	905	1874	5448	251	8	1482	16079	60132	66153	173619
B/11	8871	3429	6814	2063	478	929	3209	171	2	1079	10068	40678	30966	99886
B/12	10608	3630	7600	1902	544	1142	2931	155	3	1070	8411	31523	39377	98288
B/13	9345	817	1720	508	144	261	827	52	2	326	2195	8002	6521	21375
B/14	3862	45133	76413	49133	6273	7415	66684	2509	286	36483	313504	736174	219896	1559903
B/15	3002	11455	19639	11811	1723	2093	17697	615	4	5706	87470	390913	65981	615107
C/2	11021	72684	134494	46652	10233	19414	67988	3266	16	19882	170477	481957	474072	1501135
C/4	9484	1639	3319	1004	288	498	2044	85	26	418	6235	31857	16171	63584
C/6	6406	6254	13093	3068	834	1854	5553	286	64	1275	14031	66452	71417	184181
C/10	5960	3086	6411	1228	408	755	2178	139	24	651	6509	27674	33074	82137
C/11	6996	8746	18591	3974	1257	2885	7448	317	3	1576	20313	73246	99358	237714
C/12	11824	37058	64749	29825	6052	9247	62148	2043	11	8603	165440	507246	234832	1127254
C/13	1457	21261	36632	20456	2985	3162	32693	1203	122	10549	134596	384587	100362	748608
C/14	7213	11168	23130	4667	1248	3316	7917	333	89	2045	24802	96446	119706	294867
C/15	8499	75547	118404	81492	8491	8550	115836	4242	354	42235	512928	1012646	298370	2279095
D/1	12844	22022	56669	11040	4135	12310	20663	1011	10	2779	55568	198570	319861	704638
D/3	8004	36438	56039	32782	4783	5508	72433	2068	4	11710	276336	689612	175130	1362843
D/4	12475	39507	71624	27954	6228	9515	54180	1888	12	9390	191637	478981	253211	1144127
D/6	14985	28125	59925	12638	4262	10949	22537	910	398	3992	67391	191263	312071	714461
D/7	14514	1091	2578	513	160	308	1055	64	7	288	2778	8883	11257	28982
D/8	11078	1343	2926	857	355	430	1819	77	25	326	6429	24853	13393	52833
D/9	13703	560	1264	337	88	188	611	37	7	172	2183	8796	5705	19948
D/10	9383	2021	4922	810	274	631	1258	72	15	411	3831	11631	24704	50580
E/1	8846	4457	8747	3066	692	1020	6525	232	19	1353	24310	111388	36459	198268
E/2	9780	16746	35177	7807	2084	5002	14755	595	170	3079	48705	172063	173062	479245
E/3	1354	14640	29486	7370	1747	3279	13404	545	88	3153	44620	125039	128677	372048
E/4	9087	3067	6145	1817	442	768	4559	145	30	790	16047	72950	27065	133825
E/5	7819	86387	143977	69442	11210	13587	121566	3965	409	33019	503239	920107	352737	2259645
E/6	3921	6926	14386	3936	910	1538	8467	274	67	1352	31944	123085	61271	254156
E/7	7025	35062	61420	33585	4513	4891	53626	1729	9	14700	248844	661971	165108	1285458
E/8	1348	21927	36703	20729	3009	3061	32445	1175	101	10509	131763	393198	98091	752711
E/9	13274	18849	34234	13214	2803	4600	31483	1088	138	4423	88824[i♡]	306178	135593	641427
E/10	13734	49439	95774	27383	6308	11277	50419	1910	13	9494	177034	502787	334176	1266014

Table G2: Relative fatty acid concentrations from diet experiment (peakarea/sumarea).

Group/Fish	16:0-d31	18:3	n6-18:2	20:4	20:3	n6-20:2	22:5	n6-22:4	n6-22:2	n3-18:4	n3-20:5	n3-22:6	n9-18:1
A/1	4.897	9.623	2.908	0.783	1.711	5.762	0.249	0.053	1.124	13.084	30.474	29.333	
A/2	3.447	7.440	2.007	0.492	1.207	3.287	0.184	0.029	1.079	8.613	35.388	36.828	
A/3	3.016	5.235	2.741	0.406	0.599	4.368	0.178	0.023	1.737	16.513	47.568	17.617	
A/4	4.141	7.426	2.994	0.621	1.126	4.903	0.214	0.001	1.437	13.622	34.618	28.899	
A/5	5.450	9.389	3.497	0.766	1.590	6.165	0.274	0.051	1.494	13.810	31.195	26.319	
A/6	2.667	4.514	3.100	0.414	0.482	4.312	0.165	0.018	2.038	19.702	49.002	13.588	
A/7	2.950	5.548	2.125	0.361	0.617	3.801	0.152	0.031	1.360	13.090	48.103	21.861	
A/8	3.507	6.157	2.811	0.453	0.706	5.363	0.204	0.025	1.858	17.415	41.631	19.869	
A/9	2.945	5.556	2.393	0.482	0.848	3.989	0.159	0.034	1.358	11.863	45.213	25.161	
A/10	3.402	6.104	2.751	0.424	0.687	4.727	0.187	0.033	2.109	18.584	41.148	19.843	
A/11	2.892	5.223	2.350	0.388	0.695	4.025	0.159	0.022	1.437	14.519	49.932	18.359	
A/12	2.910	4.870	2.845	0.401	0.622	4.271	0.171	0.022	1.927	15.995	48.340	17.624	
A/14	3.016	5.655	1.910	0.338	0.630	3.430	0.136	0.030	1.297	11.288	49.268	23.003	
A/15	4.842	8.959	3.108	0.682	1.293	4.529	0.218	0.001	1.324	11.357	32.106	31.581	
B/2	3.854	7.240	3.737	0.593	0.221	3.134	0.179	0.034	1.776	27.908	26.515	24.809	
B/7	4.736	8.193	3.590	0.559	0.365	3.955	0.229	0.031	1.061	23.343	29.038	24.900	
B/8	4.959	8.714	2.863	0.446	0.495	4.457	0.200	0.045	1.029	16.719	22.512	37.561	
B/9	3.458	6.905	1.897	0.521	1.079	3.138	0.145	0.005	0.854	9.261	34.634	38.102	
B/10	3.433	6.822	2.065	0.479	0.930	3.213	0.171	0.002	1.080	10.079	40.724	31.001	
B/11	3.693	7.732	1.935	0.553	1.162	2.982	0.158	0.003	1.089	8.558	32.072	40.063	
B/12	3.822	8.047	2.377	0.674	1.221	3.869	0.243	0.009	1.525	10.269	37.436	30.508	
B/13	2.893	4.899	3.150	0.402	0.475	4.275	0.161	0.018	2.339	20.098	47.194	14.097	
B/14	1.862	3.193	1.920	0.280	0.340	2.877	0.100	0.001	0.928	14.220	63.552	10.727	
B/15	2.710	4.914	2.213	0.379	0.548	3.384	0.149	0.026	1.446	13.684	50.806	19.743	
C/2	2.578	5.220	1.579	0.453	0.783	3.215	0.134	0.041	0.657	9.806	50.102	25.432	
C/4	3.396	7.109	1.666	0.453	1.007	3.015	0.155	0.035	0.692	7.618	36.080	38.775	
C/6	3.757	7.805	1.495	0.497	0.919	2.652	0.169	0.029	0.793	7.925	33.692	40.267	
C/10	3.679	7.821	1.672	0.529	1.214	3.133	0.133	0.001	0.663	8.545	30.813	41.797	
C/11	3.287	5.744	2.646	0.537	0.820	5.513	0.181	0.001	0.763	14.676	44.998	20.832	
C/12	4.051	8.740	1.595	0.510	1.218	2.750	0.130	0.038	0.665	7.513	23.656	49.133	
C/13	3.787	7.844	1.583	0.423	1.125	2.685	0.113	0.030	0.694	8.411	32.708	40.597	
C/14	3.315	5.195	3.576	0.373	0.375	5.083	0.186	0.016	1.853	22.506	44.432	13.092	
C/15	2.248	4.412	1.546	0.349	0.514	3.291	0.117	0.010	0.682	12.261	56.181	18.389	
D/1	2.674	4.112	2.405	0.351	0.404	5.315	0.152	0.000	0.859	20.276	50.601	12.850	
D/3	3.453	6.260	2.443	0.544	0.832	4.735	0.165	0.001	0.821	16.750	41.864	22.131	
D/4	3.937	8.387	1.769	0.597	1.532	3.154	0.127	0.056	0.559	9.432	26.770	43.679	
D/6	3.764	8.895	1.770	0.552	1.063	3.640	0.221	0.024	0.994	9.585	30.650	38.841	
D/7	2.542	5.538	1.622	0.672	0.814	3.443	0.146	0.047	0.617	12.169	47.041	25.350	
D/8	2.807	6.336	1.689	0.441	0.942	3.063	0.185	0.035	0.862	10.943	44.095	28.599	
D/9	3.996	9.731	1.601	0.542	1.248	2.487	0.142	0.030	0.813	7.574	22.995	48.841	
D/10	3.759	6.152	3.019	0.420	0.500	5.426	0.190	0.021	1.373	21.963	41.609	15.568	
E/1	3.494	7.340	1.629	0.435	1.044	3.079	0.124	0.035	0.642	10.163	35.903	36.111	
E/2	3.935	7.925	1.981	0.470	0.881	3.603	0.146	0.024	0.847	11.993	33.608	34.586	
E/3	2.292	4.592	1.358	0.330	0.574	3.407	0.108	0.022	0.590	11.991	54.511	20.224	
E/4	3.823	6.372	3.073	0.496	0.601	5.380	0.175	0.018	1.461	22.271	40.719	15.610	
E/5	2.725	5.660	1.549	0.358	0.605	3.331	0.108	0.026	0.532	12.569	48.429	24.108	
E/6	2.728	4.778	2.613	0.351	0.380	4.172	0.135	0.001	1.144	19.358	51.497	12.844	
E/7	3.485	6.948	1.649	0.407	0.846	3.956	0.138	0.023	0.664	13.038	40.624	28.223	
E/8	2.939	5.337	2.060	0.437	0.717	4.908	0.170	0.022	0.690	13.848	47.734	21.139	
E/9	3.905	7.565	2.163	0.498	0.891	3.982	0.151	0.001	0.750	13.984	39.714	26.396	
E/10	3.125	8.042	1.567	0.587	1.747	2.932	0.143	0.001	0.394	7.886	28.180	45.394	
Average A	3.577	6.550	2.681	0.501	0.915	4.495	0.189	0.027	1.541	14.247	41.713	23.563	
Average B	3.542	6.666	2.575	0.489	0.684	3.528	0.173	0.017	1.313	15.414	38.448	27.151	
Average C	3.344	6.654	1.929	0.458	0.886	3.482	0.147	0.022	0.829	11.029	39.185	32.035	
Average D	3.366	6.927	2.040	0.515	0.917	3.908	0.166	0.027	0.862	13.587	38.203	29.483	
Average E	3.245	6.456	1.964	0.437	0.829	3.875	0.140	0.017	0.772	13.710	42.092	26.464	

Appendix H Protein Extraction: Feed Frequency

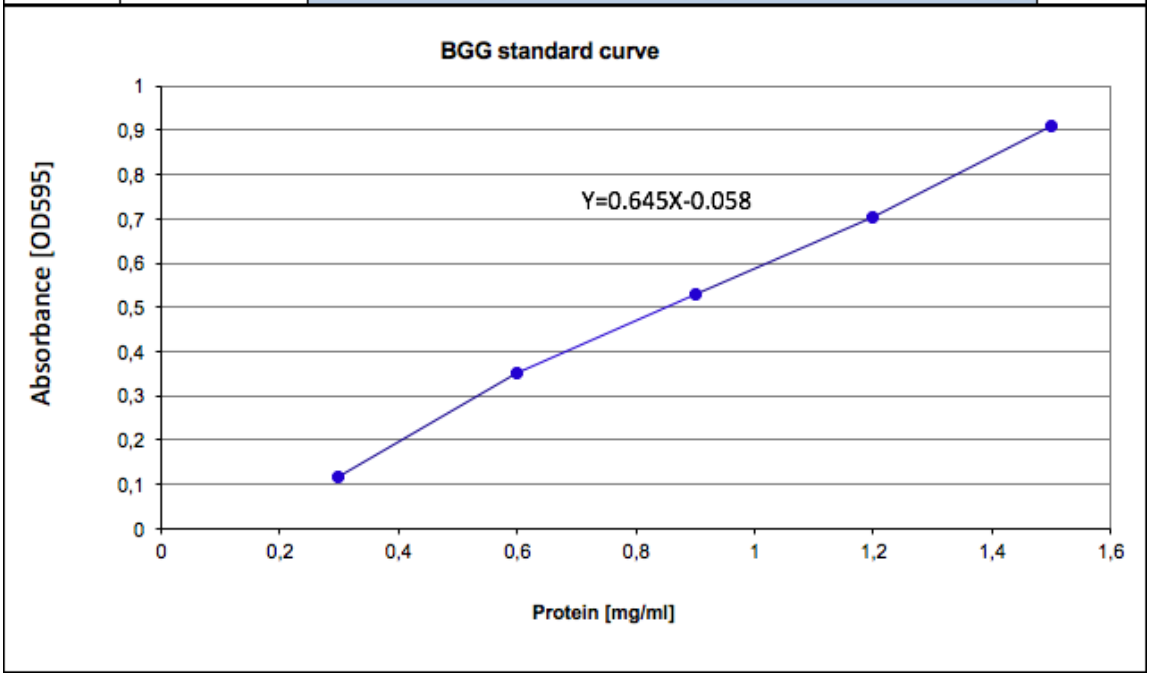
The raw data from the protein extraction are presented in the following table.

Water soluble protein												
ID	Weight [g]	pH	Extract volume [ml]	Dilution factor (1 = undiluted)	OD_{595}			protein in extract [mg/ml]				protein in extract [% av wet weight]
					a	b	c	a	b	c	snitt	
3977	4.1109	7.0	100	10	0.413	0.404	0.409	0.730	0.716	0.724	0.723	17.6 %
3978	4.3457	7.0	100	10	0.416	0.418	0.401	0.735	0.738	0.711	0.728	16.7 %
3979	4.0598	7.0	100	10	0.369	0.372	0.358	0.662	0.666	0.645	0.658	16.2 %
3980	4.0713	7.0	100	10	0.400	0.361	0.356	0.710	0.649	0.642	0.667	16.4 %
3981	4.2760	7.0	100	10	0.283	0.284	0.287	0.528	0.530	0.534	0.531	12.4 %
3983	4.1615	7.0	100	10	0.388	0.390	0.373	0.691	0.694	0.668	0.684	16.4 %
3984	4.2254	7.0	100	10	0.216	0.223	0.218	0.424	0.435	0.427	0.429	10.2 %
3985	4.1868	7.0	100	10	0.271	0.311	0.290	0.510	0.572	0.539	0.540	12.9 %
3986	4.0952	7.0	100	10	0.349	0.343	0.341	0.631	0.621	0.618	0.623	15.2 %
3987	4.1090	7.0	100	10	0.315	0.349	0.310	0.578	0.631	0.570	0.593	14.4 %
3988	4.0200	7.0	100	10	0.330	0.353	0.349	0.601	0.637	0.631	0.623	15.5 %
3989	4.2901	7.0	100	10	0.282	0.320	0.304	0.527	0.586	0.561	0.558	13.0 %
Average 3 feedings	4.1709	7.0	100	10	0.378	0.372	0.364	0.676	0.666	0.654	0.665	16.0 %
Average 1 feeding	4.1544	7.0	100	10	0.294	0.317	0.302	0.545	0.580	0.558	0.561	13.5 %

Salt soluble protein												
Sauemerke	Vekt [g]	pH	KCl [M]	Ekstraktvolum [ml]	Dilution factor (1 = undiluted)	OD_{595}			protein in extract [mg/ml]			protein in extract [% av wet weight]
						a	b	c	a	b	c	snitt	
3977	4.1109	7.0	0.6	100	5	0.270	0.318	0.312	0.508	0.583	0.573	0.555	6.7 %
3978	4.3457	7.0	0.6	100	5	0.332	0.319	0.335	0.604	0.584	0.609	0.599	6.9 %
3979	4.0598	7.0	0.6	100	5	0.344	0.358	0.339	0.623	0.645	0.615	0.628	7.7 %
3980	4.0713	7.0	0.6	100	5	0.353	0.366	0.366	0.637	0.657	0.657	0.650	8.0 %
3981	4.2760	7.0	0.6	100	5	0.384	0.392	0.337	0.685	0.697	0.612	0.665	7.8 %
3983	4.1615	7.0	0.6	100	5	0.377	0.364	0.342	0.674	0.654	0.620	0.649	7.8 %
3984	4.2254	7.0	0.6	100	5	0.223	0.201	0.232	0.435	0.401	0.449	0.429	5.1 %
3985	4.1868	7.0	0.6	100	5	0.354	0.332	0.383	0.638	0.604	0.683	0.642	7.7 %
3986	4.0952	7.0	0.6	100	5	0.388	0.365	0.398	0.691	0.655	0.707	0.684	8.4 %
3987	4.1090	7.0	0.6	100	5	0.326	0.330	0.316	0.595	0.601	0.579	0.592	7.2 %
3988	4.0200	7.0	0.6	100	5	0.310	0.309	0.304	0.570	0.569	0.561	0.567	7.0 %
3989	4.2901	7.0	0.6	100	5	0.399	0.419	0.436	0.708	0.739	0.766	0.738	8.6 %
Average 3 feedings	4.1709	7.0	0.6	100	5	0.343	0.353	0.339	0.622	0.637	0.614	0.624	7.5 %
Average 1 feeding	4.1900	7.0	0.6	100	5	0.336	0.333	0.325	0.610	0.606	0.594	0.603	7.2 %

Following is the standard curve used to record protein concentrations from the UV absorbance intensity raw data from the feed frequency experiment.

BGG standard					
tube #	mg/ml	OD_{595}			average
0	0				0
1	0.3	0.128	0.071	0.156	0.118
2	0.6	0.356	0.38	0.321	0.352
3	0.9	0.509	0.542	0.535	0.529
4	1.2	0.708	0.711	0.695	0.705
5	1.5	0.914	0.905	0.909	0.909



Protein content (used for salt or water soluble samples):

$$Protein[mg/mL] = \frac{abs}{A} - \frac{B}{A} = \frac{abs}{0.645} - \frac{0.058}{0.645}$$

$$Protein[\%] = \frac{Protein[mg/mL] * Df * V}{1000 * W}$$

Where, abs = measured absorbance (OD_{595}), A = slope of the standard curve, B = constant term of standard curve,
Df = Dillution factor (1 = undiluted), V = sample volume,
1000 = adjustment to 100%, W = sample weight [g].

Appendix I Amino Acid Analysis: Diet

Amino Acid Concentration:

$$Aminoacid[\frac{mg}{gdrysample}] = \frac{\frac{C_v * M_w}{1000} * V * Df}{1000 * w}$$

$$Serine[mg/g] = \frac{\frac{C_v * 87[g/mol]}{1000} * 10[mL] * 500}{1000 * 0.0495[g]}$$

Where, C_v = Volumetric concentration from HPLC [μmol/litre], M_w = Molar weight [g/mol], V = volume hydrolysate [mL], Df = dilution factor, w = weighed dry sample [g].

Table I1: Concentrations of amino acids in dry fish sample (mg/g), measured with HPLC. Groups A and B .

Group/Fish	Asp	Glu	Asn	His	Ser	Gln	Gly/Arg	Thr	Ala	Aba	Met	Val	Phe	Ile	Leu	Lys	sumamino
A/1	44.10	64.16	0.00	12.59	16.99	10.49	41.88	21.40	59.79	1.45	13.27	23.37	19.45	21.16	35.60	45.48	431.18
A/2	34.66	50.42	0.00	9.38	12.80	8.03	31.54	16.16	46.57	1.38	10.03	18.17	14.85	16.42	27.70	34.48	332.60
A/3	20.46	31.17	0.40	5.70	8.22	5.10	19.62	10.64	28.90	1.38	5.08	11.71	10.01	10.82	18.20	22.64	210.03
A/4	27.61	32.26	0.09	7.58	8.42	6.72	24.10	12.47	40.08	1.43	5.89	13.62	11.71	12.12	22.43	27.97	254.50
A/5	31.42	49.65	0.74	8.92	13.72	8.81	30.21	16.83	48.13	1.42	7.77	18.59	15.32	16.99	28.95	36.78	334.25
A/6	39.31	57.70	1.02	10.86	14.96	9.83	33.05	18.94	53.51	1.35	8.61	20.72	16.89	19.02	32.00	39.74	377.50
A/7	27.77	27.72	0.11	7.59	7.19	7.62	33.74	12.31	44.94	1.57	6.00	13.27	11.78	11.72	23.44	28.26	265.05
A/8	43.53	53.22	0.10	11.87	16.48	10.65	59.67	20.12	60.66	1.58	9.93	21.01	17.97	18.99	33.97	44.00	423.74
A/9	12.70	12.61	0.07	3.69	3.73	3.28	16.33	5.66	22.07	1.52	3.16	6.28	5.56	5.35	10.80	13.02	125.84
A/10	31.10	40.52	0.00	8.74	11.78	8.08	35.90	14.66	40.76	1.66	7.88	15.79	12.69	14.57	24.46	31.33	299.91
A/11	29.78	38.43	0.15	7.92	10.98	7.13	34.89	14.05	38.83	1.69	6.51	14.91	12.38	13.73	23.50	27.41	282.29
A/12	31.09	39.92	7.03	18.24	5.44	44.74	14.03	23.65	11.31	4.25	20.27	8.99	18.72	24.21	23.53	0.00	295.43
A/13	34.29	44.45	0.00	9.02	12.73	7.98	39.20	16.00	44.28	1.35	8.97	17.41	14.14	16.14	27.41	35.18	328.55
A/14	46.89	60.04	0.00	12.84	17.96	11.25	53.51	22.21	61.90	1.41	12.08	23.83	19.04	21.76	37.29	46.08	448.12
A/15	41.82	55.20	0.17	11.30	16.31	9.04	52.18	20.15	57.06	1.32	11.96	22.42	18.29	20.35	34.36	42.00	413.96
B/1	42.46	59.77	0.52	12.53	16.53	10.89	41.32	20.46	60.54	0.23	10.50	22.38	18.37	20.57	35.48	43.55	416.11
B/2	38.50	55.04	0.00	11.13	14.73	9.87	35.54	18.87	53.12	0.22	12.63	20.51	16.61	18.85	32.35	39.91	377.89
B/3	28.13	39.55	0.58	7.98	10.40	6.80	25.10	13.23	35.87	1.24	6.87	14.13	12.32	13.23	23.01	28.15	266.59
B/4	58.92	87.11	0.84	16.71	22.72	14.54	50.83	27.39	78.71	1.05	14.54	30.30	26.02	28.44	49.42	60.63	568.16
B/5	46.13	66.13	1.11	13.35	18.61	10.00	43.49	22.87	67.64	0.26	12.51	24.40	20.86	22.19	38.75	47.67	455.99
B/6	46.08	66.19	1.12	13.80	18.45	12.30	40.95	22.92	67.67	1.22	12.12	24.56	20.08	22.49	38.66	47.76	456.34
B/7	50.90	71.34	1.83	14.39	19.54	11.56	57.92	24.03	65.88	1.11	10.64	25.59	21.06	23.44	39.91	50.85	489.99
B/8	45.72	63.68	0.00	13.22	17.81	11.07	53.88	22.08	62.31	1.13	14.68	23.63	19.36	21.46	36.65	45.14	451.81
B/9	46.81	64.48	0.00	13.25	18.54	10.95	61.00	22.79	63.67	1.25	12.07	23.82	19.56	21.59	37.00	46.72	463.50
B/10	42.52	58.16	0.00	12.15	16.80	9.34	54.94	20.26	57.80	1.21	12.98	21.49	17.77	19.70	33.48	41.88	420.48
B/11	48.97	65.49	5.15	13.96	20.44	12.42	56.41	23.15	64.14	0.32	12.11	24.44	20.04	22.50	38.09	47.86	475.49
B/12	52.27	72.38	0.75	14.80	20.20	12.42	61.66	24.74	68.06	0.35	12.55	25.99	21.64	23.95	40.93	53.38	506.06
B/13	46.67	64.14	0.00	13.46	18.45	9.87	62.51	22.08	65.26	1.32	14.91	23.65	19.68	21.36	36.31	46.92	466.58
B/14	44.63	61.07	0.00	12.94	16.92	10.92	56.86	21.49	60.86	1.15	12.96	22.59	18.44	20.50	34.90	45.28	441.53
B/15	42.55	57.80	0.00	12.21	16.05	10.04	50.00	19.97	55.21	1.26	10.71	21.55	17.49	19.47	32.92	42.82	410.06

Table I2: Concentrations of amino acids in dry fish sample (mg/g), measured with HPLC. Groups C-E.

Group/Fish	Asp	Glu	Asn	His	Ser	Gln	Gly/Arg	Thr	Ala	Aba	Met	Val	Phe	Ile	Leu	Lys	sumamino
C/1	43.73	62.77	0.00	12.54	24.57	0.00	45.84	21.38	61.02	1.24	12.82	23.37	18.87	21.14	35.63	44.44	429.37
C/2	35.80	51.78	0.00	10.31	19.96	0.00	39.63	17.42	50.86	1.18	10.21	19.11	16.16	17.20	29.22	36.54	355.39
C/3	48.08	69.05	0.00	14.17	27.35	0.00	50.99	23.87	69.06	1.23	12.36	26.02	21.39	23.62	39.84	49.11	476.13
C/4	46.79	68.90	0.00	13.58	27.54	0.00	46.05	23.46	66.43	1.26	14.72	25.55	21.34	23.47	39.65	50.05	468.78
C/5	46.97	69.17	0.00	13.63	27.65	0.00	46.24	23.56	66.69	1.27	14.77	25.65	21.43	23.56	39.81	50.25	470.66
C/6	40.55	59.34	0.00	11.90	24.33	0.00	41.36	20.39	58.87	1.18	12.63	22.17	18.62	19.83	33.93	42.01	407.11
C/7	39.82	53.86	0.00	11.44	21.75	0.00	47.78	19.14	53.15	1.33	8.21	20.75	17.27	18.65	31.55	40.11	384.83
C/8	41.28	55.69	0.00	12.03	22.48	0.00	49.83	19.82	55.27	1.40	11.50	21.56	17.93	19.39	32.79	41.71	402.69
C/9	22.18	41.59	0.00	9.99	20.35	0.00	45.62	16.92	53.56	1.28	8.27	19.91	16.52	18.38	31.70	40.07	346.35
C/10	49.60	67.97	0.00	14.44	27.99	0.00	59.64	24.39	66.68	1.32	14.70	25.80	21.75	23.45	39.78	50.67	488.19
C/11	39.87	54.80	0.00	11.40	21.47	0.00	47.55	19.16	53.20	1.42	7.56	20.36	16.69	18.92	32.02	40.92	385.35
C/12	43.91	59.18	0.00	12.60	23.90	0.00	50.52	20.73	57.39	1.28	10.15	22.76	19.02	20.67	34.96	44.82	421.88
C/13	49.98	66.68	0.00	14.61	28.45	0.00	76.03	23.61	71.07	1.22	14.61	25.33	21.27	22.79	38.78	50.17	504.61
C/14	37.40	51.52	0.00	10.45	20.79	0.00	44.33	17.51	48.80	1.41	10.56	18.99	15.33	17.51	29.90	37.45	361.96
C/15	47.01	64.77	0.00	13.44	26.17	0.00	56.53	22.66	62.51	1.32	14.21	24.26	20.26	22.22	37.71	48.14	461.23
D/1	37.82	51.49	0.06	11.89	14.53	8.04	44.84	18.35	49.97	0.17	8.66	19.64	16.21	18.02	30.32	39.24	369.23
D/2	50.73	70.43	0.47	15.15	20.73	9.92	63.75	25.26	68.97	0.22	10.87	26.37	22.07	24.18	40.83	53.66	503.60
D/3	48.71	66.54	0.21	14.64	19.24	7.56	57.91	23.77	65.07	0.23	10.83	25.61	21.20	23.36	39.37	51.52	475.77
D/4	42.04	57.66	0.11	12.97	16.22	8.38	51.04	20.52	57.10	0.19	13.12	22.46	18.52	20.22	34.27	44.59	419.41
D/5	50.13	69.17	0.21	15.26	20.63	9.52	62.79	24.80	68.61	0.22	13.40	26.07	21.69	23.76	40.52	53.19	499.96
D/6	50.06	68.16	0.16	14.58	18.98	9.51	58.90	24.45	66.91	0.16	0.14	26.48	21.81	23.85	40.21	53.23	477.57
D/7	40.72	56.20	0.10	12.53	15.26	8.00	48.22	19.63	53.55	0.19	11.94	21.47	17.85	19.51	32.87	43.79	401.83
D/8	35.87	48.86	0.31	11.01	13.64	6.90	45.79	17.22	47.98	0.15	7.32	18.76	15.56	17.20	28.67	37.82	353.05
D/9	56.80	80.61	0.07	17.00	22.03	11.32	68.47	27.87	75.75	0.22	18.50	29.60	24.50	27.20	46.12	62.44	568.48
D/10	43.60	61.15	0.06	13.07	16.77	8.07	52.28	20.91	57.17	0.19	13.96	22.75	18.76	20.90	35.23	47.03	431.90
E/1	44.68	62.62	0.50	13.26	18.77	9.15	53.36	22.35	61.42	0.15	7.99	23.41	19.45	21.45	36.20	47.69	442.48
E/2	33.78	47.20	0.25	10.40	14.05	7.00	41.16	17.07	47.07	0.13	8.70	18.09	15.07	16.36	27.46	35.61	339.40
E/3	44.55	62.92	0.18	13.25	18.64	8.75	56.10	22.36	59.24	0.17	10.20	22.55	20.00	20.86	35.69	46.82	442.27
E/4	46.20	65.14	0.46	14.09	20.56	9.69	60.58	23.84	66.16	0.18	8.70	24.37	20.37	22.22	37.78	50.64	470.97
E/5	37.79	53.23	0.00	10.50	16.20	6.66	45.88	19.49	53.47	0.17	11.54	20.32	16.39	22.44	32.65	37.58	384.30
E/6	39.47	55.73	0.22	11.97	17.39	7.21	48.62	19.31	55.93	0.18	11.45	21.05	17.24	18.96	31.77	42.58	399.07
E/7	49.03	67.57	0.67	14.60	19.79	8.86	60.29	23.78	64.01	0.16	8.82	23.76	21.25	22.27	38.09	49.33	472.27
E/8	42.03	57.78	0.05	12.61	16.37	8.76	51.40	20.38	56.54	0.18	7.24	21.58	18.13	19.78	33.79	43.84	410.43
E/9	54.04	74.47	0.14	16.64	22.57	9.81	69.32	26.82	74.29	0.22	14.22	28.13	23.48	25.40	43.51	56.92	539.98
E/10	52.08	71.19	0.46	16.08	20.90	10.13	62.53	25.79	66.06	0.16	13.56	26.15	23.17	24.27	41.35	53.50	507.39

Distribution of Amino Acids in Muscle

	Group A [%]	Group B [%]	Group C [%]	Group D [%]	Group E [%]
Asp+Asn	10.303 ± 0.325	10.608 ± 0.855	10.210 ± 0.157	10.206 ± 0.151	10.147 ± 0.238
Glu+Gln	16.010 ± 1.311	16.966 ± 1.455	14.347 ± 0.317	15.932 ± 0.225	16.017 ± 0.241
His	2.808 ± 0.089	2.919 ± 0.095	2.946 ± 0.038	3.082 ± 0.067	3.007 ± 0.127
Ser	3.753 ± 0.390	3.970 ± 0.176	5.743 ± 0.120	3.970 ± 0.120	4.213 ± 0.123
Gly+Arg	11.080 ± 1.754	11.513 ± 1.697	11.433 ± 1.042	12.273 ± 0.231	12.422 ± 0.343
Thr	4.853 ± 0.112	4.870 ± 0.137	4.973 ± 0.053	4.962 ± 0.088	5.022 ± 0.082
Ala	14.200 ± 0.972	13.851 ± 0.620	14.002 ± 0.356	13.608 ± 0.241	13.705 ± 0.356
Met	2.552 ± 0.311	2.759 ± 0.318	2.793 ± 0.393	2.354 ± 1.022	2.268 ± 0.517
Val	5.274 ± 0.186	5.195 ± 0.142	5.390 ± 0.079	5.335 ± 0.104	5.198 ± 0.097
Phe	4.371 ± 0.119	4.278 ± 0.146	4.473 ± 0.098	4.419 ± 0.074	4.416 ± 0.108
Ile	4.796 ± 0.206	4.742 ± 0.132	4.903 ± 0.069	4.859 ± 0.074	4.901 ± 0.383
Leu	8.333 ± 0.237	8.107 ± 0.263	8.307 ± 0.104	8.207 ± 0.103	8.150 ± 0.166
Lys	10.393 ± 0.362	10.222 ± 0.303	10.479 ± 0.147	10.794 ± 0.186	10.533 ± 0.323

Appendix J Raw Data, Dry Matter Analysis: Feed Frequency

Raw data from dry matter analysis in the feed frequency experiment are found in the following figure.

Feedings/id:parallel	Wet weight [g]	Dry weight [g]	Water [%]
1/1:1	2.02	0.87	56.7
1/1:2	2.00	0.81	59.2
1/2:1	2.00	0.79	60.6
1/2:2	2.01	0.81	59.7
1/3:1	2.02	0.72	64.1
1/3:2	2.01	0.69	65.5
1/4:1	2.00	0.62	68.9
1/4:2	2.02	0.64	68.3
1/5:1	2.02	0.65	67.6
1/5:2	2.00	0.65	67.5
1/6:1	2.02	0.68	66.6
1/6:2	2.01	0.69	65.8
3/1:1	2.01	0.63	68.8
3/1:2	2.03	0.69	66.0
3/2:1	1.99	0.55	72.1
3/2:2	2.02	0.56	72.4
3/3:1	1.99	0.75	62.6
3/3:2	2.00	0.76	61.8
3/4:1	2.00	0.69	65.7
3/4:2	2.00	0.68	65.9
3/5:1	2.02	0.64	68.3
3/5:2	2.00	0.64	68.0
3/6:1	1.98	0.58	70.7
3/6:2	2.02	0.60	70.1
Average 1 feeding	2.01	0.72	64.2
Average 3 feedings	2.01	0.65	67.7
st.dev 1 feeding	0.01	0.08	4.1
st.dev 3 feeding	0.01	0.07	3.4

Appendix K Raw Data, Dry Matter Analysis: Diet

Table K1: Raw data from dry matter analysis from the diet experiment (Group A and B), two parallels of each sample (par.).

Group/fish	Wet par. 1 [g]	Wet par. 2 [g]	Dry par. 1 [g]	Dry par. 2 [g]	Water par.1 [%]	Water par. 2 [%]
A/1	2.128	2.026	0.806	0.762	62.1	62.4
A/2	2.050	2.005	0.758	0.746	63.1	62.8
A/3	2.080	2.693	0.824	0.833	60.4	69.1
A/4	2.157	1.999	0.799	0.737	63.0	63.1
A/5	2.089	2.058	0.801	0.792	61.6	61.5
A/6	2.015	1.991	0.759	0.749	62.3	62.4
A/7	1.965	3.057	0.717	1.114	63.5	63.6
A/8	2.031	2.070	0.766	0.734	62.3	64.6
A/9	1.996	2.029	0.748	0.766	62.5	62.3
A/10	2.067	1.999	0.765	0.740	63.0	63.0
A/11	2.056	1.932	0.762	0.714	62.9	63.0
A/12	2.125	1.981	0.835	0.777	60.7	60.8
A/13	2.004	2.026	0.751	0.757	62.5	62.7
A/14	2.016	2.034	0.708	0.713	64.9	64.9
A/15	2.014	1.995	0.719	0.712	64.3	64.3
B/1	2.035	2.029	0.801	0.800	60.6	60.6
B/2	2.005	2.020	0.757	0.763	62.2	62.2
B/3	2.030	2.029	0.810	0.805	60.1	60.3
B/4	2.033	2.011	0.710	0.704	65.1	65.0
B/5	1.995	2.021	0.718	0.722	64.0	64.3
B/6	1.995	2.047	0.746	0.766	62.6	62.6
B/7	2.004	2.025	0.730	0.738	63.6	63.5
B/8	2.002	1.999	0.742	0.739	62.9	63.0
B/9	1.991	1.998	0.747	0.748	62.5	62.6
B/10	2.017	2.002	0.761	0.756	62.3	62.2
B/11	1.994	1.997	0.748	0.748	62.5	62.5
B/12	1.986	1.983	0.707	0.706	64.4	64.4
B/13	1.989	2.005	0.768	0.768	61.4	61.7
B/14	1.981	1.971	0.750	0.746	62.1	62.2
B/15	1.994	2.013	0.778	0.782	61.0	61.2

Table K2: Raw data from dry matter analysis from the diet experiment (Group C-E), two parallels of each sample (par.).

Feedings/id:spot	Hardness 1 [N]	Hardness 2 [N]	Break[N]	Compr.break [mm]	Height [mm]	
C/1	2.000	2.005	0.763	0.759	61.9	62.1
C/2	2.038	2.024	0.779	0.767	61.8	62.1
C/3	1.992	2.017	0.766	0.768	61.5	61.9
C/4	2.055	1.988	0.729	0.709	64.5	64.4
C/5	2.004	1.965	0.701	0.689	65.0	65.0
C/6	1.924	2.038	0.704	0.750	63.4	63.2
C/7	2.016	2.017	0.781	0.788	61.2	60.9
C/8	2.056	2.014	0.792	0.773	61.5	61.6
C/9	1.966	2.021	0.759	0.779	61.4	61.5
C/10	1.978	2.006	0.755	0.770	61.8	61.6
C/11	2.050	1.986	0.772	0.750	62.4	62.3
C/12	2.024	2.052	0.778	0.791	61.5	61.5
C/13	2.050	1.988	0.775	0.745	62.2	62.5
C/14	2.032	1.958	0.738	0.704	63.7	64.1
C/15	1.958	1.979	0.712	0.718	63.6	63.7
D/1	2.006	1.988	0.800	0.796	60.1	59.9
D/2	2.021	2.004	0.731	0.729	63.8	63.6
D/3	1.996	2.004	0.772	0.780	61.3	61.1
D/4	2.032	1.989	0.774	0.764	61.9	61.6
D/5	2.015	1.993	0.758	0.739	62.4	62.9
D/6	2.015	1.995	0.767	0.757	61.9	62.1
D/7	2.022	2.020	0.780	0.786	61.4	61.1
D/8	2.003	1.963	0.763	0.724	61.9	63.1
D/9	1.978	2.017	0.601	0.618	69.6	69.4
D/10	1.994	2.011	0.717	0.701	64.1	65.1
E/1	1.993	2.012	0.723	0.729	63.7	63.8
E/2	2.019	2.000	0.856	0.847	57.6	57.6
E/3	2.001	2.014	0.734	0.736	63.3	63.4
E/4	2.073	2.018	0.744	0.724	64.1	64.1
E/5	2.005	2.044	0.822	0.842	59.0	58.8
E/6	2.033	1.996	0.772	0.797	62.0	60.1
E/7	2.000	1.998	0.751	0.749	62.5	62.5
E/8	1.977	2.028	0.775	0.802	60.8	60.5
E/9	2.024	2.005	0.734	0.729	63.7	63.7
E/10	2.020	2.011	0.757	0.754	62.5	62.5

Appendix L Texture Analysis: Feed Frequency

Table L1: Raw data from texture analysis of fish fed one time daily. Hardness at first and second bite, breaking strength, compression at break and height at contact.

Feedings/id:spot	Hardness 1 [N]	Hardness 2 [N]	Break[N]	Compr.break [mm]	Height [mm]
1/1:1	4.06	2.83	6.15	11.4	31.7
1/1:2	3.98	3.36	6.17	11.2	32.3
1/1:3	4.06	4.27	4.87	10.2	30.1
1/1:4	5.18	4.26	5.12	10.3	23.8
1/1:5	5.57	5.11	6.89	9.0	20.5
1/2:1	4.99	4.18	5.70	12.4	31.2
1/2:2	3.77	3.08	5.81	11.4	31.3
1/2:3	4.02	3.20	5.05	13.4	31.0
1/2:4	4.72	3.78	3.90	11.7	26.9
1/2:5	5.77	4.64	5.43	10.0	21.7
1/3:1	4.99	3.85	5.86	11.6	34.2
1/3:2	4.25	3.25	6.01	12.5	33.1
1/3:3	4.33	3.39	4.67	10.3	29.1
1/3:4	4.77	3.87	4.58	13.3	28.8
1/3:5	6.29	5.25	5.53	11.2	22.7
1/4:1	4.25	3.07	6.40	12.8	31.1
1/4:2	4.94	3.79	5.85	12.5	32.6
1/4:3	4.73	3.55	6.84	12.8	30.9
1/4:4	4.91	4.11	4.10	10.1	26.1
1/4:5	6.95	5.73	5.68	11.1	25.5
1/5:1	5.48	4.27	5.87	12.1	32.1
1/5:2	4.38	3.62	5.91	11.7	32.4
1/5:3	5.76	4.14	7.74	12.6	32.6
1/5:4	7.45	5.87	6.03	9.4	25.7
1/5:5	5.47	4.67	5.99	11.0	23.4
1/6:1	5.16	3.89	4.15	6.4	28.7
1/6:2	4.51	3.46	5.07	12.5	30.8
1/6:3	3.70	3.08	3.71	13.4	29.0
1/6:4	4.19	3.29	5.34	9.4	26.3
1/6:5	6.08	4.96	5.36	10.9	26.1

Table L2: Raw data from texture analysis of fish fed one time daily. Hardness at first and second bite, breaking strength, compression at break and height at contact.

Feedings/id:spot	Hardness 1 [N]	Hardness 2 [N]	Break[N]	Compr.break [mm]	Height [mm]
3/1:1	4.74	3.63	2.87	10.0	29.1
3/1:2	5.65	4.13	6.47	12.3	32.1
3/1:3	4.67	3.60	6.14	12.0	31.2
3/1:4	5.02	4.06	5.13	12.5	27.0
3/1:5	4.81	3.96	5.44	9.4	20.9
3/2:1	5.72	4.62	5.42	10.0	29.4
3/2:2	4.12	2.89	6.25	9.5	28.7
3/2:3	5.35	4.09	7.17	10.4	29.1
3/2:4	5.75	3.57	5.51	9.5	28.3
3/2:5	4.02	3.10	4.78	13.0	27.3
3/3:1	4.86	3.69	6.56	12.1	31.7
3/3:2	4.68	3.49	7.44	11.4	30.4
3/3:3	3.68	2.78	3.75	11.4	28.4
3/3:4	4.24	3.26	4.21	13.0	27.9
3/3:5	5.61	5.04	6.62	8.8	21.0
3/4:1	4.50	3.44	4.42	10.8	29.0
3/4:2	4.44	3.13	4.42	9.8	28.6
3/4:3	3.77	3.17	2.71	14.6	27.3
3/4:4	5.00	4.25	4.04	10.1	26.9
3/4:5	5.28	4.49	4.04	10.0	22.8
3/5:1	4.81	3.28	6.34	11.0	30.3
3/5:2	4.27	3.06	5.74	13.5	30.5
3/5:3	3.87	2.96	6.56	9.8	30.0
3/5:4	4.42	3.77	3.73	9.9	26.2
3/5:5	5.27	4.40	5.07	10.6	21.9
3/6:1	3.45	2.94	1.90	11.8	29.8
3/6:2	4.67	3.70	5.26	10.5	30.6
3/6:3	4.97	3.82	6.53	11.3	29.5
3/6:4	4.28	3.48	4.60	11.8	27.6
3/6:5	4.54	3.97	5.20	11.2	23.9

Appendix M Raw data Cathepsin B+L: Feed Frequency

Table M: Raw data, fluorescence intensity from cathepsin b+l proteolytic activity for fish fed one and three times daily. 3 parallels for each fish (par.). The results are subtracted with the intensity measured for a tube without substract (I^{blank}=20.57).

Feedings/fish	I par.1	I par.2	I par.3	I Average	st.dev
1/1	36.46	32.78	29.06	32.77	3.70
1/2	33.40	46.41	41.73	40.51	6.59
1/3	47.36	48.88	48.69	48.31	0.83
1/4	45.12	51.25	43.86	46.74	3.95
1/5	48.10	37.72	50.59	45.47	6.83
1/6	39.60	47.51	44.35	43.82	3.98
3/1	18.65	22.42	32.34	24.47	7.07
3/2	25.77	23.88	27.41	25.69	1.77
3/3	22.44	27.44	33.17	27.68	5.37
3/4	24.56	28.22	29.84	27.54	2.71
3/5	32.21	35.70	39.26	35.72	3.53
3/6	32.58	37.24	23.11	30.98	7.20
Average 1 feeding	41.67	44.09	43.05	42.94	4.31
Average 3 feedings	26.03	29.15	30.86	28.68	4.61
St.dev 1 feeding	6.09	7.21	7.60	5.65	2.20
St.dev 3 feedings	5.49	6.09	5.50	4.10	2.29

Appendix N Raw Data Growth: Diet

Table N1: Raw data, Growth. Average weight between week 14. 2014 and week 49. 2014, provided by the company (first 36 weeks).

Week	Group A [g]	Group B [g]	Group C [g]	Group D [g]	Group E [g]
2014/14	100.40	100.60	0.00	0.00	0.00
2014/15	110.80	111.60	0.00	0.00	0.00
2014/16	123.40	124.60	0.00	0.00	0.00
2014/17	140.70	142.60	0.00	0.00	0.00
2014/18	151.60	153.10	0.00	0.00	0.00
2014/19	174.80	177.90	81.60	0.00	93.40
2014/20	174.30	177.30	93.40	0.00	106.20
2014/21	184.80	183.70	98.00	0.00	109.00
2014/22	216.90	212.20	112.40	0.00	123.90
2014/23	231.00	230.00	120.00	0.00	132.00
2014/24	269.70	264.20	137.30	0.00	149.60
2014/25	292.00	281.00	143.00	0.00	158.00
2014/26	342.00	329.30	169.20	0.00	184.70
2014/27	379.20	366.80	192.50	0.00	207.30
2014/28	429.50	417.60	230.80	95.00	249.50
2014/29	488.60	474.70	269.50	115.00	290.70
2014/30	558.30	541.10	311.80	137.20	336.20
2014/31	641.40	620.50	363.10	167.30	386.60
2014/32	737.00	713.20	426.90	200.50	451.10
2014/33	846.90	822.30	510.80	238.80	525.20
2014/34	935.50	914.60	576.90	282.70	591.20
2014/35	1 045.40	1 030.80	664.20	300.60	669.50
2014/36	1 173.40	1 152.20	759.90	371.00	764.10
2014/37	1 300.80	1 277.40	862.90	457.80	869.60
2014/38	1 344.00	1 304.30	906.50	509.60	954.80
2014/39	1 436.20	1 400.40	988.40	574.70	1 041.70
2014/40	1 553.60	1 487.40	1 059.90	635.30	1 130.50
2014/41	1 671.50	1 572.20	1 131.60	685.50	1 209.20
2014/42	1 749.70	1 632.00	1 195.20	722.80	1 266.60
2014/43	1 827.70	1 722.40	1 266.30	756.30	1 316.60
2014/44	1 893.10	1 799.40	1 327.70	786.70	1 362.90
2014/45	1 979.70	1 897.50	1 407.40	834.70	1 421.40
2014/46	2 046.30	1 963.70	1 457.10	873.30	1 469.30
2014/47	2 128.80	2 054.30	1 539.50	919.00	1 517.90
2014/48	2 218.80	2 157.70	1 623.50	961.10	1 585.70
2014/49	2 310.00	2 247.30	1 695.70	1 013.20	1 645.00

Table N2: Raw data, growth. Average weight between week 50. 2014 and week 32. 2015, provided by the company (last 35 weeks).

Week	Group A [g]	Group B [g]	Group C [g]	Group D [g]	Group E [g]
2014/50	2 410.10	2 353.30	1 775.40	1 061.80	1 715.50
2014/51	2 491.30	2 449.70	1 853.00	1 119.00	1 774.80
2014/52	2 568.00	2 517.70	1 916.50	1 160.20	1 821.00
2015/01	2 617.80	2 578.20	1 974.20	1 207.20	1 868.70
2015/02	2 701.20	2 654.30	2 037.40	1 267.30	1 945.20
2015/03	2 794.10	2 726.00	2 093.80	1 320.70	2 013.10
2015/04	2 884.00	2 807.80	2 165.10	1 370.10	2 083.90
2015/05	2 968.80	2 875.30	2 241.40	1 411.40	2 145.00
2015/06	3 052.20	2 924.80	2 297.30	1 443.00	2 196.20
2015/07	3 129.50	2 996.40	2 358.10	1 480.90	2 267.10
2015/08	3 239.50	3 070.90	2 436.90	1 515.40	2 349.10
2015/09	3 356.50	3 154.00	2 528.00	1 580.70	2 444.10
2015/10	3 465.60	3 238.00	2 612.50	1 633.30	2 534.80
2015/11	3 562.80	3 315.20	2 691.30	1 686.10	2 610.00
2015/12	3 623.80	3 372.80	2 749.70	1 729.00	2 669.10
2015/13	3 698.30	3 439.90	2 822.60	1 782.90	2 744.70
2015/14	3 820.80	3 536.30	2 874.50	1 799.90	2 708.00
2015/15	3 925.20	3 635.50	2 959.10	1 855.30	2 784.60
2015/16	4 035.70	3 740.30	3 014.70	1 914.80	2 856.70
2015/17	4 123.30	3 818.40	3 096.90	1 985.00	2 947.60
2015/18	4 233.90	3 906.20	3 187.20	2 052.10	3 019.50
2015/19	4 340.70	3 975.70	3 208.20	2 074.40	3 043.10
2015/20	4 359.20	4 022.80	3 247.30	2 084.90	3 086.20
2015/21	4 491.40	4 143.60	3 376.80	2 164.00	3 196.70
2015/22	4 639.40	4 272.60	3 491.60	2 256.90	3 302.70
2015/23	4 816.50	4 450.80	3 651.40	2 361.10	3 435.70
2015/24	4 828.20	4 594.50	3 783.30	2 462.00	3 544.90
2015/25	4 982.80	4 626.90	3 826.10	2 486.50	3 551.00
2015/26	5 143.80	4 892.10	3 953.30	2 583.00	3 629.80
2015/27	5 427.20	5 137.00	4 138.50	2 738.30	3 805.90
2015/28	5 775.00	5 442.80	4 200.20	2 788.40	3 889.50
2015/29	5 775.00	5 496.70	4 384.70	2 962.00	4 012.50
2015/30	0.00	0.00	4 642.90	3 155.90	4 239.60
2015/31	0.00	0.00	4 912.90	3 353.90	4 463.50
2015/32	0.00	0.00	4 912.90	3 353.90	4 463.50

Appendix O Raw Data, Slaughter Data: Diet

Relative Growth Index, RGI

$$RGI = (L_t/L_s) * 100$$

L_t = observed length at age (t)
L_s = predicted age-specific standard length.

Biological and Economical Feed Convertion Ratios, BFCR and EFCR

$$BFCR = Weight_{Eaten}/(FW_{Fish}\breve{}IW_{Fish} + W_{Morts}$$

$Weight_{Eaten}$ = Total weight of feed eaten by the fish (kg), IW_{Fish} = Total initial weight of fish [kg],
FW_{Fish} = Total final weight of live fish [kg],
W_{Morts} = Total weight of dead fish over the cycle [kg]

$$EFCR = Weight_{Feed}/(FW_{Fish}\breve{}IW_{Fish})$$

WeightFeed = total weight of feed given to the unit [kg],
IWFish = Total weight of live fish at the start of the period [kg],
FWFish = Total weight of live fish at the end of the period [kg]

Coefficient of Variation, CV:

$$CV = (ST.DEV_{weight}/W_{brutto}) * 100$$

$ST.DEV_{weight}$ = Standard deviation of slaughter weights,
W_{brutto} = Average slaughter weight [kg]

Viscera [%]:

$$Viscera = (W_{netto}/W_{brutto}) * 100$$

W_{netto} = Gutted weight [g],
W_{brutto} = Slaughter weight [g]

Table O1: Raw data, slaughter data, provided by the company (first 36 weeks).

	Group A	Group B	Group C	Group D	Group E
Smolt weight	100	99	77	79	84
RGI	105	103	103	101	100
Total loss [%]	3.7	3.8	3.6	7.6	3.3
BFCR	1.15	1.16	1.27	1.26	1.3
ØFCR	1.16	1.17	1.28	1.28	1.32
Spread Slaughter Weight [CV]	21.4	20.1	20.7	22.7	19.5
Days in sea	436	444	461	479	525
Average slaughter weight	5 362	5 220	5 937	5 454	6 273
Average gutted weight	4 557	4 489	5 076	4 647	5 336
Superior [%]	98.2	98.1	97.6	97.8	98.4

Slaughter report Group A

	Group A	Global Gap nr: XXXXXXX	SALMAR

Lokalitetsnummer	33177
Fiskegruppe (stamme / opphav)	AquaGen
Smoltprodusent	Follafoss
Utsettdato	05/04/14
Dato satt på sulting	13/07/15
Er fisken GlogalGAP sertifisert?	Ja
Er fisken PIT TAG merket?	Nei
Er det brukt kitinhemmere (ektobann/Reeleze)?	Nei
Dato planlagt slaktet	
Dato virkelig utslaktet	23-Jul
Virkelig økonomisk fôrfaktor	
Innfarging (mg Asta/kg prod)	

Grå farge fylles ut av ansvarlig lokalitet
Gul farge fylles ut av ansvarlig kvalitet og kontroll
Hvit farge skal ikke røres.

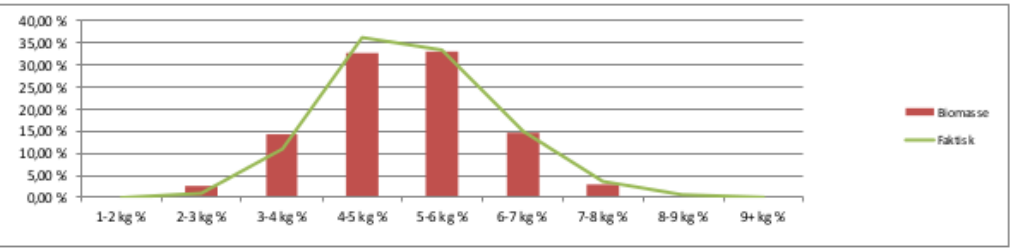
Skjema utfylt:
Dato og sign. 13.07.2015 XXX
Dato og sign. 10.08 SØ
Hvis avvik:
Dato og sign.

Kvantitative mål

Antall fisk		Snittvekt sløyd vekt (gram)			Biomasse sløyd vekt (kilo)	
Fra oppdrett	78 250	Fra oppdrett (g)		4 750	Fra oppdrett (kg)	371 688
Fra slakteri	78 573	Fra slakteri (g)		4 890	Fra slakteri (kg)	383 333
Utkast slakteri	15					
Avvik	338	Avvik		140	Avvik	11 645
Avvik i %	0.43	Avvik i %		2.95	Avvik i %	3.13

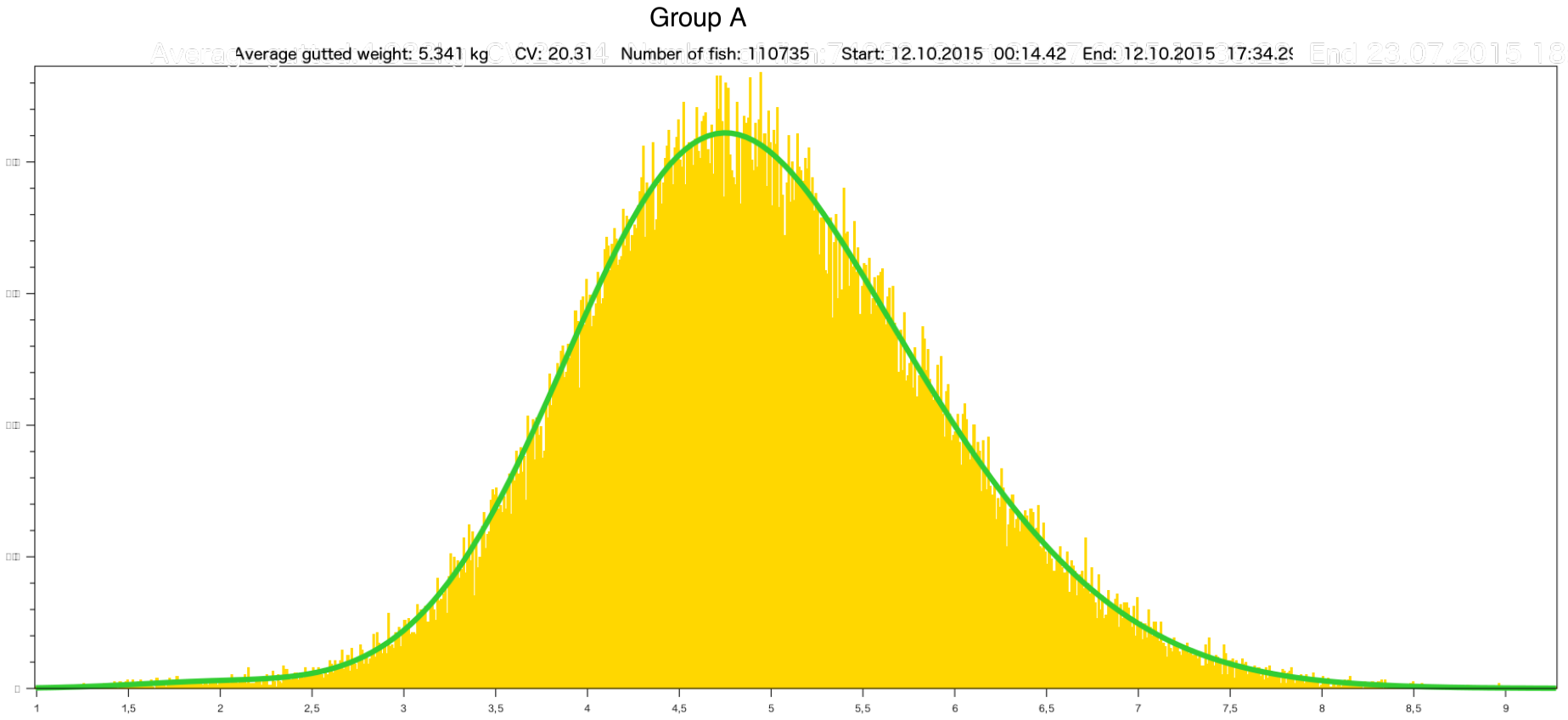
Størrelsesfordeling

Informasjon fra lokalitet			Tilbakemelding fra Prosessing	
Antatt størrelsesfordeling sløyd	**Antall**	**Biomasse**	**Størrelsesfordeling sløyd**	
1-2 kg %	0.59 %	0.19 %	1-2 kg %	0.08 %
2-3 kg %	4.87 %	2.56 %	2-3 kg %	0.90 %
3-4 kg %	19.16 %	14.12 %	3-4 kg %	10.79 %
4-5 kg %	34.43 %	32.62 %	4-5 kg %	36.07 %
5-6 kg %	28.32 %	32.79 %	5-6 kg %	33.15 %
6-7 kg %	10.66 %	14.58 %	6-7 kg %	14.87 %
7-8 kg %	1.83 %	2.88 %	7-8 kg %	3.66 %
8-9 kg %	0.14 %	0.25 %	8-9 kg %	0.48 %
9+ kg %	0.01 %	0.01 %	9+ kg %	0.00 %
Sum (%)	100.00 %	100.01 %		
Spredning (CV i %)	23.00		**Spredning (%)**	
Snittvekt (kg)	4.75			
Er fisken sortert (ja/nei)(dato)	Nei			
Dato for siste individkontroll	Delslakt midten av juni			
Har fisken svømt i Vektramme (ja/nei)	Nei			



Kvalitative mål

Fiskekvalitet				Fiskekvalitet
Andel Sup %	98.00 %			98.41 %
Andel Prod %	1.95 %			1.57 %
Årsak	Kj.mdn, sår, "pinner" og deformiteter.			
Utkast %	0.05 %			0.02 %
Årsak				
K-faktor	1.30			
Kommentar				
Farge	7.12			
Kommentar	Farge ved delslakt i juni.			
Verdi siste fett prøve (%	17.50			
Helsetilstand/ generell kommentar	God helse. Lav dødelighet. Påvist PD, AGD og HSMB i anlegget			



Slaughter weights Group A

Slaughter report Group B

Group B

Global Gap nr: XXXXXX

SALMAR

Lokalitetsnummer	33177
Fiskegruppe (stamme / opphav)	AquaGen
Smoltprodusent	Follafoss
Utsettdato	05/04/14
Dato satt på sulting	14/07/15
Er fisken GlogalGAP sertifisert?	Ja
Er fisken PIT TAG merket?	Nei
Er det brukt kitinhemmere (ektobann/Reeleze)?	Nei
Dato planlagt slaktet	
Dato virkelig utslaktet	24/07/15
Virkelig økonomisk förfaktor	
Innfarging (mg Asta/kg prod)	

Grå farge fylles ut av ansvarlig lokalitet
Gul farge fylles ut av ansvarlig kvalitet og kontroll
Hvit farge skal ikke røres.

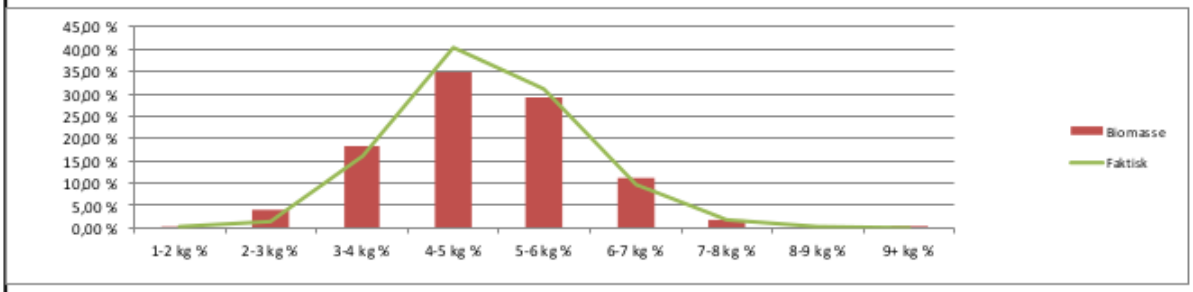
Skjema utfylt:
Dato og sign. 13.07.2015 XXXX
Dato og sign. 10.08 SØ
Hvis avvik:
Dato og sign.

Kvantitative mål

Antall fisk		Snittvekt sløyd vekt (gram)			Biomasse sløyd vekt (kilo)	
Fra oppdrett	121 520	Fra oppdrett (g)		4 515	Fra oppdrett (kg)	548 663
Fra slakteri	124 160	Fra slakteri (g)		4 640	Fra slakteri (kg)	575 569
Utkast slakteri	71					
Avvik	2 711	Avvik		125	Avvik	26 906
Avvik i %	2.23	Avvik i %		2.77	Avvik i %	4.90

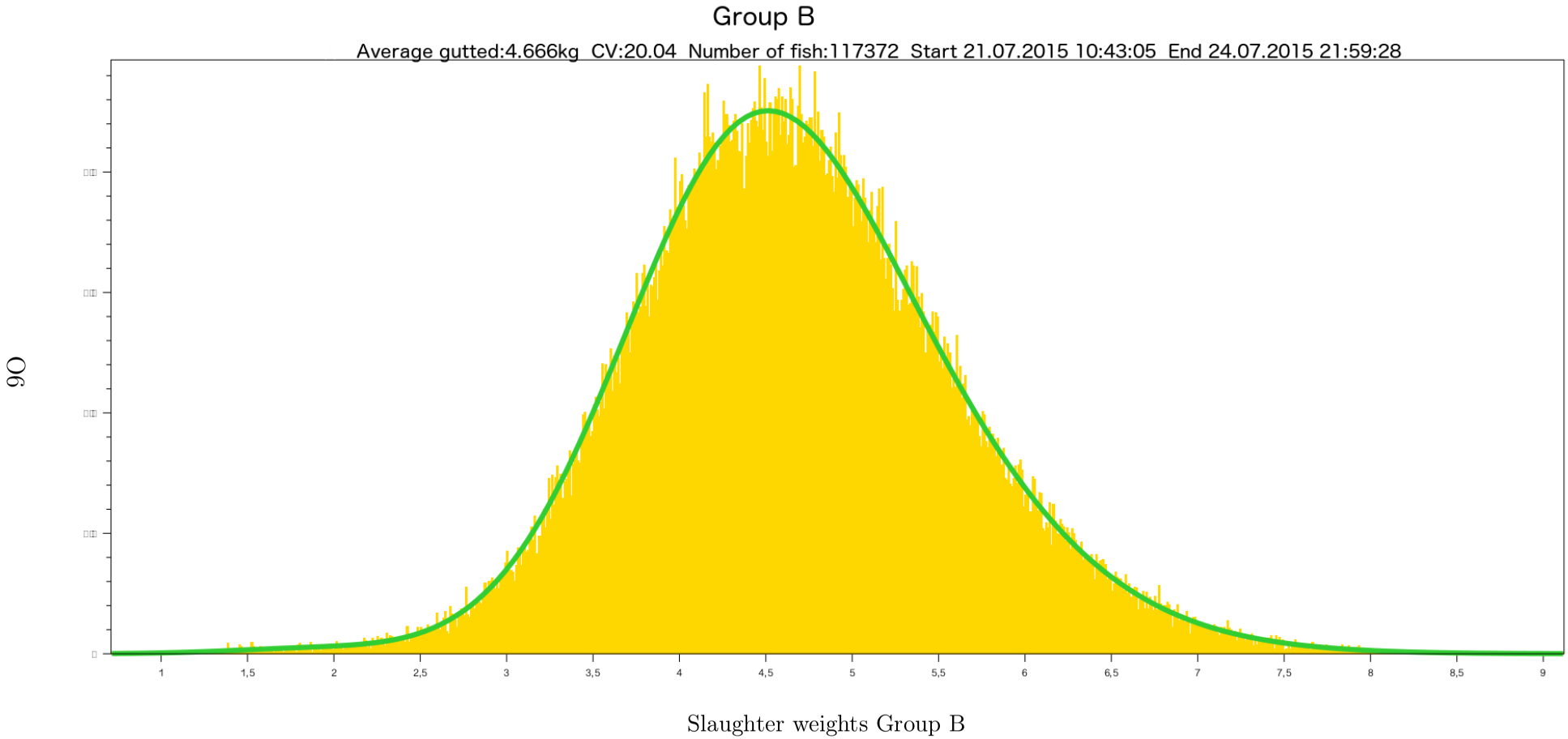
Størrelsesfordeling

Informasjon fra lokalitet			Tilbakemelding fra Prosessing	
Antatt størrelsesfordeling sløyd	Antall	Biomasse	Størrelsesfordeling sløyd	
1-2 kg %	1.15 %	0.38 %	1-2 kg %	0.10 %
2-3 kg %	7.39 %	4.09 %	2-3 kg %	1.38 %
3-4 kg %	23.54 %	18.24 %	3-4 kg %	16.00 %
4-5 kg %	34.87 %	34.75 %	4-5 kg %	40.28 %
5-6 kg %	24.08 %	29.33 %	5-6 kg %	30.87 %
6-7 kg %	7.74 %	11.14 %	6-7 kg %	9.50 %
7-8 kg %	1.15 %	1.91 %	7-8 kg %	1.69 %
8-9 kg %	0.08 %	0.15 %	8-9 kg %	0.19 %
9+ kg %	0.00 %	0.01 %	9+ kg %	0.00 %
Sum (%)	100.00 %	100.02 %		
Spredning (CV i %)	24.50		**Spredning (%)**	
Snittvekt (kg)	4.52			
Er fisken sortert (ja/nei)(dato)	Nei			
Dato for siste individkontroll	Delslakt 23.06.15			
Har fisken svømt i Vektramme (ja/nei)	Nei			



Kvalitative mål

Fiskekvalitet				Fiskekvalitet
Andel Sup %	98.50 %			98.06 %
Andel Prod %	1.45 %			1.89 %
Årsak	Kj.mdn, sår, "pinner" og deformiteter.			
Utkast %	0.05 %			0.06 %
Årsak				
K-faktor	1.30			
Farge	7.1			
Verdi siste fett prøve (%)	16,31 ved delslakt			
Helsetilstand/ generell kommentar	God helse. Lav dødelighet. Påvist PD, AGD og HSMB i anlegget.			



Slaughter weights Group B

Slaughter report Group C

	Group C	Global Gap nr: XXXXXXX	SALMAR

Lokalitetsnummer	33177
Fiskegruppe (stamme / opphav)	AquaGen
Smoltprodusent	Follafoss
Utsettdato	07/05/14
Dato satt på sulting	22/09/15
Er fisken GlogalGAP sertifisert?	Ja
Er fisken PIT TAG merket?	Nei
Er det brukt kitinhemmere (ektobann/Reeleze)?	Nei
Dato planlagt slaktet	
Dato virkelig utslaktet	28/09/15
Virkelig økonomisk förfaktor	
Innfarging (mg Asta/kg prod)	

Grå farge fylles ut av ansvarlig lokalitet
Gul farge fylles ut av ansvarlig kvalitet og kontroll
Hvit farge skal ikke røres.

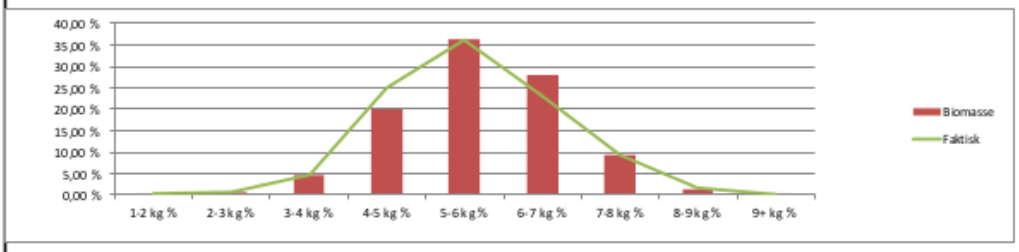
Skjema utfylt:
Dato og sign. 21.09.2015 XXX
Dato og sign. 10.10.15 SØ
Hvis avvik:
Dato og sign.

Kvantitative mål

Antall fisk		Snittvekt sløyd vekt (gram)			Biomasse sløyd vekt (kilo)	
Fra oppdrett	111 870	Fra oppdrett (g)		5 480	Fra oppdrett (kg)	613 048
Fra slakteri	107 776	Fra slakteri (g)		5 360	Fra slakteri (kg)	576 857
Utkast slakteri	97					
Avvik	-3 997	Avvik		-120	Avvik	-36 191
Avvik i %	-3.57	Avvik i %		-2.19	Avvik i %	-5.90

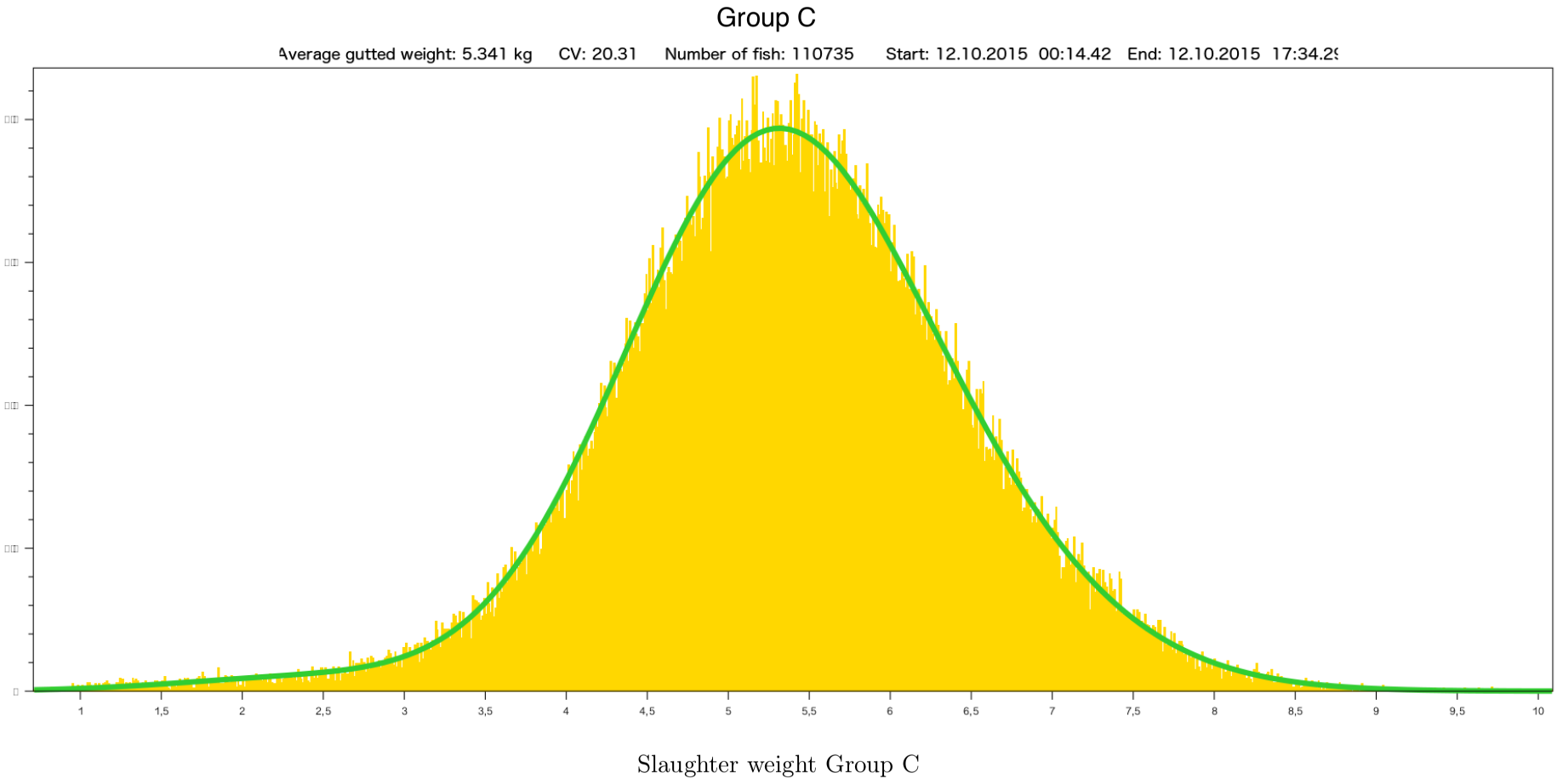
Størrelsesfordeling

Informasjon fra lokalitet			Tilbakemelding fra Prosessing	
Antatt størrelsesfordeling sløyd	**Antall**	**Biomasse**	**Størrelsesfordeling sløyd**	
1-2 kg %	0.06 %	0.02 %	1-2 kg %	0.16 %
2-3 kg %	0.96 %	0.44 %	2-3 kg %	0.64 %
3-4 kg %	7.29 %	4.65 %	3-4 kg %	4.44 %
4-5 kg %	24.36 %	20.01 %	4-5 kg %	25.00 %
5-6 kg %	36.01 %	36.14 %	5-6 kg %	36.07 %
6-7 kg %	23.58 %	27.97 %	6-7 kg %	22.78 %
7-8 kg %	6.83 %	9.34 %	7-8 kg %	9.25 %
8-9 kg %	0.87 %	1.35 %	8-9 kg %	1.60 %
9+ kg %	0.05 %	0.09 %	9+ kg %	0.06 %
Sum (%)	100.00 %	100.00 %		
Spredning (CV i %)	19.50		**Spredning (%)**	
Snittvekt (kg)	5.48			
Er fisken sortert (ja/nei)(dato)	Nei (ristslakt august)			
Dato for siste individkontroll	11/09/2015			
Har fisken svømt i Vektramme (ja/nei)	Nei			



Kvalitative mål

Fiskekvalitet			Fiskekvalitet	
Andel Sup %	93.00 %			95.21 %
Andel Prod %	4.50 %			4.70 %
Årsak	Kj.mdn, sår, "pinner" og			A 1,43 B 3,27
Utkast %	2.50 %			0.09 %
Årsak	Kj.mdn, sår, "pinner" og deformiteter.			
K-faktor	1.30			
Farge	7.1			
Kommentar	Siste fargeprøve: 12.08.15			
Verdi siste fett prøve (%)	16,2 - tatt 12.08.15			
Helsetilstand/ generell kommentar	God helse. Lav dødelighet. Påvist PD, AGD og HSMB i anlegget. Ikke klinisk. En del lus - siste telling: 1,55 holus og 3,35 bev.			



Slaughter weight Group C

Slaughter report Group D

	Group D	Global Gap nr: XXXXXXX	SALMAR

Lokalitetsnummer	33177
Fiskegruppe (stamme / opphav)	AquaGen
Smoltprodusent	Follafoss
Utsettdato	07/07/14
Dato satt på sulting	21/10/15
Er fisken GlogalGAP sertifisert?	Ja
Er fisken PIT TAG merket?	Nei
Er det brukt kitinhemmere (ektobann/Ræleze)?	Nei
Dato planlagt slaktet	
Dato virkelig utslaktet	02/11/15
Virkelig økonomisk förfaktor	
Innfarging (mg Asta/kg prod)	

Grå farge fylles ut av ansvarlig lokalitet
Gul farge fylles ut av ansvarlig kvalitet og kontroll
Hvit farge skal ikke røres.

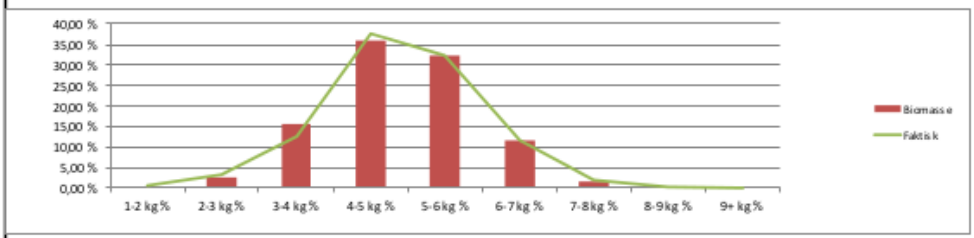
Skjema utfylt:
Dato og sign. 21.10.2015 XXX
Dato og sign. 03.11.15 SØ
Hvis avvik:
Dato og sign.

Kvantitative mål

Antall fisk		Snittvekt sløyd vekt (gram)			Biomasse sløyd vekt (kilo)	
Fra oppdrett	172 500	Fra oppdrett (g)		4 650	Fra oppdrett (kg)	802 125
Fra slakteri	171 640	Fra slakteri (g)		4 650	Fra slakteri (kg)	798 091
Utkast slakteri	124					
Avvik	-736	Avvik			Avvik	-4 034
Avvik i %	-0.43	Avvik i %			Avvik i %	-0.50

Størrelsesfordeling

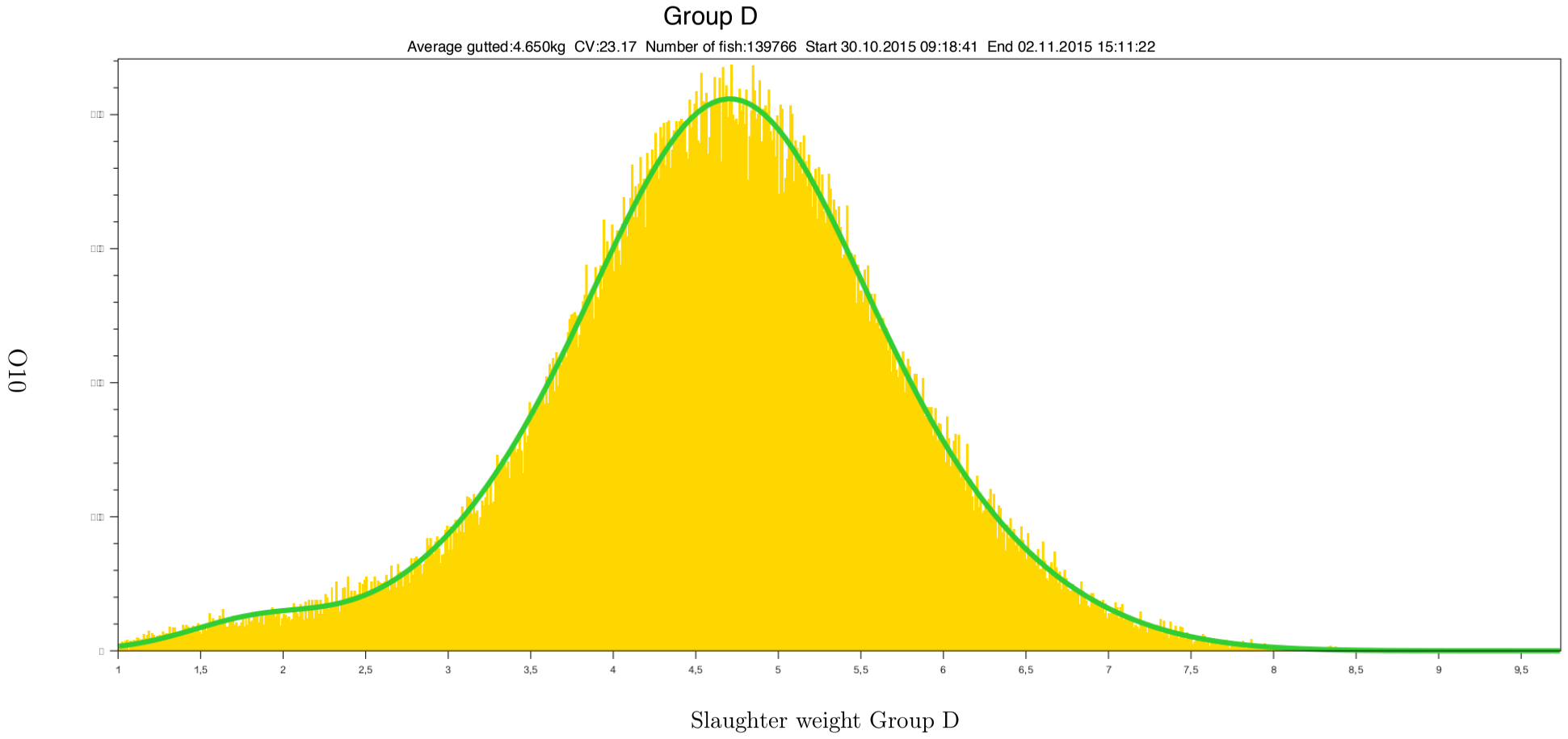
Informasjon fra lokalitet			Tilbakemelding fra Prosessing	
Antatt størrelsesfordeling sløyd	Antall	Biomasse	Størrelsesfordeling sløyd	
1-2 kg %	0.48 %	0.15 %	1-2 kg %	0.47 %
2-3 kg %	4.86 %	2.61 %	2-3 kg %	3.20 %
3-4 kg %	20.92 %	15.75 %	3-4 kg %	12.65 %
4-5 kg %	37.13 %	35.93 %	4-5 kg %	37.57 %
5-6 kg %	27.26 %	32.25 %	5-6 kg %	32.28 %
6-7 kg %	8.27 %	11.56 %	6-7 kg %	11.70 %
7-8 kg %	1.03 %	1.66 %	7-8 kg %	1.98 %
8-9 kg %	0.05 %	0.09 %	8-9 kg %	0.14 %
9+ kg %	0.00 %	0.00 %	9+ kg %	0.01 %
Sum (%)	100.00 %	100.00 %		
Spredning (CV i %)	22.00		**Spredning (%)**	
Snittvekt (kg)	4.65			
Er fisken sortert (ja/nei)(dato)	Nei			
Dato for siste individkontroll	18/10/2015			
Har fisken svømt i Vektramme (ja/nei)	Nei			



Kvalitative mål

Informasjon fra lokalitet			Tilbakemelding fra Prosessing	
Speilbergscore			Ant. målinger	
Melaningrad 0				
Melaningrad 1			Melanin dyp (grad3)	
Melaningrad 2			Melanin overflate (grad 2)	
Melaningrad 3			Melanin totalt	

Fiskekvalitet			Fiskekvalitet	
Andel Sup %	95.00 %			97.54 %
Andel Prod %	4.00 %			2.39 %
Årsak	Kj.mdn, sår, "pinner" og			A 1,69 B 0,70
Utkast %	1.00 %			0.07 %
Årsak	Kj.mdn, sår, "pinner" og deformiteter.			
K-faktor	1.25			
Farge	6.6			
Verdi siste fett prøve (%)	15.20			
Helsetilstand/ generell kommentar	God helse. Lav dødelighet. Påvist PD, AGD og HSMB i anlegget. Ikke klinisk. Mye lus.			


Group D
Average gutted:4.650kg CV:23.17 Number of fish:139766 Start 30.10.2015 09:18:41 End 02.11.2015 15:11:22
O10
1
1,5
2
2,5
3
3,5
4
4,5
5
5,5
6
6,5
7
7,5
8
8,5
9
9,5
Slaughter weight Group D

Slaughter report Group E

	Group E	Global Gap nr: XXXXXX	SALMAR

Lokalitetsnummer	30297
Fiskegruppe (stamme / opphav)	AquaGen
Smoltprodusent	Follafoss
Utsettdato	05/05/14
Dato satt på sulting	06/10/15
Er fisken GlogaGAP sertifisert?	Ja
Er fisken PIT TAG merket?	Nei
Er det brukt kitinhemmere (ektobann/Reekeze)?	Nei
Dato planlagt slaktet	
Dato virkelig utslaktet	12/10/15
Virkelig økonomisk fôrfaktor	
Innfarging (mg Asta/kg prod)	

Grå farge fylles ut av ansvarlig lokalitet
Gul farge fylles ut av ansvarlig kvalitet og kontroll
Hvit farge skal ikke røres.

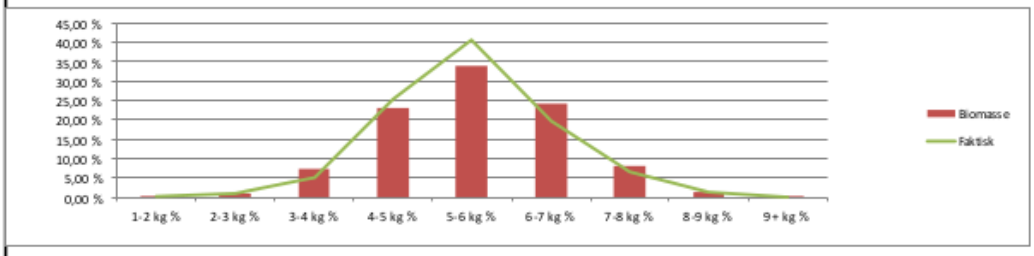
Skjema utfylt:
Dato og sign. 06.10.15 XXXX
Dato og sign. 14.10.15 SØ
Hvis avvik:
Dato og sign.

Kvantitative mål

Antall fisk		Snittvekt sløyd vekt (gram)		Biomasse sløyd vekt (kilo)	
Fra oppdrett	121 500	Fra oppdrett (g)	5 270	Fra oppdrett (kg)	640 305
Fra slakteri	117 696	Fra slakteri (g)	5 340		628 471
Utkast slakteri	82				
Avvik	-3 722	Avvik	70	Avvik	-11 834
Avvik i %	-3.06	Avvik i %	1.33	Avvik i %	-1.85

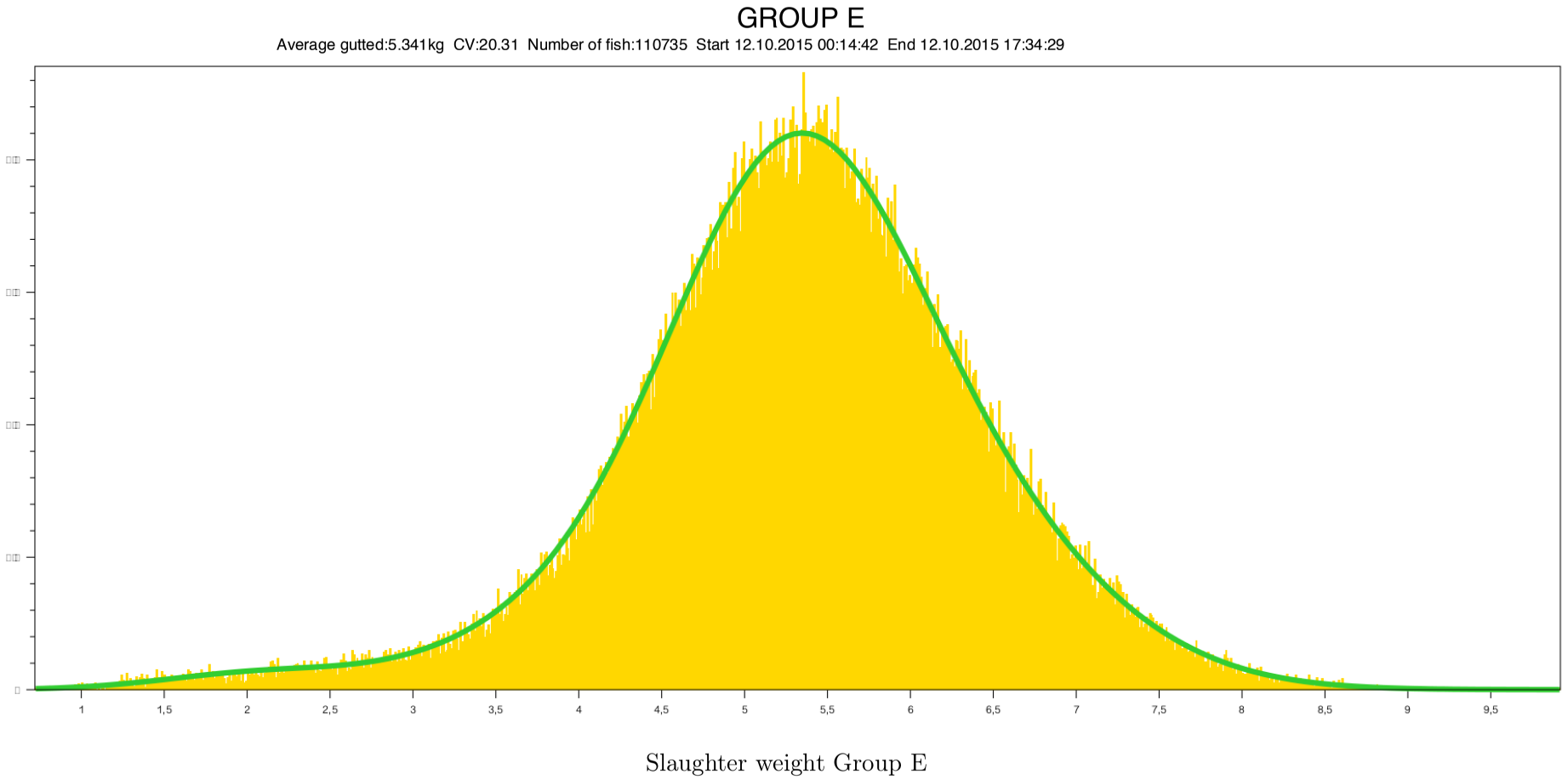
Størrelsesfordeling

Informasjon fra lokalitet			Tilbakemelding fra Prosessing	
Antatt størrelsesfordeling sløyd	**Antall**	**Biomasse**	**Størrelsesfordeling sløyd**	
1-2 kg %	0.24 %	0.07 %	1-2 kg %	0.23 %
2-3 kg %	2.27 %	1.08 %	2-3 kg %	0.93 %
3-4 kg %	11.16 %	7.41 %	3-4 kg %	5.02 %
4-5 kg %	27.13 %	23.16 %	4-5 kg %	25.35 %
5-6 kg %	32.76 %	34.19 %	5-6 kg %	40.74 %
6-7 kg %	19.66 %	24.25 %	6-7 kg %	19.73 %
7-8 kg %	5.86 %	8.33 %	7-8 kg %	6.66 %
8-9 kg %	0.86 %	1.39 %	8-9 kg %	1.24 %
9+ kg %	0.06 %	0.12 %	9+ kg %	0.10 %
Sum (%)	100.00 %	100.00 %		
Spredning (CV i %)	22.00		**Spredning (%)**	
Snittvekt (kg)	5.27			
Er fisken sortert (ja/nei)(dato)	Nei			
Dato for siste individkontroll	12/09/2015			
Har fisken svømt i Vektramme (ja/nei)	Nei			



Kvalitative mål

Fiskekvalitet			Fiskekvalitet	
Andel Sup %	93.00 %			97.23 %
Andel Prod %	2.00 %			2.61 %
Årsak	Kj.mdn, sår, pinner			A 1,24 B 1,37
Utkast %	5.00 %			0.07 %
Årsak	Kj.mdn, sår, pinner			
K-faktor	1.25			
Farge	6.9			
Kommentar	Siste fett- og fargeprøve: 21.09.15			
Verdi siste fett prøve (%)	15.40			
Helsetilstand/ generell kommentar	God helse. Lav dødelighet. Påvist PD, AGD og HSMB i anlegget. Mye lus.			



O12

Slaughter weight Group E