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Effects of functional ingredients from yeast in diets for Atlantic salmon (*Salmo salar*), from two genetic backgrounds, on growth performance and nutrient utilization

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Master of Science in Aquaculture

Acknowledgments

This master's thesis concludes my two years as an aquaculture master student at NMBU.
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Reidun Lund

Abstract

The overall aim of this thesis was to determine effects of functional ingredients in diets for Atlantic salmon, extracted from non- *saccharomyces* yeast, on growth performance and feed utilization. A 13 week (7 weeks in freshwater and 6 weeks in seawater) long feeding trial on pre- and post-smolt from two family groups (Gain and Prime) was conducted. Triple groups of salmon (initial weight ~ 30 grams) were fed one control diet containing a commercial like basal diet, the two other diets (diet 2 and diet 3) were added 0.1 % yeast. There were found no significant (p>0.05) differences in feed intake, growth rate of feed efficiency between diets within the same family. However, final weight measurements show that fish fed diet 3 had grown, statistically significantly larger than fish from the control group, in both families. Significant differences between Gain and Prime were relatively large (p<0.001). Gain had the highest growth rates but also the highest FCR (lower feed efficiency) in FW. Indicating positive effects of selective breeding programs for selection of improved growth in Atlantic salmon. However, opportunities for directly selection for feed efficiency should be explored, and not indirectly selected for via growth. Despite large family effects on all performance indicators in FW, family effect was not significant on ADC of crude protein, phosphorus and protein retention. Digestibility or nutrient retention could consequently not explain why the fish with the highest feed intake had lower feed efficiency in FW.

Sammendrag

Det overordnede målet med denne oppgaven var å bestemme effekten av funksjonelle ingredienser i dietter for Atlanterhavslaks på vekst og fôrutnyttelse, ekstrahert fra ikke-*saccharomyces* gjær. Forsøksperioden varte i 13 uker (7 uker i ferskvann og 6 uker i sjøvann) og fôret ble testet på laks før og etter smoltifisering, samt på to forskjellige familiegrupper (Gain and Prime). Laksen ble delt inn triplikater (startvekt ~ 30 gram) for hver diett i hver familiegruppe. Kontrollgruppen fikk en kontrolldiett som inneholdt en kommersiell basaldiett, de to andre diettene (diett 2 og diett 3) ble tilsatt 0.1% gjær. Ingen signifikante ($p > 0.05$) forskjeller ble funnet i fôrinntak, vekstrate eller fôreffektivitet mellom dietter i samme familie. Imidlertid viste sluttveken at fisk fôret med diett 3 hadde vokst, statistisk signifikant, mer enn fisk fra kontrollgruppen. Dette gjald for både Gain og Prime. De signifikante forskjeller mellom frisk fra Gain og Prime var relativt store ($p < 0.001$), gain hadde de høyeste vekstratene, men hadde også høyere FCR (lavere fôreffektivitet) i ferskvann. Dette Indikerer positive effekter av avlsprogram for seleksjon av Atlanterhavslaks på vekst. I midlertidig bør muligheter for direkte seleksjon for fôreffektivitet utforskes, og ikke indirekte selekteres for via vekst. Til tross for store familieeffekter funnet i ferskvann, var familieeffekten ikke signifikant på ADC av protein, fosfor og proteinretensjon. Følgelig kunne ikke fornøyelighet eller retensjon av næringsstoffer forklare hvorfor fisken med høyest fôrinntak hadde lavere fôreffektivitet i ferskvann.

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1 Introduction

Atlantic salmon is one of the most successful aquaculture species worldwide, having a higher production growth than aquaculture in general (Iversen et al., 2020). Norway is the largest producer of Atlantic salmon (*Salmo salar*) in the world. In 2019, 1.36 million tonnes of Atlantic salmon were produced (Figure 1.1), that is an increase of 5.9 % from the previous year (Statistics Norway, 2020). Several factors have been the basis of this success, such as breeding programs, enhanced production technology, vaccines, disease control and improved feed. The introduction of extrusion and vacuum-coating technology in the 1990s also allowed for a higher inclusion level of lipids in diets for Atlantic salmon. Making it possible to increase dietary energy levels, in order to reduce feed conversion ratios (Torrissen et al., 2011). Another important factor for the success of Norwegian salmon production has to do with the fact that Atlantic salmon are produced in relatively few countries. Only five countries contributed to 96 % of the production in 2015, where Norway accounted for 55.3 % (Iversen et al., 2020). Only in the last thirteen years, salmon production in Norway have more than doubled. It is expected that the production will continue to increase in the coming years, as the demand for aquatic food resourced likely will increase in line with the growing world population (FAO, 2020).

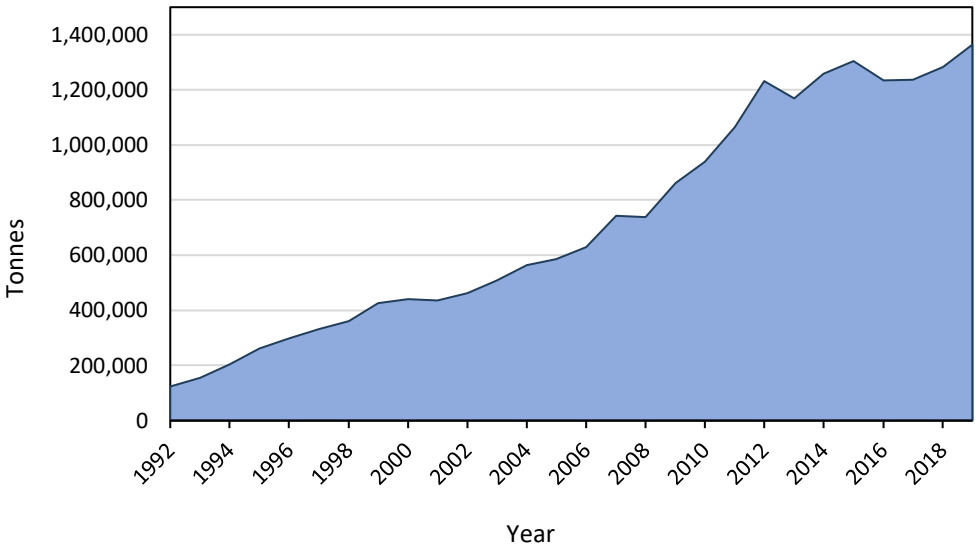

Figure 1.1: Amount (tonnes) of sold Atlantic salmon (*Salmo salar*) in Norway, from 1992 to 2019.

The rapid growth in the industry has led to a rise in stress and increased the risk of disease outbreaks. Consequently, this will lead to production loss and impediment of profitability and sustainability of the aquaculture industry, through negative effects on survival, feed utilization and weight gain (Torrecillas et al., 2007). In order to increase production to keep up with the growing demand, it is necessary to have a sustainable and robust production. This implies fish that can withstand disease and stress, without compromising growth performance and feed utilization. Good nutrition in salmon production is essential to produce a healthy, high quality product in an economical way. Costs connected to feed are the biggest expenses for salmon producers (Figure 1.2). Around 45 % of average production cost per kilo salmonid produced, are associated with costs connected to feed, making the need for high quality feed ingredients that support high performance and resilience (Directorate of Fisheries, 2020). Functional feeds are considered an effective tool to develop diets with a balanced nutrition, supplemented with feed additives for improved health and disease resistance. Among functional ingredients used in aquaculture, yeast cell wall components such as β-glucans and mannanoligosaccharides (MOS) are two of the promising immunostimulating ingredients used (Meena et al., 2013).

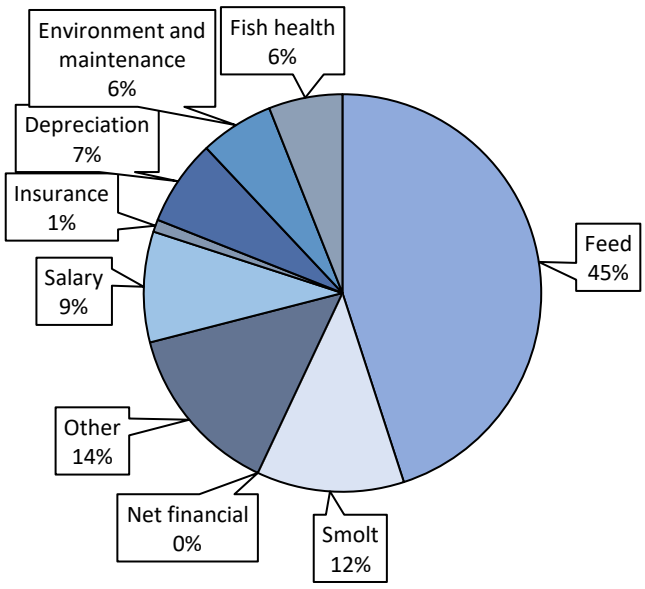

Figure 1.2: Production costs per. kg salmonoid fish produced in Norway in 2019 (Directorate of Fisheries, 2020).

The objective of this thesis will be to determine if growth performance and nutrient utilization of Atlantic salmon, from two different genetic backgrounds, can be enhanced when given functional ingredients extracted from non- *saccharomyces* yeast, in both freshwater (FW) and seawater (SW). Two yeast products, which due to confidentiality, containing unknown content and composition will be given to fish during each phase an Atlantic salmon undergoes during a life/ production cycle. In order to determine possible effects, it will be performed measurements on feed intake, growth rate, ability to utilize nutrients, digestibility and retention of protein.

2 Background

The background of this thesis will focus on breeding and breeding programs, Atlantic salmon's digestive system and functional ingredients, with focus on yeast and yeast cell wall extractions with growth and immunostimulating properties.

2.1 **Breeding**

Breeding is the process of selective mating of fish, or other animals, with desirable genetic traits in order to enhance these traits in future generations (Gjedrem & Baranski, 2010). In the 1970s a national selective breeding program on Atlantic salmon were started by AKVAFORSK in Norway. They collected eggs from more than 40 Norwegian rivers (Thodesen & Gjedrem, 2006). Initially, the focus was mainly on growth performance when selecting fish for broodstock candidates. Later on, the breeding strategies changed and became more complex, with focus on late sexual maturation, resistance for diseases, filet colour and fat distribution, in addition to growth. High heritability for economically important traits, combined with hight fecundity for both males and females and short generation intervals (around 4 years for Atlantic salmon) are important for the success of aquaculture breeding programs (Gjedrem et al., 2012). These factors can also help to explain the high genetic gain found in salmon breeding. Genetic gain is defined as the amount of increase in performance, achieved through selection, over a given time (often a generation interval). Measured by the difference in breeding value between a population and the populations offspring (Xu et al., 2017). This can be illustrated by a breeding stair, where each step represents a generation interval, and the height of the step is the genetic gain obtained by selection of favourable traits in the prior generation (Figure 2.1).

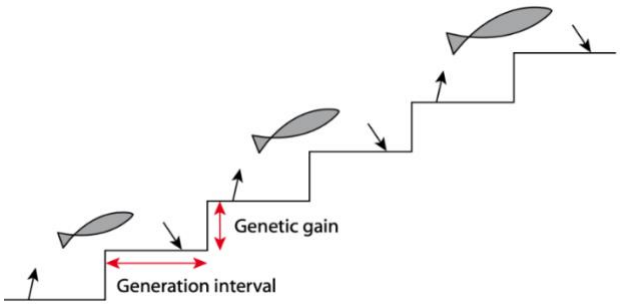

Figure 2.1: Breeding stair where the steps represent a generation interval, and the rise is the progress generated by the selection.

In Norway, selective breeding programs on Atlantic salmon have been crucial for the success of salmon farming. Today the industry is depending on genetically improved fish from breeding programs to stray competitive (Gjedrem & Baranski, 2010). Breeding is also an important part of increasing the future aquaculture production, by improve biological productivity of farmed species (Gjedrem et al., 2012). Small improvements on feed effectivity will have an impact on production cost, as well as better utilization of resources available (Kolstad et al., 2004). Genetic selection for improved feed efficiency mainly targets growth, but there have been reports on positive correlations between feed efficiency and growth rate (Janssen et al., 2017). Feed efficiency, often expressed by Feed Conversion Ratio (FCR), is a biological and mathematical way of illustrating the relationship between feed intake and mass produced (Kolstad et al., 2004). According to a study by Thodesen et al. (1999) Atlantic salmon demonstrated high genetic gain on growth rate after 5 generations of selection, compared with non-selected offspring from wild-caught salmon. The salmon selected for increased growth had a 113 % improved growth rate. In addition, they also found a favourable correlation response between FCR (-20 %) and protein retention (9 %). Implying that Atlantic salmon selected for improved growth, may improve both FCR and protein retention. There have also been detected differences in feed efficiency between families of Atlantic salmon. 10 full- sibling (offspring have the same male and female parents) families reared under the same conditions showed variation in feed efficiency, feed intake, growth and energy retention. Effect of families explained 77% of the variation in feed efficiency (Kolstad et al., 2004).

Although improved feed utilization is very important in salmon production, both economically and environmentally, there is no direct selection for this in breeding programs today. Feed efficiency has been indirectly selected for, by selecting for increased growth, with the assumption that it will lead to improved feed utilization (Thodesen et al., 2001). When recording feed efficiency, it is necessary to measure feed intake, which is challenging to do individually for fish. Because it is normally a lot of individuals living in large tanks, fed by dispersing feed into the water. However, there are many good ways to determine individual growth, making it easier for breeders to select for this trait (Dvergedal et al., 2019).

Today one of the major breeding companies in Norway is AquaGen, they were established in 1992. AquaGen initially uses a traditional family- based selection, with over 20 measurable properties of the broodstock. The traits selected for are connected to growth in both fresh- and sea water, filet yield and colour, as well as properties related to resistance to specific diseases and stress (AquaGen, 2020b). To conduct a more precise selection the broodstock candidates at AquaGen undergo a DNA- analysis to locate gene markers, so called quantitative trait locus (QTL). QTL is a section of DNA associated with specific phenotypical traits (Khatkar et al., 2004). This double selection method makes it possible to select the best individuals from the already selected best families.

GAIN, short for GEN- innOva® is the most advanced and precise product AquaGen offers. The product was launched in 2016 and achieved through two selection methods combined, both QTL and genomic selection (AquaGen, 2020a). Genomic selection means selecting individuals based on breeding values found by marker-assistant selection that covers the whole genome (Goddard & Hayes, 2007). That is, selection of animals based on their whole DNA-profile. Gains breeding goal is to produce salmon with extra resistance against sea lice, handling tolerance and improved growth performance to shorten the sea water period. According to AquaGen (2020a), Gain fish can have 1-2 months shorter time in the sea cages. Because improved growth will provide increased production capacity and a more cost effective production, shorter production time will also reduce the risk for waterborne infections. Figure 2.2 illustrates the differences in weight between Gain and salmon without genomic selection, living in the same sea cage.

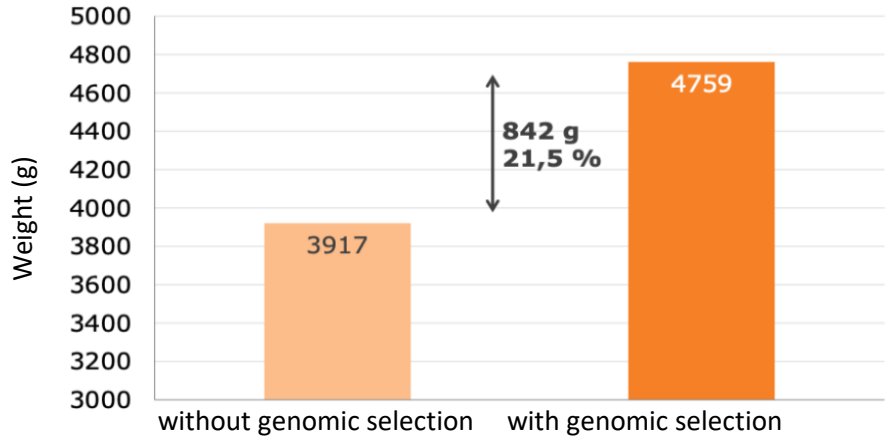

Figure 2.2: Weight measurements from two different product lines, one with genomic selection compared with one without, in the same sea cage (AquaGen, 2020a).

AquaGen produces, in addition to Gain, several other salmon roes. One of these products is QTL-InnOva® Prime (AquaGen, 2021). The major difference between Gain and Prime are that Gain uses genomic selection in combination with QTL. Genomic selection is a more comprehensive method than QTL, because it is better adapted to traits that are controlled by many genes. Whereas QTL is used for traits that are determined by fewer genes.

2.2 The digestive system

The digestive system of Atlantic salmon includes the mouth, esophagus, a U-shaped stomach, the pyloric caeca and the intestine. The intestine is often divided into mid- and distal intestine, and the transition is characterized by an increase in diameter (Navarrete et al., 2009). The digestive systems are often referred to as gastrointestinal tract (GIT), and its job is to ingest food and convert it into energy for basal metabolism, activity, maintenance and growth. Other organs involved in the process of digesting and absorption of food are the pancreas, gall bladder and liver. Food that is not digested pass through the intestine as waste products and is expelled as faeces (Sjaastad et al., 2010).

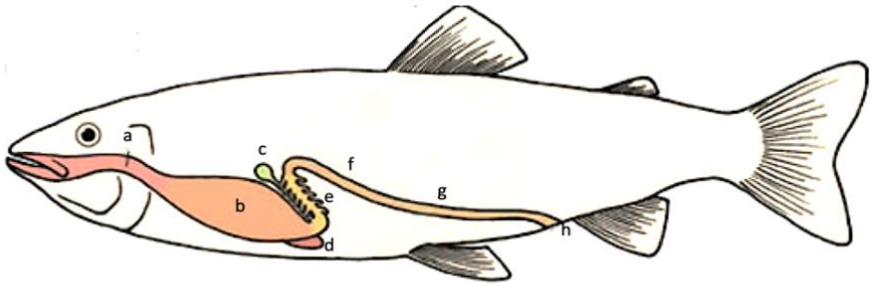

Figure 2.3: Schematic illustration of the digestive system from a salmonoid. Where a) is the esophagus, b) stomach, c) gall bladder, d) spleen, e) pyloric caeca, f) mid- intestine, g) distal-intestine and h) anus.

Food is ingested via the mouth and transported by the esophagus to the stomach, were the digestive processes starts. A highly acidic digesta, also called chyme, is fed into the intestine from the stomach at a controlled rate through the pyloric sphincter. In the intestine the digesta is mixed with secretions (digestive enzymes) from the diffuse pancreas and bile transported from the gallbladder (Krogdahl et al., 2015). Digestive enzymes break down complex dietary nutrients into components available for absorption across the intestinal wall. Not all components are digestible but pass through the GIT to be excreted as feces. Feces includes indigestible feed, metabolic products, gut epithelial cells and digestive enzymes (Nates, 2015). In Atlantic salmon, 70 % of the total nutrient absorption occur in the first segment of the mid-intestine with the pyloric caeca. Still, nearly the entire length of the intestine has a functional brush-border capable to transport nutrients across the membrane (Bakke-McKellep et al., 2000). After digestion, nutrient components are metabolized in order to ensure energy supply and molecules necessary for the body to function. A distinction is made between catabolism and anabolism, where catabolism is biochemical degradation processes in with larger organic molecules are broken down into smaller compounds, with the simultaneous release of energy. Anabolism is biochemical building process or synthetic reaction in organism, in with large molecules are made from simpler compounds. Basal metabolism refers to the minimum levels of catabolism and anabolism in a cell, needed to obtain the structure and function of organs and tissue (Baldwin et al., 1980; Hardy & Halver, 2002). Maintenance cost are the sum of energy, received through feed, to cover energy losses associated with basal metabolism and heat increment. This restricts the fraction of digested nutrients available for growth (Baldwin et al., 1980). Better digestibility will lead to lower

feceal losses After feed is digested the main losses of nutrients and energy are branchial and urinary losses. The net utilized material remaining represents energy available for growth, basal metabolism and voluntary activity (Figure 2.4) (Nates, 2015).

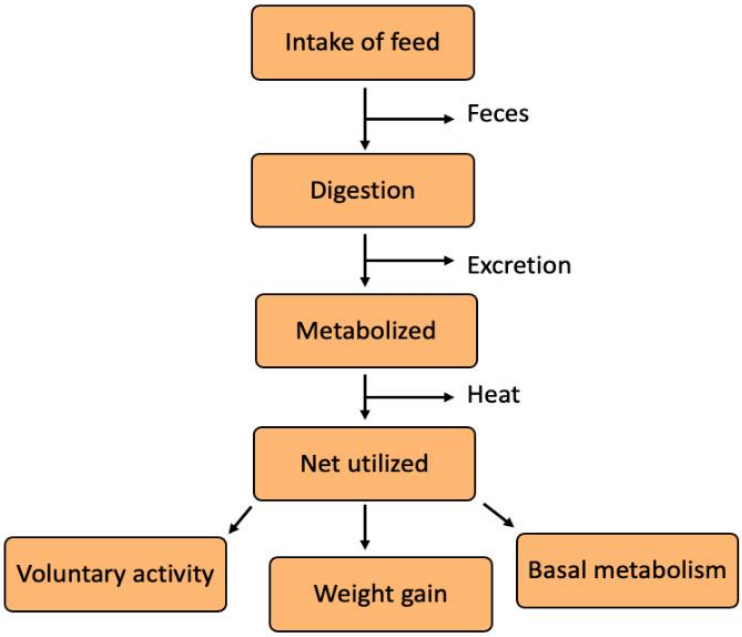

Figure 2.4: Schematic overview of distribution of dietary feed intake in a fish (Adapted from Nates, 2015).

Atlantic salmon are carnivores, and their digestive system is adapted accordingly. They have a short intestine, compared to herbivores, only about 0,8 times their own body length. Feed should thus only contain small amounts of indigestible material. The pancreas is not readily visible in Atlantic salmon, it is diffusely scattered in the fat and connective tissue around the pyloric caeca. The gall bladder extends from the middle lobe of the liver and the bile duct can usually be traced to the upper midgut in larger individuals. The mucosa is the mucous membrane lining towards the intestinal lumen, it consists of the epithelium, the lamina propria, the stratum compactum and the stratum granulosum (Løkka et al., 2013). The intestinal mucosa has two major functions. It is responsible for nutrient and fluid absorption and to create a selective barrier towards the external environment, in order to protect the host from pathogens. Along the GIT the mucosa has a complex folding pattern with a brush-border, increasing the surface area of the intestine (Bakke-McKellep et al., 2000; Hellberg et al., 2013). GIT can be described as an ecosystem of interactions between micro-organisms and the host organism. Micro-organisms, usually called microbiota, has an important role in

intestinal development, homeostasis and health of the fish (Navarrete et al., 2009). Fish have generally a diverse microbiota, but it varies based on species, life cycle, diet and genetic background (Martin et al., 2016).

In addition, Atlantic salmon does not utilize carbohydrates efficiently, due to a low alpha-amylase (enzyme that hydrolyses starch) activity in the intestine. A mutation near the active site on the enzyme makes it difficult to form bindings with the substrate. Binding can still occur, but at a slower rate (Frøystad et al., 2006). As a result, feed for Atlantic salmon should not include large levels of starch, but rather fat as their main source for energy. Salmon also utilized protein for metabolic functions, but the goal is to use it primarily for growth. Making the need for fat around 30 %. Carnivorous fish also need a high protein content in the diets, adult salmon need between 55 % crud protein during the fresh water stage (Grisdale-Helland & Helland, 1997). Utilization of protein is likely dependent on the function and morphology of the GIT (Lemieux et al., 1999). It can be measured by calculation apparent digestibility coefficient, which is defined as the amount of feed that is absorbed and not excreted as feces, without correcting for endogenous fecal excretions (Hardy & Halver, 2002). Enhanced digestibility of protein will potentially improve the feed efficiency. Since costs linked to feed are an important factor for economic success in salmon production, there is a focus on understanding how feed resources can best be utilized. Therefore, there is a need to understand how feed additives potentially affect the health and productivity of the salmon. Diets are being reformulated and new ingredients are added continually to optimize growth to further develop the industry.

2.3 **Functional ingredients**

Proper nutrition is a critical factor for successful aquaculture production. A complete diet meets the needs of the target species, is nutritionally balanced and the ingredients have a low production cost (Bharathi et al., 2019). In recent years, there has been an increased focus on the use of functional ingredients with immunostimulating properties in diets for farmed animals. Functional ingredients or functional feeds are used to describe a type of added compounds that will improve animals' health and growth performance. The ingredient is added in addition to the basic nutritional requirement of the target species (Martin & Król, 2017). These feed additives can be quite diverse in nature and characteristic, but their

application into the diets targets a specific purpose. They have also proved to be biocompatible, biodegradable, environmental friendly, as well as safe for both humans and animals to ingest (Encarnação, 2016; Van Hai & Fotedar, 2009). Functional ingredients include probiotics, prebiotics, enzymes, organic acids and immune stimulants that improve intestinal health, stress and their ability to resist diseases. Other additives can improve growth performance and utilization by enhancing digestibility or neutralize antinutrients (Bharathi et al., 2019; Feldmann, 2011; Montalban-Arques et al., 2015). Consequently, proper diet formulation can be used as a disease preventor in a more cost effective way than for example antibiotics. Diets are formulated with functional ingredients to ensure production of high-quality fish, that will lead to improve aquaculture profit beyond traditional feeds used today (Montalban-Arques et al., 2015).

Various kinds of ingredients have been studied and their suitability as immunostimulant have been tested, but only few are found to be suitable for use in aquaculture. One such group of functional ingredients are prebiotics. Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating growth or by acting as food for microbes already resident in the host gut, in an attempt to improve the host health (Gibson & Roberfroid, 1995). In aquaculture, the most commonly used prebiotic are polysaccharides derived from yeast. The most extensively studies and proposed for various applications are mannanoligosaccharides (MOS), inulins, β-glucans and fructooligosaccarides (FOS) (Grisdale-Helland et al., 2008; Mohan et al., 2019).

2.4 **Yeast**

Yeast are unicellular eukaryotic organisms. They are among the smallest eukaryotic cells with diameters ranging from 5 to 10 um. Yeast cells are very diverse, with over 1500 species, widely used in fields of life science, medicine and biotechnology (Feldmann, 2011; Osumi, 1998). The exterior of the yeast cell consists of a cell wall and a plasma membrane with a space between, called periplasm (Stewart, 2017). This structure provides protection from the surrounding environment, such as changes in osmotic pressure and mechanical treatment (Aguilar-Uscanga & François, 2003). Approximately 26-32 % of the yeast dry weight is made up of the cell wall, which consist mainly of polysaccharides. The polysaccharides mannans make up 25-50 %, glucans 30-60 % and chitin 5-10 % of the cell wall. Figure 2.5 represents a schematic

overview of the yeast cell wall components and structure. The yeast cell wall is built from the covalently linked complex of 1.3 β-glucan, 1.6 β-glucan and chitin, while the surface and outer layer consist of α-mannans attached to a protein backbone which forms a mannoprotein complex (Kogan & Kocher, 2007). The chemical composition of the cell wall will vary depending on species, fermentation substrate, growth conditions and method used for analysis (Papatryphon et al., 1999). β-D-glucans and chitin are responsible for maintaining the rigidity and structural support of the cell wall. Mannoprotein (including α-D-mannans) are responsible for the cell-cell recognition and interaction with the surrounding environment (Ruiz-Herrera, 1991).

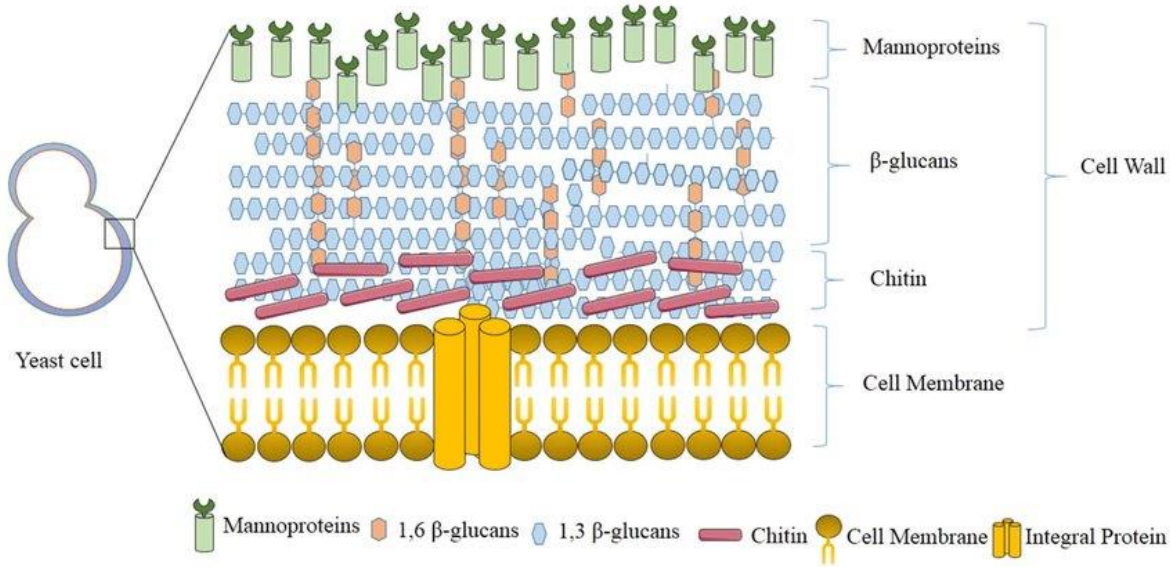

Figure 2.5: Schematic overview of the structural organization and composition of yeast cell wall (Anwar et al., 2017).

Yeast is commonly used as a functional ingredient in aquaculture feed. It is used as a growth promotor and immunostimulant (Øverland & Skrede, 2017). Both β-D-glucans and α-D-mannans have shown the ability to modulate the immune system of various living species, by interactions with different immunocompetent cells (Kogan & Kocher, 2007). It has also shown to have a positive effect on metabolic functions and gut homeostasis, as well as regulatory effects on the microbiota (Hoseinifar et al., 2015; Ringø et al., 2011). This realization has made the use of yeast derivatives in feeds common practice in aquaculture industry. There is evidence that β-glucans given through diet enhances immune responses and survival of the host organism after a pathogen infection in Atlantic salmon (Bridle et al., 2005). They are

responsible for a multitude of actions to enhance the immune system because of their ability to bind directly to macrophages and other white blood cells, in order to activate them. Activation of macrophages will in turn improve immune functions, such as phagocytosis, release of cytokines, interferons, lysozymes and leukocyte migration (Gantner et al., 2003; Vallejos-Vidal et al., 2016). Immune modulations of β-glucans have been demonstrated in salmonids, through the expression of pro inflammatory cytokines such as TNFα and IL-8. They can also enhance immune responses, such as bacteria killing activity (Lauridsen & Buchmann, 2010; Morales-Lange et al., 2015).

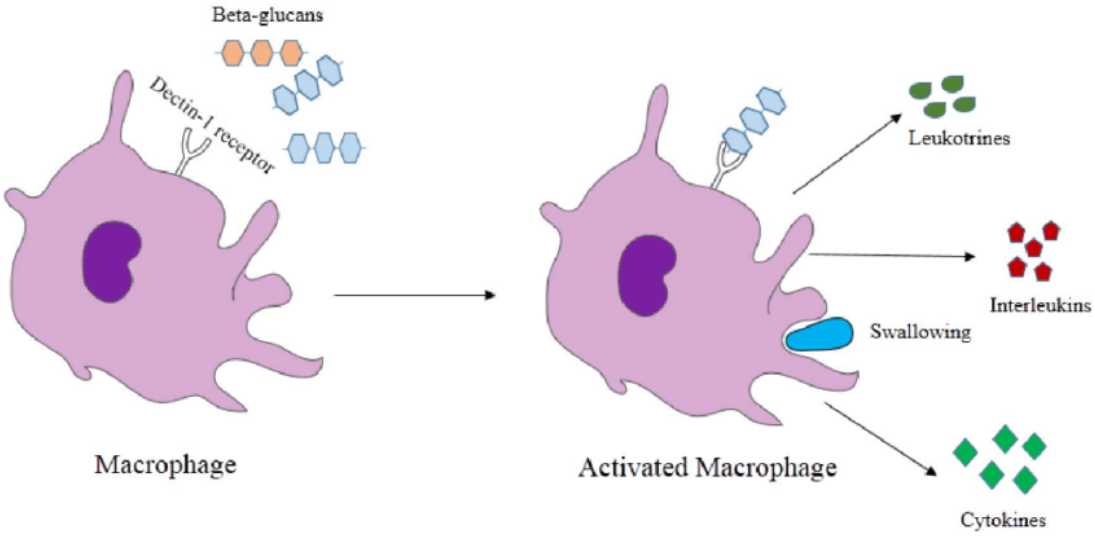

Figure 2.6: Activation of macrophages by β- glucans binding to a dectin- 1 receptor, stimulating phagocytosis and release of cytokines, interleukins and leukotrienes (Anwar et al., 2017)

MOS is another yeast cell wall derived feed ingredient that are known to improve digestion and gut health, by binding to glycoprotein receptors on pathogens and lessening the impact of colonization. MOS can also function as a prebiotic by stimulation growth of beneficial bacteria in the gut. Purified MOS have also been shown to improve growth and feed efficiency in Atlantic salmon (Grisdale-Helland et al., 2008; Torrecillas et al., 2014). Significantly improved performance and immune status were found in rainbow trout fed MOS in a sea cage trial. Weight gained improved 13 %, FCR and mortality was reduced, and improvements in the indicators of immune status for fish fed MOS, compared with the control group (Staykov et al., 2007).

A critical phase of salmon production is during SW transfer, when the fish undergoes smoltification. Atlantic salmon are anadromous fish and goes through a number of changes during transition to SW. Smoltification includes a number of developmental changes in physiology, biochemistry, morphology and behaviour, making the fish more vulnerable to stress, physical damage and infectious diseases (Björnsson et al., 2011). Changes include alteration in metabolism, osmoregulation, oxygen transport growth, colour and behaviour, to maximize success in marine environments (Stefansson et al., 2008). According to the Norwegian Directorate of Fisheries (2019), cost related to smoltification are the second largest expense, after feed costs. Use of functional feeds could be a part of the solution for challenges connected to smoltification and SW transfer. A study by Sahlmann et al., (2019) evaluated the effect of adding 25 % inclusion of the yeast Candida utilis to Atlantic salmon on growth performance and overall health, pre and post SW transfer. Yeast inclusion improved feed intake and growth. It also triggered a lower secretion of cytokines in the distal intestine. Fish fed yeast also modified immunosuppressive responses related to SW acclimation. Immune-stimulating ingredients can be used prior to situations were fish generally experience stress and impaired performance, in order to improve health and performance. For anadromes fish smoltification is a typical stressful situation, where they are exposed to a new environment, other pathogens, handling in addition to all the developmental changes (Raa, 2000).

However, reported effects of yeast polysaccharides on immunity, survival rate and growth performance in fish, are inconsistent. Effects have been widely studied on salmonoid families with varying results (Mohan et al., 2019). This could be due to factors such as type of yeast, concentrations added, feeding duration, fermentation conditions and downstream processing. Factors related to the recipient of the yeast-product also influence the health promoting effects. Including, fish species, size, age and environment (Øverland & Skrede, 2017).

3 Material and method

The experiment consisted of a FW phase and a SW phase. The Freshwater phase was carried out at Center for Fish Research at NMBU, while the seawater phase was conducted at the Norwegian Institute of Water Research (NIVA) at Solbergstranda. Diets was produced at Centre for Feed Technology (FôrTek) at NMBU.

3.1 Experimental diets

In both FW and SW water, fish was fed three different diets. One control diet containing a commercial like basal diet without functional ingredients. The two others were added 0.1 % of a product from non-*saccharomyces* yeast, delivered by Lallemand and will be referred to as Yeast 1 and Yeast 2 during this thesis. Diets were formulated based on the nutrient requirements of Atlantic and are shown in table 3.1. Diets also contained around 0,08 mg/g yttrium oxide (Y_2O_3) as an indigestible marker for determination of apparent digestibility coefficient (ADC) of crude protein.

- Diet 1: control
- Diet 2: Yeast 1
- Diet 3: Yeast 2

Each tank was given one of the three diets throughout both periods of the experiment. The diets were produced at FôrTek at NMBU, as described in Weththasinghe et al. (2021). The macro ingredients including fishmeal, soy protein concentrate, wheat gluten and wheat were measured and mixed with an ISDECA mixer (60-l paddle mixer), before it was ground in a Hammer mill. The ground macro ingredients were then added the premix (vitamineral-p-AA-kolin). All the diets were extruded (3 mm pellet size) with a Bühler twin-screw. The pellets were dried and cooled before they were vacuum coted (Gentle Vaccum Coater-80 prototype) with fish and rapeseed oil.

Table 3.1: Diet formulation (%) for the basal diet.

Ingredient	%
Fish meal[a]	39,00
Soy protein concentrate[b]	24,67
wheat gluten[c]	3,34
Wheat[d]	11,80
Fish oil[e]	8,09
Rapeseed oil[f]	9,00
Premix[g]	2,85
Yttrium oxide[h]	0,008

[a]LT Fish meal: Norsildmel AS, Bergen, Norway

[b]Soy protein concentrate: Tradkon SPC HC-200, Sojaprotein, Becej, Serbia

[c]Wheat gluten: Amilina AB, Panevezys, Lithuania

[d]Wheat: Norgesmøllene, Bergen, Norway

[e]Fish oil: (28 % EPA + DHA), Nordsilmel AS, Bergen, Norway

[f]Rapeseed oil: AAK, Karlshamn, Sweden

[g]Premix: (vitamineral-p-AA-kolin), BioMar AS, Norway

[h]Yttrium oxide (Y_2O_3): Metal Rare Earth Limited, Shenzen, China

3.2 Water stability

Water stability and sinking velocity was measured on oil-coated pellets. Sinking velocity was determined by measuring the time required for 1 pellet to sink 1 meter, in 17°C tap-water. It was performed on 10 pellets per diet, and the mean per diet was calculated. Water stability were measured during 30 and 60 minutes of incubation in distilled water (20°C) before drying in 104°C for at least 18 hours, as explained in Baeverfjord et al. (2006). The sample size was 20 grams, and the measurements were done in triplicates. Water stability was calculated as the DM retained (%) in the basket after incubation divided by the Dm before incubation.

3.3 Experimental fish

The fish used in the experiment was delivered from AquaGen on 16th of March 2020. 2060 eggs of the species Atlantic salmon were delivered to the centre of fish experiments at NMBU. The fish came from two different genetic backgrounds (Gain and Prime), with possibly large growth performance, but different levels of resistance. Upon arrival the eggs were disinfected

using buffodin and placed on hatchery trays. Hatching begun on 28th of march. The fish were transferred to start-feeding tanks on 18th of May. The water temperature was around 8.4°C and were gradually increased during the next days until it reached 15°C. Fish were kept under 24-hour light and continuous feeding until vaccination. Vaccination happened with Elanco, prior to experimental tank transferer. 21st of September the fish were graded and transferred to the experimental tanks. The start weight at the bigging of the experiment where 34 grams (Prime) and 39 grams (Gain).

3.4 Fish experiment

The experimental procedures were performed according to the national guidelines for care and use of animals. The freshwater phase of the experiment was performed at the Centre of Fish Experiments at NMBU. It lasted for 7 weeks, from 21st of September 2020 to 10th of November 2020. Fish were sampled after 7 days, and again after 54 days, before SW transfer. 20 fish from each tank were transferred to Norwegian Institute of Water Research (NIVA) at Solbergstranda. They were transported in transparent bags with 15 litres of water with 5 mg/L Aqui-S. The seawater phase lasted for 41 days, from 10th of November 2020 to 15th of December 2020.

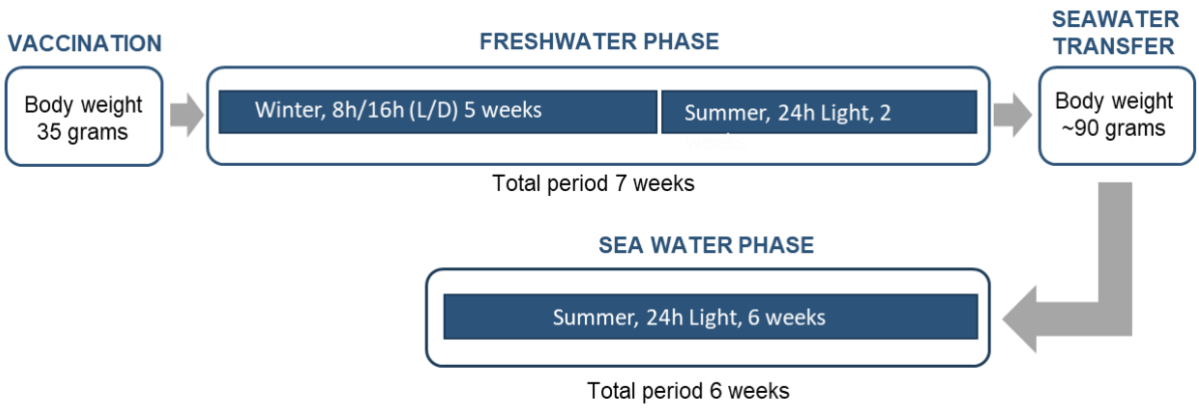

Figure 3.1: Experimental set up, showing approximately weight for seawater transfer, light regime and time period.

3.5 Experimental design

A total of 18 experimental tanks were used in the FW phase, containing 60 fish per tank. The tanks were made of fiberglass (300 litres) and the centre uses a Recirculatory Aquaculture System (RAS). 9 tanks contained fish from the genetic group GAIN and 9 contained the PRIME group. Each of the diets was given to 3 tanks per family, making it 6 treatments in total. The distribution of treatments was randomized. All parameters were measured and recorded every day to maintain standardisation during the experiment. All measurements were performed in the outlet water with an OxyGuard. In the FW phase, water temperature was on average between 14°C and 16°C, and water flow around 5 seconds per litre. Fish were exposed to 8-hour light and 16 hours dark for 5 weeks, followed by 24-hour light for the last 2 weeks. Throughout the experimental period, oxygen saturation should be minimum 80 %, and the water flow was increased according to oxygen saturation. At NIVA, fish were distributed to 18 tanks with the same design as the FW phase. The water was pumped from 60-meters depth, and the centre uses a flow through system. After transfer the fish was exposed to 10 ppt salt, the amount of SW was gradually increased every second day until full salinity was achieved after 12 days. The facility uses fiberglass tanks (300 litre) and a flowthrough system. All the tanks were supplemented with air from aeration stones. In the SW phase temperatures were on 12.5°C and the fish were exposed to 24 hours of light during this phase of the experiment.

3.6 Feeding

Triplicate tanks per family were given one of three diets. The fish was fed using automatic belt feeders for 6 hours a day, from 07:30 to 13:30. The feed was evenly distributed on the belts, and the fish were fed 110 % of expected feed intake. Feed intake was measured according to Helland et al. (1996), by collection of uneaten feed once a day, from a wedge wire screen in the outlet water. Amounts where weighed and stored in a freezer, in order to determine daily feed intake, FCR and to adjust feeding for next day.

3.7 Sampling

In connection with sampling the aesthetic MS 222 was used to anesthetize the fish. 6 fish per tank (108 in total) were netted into a bucket, containing 2 grams of MS 222 per 10 litres of water. After anaesthesia the fish were killed by a blow to the head, before they were weighed and measured (standard). Then a blood sample was taken, before the fish was dissected. Samples from gills (second one from the front), muscle, liver, head kidney, spleen, distal intestine and digesta were taken. At the beginning of the experiment five fish per tank were randomly sampled and stored at -20°C. The same process was done at the end of the FW phase in order to conduct a whole-body analysis.

3.8 Analytical methods

Apparent digestibility of protein was measured using an indigestible marker, as described in Austreng (1978), except that yttrium oxide were used instead of chromic oxide. Amount of marker were measured in the diet to amount found in faeces. Faeces was collected from the distal intestine in dissected frozen fish, before it was freeze-dried prior to chemical analyses. Amount of yttrium was determined using a microwave plasma spectrometer. The diets were analysed for DM, nitrogen and phosphorus.

Whole-body composition was analysed on five fish (per tank) from the beginning and end of the FW phase. Prior to this the remains of digesta from the intestine and stomach were removed by dissecting the fish. Accordingly, the fish were weight before it was homogenized in a kitchen grinder (Brown Power Pluss 1300). The grinder was washed between each tank and the mass was run through the grinder twice to obtain a representative sample. About 100 ml of the mince was sampled and freeze-dried before it was analysed. Quantitative determination of nitrogen was found using the Kjeldahl method. Crude protein was then calculated based on the level of nitrogen obtained multiplied by 6.25 (nitrogen to protein conversion factor). Total phosphorus content was analysed by a spectrophotometric kit.

Quantitative Polymerase Chain Reaction (qPCR) were used to detect possible infection of *Moritella viscosa* in samples taken from the gills. MvOmp1 was used as a target gene.

3.9 Calculations

Feed intake is expressed in gram DM per fish and were calculated as followed:

$$Feed\ intake\ per\ tank = Total\ feed * Feed\ DM - \frac{(uneaten\ feed * uneaten\ feed\ DM)}{Recovery\ value}$$

$$Feed\ intake\ per\ fish = \frac{Feed\ intake\ per\ tank}{Number\ of\ fish\ in\ the\ tank}$$

Feed utilization was evaluated by FCR, calculated by individual feed intake and weight increase.

$$Weight\ increase = final\ body\ weight\ (FBW) - initial\ body\ weight\ (IBW)$$

$$FCR = \frac{Feed\ intake}{Weight\ increase}$$

Growth performance were evaluated by specific growth rate (SGR), which is the average increase in body weight per day, expressed in percent. Δt is number of experimental days. Relatively weight gain (RWG) was also calculated:

$$SGR = \frac{\ln(FBW) - \ln(IBW)}{\Delta t} * 100\ \%$$

$$RWG = \frac{(FBW - IBW)}{IBW} * 100\ \%$$

Apparent digestibility coefficient (ADC) of nutrients was calculated, where *m* are marker and *n* are nutrient:

$$ADC = 100 - (100 * (\frac{consentration_m in\ diet}{consentration_m\ in\ feces} * \frac{consentration_n\ in\ feces}{consentration_n\ in\ diet}))$$

Nutrient retention is expressed in % intake, and *n* are nutrient.

$$Nutrient\ retention = 100 * (\frac{(FBW * final\ consentration_n) - (IBW * initial\ consentration_n)}{(Feed\ intake\ per\ fish * nutrient\ content\ in\ feed)})/100$$

3.10 **Statistical analysis**

The results were statistically analysed using a two-way analysis of variance (ANOVA) test in R studios. It was tested for interaction effects between genotype and environment, but there were no significant results and environments (FW and SW) were thus analysed separately. It was also tested for interaction effect between genotype and diet, this was not found significant and was thus removed from the statistical model. A post-hoc test was used to uncover the difference between group means when the ANOVA test were significant. Tukeys multiple comparison test in R studios were used. Differences were considered significant if $p < .05$, and results are presented as mean $\pm$ standard error mean (SEM) in tables and standard deviation (SD) was used in figures.

The model used:

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

Y_{ij}= Responsvariable

μ = overall mean response

α_i= Effects of family

β_j= Effect of diet

ε_{ij}= Error

4 Results

The results are divided into a FW and a SW section, as well as a section about diet analysis and water stability of the pellets. Results are based on growth data obtained at the beginning and end of each period and daily registration of feed intake. Note that digestibility was only examined on fish sampled in the FW phase.

4.1 Diet

Analyse of diet composition is shown in table 4.1. It was performed two replications per diet, and results are based on the average of these. Except for amino acids, which are only based on analysis from the control diet (Table 4.2). All components are relatively consistent and there is little difference between the diets, as expected.

Table 4.1: Analysis of chemical composition of experimental diets.

Nutrient	Control diet	Diet 2	Diet 3
Dry matter (%)	90.9	94.8	93.3
Ash (%)	8.4	9.1	8.8
Crude protein (%)	51.7	53.3	52.4
Crude fat (%)	14.9	15.9	14.5
Nitrogen (%)	7.5	8.1	7.8
Phosphorus (mg g^{-1})	16.1	16.4	16.6
Yttrium (mg g^{-1})	0.085	0.082	0.089

Table 4.2: Analysis of amino acid composition in control diet

Amino acids	Control diet
Essential amino acids	g kg DM^{-1}
Arginine	26.0
Histidine	12.1
Isoleucine	17.3
Leucine	30.8
Lysine	29.1
Methionine	9.5
Phenylalanine	18.8
Threonine	16.0
Valine	15.4
Non-essential amino acids	
Alanine	20.0
Aspartic acid	39.5
Glycine	18.9
Glutamic acid	78.1
Cysteine	4.2
Tyrosine	11.6
Proline	20.1
Serine	17.6
Sum amino acids	384.8

Values are water corrected

In addition to diet composition analysis, the water stability and sinking velocity of oil-coated pellets were tested. The physical quality of the pellets did not show any large numerically differences among the diets. Sinking velocity were on average around 9 seconds per meter for all three diets. The water stability ranged from 80 to 90 % after 30 minutes in the water bath and dropped down to approximately 70 % after 60 minutes (Figure 4.1). Water stability for diet 3 incubated in 30 mins were numerically lower than the other two diets, whit a relatively larger SD. After 60 minutes incubation there were little variation among diets.

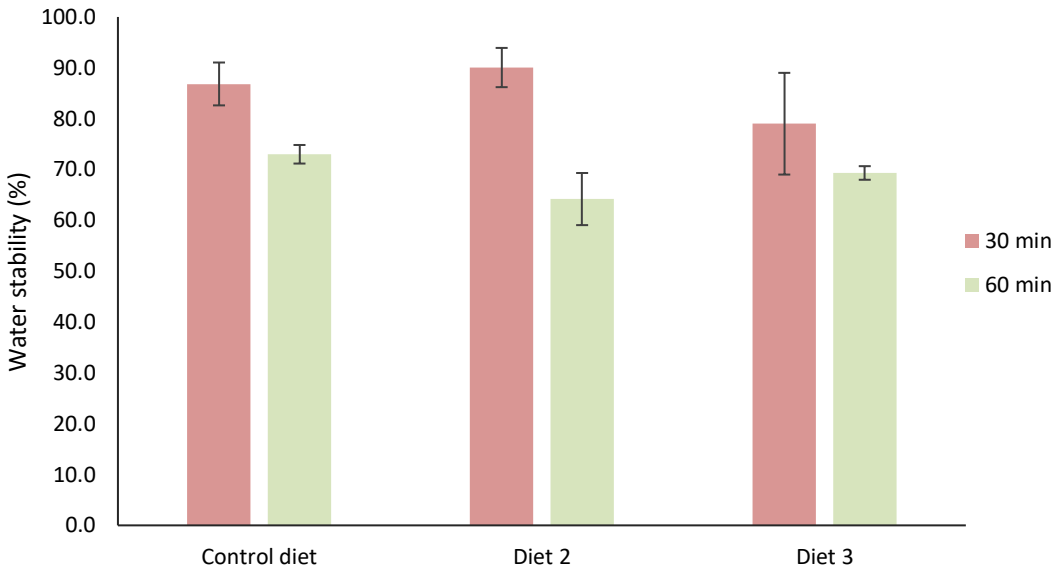

Figure 4.1: Water stability (%) for experimental diets (oil-coated pellets) after 30- and 60-minutes incubation 20°C distilled water. Data are presented as mean of triplicates per diet, and error bars are standard deviation.

4.2 Freshwater phase

In total 1080 experimental fish participated in this phase of the experiment. The fish were weighed at the beginning and end of the experimental period. Only one fish died, and the fish had no external injuries, but swam abnormal near the water surface.

4.2.1 Growth performance

Growth performance indicators of fish fed experimental diets in the freshwater phase are shown in table 4.3. Initial average weight for Gain were 39.6 grams. Prime weighed on average 34.2 grams. On the final sampling, Gain weighed 110.3 grams and Prime 80.2 grams. This gives an average weight increase of 70.5 grams and 46.0 grams, respectively. There was little difference in growth between diets within the same family, and the same trend could be seen in feed intake, SGR, RWG and FCR. There were observed no significant differences between diets. Between the families however, there were relatively large differences in all performance indicators measured.

Table 4.3: Performance indicators of Atlantic salmon (*Salmo salar*) fed a control diet and two experimental diets with functional ingredients derived from yeast cell walls, reared in freshwater.

	Gain			Prime				Diet	Family
Performance indicator	Control	Diet 2	Diet 3	Control	Diet 2	Diet 3	SEM	*P*-value	*P*-value
Initial body weight (g)	39.7[a]	39.6[a]	39.6[a]	34.1[b]	34.3[b]	34.3[b]	0.12	0.701	<.001*
Final Body weight (g)	109.6[a]	110.4[a]	111.0[a]	78.1[b]	81.9[b]	80.6[b]	2.92	0.362	<.001*
Weight increase (g)	69.9[a]	70.1[a]	71.4[a]	44.0[b]	47.6[b]	46.4[b]	3.12	0.480	<.001*
Feed intake (g DM)	48.1[a]	49.0[a]	48.7[a]	29.4[b]	32.0[b]	31.3[b]	1.70	0.223	<.001*
Relative weight Gain (%)	176.1[a]	178.6[a]	180.5[a]	128.9[b]	138.8[b]	135.4[b]	8.60	0.384	<.001*

Initial body weight: biomass in tank divided by number of fish

Final body weight: biomass in tank divided by number of fish

Feed intake: grams dry matter per fish

SEM: pooled standard error mean

Values in the same row with different letters are statistically significant

* indicates statistically significant *p*-values.

Figure 4.2 shows the SGR after the freshwater period, each bar represents one of the six treatments. Results show a relatively large difference in SGR between families (p= <0.001), were fish from genetic background Gain have a higher daily growth performance during the period. The control diet, in both families, had the lowest SGR. However, there was no significant difference in SGR between diets (p= 0.479). The same trend is observed for FCR, no significant difference between diets (p= 0.836) but differences between the two families (p= 0.023).

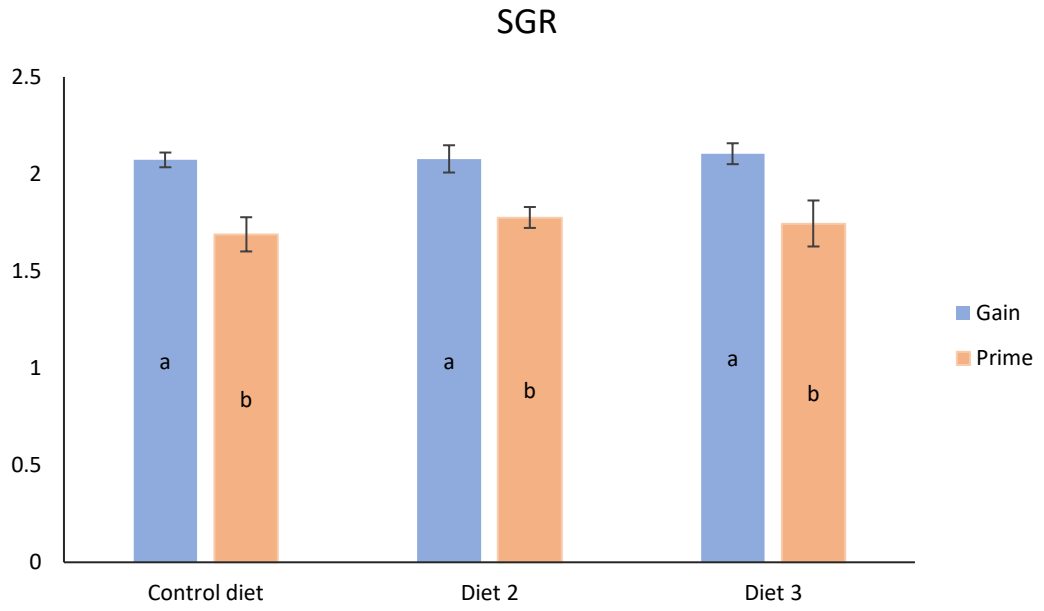

Figure 4.2: Specific growth rate (SGR) of Atlantic salmon (*Salmo salar*) fed one control diet and two experimental diets with functional ingredients extracted from yeast cell wall, reared in freshwater. Different letters are considered significant, n=3, ± SD.

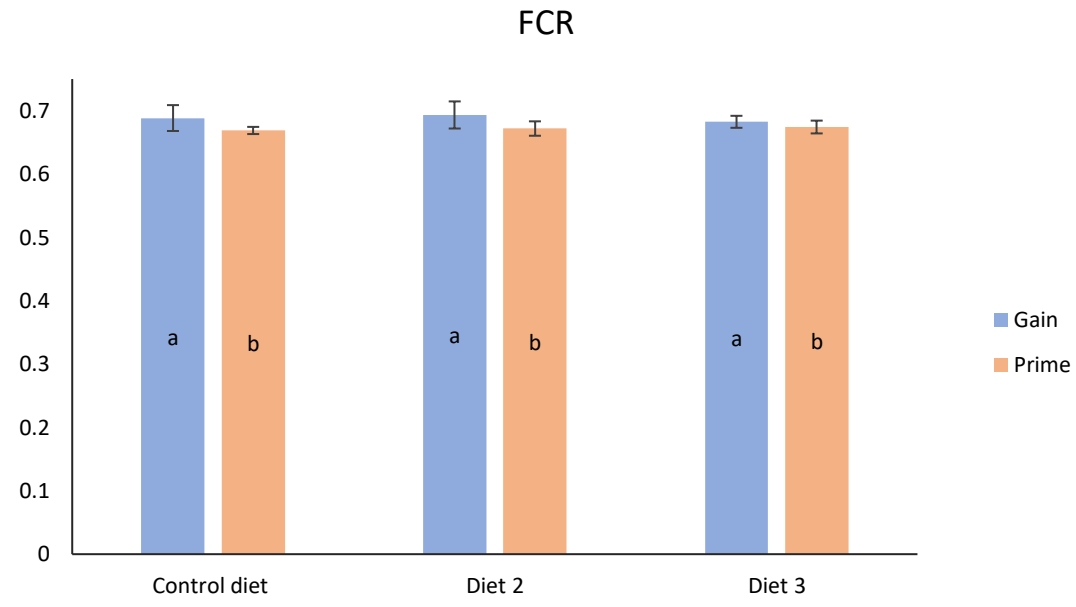

Figure 4.3: Feed conversion ratio (FCR) of Atlantic salmon (*Salmo salar*) fed one control diet and two experimental diets with functional ingredients extracted from yeast cell wall, reared in freshwater. Different letters are considered significant, n=3, ± SD

4.2.2 Digestibility

Apparent digestible coefficient (ADC) for and retention was calculated for fish from the freshwater phase after 7 weeks on the experimental diets. ADC of crude protein did not differ from the control diet, or between families. No statistical differences were detected for ADC, protein- and phosphorous retention.

Table 4.4: Apparent digestibility coefficient (ADC) for crude protein, protein- and phosphorus retention for Atlantic salmon (*Salmo salar*) fed one control diet and two experimental diets containing functional ingredients extracted from yeast cell wall, reared in freshwater.

	Gain			Prime				Diet	Family
	Control	Diet 2	Diet 3	Control	Diet 2	Diet 3	SEM	*P*-value	*P*-value
ADC of Crude Protein (%)	67.0	65.6	63.2	63.1	65.7	62.0	3.21	0.370	0.392
Protein Retention (%)	50.4	48.8	48.8	50.7	48.7	50.3	1.91	0.307	0.555
Phosphorus Retention (%)	34.7	31.4	32.7	27.5	34.7	32.7	8.93	0.914	0.758

ADC: Apparent digestibility coefficient

SEM: pooled standard error mean

4.3 Seawater phase

360 experimental fish participated in this phase of the experiment. Fish were weight upon transfer to sea water tanks and during sampling on day 35. During Monday 30 of November and 1 of December a total of 7 fish died, collected from tank 4 (control diet). Dead fish had all external wounds along the lateral line, see figure 4.5. It was later confirmed by qPCR analysis that they were infected with the bacteria *M. viscosa*, often referred to as winter-ulcer disease. Due to the confirmation of *M. viscosa,* gill samples from fish in all tanks were analysed and all samples tested positive for the bacteria.

Figure 4.4: Dead Atlantic salmon (Salmo salar) infected by Moritella viscosa (Photo: Jon Øvrum Hansen).

4.3.1 Growth performance

On average Gain weighed 111.1 grams at the beginning, and 152.8 grams on the final day. Prime had a start weight of 80.2 grams and a final weight of 118.0 grams. This gives a weight increase of 30.0 grams and 26.0 grams, respectively. Fish from diet 3 had a significantly higher final body weight than fish from the control group within both families. Feed intake were significantly higher in fish from family group Gain and insignificant among diets.

Table 4.5: Performance indicators of Atlantic salmon (*Salmo salar*) fed a control diet and two experimental diets with functional ingredients derived from yeast cell walls, reared in seawater.

	Gain			Prime				Diet	Family
Performance indicator	Control	Diet 2	Diet 3	Control	Diet 2	Diet 3	SEM	*P*-value	*P*-value
Initial body weight (g)	109.3^{a}	110.9^{a}	112.9^{a}	78.3^{b}	81.3^{b}	81.1^{b}	4.52	0.466	<.001*
Final Body weight (g)	149.0^{ax}	150.5^{axy}	158.9^{acy}	114.1^{bx}	118.2^{bxy}	121.9^{by}	5.79	0.05*	<.001*
Weight increase (g)	39.7	39.6	46.0	35.8	36.9	40.8	3.17	0.113	0.116
Feed intake (g DM)	28.4^{a}	28.9^{a}	32.7^{a}	25.7^{b}	24.9^{b}	27.2^{b}	3.00	0.160	0.011*
Relative weight gain (%)	36.3^{a}	35.7^{a}	40.8^{a}	45.8^{b}	46.0^{b}	50.4^{b}	4.95	0.364	0.005*

Initial body weight: biomass in tank divided by number of fish

Final body weight: biomass in tank divided by number of fish

Feed intake: grams dry matter per fish

SEM: pooled standard error mean

Values in the same row with different letters are statistically significant

* indicates statistically significant *p*-values.

During the SW period Prime had a statistically significant higher SGR (p= 0.004) than Gain, see figure 4.5. Differences in SGR were insignificant between diets (p= 0.354). Unlike the freshwater phase, diet 3 had the highest growth rate for both families. However, the difference is not statistically significant. The control diet and diet 2 have approximately equal SGR for both families. There is generally larger variation in FCR between families and diets during the sea water phase, but no significant difference between diets (p= 0.768) or genetic background (p= 0.310).

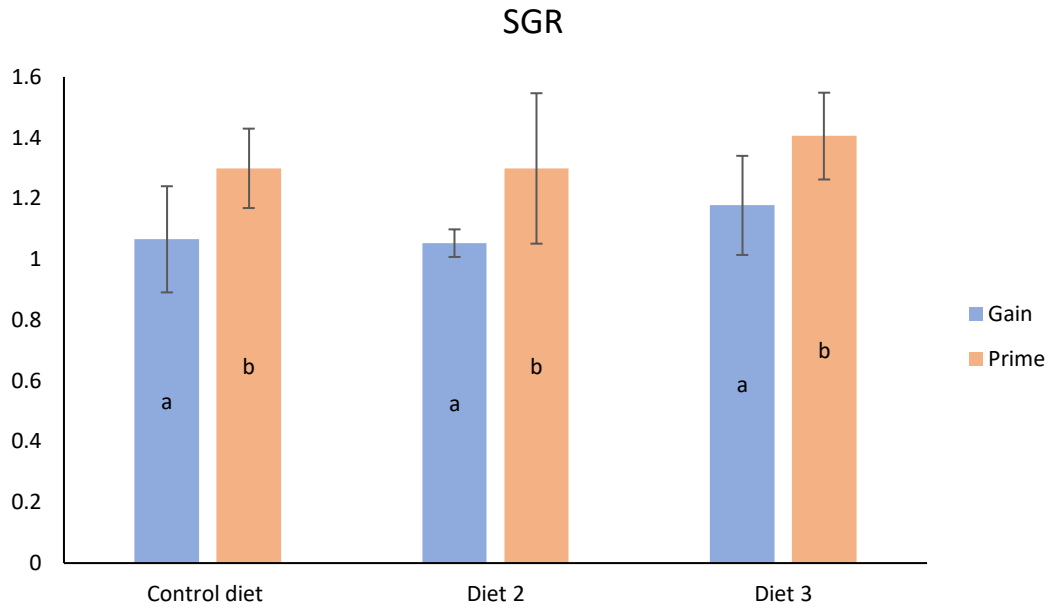

Figure 4.5: Specific growth rate (SGR) of Atlantic salmon (*Salmo salar*) fed one control diet and two experimental diets with functional ingredients extracted from yeast cell wall, reared in sea water. Different letters are considered significant, n=3, ± SD.

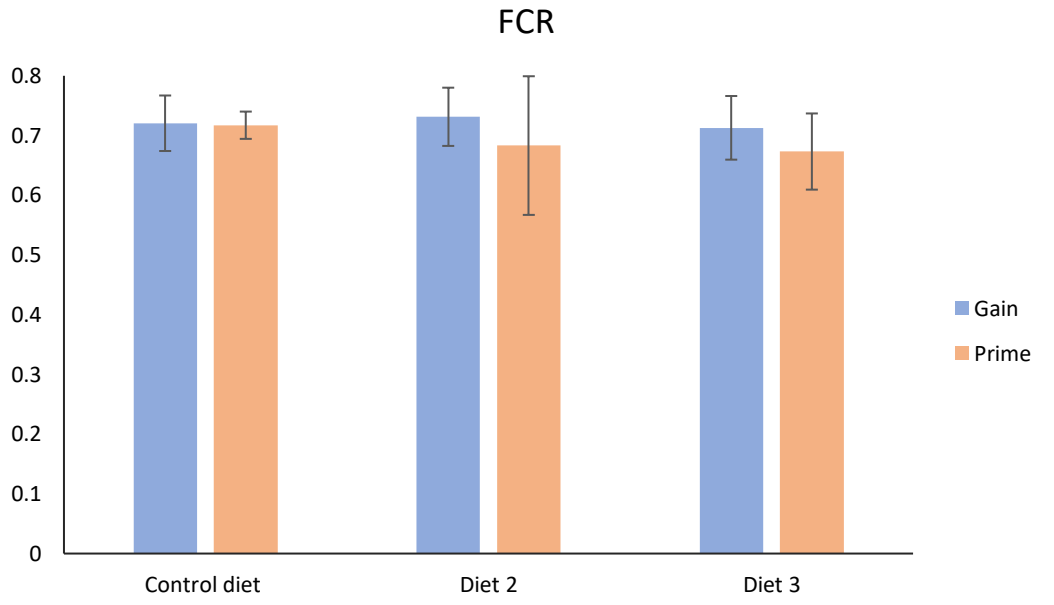

Figure 4.6: Feed conversion ratio (FCR) of Atlantic salmon (*Salmo salar*) fed one control diet and two experimental diets with functional ingredients extracted from yeast cell wall, reared in sea water. n=3, ± SD.

5 Discussion

The objective of this thesis was to determine if growth performance and nutrient utilization of Atlantic salmon, from two different genetic backgrounds, can be enhanced when given functional ingredients extracted from non-*saccharomyces* yeast, in both FW and SW. Two yeast products containing different functional ingredients were given to Atlantic salmon via their diet. Effects of the yeast products were evaluated by measuring weight increase, feed intake, SGR, RWG and FCR, in addition to ADC of crude protein and protein retention.

5.1 Performance indicators

5.1.1 Mortality

Fish were generally healthy and little mortality occurred during the experiment. Out of 1080 experimental fish only 1 fish died during the FW period of the experiment, this is considered unusually low. This is because of the Centre of fish research, were there have never been registered infectious or environmentally diseases. Which in turn is due to that the facility bases its stock on roe that can be disinfected in contrast to live fish. All lines that the centre accept have been screened and are disease free (NMBU, 2017).

The SW phase consisted of 360 experimental in total and 7 fish died during the period. This represents a mortality rate of 1.94 %, which is still considered low. All dead fish showed symptoms of winter-ulcer disease, and it was later confirmed that they were infected with the bacteria *M. viscosa*. Interestingly, all dead fish came from the same tank, and has thus been fed the same diet. In this case, the dead fish belong to the control diet and have consequently not been fed functional ingredients. These are interesting observations, when it is known that yeast can have positive effect on fish exposed to bacterial infections. Yeast contains several components that can protect against bacterial infections and improve immune response and GIT barrier functions and disease resistance (Mohan et al., 2019). At the same time, the objective of this experiment is not to investigate how functional ingredients affected salmon susceptibility to pathogenic bacteria, such as *M. viscosa.*

On the other hand, there may be a completely natural reason why winter-ulcer were discovered in this tank. During the SW period a screen near the outlet pipe detached, and the fish were removed from the tank in order to fix the problem. It is well known that injuries related to handling and/or treatments can make it easier for fish to be affected by the bacteria. Fish are exposed to waterborne pathogens all the time, and in order to cause an infection the pathogens need to gain access to the host by penetrate its primary barriers. Routes of bacterial entry to the host are through the mucosal surface of the skin, GIT and gills (Løvoll et al., 2009). The bacteria are rarely the underlying cause of wounds, fish usually develop wounds after the skin has been damaged by an external factor (MarineHealth, 2018). The fact that the bacteria was detected in all tanks supports the claim that damage in skin after handling was the reason that only fish from one tank died from *M. viscosa*. Results indicates that handling poses an increased risk associated with development of wounds by the bacteria *M. viscosa*, and gills and wounds likely represents an entry site for bacteria.

5.1.2 Feed intake

Palatability is not affected by adding functional ingredients from yeast cell wall to the diets. This is illustrated by similar feed intake among all the diets. Yeast contains feed enhancing properties, that have been proven to act as taste enhancers for fish (Kasumyan & Døving, 2003). A study by Sahlmann et al. (2019) evaluated effects of adding 25 % yeast to Atlantic salmon diets, results showed that fish fed a yeast diet achieved higher feed intake and growth rate than the control group, in both FW and SW. One reason why this is not observed, may be because small amounts (0.1 %) of the yeast product have been used in the diets. The yeast product used in this experiment were also added as a possible functional ingredient and not as a protein source. Another reason may be that the components added is not taste enhancers for Atlantic salmon. Taste preferences are species dependent and Atlantic salmon is considered a selective feeder compared to other salmonoids (Kasumyan & Døving, 2003).

Feed intake was, however, different between the two families (Gain and Prime) during the FW and SW phase. Where Gain had a significantly higher feed intake than Prime. Genetic variations on feed intake between families of Atlantic salmon, have been observed in a study by Thodesen et al. (1999) were family effect explained 31-32 % of the variation. After SW transfer it is normal for fish to eat less, and it can take weeks before they exhibit typical

feeding behavior (Sahlmann et al., 2019). This trend could also be seen for fish in this experiment. As mentioned, there were no significant difference in feed intake between diets. Anyway, it is still worth mentioning that the *p*-value is relatively low (0.160), many studies refer to *p*-values between 0.05 and 0.1 as a trend. Fish from both families fed diet 3, had the highest feed intake in both phases of the experiment. A *p*-value of 0.160 means that there is an 84 % probability that there is a difference in feed intake between fish given control diets and diets containing yeast products. These are interesting observations, as the hypothesis tested suggest that yeast products can lead to increased growth, and growth is positively linked to feed intake. Perhaps Yeast 2 contains functional ingredients that are beneficial for Atlantic salmon during smoltification. There are many strains of yeast that contain bioactive components, such as β-glucans, α-mannans, chitin and nucleotides that can be beneficial for salmon during SW transfer. These immune-stimulants can improve health and performance in fish, if used prior to situations that are known to be stressful. For example, handling, change of environment, increased exposure to photogenetic organisms and developmental phases such as smoltification for salmon (Klis et al., 2002; Raa, 2000). And thus be an explanation for why fish fed with diet 3 (Yeast 2) had a statistically significant higher final body weight.

Transition from FW to SW exposes salmon to large environmental changes, leading to challenges with osmotic changes and exposure to other types of bacteria (Sahlmann et al., 2019). A study by Arnesen et al. (1998) observed low feed intake 8 days after SW transfer for Atlantic salmon. This is a relatively short acclimation period compared to other findings. Jørgensen and Jobling (1994) detected normal feeding behavior 14 days after SW transfer, with temperatures over 7°C. Other studies concluded that it could take up to 35-50 days before fish display normal appetite (McCarthy et al., 1996; Usher et al., 1991). Based on this, determination of how long it will take before salmon, that have undergone smoltification and transfer to SW, display normal behavior is difficult. The experimental period in SW only lasted for 6 weeks in this experiment, including general low feed intake during the two first weeks. It would have been interesting to see if a trend could be observed, if the experiment had a longer duration.

Fish feeds need to be water stable in order to stay compact until ingestion and in order to minimize the amount of nutrients leaching into the water. Higher water stability is associated

with decreased leaching of nutrients when the pellets are soaked in water. The water stability of pellets was similar for the three diets in this experiment. This was expected as they are formulated and produced in exactly the same way, with the exception of 0.1 % yeast in diet 2 and 3. The yeast product was not expected to have any effect on the water stability, and this was confirmed by the results. Compared to the control diet in a study by Weththasinghe et al. (2021), with similar feed formulation as in this experiment, values for 30 min incubation are almost equal. The water stability of pellets incubated in 60 minutes were somewhat lower.
Feed intake and growth rate can also be influenced by the water stability of the pellet (Houlihan et al., 2008). It has been suggested that diets with high water stability gives longer gastric retention time, resulting in lower feed intake (Baeverfjord et al., 2006; Aas et al., 2011). Because there were no significant effects of diets on feed intake and variation in water stability were relatively small, it cannot be argued that water stability effected feed intake or growth rate.

5.1.3 Specific growth rate

The overall SGR in this experiment was lower than standardised growth tables for Atlantic salmon, in freshwater (Melberg & Davidrajuh, 2009). Normally an Atlantic salmon reared in 14-16°C with a body weight between 30 and 60 grams, will have an SGR of around 2.6 (Melberg & Davidrajuh, 2009). When the fish were transferred to experimental tanks, Gain had on average 5 grams higher initial body weight than Prime. The difference in initial body weight where also considered statistically significant. It is still relevant to compare the two, as the fish were hatched on the same day and reared under the same conditions until the beginning of the experiment. Throughout the freshwater period Gain grew faster than Prime and weight on average 25 grams more when transferred to seawater. This is clearly illustrated when comparing SGR, where Gain (2.08) had a significant larger value than Prime (1.74). This means that Gain grew on average 20 % more than Prime every day. AquaGens experiment on fish with (Gain) and without genomic selection gave a 21.5 % higher weight gain (Figure 2.2), this corresponds well with results in this experiment. SGR for Atlantic salmon increases with increasing temperature, up to 16°C, and decreased with increasing body weight (Austreng et al., 1987). A smaller fish will normally have a higher SGR, because they have a higher growth potential due to their low body weight. Prime had a lower initial body weight, and still had lower SGR in FW. Because they were reared under the same conditions and fed the same diets

as Gain, this difference is most likely due to genetic differences. Effects of genetic background on growth have been observed in Thodesen et al. (2001) and Kolstad et al. (2004). Growth can be improved by selective breeding, as shown by Thodesen et al. (1999). One of AquaGens breeding goals for Gain is improved growth performance, the fish are selected for fast growth, and explain much of the genetic variations in this experiment.

Interestingly, after 6 weeks in SW Prime had a higher SGR and grew on average 21 % more than Gain every day. Perhaps SGR is not the best growth indicator to use in a situation like this, where one group of fish is around 30 grams larger than the other. Based on this RWG were also calculated to try to even out the differenced in initial body weight between Gain and Prime. RWG explains how much the weight increases in relation to the starting point, ie initial body weight. While SGR refers to the average percentage increase in weight per day. This shift in SGR can most likely be explained by the fact that Gain had a higher initial body weight at SW transfer, and thus a lower growth potential. Nevertheless, Prime had the largest RWG and there was still a significant difference between families and insignificant between diets. This means that even when trying to account for different initial weights, Prime grew faster than Gain. Still, fish from family Gain had the largest weight increase during this period, but this is not considered significant.

There was a positive correlation between feed intake and SGR in the FW phase, for both families. This corresponds well with results from earlier experiments on Atlantic salmon (Thodesen et al., 2001). Increased feed intake leads to improved growth by increasing the amount of metabolizes energy available for growth (McCarthy, 1983). This may not have been the case during the SW period, Gain had the highest feed intake and the lowest SGR and RWG. But there were found no significant difference in feed intake for Gain and Prime. Another interesting observation in the SW phase, was the statistically significant difference between diets on final body weight. Overall fish from the control group had the lowest body weight at final sampling and fish given diet 3 had the highest. This can possibly be explained by a higher feed intake. Studies have shown that yeast given before and after smoltification gave better growth performance and feeding behaviour (Sahlmann et al., 2019). As mentioned earlier, fish given diet 3 ate more than fish given control diet and diet 2, although there was no significant difference, it was still mentioned as a notable low *p*-value.

The results from FW laid the foundation for the SW phase, so even though no significant differences in SGR and RWG were observed among diets, differences between diets increased in the latter phase. By comparing standard deviations and SEM from the two phases, there is a difference in agreement between the observations. The results from FW have lower SD and SEM and thus only deviate slightly from the average, compared with the SW results. This tell us that there is a larger spread in the observations.

5.1.4 Feed conversion ratio

Feed efficiency is the ratio between feed intake and growth, and a faster growing animal will likely utilize feed more efficiently because they have more nutrients available to use for growth (Gjedrem & Baranski, 2010). This was not observed in the FW phase of this experiment, were Gain had the highest feed intake and growth but also the highest FCR. Differences between the two family groups were considered significant. This means that fish from family group Prime needs less feed to gain one kilo body weight. Genetic variations in feed efficiency have been observed within species in several studies. In a study by Kolstad et al. (2004) 10 full-sibling families reared under the same conditions showed variation in feed efficiency, feed intake, growth and energy retention. Effect of families explained 77% of the variation in feed efficiency. The same results were observed by Thodesen et al. (2001), were family explained 31-32 % of the variation on feed intake, growth and feed efficiency. The study also found a favourable correlating between growth and feed efficiency, where increased growth reduced the FCR for Atlantic salmon. As mentioned, the opposite was observed in the FW phase of this experiment. Indicating that feed efficiency is a complex trait whit other underlying mechanism causing variation, in addition to growth. Feed efficiency can partly be explained by feed intake, digestibility, absorption, utilization, metabolism, growth and activity level (Byerly, 1967; Gjedrem, 2005). Genetic selection for improved feed efficiency mainly targets growth, but there have been reports on positive correlations between feed efficiency and growth rate (Janssen et al., 2017). Implying that Atlantic salmon selected for improved growth, may improve FCR. Gain have been selected for improved growth, with promising results on weight gain. This shows that selection for increased growth does not necessarily lead to better feed efficiency. The amount of energy left for growth in an animal are dependent on feed intake, the digestibility of the nutrients ingested, as well as the animals' metabolic efficiencies.

Changes in protein synthesis and degradation, due to differences in maintenance costs, can explain the varying amount of energy spent on growth, leading to variations in feed efficiency not attributed to growth (Dvergedal et al., 2019).

Results from the SW phase showed no significant difference between the two family groups, even though Prime had higher SGR and RWG. This supports the claim that other mechanisms contributing to variation in FCR. Growth and feed efficiency are correlated, but growth does not explain all the variation. FCR is the ratio between feed intake and growth, but when measuring growth performance indicators, such as SGR and RWG, feed intake is not a part of the formula. A higher feed intake may not necessarily indicate a more efficient animal. Digestibility should also be included when analysing growth performance, because increased disability will potentially improve the feed efficiency. An increase in digestibility implies that more of the nutrients ingested are available for the animal, rather than being lost through the feces. On the other hand, studies have indicated that genetic selection for increased growth might lead to a reduction in digestibility (Dvergedal et al., 2019). This was explained by the fact that growth is positively linked to high feed intake, which in turn leads to increased passage rate in the GIT and thus a reduction in digestibility. ADC of crude protein were measured in this experiment, after 7 weeks in FW. The results showed no statistically significant differences in neither diet nor family group. ADC values were generally lower than expected and reasons for this will be discussed later. It is therefore difficult to draw any conclusions about connections between feed intake, growth, feed efficiency in context with digestibility.

After 7 weeks in FW and 6 weeks in SW there was no significant effects ($p > 0.05$) of yeast products on FCR. The same have been observed in other studies, Pooramini et al. (2009) found no effect on growth or FCR in rainbow trout given yeast as a probiotic during 25 days in FW. The same was observed in another study where diets were supplemented with different amounts of MOS. No significant effect on FCR were found, but fish still had better growth (Yilmaz et al., 2007). Functional feeds can improve growth performance and utilization of nutrients by enhancing digestibility by promotion of digestive enzymes that could lead to a degrade of more nutrients, or by neutralize antinutrients in the feed (Bharathi, 2019; Montalban-Arques, 2015; De Schrijver, 2000). Dietary supplementation of the prebiotic FOS

had no effect on feed intake, growth or digestibility, but showed a 5 % higher feed efficiency than the control group. In the same experiment fish given MOS extracted for yeast tended to have a better overall feed efficiency ratio than the control group (p = 0.08) (Grisdale-Helland et al., 2008). The differences in FCR among diets in the FW were almost identical, with a SEM of 0.014. This was also the case with the other performance indicators measured, indicating that the yeast products supplemented in diet 2 and 3 had no detectable effect on Atlantic salmon reared in FW. As mentioned, were there no effects on FCR in the SW phase eighter. However, diet 3 had the most favorable FCR values, even though they were not significant. This is still mentioned because overall diet 3 haad the "best" performance parameters throughout the phase, feed intake and weight increase values are close to a trend.

5.1.5 Digestibility and retention

Apparent protein and phosphorus retention were not affected by the dietary addition of yeast in this experiment. Values were relatively stable at approximately 50 % for protein and varied a little more for phosphorus at between 27 and 34 %. Although many differences between the two family groups have been discovered in this experiment, this was not the case for either ADC, protein retention or phosphorus retention. FCR and nutrient retention are useful measurements to evaluate an animal's efficiency. An optimal result would be low FCR and high retention levels. Nutrient retention gives a measurement of how much of the dietary nutrient that is retained by the fish (Einen & Roem, 1997). Gain's improved growth and Prime's feed efficiency cannot be explained by different retention levels of protein. Increased growth does not necessarily have to mean increased protein levels in the fish. The deposition will differentiate between protein, lipids, water and minerals (Bureau et al., 2000). Gain had the highest growth rate and feed intake in FW but not higher protein retention, indicating that perhaps much of the weight gained are in lipids and/or water.

In this study digestibility was expressed by ADC of crude protein. Yeast products can enhance digestive capacity by improving gastrointestinal morphology and thereby increasing the absorptive surface. There were not observed increased digestibility in fish given yeast products in this experiment. And no correlations between growth, feed intake or feed efficiency with digestibility could be detected. There were, however, no significant difference

in ADC of crude protein between the two families. Digestibility can consequently not explain why the fish with the highest feed intake had lower feed efficiency.

The overall ADC results in this experiment were not corresponding with studies performed on similar diets. ADC on crude protein in a study by Weththasinghe et al. (2021) were 87,2 %. None of the treatments in this study surpassed 70 %, they were on average around 65 %. One reason for this could be the method used for collection of faeces. Digesta was removed from the distal intestine by dissection of semi-frozen fish. During sampling it was observed that some mucus was collected together with the digesta, as this was close to impossible to avoid. Accuracy of ADC depends largely on capacity to collect faeces method (Percival et al., 2001). Stripping and dissection of feaces eliminates nutrient leaching and thus over-estimation of ADC, these methods have been reported to give reliable estimates of ADC (Percival et al., 2001; Shomorin et al., 2019). Contamination of mucus with the faeces sample can underestimate the digestibility (Percival et al., 2001). Intestinal dissection also has the disadvantage that contamination with endogenous material can occur. Which also gives and underestimation of ADC, especially on protein (Bureau et al., 1999). It is also important to avoid sampling material from areas in the intestine where absorption is not complete. A study by Austreng (1978) suggested limiting collection of faeces to the posterior part of the intestine, to achieve the highest ADC of protein. Samples in this experiment were only collected from the distal intestine and should therefore not be significantly affected by unabsorbed protein.

Exactly the same Fish meal and soy protein concentrate, from the same supplier and batch, gave a higher ADC in a previous experiment (Weththasinghe et al., 2021). Thus, the low digestibility levels are, with relatively high certainty, due to the method uses for collection of feces. Firstly, there were contamination of mucus in the faecal sample and secondly, when fish de-freezes more mucus will be released form the intestine.

6 Conclusion

Growth performance and nutrient utilization can be enhanced in fish when given functional ingredients. A common functional ingredient used in aquaculture are polysaccharides derived from yeast, and the most extensively studied are β-glucans and MOS. In this experiment, Feed intake, growth rate and feed efficiency were not affected by adding 0.1 % of eighter Yeast 1 or Yeast 2 in diets for Atlantic salmon. Nevertheless, increased differences were found between FW and SW, were feed intake and weight gain approached what many defines as a trend. Although the differences were not concluded to be significant, the largest differences were between fish fed diet 3 and the control group. Final weight measurements showed that fish fed diet 3 had grown statistically significantly larger than fish from the control group, in both families. It would have been interesting to see if any of the observations could have been amplified if the experimental period in SW were longer.

There were not found increased digestibility in fish given yeast products in this experiment. Apparent protein and phosphorus retention were also not affected by the dietary addition of yeast. Correlations between growth, feed intake or feed efficiency with digestibility or retention could thus not be detected.

During the experiment large differences between family groups have been observed. This shows the effect and importance of selective breeding programs for producers of Atlantic salmon. There were, however, no significant difference in ADC of crude protein, protein- or phosphorus retention between the two families. Interestingly enough, Gain had the highest growth rates and the highest FCR (lower feed efficiency) in FW. Digestibility can consequently not explain why the fish with the highest feed intake had lower feed efficiency in. It is common to select indirectly for feed efficiency by selection for increased growth. Because cost related to feed, make up 45 % of total cost in salmon production, feed efficiency is an important trait to select for economically, but also environmentally. Direct selection for feed efficiency is important and should be emphasized.

7 Reference list

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