Norwegian University of Life Sciences

**Master's Thesis 2023 60 ECTS**
Faculty of Biosciences

# Filamentous fungi (*Paecilomyces variotii*) as an alternative sustainable ingredient in diets for Atlantic salmon (*Salmo salar*): effects on pellet quality, growth performance, nutrient digestibility and utilization, and immune-related biomarkers in the distal intestine during the freshwater phase.

Sana Javed
Master of Science in Aquaculture

# Filamentous fungi (*Paecilomyces variotii)* as an alternative sustainable ingredient in diets for Atlantic salmon *(Salmo salar*): effects on pellet quality, growth performance, nutrient digestibility and utilization, and immune-related biomarkers in the distal intestine during the freshwater phase.


## Sana Javed

**Main Supervisor: Jamie Marie Hooft, NMBU, Faculty of Biosciences**

**Assistant supervisor: Prof. Liv Torunn Mydland, NMBU, Faculty of Biosciences**

**Assistant supervisor: Prof. Margareth Øverland, NMBU, Faculty of Biosciences**



**Credits: 60**

**Program/Education: Master of Science in Aquaculture**

**Place of publication: Ås, Norway**

**Year of publication: 2023**



**Norwegian University of Life Science (NMBU)**

**Faculty of Biosciences**

**Department of Animal and Aquacultural Sciences**

# Acknowledgment

In the name of ALLAH, the most beneficent and most Merciful. First, I will thank ALLAH ALMIGHTY for giving me the health and courage to fulfill my master's thesis. I will take this opportunity to thank Prof. Margareth Øverland who gave me this chance to be a part of this project. She is really kind, supportive, and believes in the capacity building of her students. I hope she continues to spread kindness and warmth with her personality. I would especially like to thank my main supervisor Jamie Marie Hooft for her feedback, support, and guidance throughout this process. She is truly a mentor and I have learned so much from her. I would also thank my co-supervisor Prof. Liv Torunn for her support. I would also like to thank Veronica who really helped me in understanding and learning new lab techniques. I would also like to thank Byron and Tamara for their help. I will also thank my mother for her prayers. Finally, I will also thank my husband and kids for being so supportive.

# Table of Contents:

# List of Abbreviations

| | |
|---|---|
| **ADCs** | Apparent digestibility coefficients |
| **ANFs** | Anti-nutritional factors |
| **CP** | Crude Protein |
| **DI** | Distal Intestine |
| **e-cad 1** | E-cadherin 1 |
| **EAAs** | Essential Amino acids |
| **EPS** | Exopolysaccharides |
| **FAO** | The Food and Agriculture Organization (FAO) |
| **FCR** | Feed conversion ratio |
| **FF** | Filamentous fungi |
| **GALT** | Gut-associated lymphoid tissue |
| **GIT** | Gastrointestinal tract |
| ***ifnγ*** | Interferon gamma |
| ***il10*** | Interleukin-10 |
| ***inos*** | Inducible nitric oxide synthase |
| **il1b** | Interleukin 1 beta |
| **MALTs** | Mucosa-associated lymphoid tissues |
| **MIs** | Microbial Ingredients |
| **PUFAs** | Polyunsaturated fatty acids |
| **qPCR** | Quantitative polymerase chain reaction |
| **RAS** | Recirculating Aquaculture System |
| **SBM** | Soybean meal |
| **SBMIE** | Soybean meal-induced enteritis |
| **SGR** | Specific growth rate |
| **TAAs** | Total Amino acids |
| ***tgfb*** | Transforming growth factor beta |

# Abstract

Filamentous fungi (FF) are promising microbial ingredients (MI) for use in aquaculture feeds due to their high protein content and bioactive components with potential health beneficial effects. This study aimed to assess the effects of *Paecilomyces variotii* produced from sulfite stillage obtained from forest by-products on pellet quality, growth performance, nutrient digestibility and utilization, and expression of immune-related biomarkers in the distal intestine of Atlantic salmon (*Salmo salar*) reared in freshwater. Four isonitrogenous, isolipidic, and isoenergetic diets were formulated. Diet 1 was a control diet formulated with fish meal, soy protein concentrate, and wheat gluten meal. Diets 2, 3, and 4 were formulated so that *P. variotii* replaced 5%, 10%, and 20% of the crude protein content of the diets, respectively. As the inclusion level of *P. variotii* increased, there were changes in physical pellet quality, with linear and/or quadratic decreases in pellet length, width, expansion, and durability, and linear and quadratic increases in water activity, bulk density, sinking velocity, and water stability index. The changes in pellet quality were attributed to the high fiber and β-glucan content of *P. variotii*. Although weight gain, growth rate, and feed intake were not significantly different among fish fed the experimental diets, there was a linear improvement in feed conversion ratio with increasing inclusion level of *P. variotii*. The apparent digestibility coefficients for crude protein and gross energy decreased linearly with increasing dietary levels *of P. variotii*, as did those for essential and non-essential amino acids. However, there was a linear increase in nitrogen, energy, and mineral retention efficiencies with an increasing inclusion level of *P. variotii*. Furthermore, it was found that *P. variotii* exhibits immunomodulatory effects in the distal intestine. Based on these results, it can be concluded that *P. variotii* has high potential as an alternative protein ingredient in salmon feeds.

# Chapter 1: Introduction

## 1.1 Status of global aquaculture

Aquaculture has emerged as the fastest growing food-producing sector on a global scale and currently accounts for more than half of the world's seafood production. The Food and Agriculture Organization (FAO) reported that aquaculture production has expanded in the past seven decades from 19 million tonnes in 1950 to 179 million tonnes in 2018. Figure 1 shows the production in million tonnes of both capture fisheries and aquaculture from 1950 to 2020 (FAO, 2022).

From 1990 to 2020, the global aquaculture industry experienced an average yearly growth rate of 6.7%, resulting in the production of nearly 88 million tonnes of aquatic animals in 2020. With demand for protein sources on the rise, the aquaculture sector faces mounting pressure as capture fisheries struggle to keep pace. As of 2020, worldwide Atlantic salmon production reached 2.7 million tonnes, with Norway ranking as the largest producer (FAO, 2022; Reverter et al., 2014).

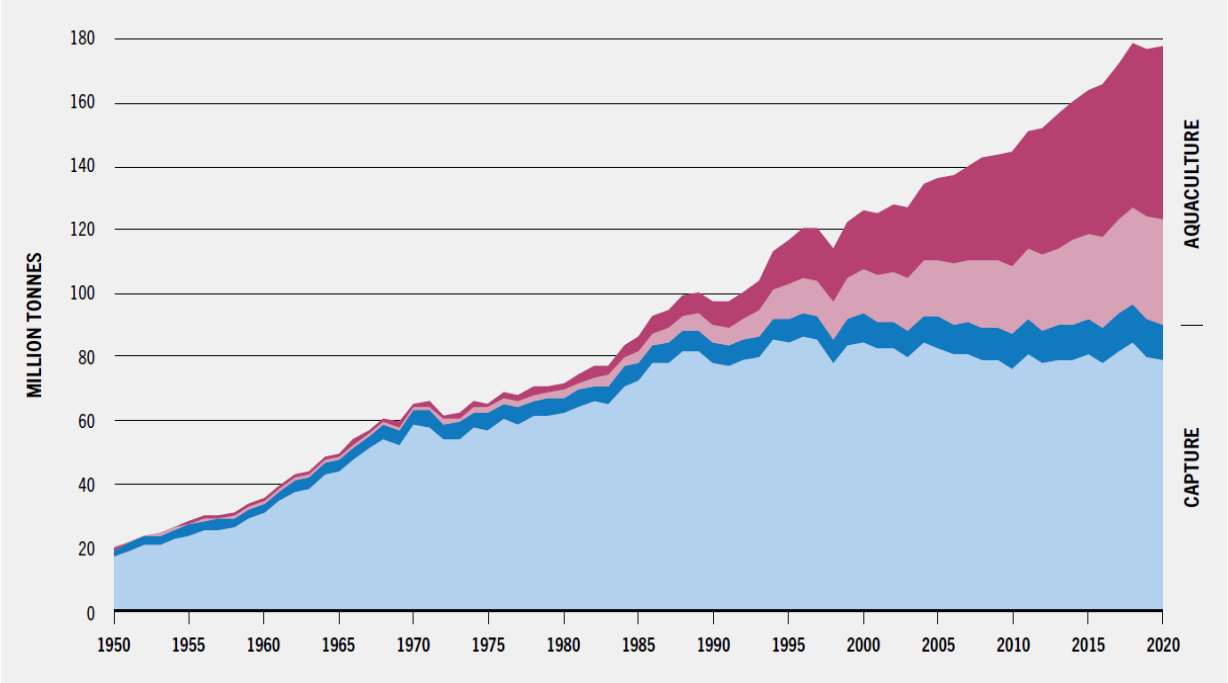


*Figure 1 : The world's capture fisheries and aquaculture production from 1950 until 2020 expressed in live weight equivalent. The production in inland waters is represented by dark blue and dark pink colors, while production in marine waters is shown in light blue and light pink colors (FAO, 2022).*

The world is currently grappling with significant challenges related to climate change and population growth. One of the sectors most affected by climate change is agriculture, and to

limit global warming to no more than 1.5°C per year, it will be necessary to achieve net zero greenhouse gas emissions by 2050. Furthermore, food systems, including aquaculture, must be expanded to accommodate a projected population of 9.7 billion people by the same year (Hunter et al., 2017).

When it comes to generating animal protein, sustainable production of Atlantic salmon is a highly efficient option that also results in a lower carbon footprint than other forms of meat production. According to a report by Ukrainian importers of fish and seafood (UIFSA) in 2020, fish have the lowest feed conversion ratio among various protein sources (UIFSA, 2020). However, intensive aquaculture practices can have adverse effects such as water pollution, habitat destruction, and the proliferation of diseases and parasites. To ensure that the expansion of aquaculture production does not harm biodiversity or contribute to emissions, it is crucial to undertake upscaling and development in a responsible manner. Additionally, the use of resources must align with national and international sustainability commitments (Bellona, 2022).

## 1.2 Status of Norwegian salmon farming

Norway is amongst the top ten countries with the largest aquaculture production (i.e., 1.2 million tons annually) and it is estimated to increase to 5 million tons by 2050 (Solberg et al., 2021). Norway is the world's largest producer of farmed Atlantic salmon *(Salmo salar)*. The sector has been expanding rapidly in recent years, with exports reaching record levels in 2021. According to the Norwegian Seafood Council, the country exported 3.1 million tons of seafood worth NOK 120.8 billion in 2021, of which salmon accounted for 67.4% of the total value (Norwegian Seafood Council, 2022).

Norwegian salmon farming has been successful in meeting the growing global demand for high-quality seafood, primarily due to the country's favourable natural conditions, which are conducive to salmon farming. These include cold, clean, and nutrient-rich seawater, a long coastline with numerous fjords, and a stable political and economic environment (Cadillo-Benalcazar et al., 2020).

In recent years, Norwegian salmon farming has come under scrutiny due to concerns over the environmental impact of the industry. The farming of large quantities of salmon in confined spaces can lead to pollution of the surrounding water, and the use of antibiotics and other chemicals can pose a risk to wild fish populations. Moreover, fish welfare is a major challenge

for the salmon industry, with issues such as cleaner fish usage, escapements, and mortality caused by salmon lice and diseases (Bjørnevik & Nilsen, 2021). Salmon lice is a significant cause of mortality and a major biological challenge for the industry (Hoddevik, 2020).

To address these concerns, the Norwegian government has introduced stricter regulations to ensure that salmon farming is carried out in an environmentally sustainable manner. These regulations cover issues such as the use of antibiotics, the discharge of effluent, and the control of sea lice infestations (Maroni, 2000). Moreover, to cope up with environmental challenges, new technological developments are being supported by the government by giving developmental licenses (Moe Føre et al., 2022).

However, as the number of licenses for sea-based aquaculture reaches its maximum, there is a growing trend towards land-based facilities. Recirculating aquaculture systems (RAS) provide ideal conditions for fish farming, including controlled environments that regulate factors such as water temperature, quality, and cleanliness through methods such as aeration, particle removal, pH regulation, disinfection, and $CO_2$ removal (Lekang, 2020). Because of their controlled environment, land-based facilities don't require cleaner fish and use fewer chemicals for sea lice treatment. Moreover, the absence of fish escapement means that these facilities have minimal genetic mixing with wild fish, less nutrient leakage and greater environmental sustainability. Land-based facilities are also more flexible in their location, as they do not depend on proximity to the coastline and eliminate weather threats that sea-based farming faces due to their enclosure (Bjørndal & Tusvik, 2017; Lekang, 2020).

Continuous development of RAS technology has led to improved fish farming practices. However, any technical issues or problems with water quality in land-based systems could have severe consequences for the fish (Bjørdal et al., 2018).

New technologies, such as offshore cages, may emerge as competitors to RAS in the long run, as the ocean provides ample opportunities for establishing such systems. Offshore cages enable fish to be produced far away from the coastline, where they can withstand harsh weather conditions. This approach allows for large-scale production and could potentially provide a solution for preventing salmon lice (Ecotone, n.d.; Vatlestad, 2017).

## 1.3 Historical development of the salmon feed industry

The aquaculture sector's growth has largely relied on the rapid expansion and progress of the aquaculture feed industry. Fish feed consists of ingredients that contribute to essential nutrients

required by the fish. To meet the increasing demand for aquaculture feed, we must rely on a variety of ingredients, particularly those that can be sustainably produced and keep pace with the growth of the aquaculture sector (Glencross et al., 2007).

Historically, the feed used in fish farming relied heavily on marine resources, particularly pelagic fish species like anchovies, sardines, herring, and mackerel. However, due to costliness, stagnation in forage, sustainability concerns, and restricted availability of fish meal and fish oil, plant-based protein sources have become more appealing for use in aquaculture feeds (Tacon and Metian, 2015).

However, plant-based proteins have issues surrounding sustainability and nutritional composition (Fry et al., 2016). They have nutritional limitations (i.e., high fiber content, imbalanced amino-acid profiles and lack of n-3 long-chain polyunsaturated fatty acids). In some cases, such as soybean meal (SBM), they contain anti-nutritional factors (ANFs) (Krogdahl et al., 2010) causing SBM-induced enteritis (SBMIE) in the distal intestine which affects fish health and growth (Baeverfjord and Krogdhal., 1996; Uran et al., 2009; Urán et al., 2008; Hardy., 2010). Another serious concern related to plant-based protein is food-feed competition as most of them can be directly consumed by humans (Ytrestøyl et al., 2015). Furthermore, the intensification of crop production, related usage of large land areas, clean water, and transportation (i.e., soya from Brazil) have severe environmental impacts as they lead to an increase in GHG emissions (Øverland et al., 2013; Øverland and Skrede, 2017).

The ingredients that are mostly used nowadays in fish feed are mostly from land animals (terrestrial), aquatic animals, or from plant origin (soybean, canola, lupin) as shown in Figure 2.

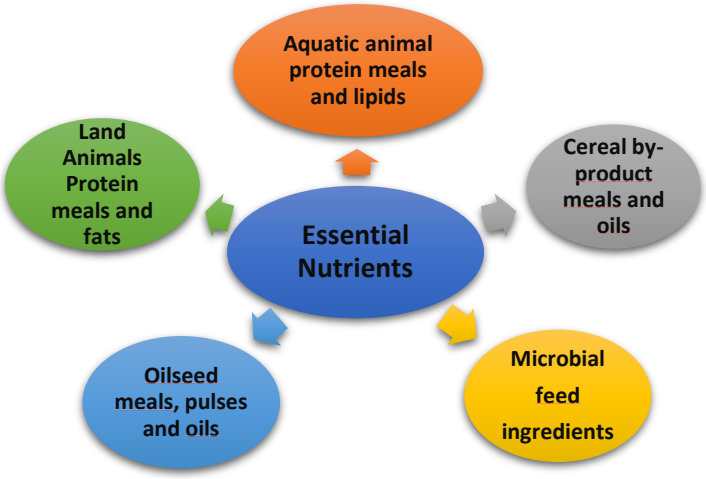


*Figure 2 : Sources of Essential Nutrients (Glencross et al., 2007)*

Moreover, plant-based protein sources such as soy protein concentrate, wheat gluten, wheat, corn gluten meal, and sunflower meal are widely utilized, constituting 40.5% of commercial salmon feed formulations in 2020 (Figure 2). In 2020, soy protein concentrate (SPC) was the primary ingredient used in the largest quantity, accounting for roughly 21% of feed ingredients, while rapeseed oil made up 18% of the ingredients. Micro-ingredients such as vitamin and mineral premixes, phosphorus sources, astaxanthin, and crystalline amino acids were also used. Salmon feeds also incorporated 0.4% or 8,126 tonnes of novel ingredients like single-cell proteins, insect meal, fermented products, and microalgae, which can be produced from waste streams, making a significant contribution to sustainability and a circular economy (Aas et al., 2022a).

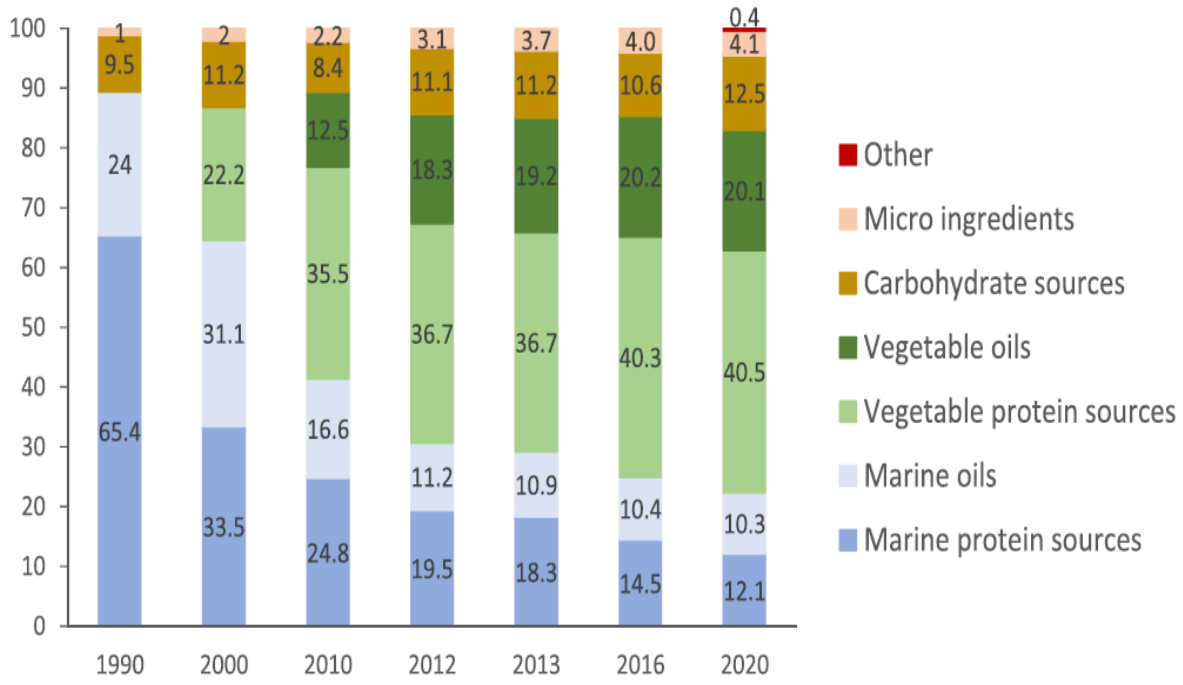


*Figure 3: Sources of feed ingredients (% of feed) in Norwegian salmon feed in 2020 compared to previous years (Aas et al., 2019; Ytrestøyl et al., 2015). Micro ingredients include vitamin- and mineral premixes, phosphorus sources, astaxanthin, and crystalline amino acids. 'Other' includes insect meal, single-cell protein, fermented products, and micellaneous. (Aas et al., 2022a)*

These modifications in usage of feed ingredients are fueled by several factors, including the availability of ingredients, price, consumer preferences (increased interest in sustainable production), climate change, and conflicts that can impact commodity trade. However, the main challenges with these innovative ingredients are their cost and scaling up production to significant amounts (Aas et al., 2022a).

Nearly 2 million tonnes of feed ingredients were used in Norwegian salmon feed in 2020 to produce almost 1.5 million tonnes of salmon. Feed in Norway was produced from 22.4% marine ingredients (proteins and oils), but amongst them, only 8.3% were produced in Norway with the rest (91.7%) being imported (Aas et al., 2022a). This leads to climate emissions of almost 75-80% per unit of salmon delivered to market. So, the need for sustainable ingredients that can be produced locally is really in demand (Winther et al., 2020). Therefore, MIs and insects are gaining much attention in recent times (Van Huis, A. 2013; Jones et al., 2020)

## 1.4 Alternative feed ingredients

The Norwegian government has stated that by 2030 all the ingredients used in fish feed must come from sustainable sources. However, the ongoing war between Russia and Ukraine has triggered a severe shortage in the global food supply, resulting in skyrocketing prices. This is because both Ukraine and Russia have emerged as major producers of wheat and other vegetables used for human consumption and animal feed. As a result, the use of feed ingredients in salmon feed may undergo a rapid transformation (Aas et al., 2022b).

Considerable research efforts have been invested in the development of sustainable alternative feed ingredients which contribute to the circular bioeconomy such as marine feed resources which includes mussels, Calanus, mesopelagic fish, Antarctic and Arctic krill, marine macro-algae (seaweeds and kelps), plant-based by-products, microbial feed ingredients (bacteria, protists (fungi), yeasts, microalgae), insects, and by-products from the fish and animal industry (Albrektsen et al., 2022).

Each alternative has the potential for being used in feed, however, each one also has inherent barriers which limit its production at an industrial level. Some of the challenges which can potentially hinder the utilization of raw materials are regulatory barriers such as certification, lack of research and knowledge, economic and technological barriers (Almås et al., 2020).

However, the prevailing scarcity and high cost of ingredients are compelling factors that may accelerate efforts to overcome these obstacles and promote the use of alternative ingredients (Albrektsen et al., 2022; Eidem and Melås, 2021). A crucial challenge towards 2030 will be to deliver constantly available, sufficient volumes and nutritionally correct raw materials for fish feed, while at the same time managing to cut greenhouse gas emissions and increase sustainability (Bellona, 2022).

# Chapter 2: Background

## 2.1 Microbial Ingredients

MIs are also termed single-cell proteins (SCPs), or natural protein concentrate and includes yeast, fungi, algae, and bacteria (Nasseri et al., 2011; Øverland and Skrede., 2017). MIs are dried forms of micro-organisms and can be regarded as sustainable high-quality alternative ingredients in fish feeds because they can convert the low-value, non-food organic waste streams from forestry, agriculture, and food manufacturing industries into high-value nutrients. These materials are considered as waste. However, they can be used as energy sources for microbes during fermentation to produce MIs which makes them cost-effective and sustainable alternative for aquafeed (Bajpai, 2007). Variation in feedstocks have opened exploration of different processes involving different ways of growth of MI (i.e., autotrophs, photoautotrophs, chemoautotrophs, methylotrophs, heterotrophs and mixotrophs) (Jones et al., 2020).

### 2.1.1 Lignocellulosic biomass

MIs require substrate to grow (carbon and energy). Traditionally, use of first-generation biomass was common, but for the sustainable production of MIs, the source of this substrate must be low-cost (economically viable) and should not compete with human food and have low impact on environment and biodiversity. One of the plentiful and renewable organic sources is second-generation lignocellulosic material produced through forestry, agricultural practices, timber, pulp and paper, and many agro industries (Albrektsen et al., 2022).

Some other feedstocks that have been recently in focus are corn stover, apple pomace, sugarcane bagasse, rice polishings, rice husks, maize cobs, maize fibre and citrus waste (Bhalla and Joshi, 1994; Pandey et al., 2000; Rajoka et al., 2006; Robinson and Nigam, 2003; Singh et al., 1988; Villa Bôas et al., 2002; Yakoub Khan et al., 1992; Zhang et al., 2006). However, the availability of feedstocks is dependent on factors likes types of agricultural practice, climate and technological development (Bajpai, 2007).

### 2.1.2 Processing of lignocellulosic biomass

Lignocelluloses is the main structural component of plant biomass consisting of cellulose, hemicelluloses and lignin that are chemically bonded by covalent linkages and non-covalent

forces (Malherbe and Cloete, 2002). Therefore, it is vital to process them before they are used in MIs production. The first step is pre-treatment which involves the hydrolysis of lignocellulose into fermentable sugars by chemical, physical, physico-chemical and biological methods (Mosier et al., 2005; Chandra et al., 2007; Van Dyk and Pletschke, 2012). At this step, lignin is removed, and the availability of cellulose and hemicellulose sugars is increased by making them porous. This method is cost and energy effective, as it gives high input of all degradable carbohydrates and fewer by-products that are inhibitory for further hydrolysis (Hahn-Hägerdal et al., 2007; Kumar et al., 2009).

Enzymatic hydrolysis is used to convert oligomeric sugars (cellulose) into monomeric sugars. These monomeric sugars are then subjected to fermentation using specialized strains of MIs, such as yeast or fungi, resulting in the production of microbial mass. The fermentation process is usually carried out in a fermenter, which provides aseptic conditions and allows for control over oxygen levels, pH, and temperature. Fermentation can be performed using different strategies, such as batch/fed-batch, continuous, and repeated fed-batch methods (Agboola, J. O. (2022)).

In batch fermentation, a fixed substrate concentration is added, and microbial inoculum is cultured over it. Fed-batch fermentation starts with a batch mode, but the medium is replaced continuously or sequentially. Continuous fermentation involves the constant addition of feed while the microbial mass is continuously removed (Lapena, 2009).

After fermentation, downstream processing is used to produce high-quality protein while preserving the nutritional and functional values of the single cell protein (Øverland and Skrede, 2016). Different methods of drying, such as spray-drying, drum-drying, oven-drying, and freeze-drying, can be used (Chen et al., 2015). However, the type of drying method employed can affect the nutrient quality of the final product, as reported by Hansen et al. (2021a) where drying at 250°C reduced the protein digestibility of *S.cerevisiae* compared to spray-drying at 180°C.

### 2.1.3 Potential of microbial ingredients for use in aquafeeds

MIs are selected based on certain characteristics (i.e., rich in proteins, low in fat, non-pathogenic to humans, animals and plants, lack toxic compounds, short generation time and a balanced amino-acid profile). Most of them have high levels of threonine and tryptophan. They

also have free amino acids, lipids, carbohydrates, minerals, and vitamins (Nitayavardhana et al., 2013; Alriksson et al., 2014; UniBio A/S, 2014).

Moreover, MIs are attractive from a sustainability point of view because they have a high growth rate, do not require agricultural land (produced in bioreactors), are least affected by climate change, use little fresh water, and can be produced from non-food biomass and therefore do not compete with human food resources (Bajpai., 2007).

Methanotroph-based MI products have performed well in many tests on Atlantic salmon. Salmon fed diets containing bacterial protein meal (BPM) exhibited higher growth rates and feed efficiency ratios, despite lower nutrient digestibility (Aas et al., 2006). *M. capsulatus* MI can constitute up to 52% and 38% of dietary protein in salmon and trout diets, respectively, without any negative consequences (Øverland et al., 2010). KnipBio Meal (*Methylobacterium extorquens*) can replace up to 55% of fishmeal in salmon diets and up to 10% of soybean meal in trout diets without impacting growth (Tlust et al., 2017; Hardy et al., 2018).

When it comes to yeast, most of the research has been done on *Saccharomyces cerevisiae* as it can partially replace protein from fish meal in Atlantic Salmon diets. But, now many other species such as *C.jadanii, Kluyveromyces marxianus,* and *Wicherhamomyces anomalus* are also in focus as they can also replace up to 40% of protein from a fish meal in Atlantic Salmon without compromising growth performance (Øverland et al., 2013).

In recent years, inactivated yeast cells have gained much popularity as functional and nutritional feed resource in aquafeeds mainly because of their positive impact on fish growth and health (Agboola et al., 2021b; Meena et al., 2013; Reyes-Becerril et al., 2008a; Robertsen et al., 1990; Sahlmann et al., 2019; Sarlin & Philip, 2011; Torrecillas et al., 2014). They have low amounts of lipids, 40-55% of crude protein content, and almost 2-5% of unsaturated fatty acids (Halasz & Laztity, 1991). Nucleic acid (NA) concentration levels are high (10-15%), but salmonids have the ability to metabolize high levels of dietary NA, as the urolytic pathway is well-developed (Agboola et al., 2021a). Furthermore, it has been reported that NA can be utilized for the synthesis of essential amino acids (EAAs) in rainbow trout (Rumsey et al., 1992).

Cell wall of the yeast is rigid, and it affects the digestibility and nutrient utilization as intracellular nutrients are not available for digestion. To deal with this, there are cell disruption technologies that can be used (i.e., chemical (acidic/alkaline treatments), physical, enzymatic (usage of endogenous or exogenous enzymes) and mechanical methods (mechanical forces) (Nasseri et al., 2011).

Several studies have convincingly demonstrated the immunomodulatory effects of yeasts which are commonly attributed to their cell wall components, namely β-glucans, and mannan-oligosaccharides (MOS). Due to a ban on prophylactic growth-promoting antibiotics in animal feed by both the EU and the US, yeast derivatives have become a popular alternative. β-glucans have been demonstrated to enhance the immune response and improve the survival of fish after pathogen infection in different species including Atlantic salmon (Robertsen et al., 1990; Bridle et al., 2005), rainbow trout (Siwicki et al., 2004; Guselle et al., 2007), and European seabass (Bonaldo et al., 2007). The mode of action for β-glucans is through the dectin-1 receptor, which is highly expressed on the surface of immune cells such as dendritic cells, neutrophils, eosinophils, macrophages, monocytes, and some T-cells (Volman et al., 2008). When β-glucans bind to the dectin-1 receptor, it activates intracellular signaling and leads to cytokine production, phagocytosis, and respiratory burst, ultimately supporting immune function. Additionally, yeast-derived β-glucans have been shown to adsorb or bind toxins, viruses, and pathogenic bacteria (Volman et al., 2008).

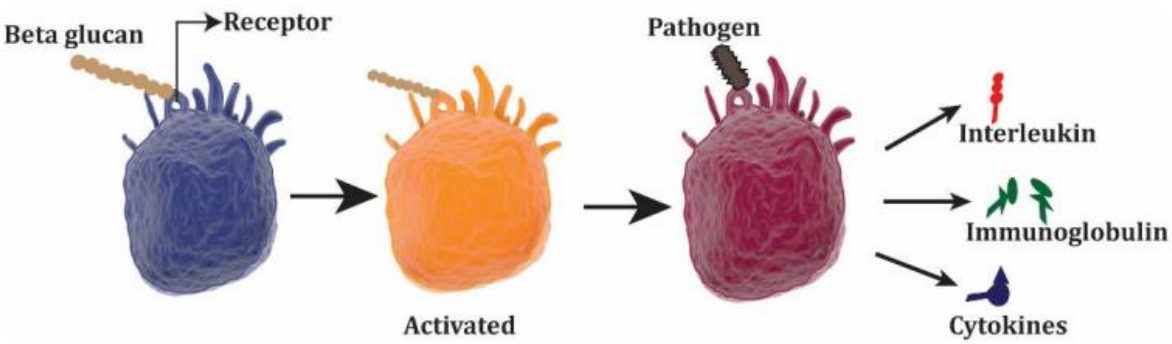


*Figure 4: β-glucan activation of the immune system. β-glucan activates the macrophage by binding to the dectin-1 surface receptor (Agboola, J. O. (2022); Bell et al., 2018)*

Similarly, MOS can positively influence the health and growth performance of fish, exerting its effects through its ability to bind to enteropathogenic bacteria, thereby preventing host colonization (Torrecillas et al., 2014; Agboola, J.O. (2022)). However, some studies have also found no effect on feed intake, growth rate, and immune response (Agboola et al., 2021b). Therefore, it is dependent on a lot of factors i.e., type of fish species, replacement level, the molecular structure of β-glucan and MOS, culture condition, feeding rate, growth stage, and general health of fish (Torrecillas et al., 2014).

## 2.2 Filamentous Fungi

Filamentous fungi (FF) have been identified as a potential alternative source of single-cell protein (SCP) for aquafeeds. Fungal microbial ingredients can be used as single-cell protein (SCP) if they contain more than 300 g/kg of protein (on a dry basis). Like yeast, FF are robust organisms. They can survive over a wide range of growth conditions (lower pH than bacteria, pH=4.7). Since they can also grow on a broad range of substrates and can take low concentrations of substrate, they can be exploited in biorefineries for various waste streams. (Bajpai, 2007).

The studies by Karimi et al. (2018, 2019, 2021) evaluate the potential of FF as a source of protein, lipid, and minerals in fish feed. According to Karimi et al. (2019) fungal biomass cultivated on vinasse, a waste material from ethanol production, has a high protein content, ranging from 44.7 to 55.6%, with a good balance of essential amino acids. The lipid content of fungal biomass ranged from 3.5% to 7.0%. Additionally, fungal biomass was found to contain significant levels of minerals such as potassium, magnesium, and calcium, as well as trace elements such as iron and zinc. These findings suggest that fungal biomass cultivated on vinasse has the potential to be a sustainable and cost-effective alternative nutrient source for fish feed.

Another study evaluated the nutritional composition of pure filamentous fungal biomass from the cultivation of *Aspergillus oryzae*, *Neurospora intermedia*, and *Rhizopus oryzae*, as a novel ingredient for fish feed (Karimi et al., 2021). The study found that FF have a high protein content (up to 62.2%), with a good balance of essential amino acids, particularly lysine and methionine. According to Karimi et al. (2018) fungal biomass also contains significant levels of fiber, up to 30% dry weight, as well as minerals such as calcium, phosphorus, and potassium, and vitamins such as thiamine, riboflavin, and niacin. Moreover, certain fungal species can produce bioactive compounds such as polysaccharides and antioxidants, which could potentially have health benefits for fish.

Both studies (Karimi et al., 2019; Karimi et al., 2021) have reported that fungal biomass have high content of polyunsaturated fatty acids (PUFAs) such as linolenic acid (LA), α-linolenic acid (ALA) and they are precursors for omega-3 and omega-6 fatty acids. Atlantic salmon can convert ALA into long chain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) which plays a key role in fish growth and health (Asadollahzadeh et al., 2018; Karimi et al., 2021).

Yeasts mostly assimilate C6 sugars whereas FF such as *P. variotii* can assimilate both C6 and C5 sugars as well as organic acids and glycerol. Moreover, they are easier to separate from a substrate than yeast.

FF from lignocellulosic residues have a wide variety of applications in many industries. That is why they are very important in creating a circular economy. FF secrete a wide range of enzymes enabling them to degrade cellulose, hemicellulose, lignin, starch, and protein into their monomers. The monomers are then metabolized and valuable products such as organic acids and ethanol are generated. FF can be consumed as food, a pure fungal biomass known as mycoprotein. Examples of FF-based fermented foods include tempeh, soy sauce, and miso. Fermentation by FF improves nutritional benefits (tempe has higher protein, vitamin and digestibility than soybean and lower ANF (Meyer et al., 2020; Wikanderi et al.,2022; Karimi et al., 2023).

## 2.3 History of Pekilo

PEKILO® is the trade name for the filamentous fungus *Paecilomyces variotii.* It was initially discovered in 1963 within the Finnish pulp and paper industry. Researchers noted that *Aspergillus niger* could grow on sulfite liquor, a by-product of paper production from wood. The need to bridge the protein gap prompted the exploration of single-cell protein technologies, leading to the initiation of a screening program in 1965, which evaluated over 300 micro-fungi to determine the most suitable organism. At the time, bacterial and yeast MIs were the primary focus of research, but it was ultimately decided that mycelium from FF would be simpler to filter out from dilute spent liquors. Thus, the approach was narrowed down to FF.

Initial screening in flasks led to the shortlisting of 17 fungi, which were then tested in 100 L fermenters and used in preliminary animal feeding trials in 1967. Later, 12 fungi were evaluated in a 3,000 L fermenter, producing sufficient product for chemical and biological testing and a feeding trial in rats. Researchers soon recognized that the fermentation process had to be continuous to be economically viable.

After conducting a comprehensive assessment of the biological and chemical characteristics of the fungal cell mass or mycoprotein, as well as the fungi's performance in continuous fermentation and downstream processing, *P. variotii* was determined to be the most suitable fungus for the PEKILO® process.

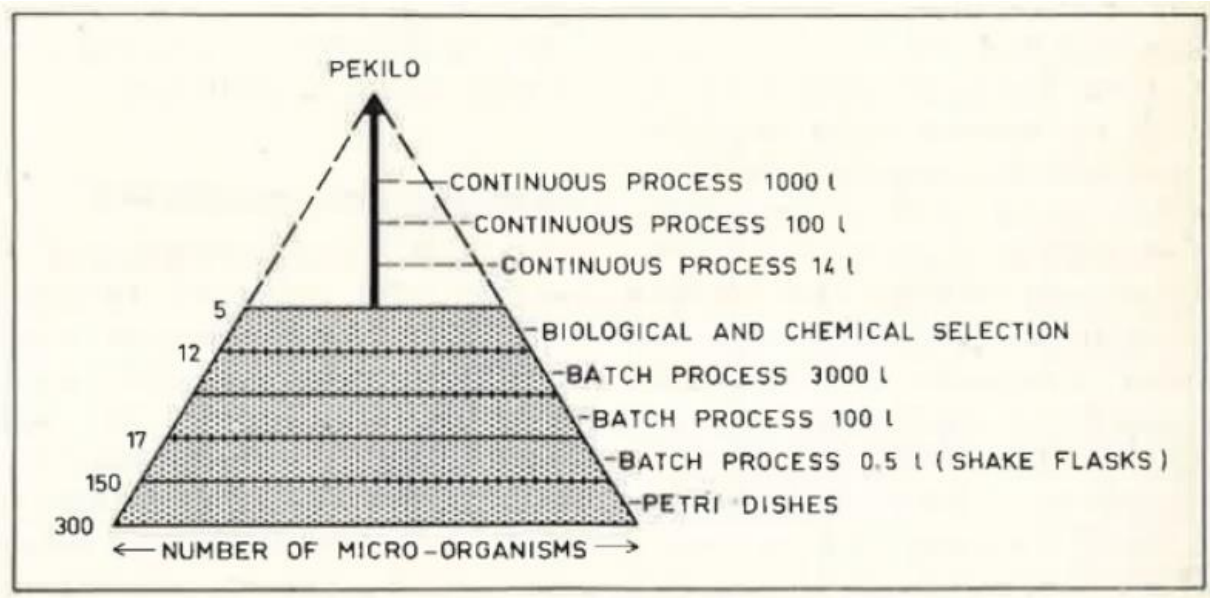


*Figure 5: Screening program that was used to uncover the ideal protein-producing fungus P. variotii.*

The original patent for the PEKILO® process was filed in 1970, and the Pekilo trademark was subsequently registered. By 1971, a 1000 L fermenter capable of continuous fermentation had been developed, allowing to produce tons of mycoprotein for feeding trials in pigs, calves, laying hens, and broilers. In the same year, Pekilo was approved as a feed ingredient in Finland. A 15,000 L pilot fermenter was also developed, which could produce 30 kg of Pekilo per hour and run for over 3000 hours consecutively, using feedstock piped from the pulp mill. Research and development efforts eventually led to the establishment of two industrial-scale Pekilo plants in Finland, each with an annual production capacity of 10,000 tons.

Unfortunately, the Pekilo process came to an end in 1991 due to the discontinuation of sulfite pulping, which resulted in a lack of suitable side streams. Additionally, the protein gap failed to materialize, and there was no demand for alternatives to soy or animal protein at that time.

However, in 2017, the PEKILO® process was revived with the emergence of new biorefinery concepts producing dilute side streams. *P. variotii* was found to thrive in these streams (EniferBio, 2022).

## 2.4 Production process of modern-day Pekilo

Figure 6 depicts the contemporary PEKILO® process, which utilizes sulfite stillage from Borregaard's ethanol production as its source material. The primary carbon sources in the stillage are xylose, acetic acid, arabinose, and a minor amount of glycerol from the yeast ethanol

process. While traditional spent liquors were obtained from the sulfite pulping process, more varied by-products from biorefineries are now used.

Following clarification and sterilization, a continuous stream of nutrients and spent liquor is pumped into a fermenter, where the solution is blended and aerated at a constant pH and temperature. One significant advantage of this approach is that specialized separators are not necessary since the final product is fibrous and can be easily filtered. The resulting biomass is then continuously extracted from the fermenter, along with the broth. Subsequently, the biomass is filtered, washed, and dewatered via mechanical pressing before being dried, thus conserving energy. Separation of the fungus mycelium is accomplished through a fibrous filtering process, aided by an ordinary drum filter. This method offers an advantage over yeast processes, as the amount of wash water needed is minimal.

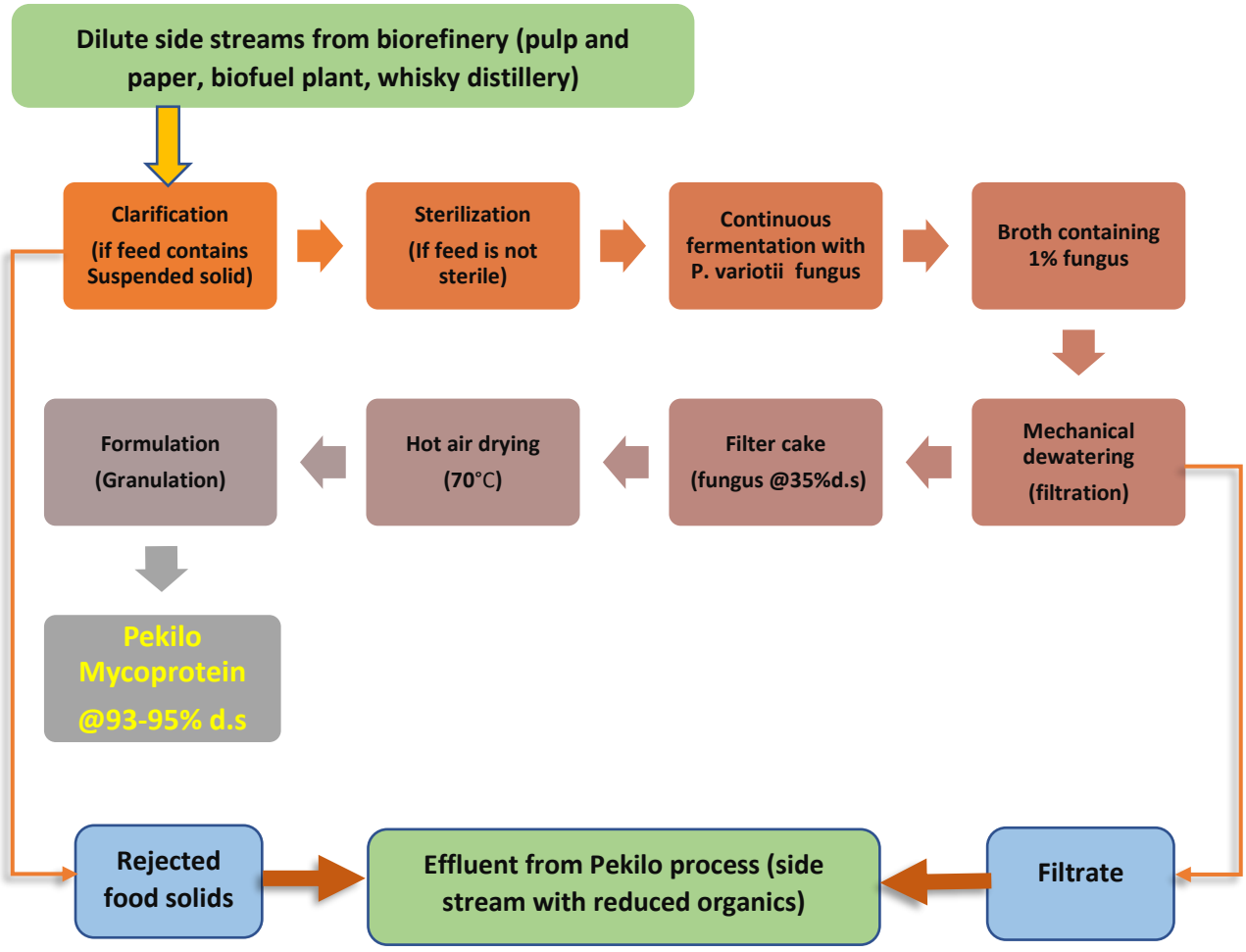


*Figure 6: Modern-day PEKILO® production process by eniferBio*

Biomass is transferred from filter to mechanical press where it's dewatered and content of d.s. 30-35% is obtained. Possibility of pressing saves energy costs because the amount of water to

be evaporated at the end is less. Investment and operation costs lower compared to other SCP. Drying is not expensive since a considerable amount of water can be removed by mechanical pressing.

## 2.5 Potential of Pekilo *(P. variotii)* as an alternative protein ingredient

The nutritive value of *P. variotii* is equivalent to soybean meal and skim milk powder (Koivurinta et al., 1979; Näsi, 1982). It contains almost 60-70% crude protein including nucleotides and 10 to 15% β-glucan. It also has a favourable amino-acid profile. Therefore, Pekilo can effectively replace soy and marine proteins in aquaculture feeds.

In the 1970s and 1980s, research efforts demonstrated that Pekilo *(P. variotii)* could be used as a protein source in feeds for terrestrial species. For instance, Alaviuhkola et al. (1975) successfully replaced digestible protein from skim milk powder with *P. variotii* in diets for growing-finishing pigs without any significant effects on growth or carcass characteristics. Similarly, Barber et al. (1977) found no significant impact on the performance of growing pigs when fish meal was replaced with *P. variotii*. Replacement of fish meal and soybean meal with15% inclusion of *P. variotii* in diets did not affect weight gain, feed consumption, feed efficiency, or reproductive performance of breeding cows (Järvinen et al., 1980). In laying hens, the replacement of crude protein from fish meal and soybean meal with *P. variotii* did not significantly affect laying rate or egg weight, and the feed intake was higher than in hens fed a diet containing fish meal and soybean meal (Näsi, 1982). Although limited, recent research has shown that *P. variotii* has a high digestibility of 85% in Atlantic salmon, suggesting that it may be of high nutritive value for salmonid species (Dhalberg, A. 2019).

Apart from its potential use as a protein source in aquaculture feeds, *P. variotii* may have advantageous health-promoting effects. While yeasts have been extensively studied for their functional properties in fish, the beneficial effects of other SCPs, such as filamentous fungi, are less defined. Recent research by Osaku et al. (2018) demonstrated that *P. variotti's* exopolysaccharide (EPS) β-(1→6)-glucan has the potential to stimulate the secretion of pro-inflammatory cytokines by murine macrophages, suggesting immunomodulatory properties *in vivo*. Similarly, an EPS derived from *P. lilacinus* was found to have immunostimulatory effects on a murine macrophage cell line, as evidenced by the secretion of interleukin (IL-1β), tumor necrosis factor (TNF-α), and nitric oxide (NO) (He et al., 2019). Therefore, the inclusion *of P. variotii* in aquaculture feeds could potentially have immunostimulatory effects in fish. However, there is a need for systematic evaluation of the effects of *P. variotii* on fish health.

## 2.6 Gut health of Atlantic salmon

The gastrointestinal tract (GIT) serves as a multifunctional organ involved in immune responses and serves as the primary site of interaction between environmental microorganisms and antigens and the host (Montalto et al., 2009). The presence of gut microbiota within the GIT is crucial for the development, maintenance, and defense against pathogenic challenges, playing a vital role in the maturation of both the innate and adaptive immune responses of the host (Koch et al., 2021; Montalto et al., 2009). Abundant immune cells, including B and T lymphocytes, macrophages, and granulocytes, are present in the gut mucosa and can initiate localized immune responses (Press & Evensen, 1999).

The gut-associated lymphoid tissue (GALT) is one of the main mucosa-associated lymphoid tissues (MALTs) found in teleosts, alongside the skin-associated lymphoid tissue, gill-associated lymphoid tissue, and nasopharynx-associated lymphoid tissue. GALT consists of both innate and adaptive immune cells and molecules that work together to maintain mucosal homeostasis (Salinas, 2015). GALT plays a crucial role as the protective immune system within the GIT, defending against pathogenic agents that breach the mechanical barrier of the intestinal lumen. Chemokines secreted within GALT participate in regulating immune responses in the intestine (Caballero, 2005; Neu, 2008).

The DI, functioning as MALT (Cesta, 2006; Jung-Schroers et al., 2018), plays a critical role in regulating both local and systemic physiological responses by controlling the production of pro- and anti-inflammatory cytokines, coordinating antigen-presenting cells, and influencing the activity of effector molecules (Morales-Lange et al., 2022).

The use of plant-based salmon feed has been associated with gut health issues such as intestinal inflammation and lipid malabsorption, which in turn increase susceptibility to various diseases (Li et al., 2019). Studies have demonstrated that incorporating plant-based protein sources like soybean meal and pea protein concentrate into diets for farmed Atlantic salmon can lead to proliferative or inflammatory conditions in the intestinal mucosa. Some antinutritional factors present in certain plant-based protein sources pose challenges to the function and health of the fish's gut (Agboola et al., 2021; Gajardo et al., 2017; Penn et al., 2011).

However, functional ingredients are believed to have beneficial effects within this region (Wang et al., 2020) as they can directly stimulate the innate immune system or enhance the growth of gut microbiota (Dawood et al., 2018). These additives promote the natural defense mechanisms of fish by stimulating the immune system, increasing immune resistance,

promoting the proliferation of gut microflora, and enhancing specific or nonspecific defense mechanisms (Assefa & Abunna, 2018).

## 2.7 Ingredient development and characterization

For any new ingredient to be used in fish feeds, it is important to assess them against a standard evaluation criterion based on nutritional response parameters. Glencross et al. (2020) has proposed seven steps that are required in the validation of new ingredients (Figure 7). These steps reduce the risk of feed failing and make the ingredient selection process easier.

The first step is a characterization that deals with the nutritional composition, origin (taxonomy), processing, storage history, etc. The second one is palatability, which is a very critical quality of the selected ingredient as it determines the level of feed intake and affects the growth performance of fish. The third step is assessment of digestibility of nutrients and energy available to the fish by the ingredients or diets containing them. There are different methods to calculate it, one of them is to calculate the difference of nutrients in feed and faeces. Next step is to calculate nutrient utilization which gives an estimate of energy and nutrients retained in the body of fish. The fifth step is to calculate the impact of ingredients on their immune system and general health. The sixth step is functionality and involves evaluation of physiochemical properties of the ingredients on the extrusion process. Lipid content, bulk density, ability to absorb water are few of the physical properties that can alter the extrusion process for manufacturing feed pellets. The last one is the assessment of sensory qualities of the ingredient which includes smell, taste and color (Glencross et al., 2020).

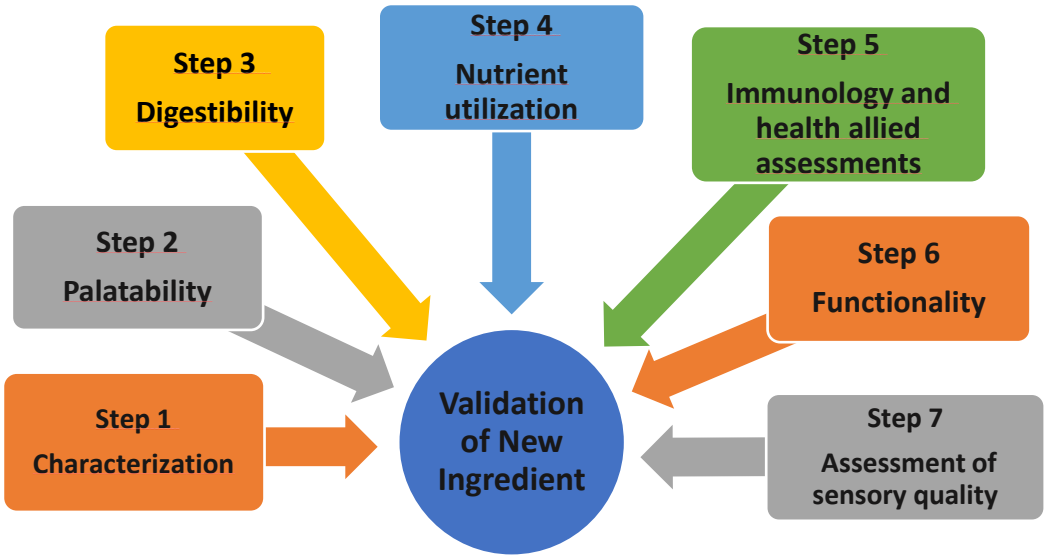


*Figure 7: Seven steps proposed by Glencross et al. (2020) for validation of a new ingredient in aquafeed.*

In this study, the first six steps have been evaluated for the selected ingredient described in detail below. The selected ingredient belongs to the category of MIs and is a FF (*P. variotii*) having a trademark name PEKILO®.

## 2.8 Hypothesis

- FF *(P. variotii)*, will effectively replace up to 20% of the crude protein content of the diet for Atlantic salmon, reared in freshwater, without affecting pellet quality.
- Diets containing FF *(P. variotii)* will improve the growth performance and nutrient utilization and will modulate the nutrient digestibility and immune-related biomarkers in the distal intestine of Atlantic salmon during the freshwater phase.

## 2.9 General Objective

To characterize the effects of diets containing FF *(P. variotii)* on pellet quality, growth performance, nutrient digestibility and utilization, and immune-related biomarkers in the distal intestine of Atlantic salmon during the freshwater phase.

### 2.9.1 Specific Objectives

1. To evaluate the effects of FF *(P. variotii)* on physical pellet quality parameters including durability, hardness, expansion, sinking velocity, water activity, and water stability of the experimental diets.
2. To evaluate the effects of diets containing FF *(P. variotii)* (up to 20% replacement of the crude protein content of the diet) on the growth performance (weight gain, feed conversion ratio, and growth rate) of Atlantic salmon during the freshwater phase.
3. To assess the impact of the inclusion of increasing, graded levels of FF *(P. variotii)* on nutrient digestibility and utilization efficiency in Atlantic salmon reared in freshwater.
4. To evaluate the effect of diets containing FF *(P. variotii*) on the gene expression of immune-related biomarkers (IL-1β, IFN-γ, iNOS, IL-10, TGF-β, and E-cadherin) in the distal intestine of Atlantic salmon during the freshwater phase.
5. To integrate all of the information from the pellet quality analysis, growth performance, digestibility, nutrient utilization, and gene expression of immune-related biomarkers in order to evaluate the potential of *P. variotii* as an alternative ingredient in salmon feeds.

# Chapter 3: Materials and Methods

## 3.1 Experimental diets

*P. variotii* for the experimental diets was provided by eniferBio, a Finnish biotech start-up company with unique expertise in the development of fungal bioprocesses. eniferBio targets widely available complex and diluted organic streams, the refining and/or disposal of which has previously provided little added value or resulted in negative net cost. In this project, the eniferBio team was responsible for the optimization and operation of a small-scale (100 L) pilot process to produce *P. variotii* from Nordic Forest and biorefining industry-side streams.

Four isonitrogenous, isolipidic, and isoenergetic experimental diets were formulated to investigate the nutritional value of *P. variotii* in feeds for Atlantic salmon. The diets were formulated to meet or exceed the nutritional requirements of Atlantic salmon according to NRC (2011). Diet 1 was a control diet formulated with fish meal, soy protein concentrate, and wheat gluten meal as protein ingredients. Diets 2, 3, and 4 were formulated such that *P. variotii* replaced 5, 10, and 20% of the crude protein content of the diets, respectively. Increasing the inclusion of *P. variotii* in the diets was achieved by reducing the protein contributions of the major ingredients including fish meal, wheat gluten meal (WGM), and soy protein concentrate (SPC).

The experimental diets were analyzed for chemical composition at Labtek (NMBU), including analysis of dry matter (DM), the content of ash, crude protein, crude lipids, gross energy, essential amino acids, non-essential amino acids, and several minerals (calcium, sulphur, sodium, magnesium, potassium, and phosphorus). The nutritional composition of the major protein ingredients is shown in Table 1. The formulation of the experimental diets and the analyzed nutritional composition of the diets is presented in Table 2. The diets were manufactured by extrusion at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden.

**Table 1.** Nutritional composition of the ingredients including *P.variotii*, fishmeal, soy protein concentrate (SPC), and wheat gluten meal (WGM) used to formulate the experimental diets.

| **Nutrient (g/kg DM)** | ***P.variotii*[a]** | **Fishmeal[b]** | **SPC[c]** | **WGM[d]** |
|---|---|---|---|---|
| Dry matter | 925.2 | 919.7 | 920.3 | 917.7 |
| Crude protein | 625.1 | 751.8 | 610.0 | 827.8 |
| Crude lipids | 7.5 | 77.0 | 15.1 | 5.8 |
| Ash | 92.3 | 178.8 | 73.7 | 5.8 |
| Gross energy (MJ/kg DM) | 21.0 | 20.5 | 20.3 | 23.5 |
| **Minerals (g/kg DM)** | | | | |
| Sulphur | 6.4 | 8.5 | 4.2 | 6.6 |
| Phosphorous | 20.9 | 24.5 | 8.4 | 1.9 |
| Calcium | 12.5 | 46.2 | 4.1 | 0.4 |
| Magnesium | 0.9 | 2.7 | 4.1 | 0.2 |
| Potassium | 3.4 | 13.5 | 23.9 | 0.8 |
| Sodium | 0.1 | 14.4 | 0.1 | 0.0 |
| **Essential amino acids (g/kg DM)** | | | | |
| Arginine | 26.2 | 36.0 | 35.6 | 20.7 |
| Histidine | 13.7 | 18.3 | 17.4 | 19.2 |
| Isoleucine | 21.1 | 26.9 | 26.6 | 27.4 |
| Leucine | 33.4 | 46.5 | 42.6 | 50.6 |
| Lysine | 33.8 | 54.8 | 27.4 | 12.1 |
| Methionine | 7.5 | 14.3 | 6.1 | 10.8 |
| Phenylalanine | 15.2 | 19.1 | 20.2 | 33.0 |
| Threonine | 19.9 | 25.3 | 22.0 | 16.3 |
| Valine | 20.6 | 25.1 | 21.9 | 23.6 |
| Tryptophan | 5.3 | 5.8 | 6.5 | 5.3 |
| **Non-essential amino acids (g/kg DM)** | | | | |
| Alanine | 23.9 | 36.5 | 21.4 | 17.3 |
| Aspartic acid | 30.7 | 51.7 | 50.2 | 17.3 |
| Glycine | 18.2 | 37.1 | 19.0 | 20.4 |
| Glutamic acid | 51.8 | 92.4 | 102.8 | 290.1 |
| Cysteine | 3.3 | 5.1 | 7.2 | 13.2 |
| Tyrosine | 17.5 | 13.8 | 14.4 | 18.4 |
| Proline | 19.2 | 25.8 | 26.2 | 87.3 |
| Serine | 16.9 | 20.3 | 23.6 | 29.2 |
| **Sum amino acids** | 372.8 | 549.0 | 484.6 | 706.8 |

[a]*P.variotii*, eniferBio, Espoo, Finland.
[b]LT-fishmeal, Norsildmel AS, Bergen, Norway.
[c]Soy protein concentrate, Hamlet Protein A/S, Horsens, Denmark.
[d]Wheat gluten meal, Lantmännen Reppe AB, Lidköping, Sweden.

**Table 2.** Ingredient and analyzed nutritional composition of the experimental diets containing increasing levels of *P.variotii*.

| **Ingredients (g/kg diet)** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** |
|---|---|---|---|---|
| Fishmeal[a] | 340.0 | 335.0 | 325.0 | 315.0 |
| *P.variotii*[b] | 0 | 40.4 | 79.5 | 159.0 |
| Wheat gluten meal[c] | 90.0 | 85.0 | 70.0 | 65.0 |
| Soy protein concentrate[d] | 235.4 | 205.0 | 190.9 | 126.4 |
| Wheat flour[e] | 160.0 | 160.0 | 160.0 | 160.0 |
| Rapeseed oil[f] | 120.0 | 120.0 | 120.0 | 120.0 |
| Fish oil[g] | 40.0 | 40.0 | 40.0 | 40.0 |
| Vitamin and mineral premix[h] | 3.0 | 3.0 | 3.0 | 3.0 |
| L-lysine[i] | 5.0 | 5.0 | 5.0 | 5.0 |
| DL-methionine[j] | 3.0 | 3.0 | 3.0 | 3.0 |
| L-threonine[k] | 2.0 | 2.0 | 2.0 | 2.0 |
| Choline chloride[l] | 1.5 | 1.5 | 1.5 | 1.5 |
| Yttrium oxide[m] | 0.1 | 0.1 | 0.1 | 0.1 |
| Total | 1000 | 1000 | 1000 | 1000 |
| **Analyzed chemical composition (g/kg DM)** | | | | |
| Dry matter | 943.4 | 931.0 | 930.0 | 920.3 |
| Crude protein | 503.9 | 506.9 | 499.7 | 500.7 |
| Crude lipids | 174.1 | 174.3 | 171.7 | 187.1 |
| Ash | 82.8 | 81.5 | 81.8 | 77.8 |
| Gross energy (MJ/kg DM) | 23.6 | 23.8 | 23.8 | 23.7 |
| **Minerals (g/kg DM)** | | | | |
| Sulphur | 5.5 | 5.6 | 5.5 | 5.2 |
| Phosphorous | 11.8 | 11.6 | 12.5 | 12.9 |
| Calcium | 15.1 | 14.9 | 15.8 | 16.7 |
| Magnesium | 2.0 | 1.8 | 1.8 | 1.6 |
| Potassium | 11.5 | 10.7 | 10.5 | 9.0 |
| Sodium | 5.0 | 4.9 | 4.9 | 4.8 |
| **Essential amino acids (g/kg DM)**[n] | | | | |
| Arginine | 22.9 | 24.7 | 25.5 | 20.2 |
| Histidine | 13.2 | 12.5 | 11.7 | 12.7 |
| Isoleucine | 19.1 | 18.4 | 17.9 | 17.8 |
| Leucine | 32.3 | 31.1 | 30.5 | 29.5 |
| Lysine | 33.5 | 33.8 | 33.1 | 32.3 |
| Methionine | 14.6 | 14.5 | 13.8 | 10.5 |
| Phenylalanine | 16.4 | 14.5 | 14.3 | 13.7 |
| Threonine | 19.0 | 18.7 | 17.9 | 17.8 |
| Valine | 17.3 | 16.9 | 16.7 | 16.1 |
| Tryptophan | 4.7 | 4.7 | 4.8 | 4.2 |
| **Non-essential amino acids (g/kg DM)**[n] | | | | |
| Alanine | 20.1 | 19.6 | 19.7 | 19.6 |
| Aspartic acid | 34.5 | 34.1 | 32.3 | 30.4 |
| Glycine | 20.2 | 20.1 | 19.3 | 19.0 |
| Glutamic acid | 90.8 | 86.9 | 79.7 | 73.7 |
| Cysteine | 4.9 | 4.5 | 4.1 | 4.2 |
| Tyrosine | 10.9 | 9.3 | 9.0 | 9.6 |
| Proline | 27.2 | 23.9 | 24.5 | 23.0 |
| Serine | 18.2 | 18.1 | 15.6 | 16.2 |
| **Sum amino acids**[o] | 415.2 | 401.5 | 385.6 | 366.5 |

[a]LT-fishmeal, Norsildmel AS, Bergen, Norway.

[b]*P.variotii,* eniferBio, Espoo, Finland.

[c]Wheat gluten meal, Lantmännen Reppe AB, Lidköping, Sweden.

[d]Soy protein concentrate, Hamlet Protein A/S, Horsens, Denmark.

[e]Wheat flour, Garant, Axfood AB, Stockholm, Sweden.
[f]Rapeseed oil, Avena Nordic Grain, Finland.
[g]Fish oil, Ab Salmonfarm Oy, Kasnäs, Finland.
[h]Provides per kg of diet: vitamin A, 6800 IU; vitamin D3, 3000 IU; vitamin E, 260 mg; vitamin K3, 20 mg; vitamin B1, 18 mg; vitamin B2, 26mg; Pantothenic acid, 80 mg; vitamin B6, 17 mg; vitamin B12, 60 mcg; Nicotinic acid, 150 mg; Folic acid, 10 mg; Biotin, 791 mcg; Vitamin C, 270 mg; Inositol, 495 mg; Zinc oxide, 75 mg; Iodine, 3.2 mg; Copper, 3.9 mg; Manganese, 4.92 mg; Zinc chelate, 37.5 mg; Citric acid, 0.54 mg; BHT (Butylated hydroxytoluene), 1.6 mg; Propyl gallate, 0.84 mg; BHA (Butylated hydroxyanisole), 1 m; Silicic acid, 12.45 mg; Calcium carbonate, 0.053 %.
[i]L-lysine, feed grade (Biolys®), Evonik, Essen, Germany.
[j]DL-methionine, Evonik, Essen, Germany.
[k]L-threonine, feed grade, Evonik, Essen, Germany.
[l]Choline chloride, MIAVIT GmbH, Essen, Germany.
[m]Yttrium oxide ($Y_2O_3$), Sigma-Aldrich, Vienna, Austria.
[n]Water corrected values.
[o]Sum of essential and non-essential amino acids without tryptophan.

## 3.2 Experimental procedure and duration

The experiment was started in mid-August of 2022 (week 33) and lasted for a duration of nine weeks. Fish were fed with a commercial salmon feed prior to the start of the trial. At the start of the experiment, fish were batch weighed for determination of initial body weight and to ensure that equal total biomass was allocated to each tank. Fish were batch weighed again at the end of the experiment for the determination of weight gain and growth rate, expressed as specific growth rate (SGR) and thermal-unit growth coefficient (TGC).

Fish were fed with the experimental diets *ad libitum* (i.e., 10% excess) with electrically driven belt feeders for twelve hours per day (07:00 to 13:00 and from 18:00 to 24:00). The amount of feed was weighed and adjusted daily based on the expected fish biomass in the tanks. Daily feed intake of each tank was quantified according to Helland et al. (1996) by collection of uneaten feed using wedge wire screens as described by Shomorin et al. (2019). Feed conversion ratio (FCR) was also determined. Recovery test was also done for each experimental diet with all corresponding tanks according to Helland et al. (1996). This was done to ensure correct calculations of eaten and uneaten feed.

## 3.3 Experimental fish, housing, and animal welfare

The trial was carried out at the Center for Sustainable Aquaculture at the Norwegian University of Life Sciences (NMBU, Ås, Norway). A total of 480 Atlantic salmon (with an initial average body weight of 24 g) were randomly distributed into 12 fiber glass tanks (40 fish/tank) with three replicate tanks per diet (Table 3). Each tank had a capacity of 300 L. Fish were kept in

recirculated fresh water at approximately 14-15 °C and exposed to a 24-h light regime. Water quality parameters (dissolved oxygen level, temperature, salinity, and pH) and unusual incidents were recorded regularly according to standard routines. In general, flow rate was adjusted to maintain the oxygen concentration above 75% and dissolved oxygen levels were kept above 7.0 mg $L^{-1}$ in the outlet water. Water supply was maintained at approximately 8.5 L/min.

The welfare of the fish (housing and handling) was in accordance with the national guidelines for the care and use of animals (The Norwegian Animal Welfare Act and environmental Regulation and Animal Experimentation). Prior to weighing, sampling, and stripping, fish were anesthetized with tricaine methane sulfonate (MS-222; 15 mg $L^{-1}$).

**Table 3**. Randomization of the experimental treatments to all the tanks.

| Tank No | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet No | 4 | 2 | 3 | 1 | 3 | 1 | 4 | 2 | 1 | 3 | 2 | 4 |

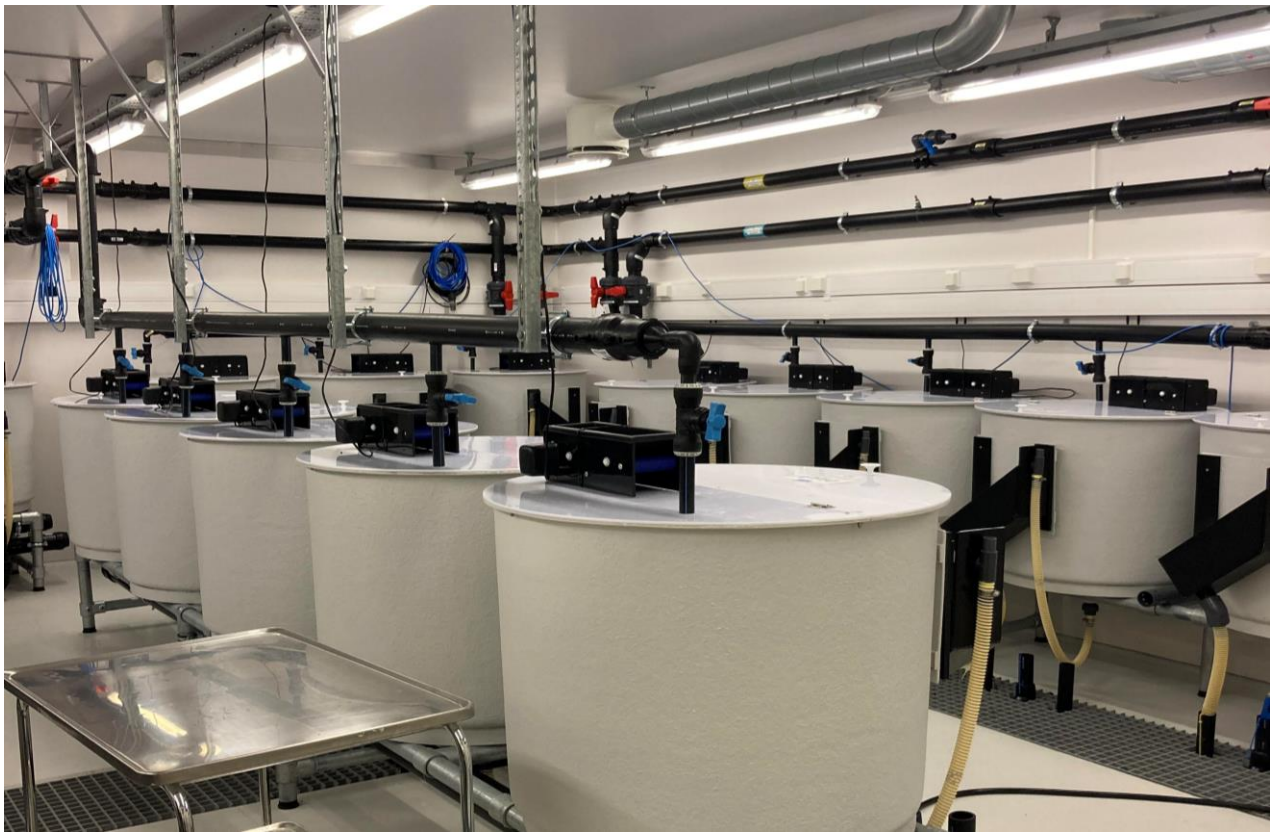

*Figure 8: Experimental setup at the Center for Sustainable Aquaculture, NMBU.*

## 3.4 Sampling procedure

Body lengths and weights were recorded for all sampled fish. At the start of the experiment, a pooled sample of 20 representative fish from the holding tank was collected for body chemical composition. Following the 9-week experimental period, six fish per tank were randomly sampled and anesthetized with MS-222 (15 mg $L^{-1}$). Blood was collected from the caudal vein and kept on ice until centrifugation. Serum was then aliquoted into sterile Eppendorf tubes and kept on dry ice until storage at – 80 °C. The gastrointestinal tract was removed, cleaned free of associated adipose tissue, and divided into the proximal (PI), mid (MI), and distal (DI) intestine. The PI is defined as the section between the stomach's pyloric sphincter and the distal most caecum, while the MI is the section between the distal caecum and the increase in diameter indicating the start of the DI. The DI is defined as the section between the distal end of the MI and the anus and is distinguished by an increased diameter and pronounced circular striation of the mucosa (Krogdahl and Bakke-McKellep, 2005). The distal intestine was opened longitudinally, and the contents were collected in cryotubes for snap freezing. A small section of the DI closest to the anus was collected and placed in RNAlater for 24 hours at 4 °C and then stored at – 80 °C. The remainder of the DI was Swiss rolled and fixed in 4% phosphate-buffered formalin for 24 hours before storage in 70% ethanol until further processing. The remaining fish in each tank were carefully stripped for fecal collection from the posterior intestine according to Austreng (1978). The feces from all fish in each tank was pooled, weighed, and stored at – 20 °C until freeze drying.

Following the initial sampling, fish were fasted for 72 hours. Subsequently, a pooled sample of five fish per tank were collected for whole-body chemical composition. Fish were stored at – 20 °C until processing and freeze drying. After an additional week of feeding, the remaining fish were carefully stripped for fecal collection a second time. The timeline of the experiment is depicted in Figure 9.

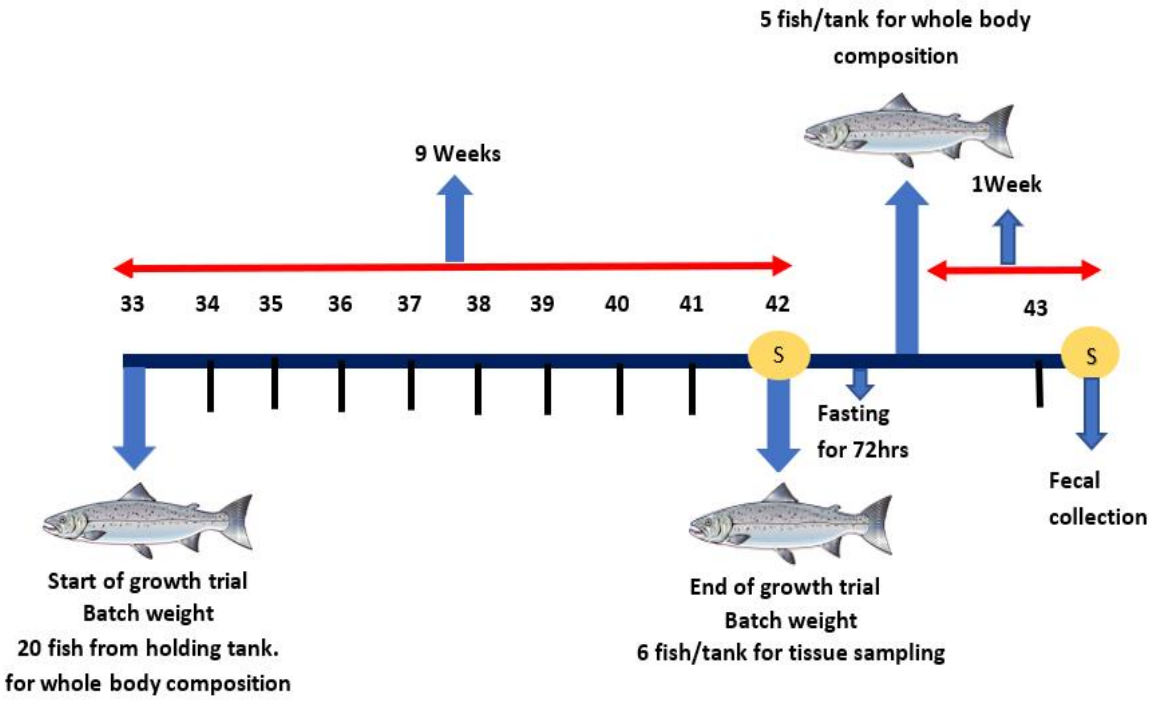


*Figure 9: Schematic representation of the timeline and design of the experiment. "S" denotes stripping.*

## 3.5 Physical pellet quality analysis

Pellet analysis is a widely used method for assessing the quality of fish feed pellets. This evaluation can assist in optimizing the formulation and processing of fish feed to ensure that fish consume the feed, efficiently utilize its nutrients, and attain optimal growth performance. To assess the physical quality of oil-coated pellets, several parameters were measured, including durability, hardness, water stability index, bulk density, water activity, and sinking velocity. The specific methodology for each test is described in detail below.

### 3.5.1 Durability

The durability of pellets was measured in triplicate using a DORIS Pellet tester (AKVAsmart, Bryne, Norway) (Figure 10). Testing of durability required approximately 100 g of pre-sieved pellets per replicate. Briefly, the pellets were placed in the DORIS tester and subjected to a screw conveyor and rotating fan. The weight of the sample after passing through the DORIS tester was recorded (initial weight of pellets). Subsequently, samples were sieved for 60 sec at an amplitude of 0.5 Hz using a Retsch Vibratory Sieve Shaker (AS 200 Control, Haan, Germany) (Figure 11). Weights of the selected empty sieves (2.5 mm, 2 mm, and 1 mm) and the bottom dustpan were recorded. Following sieving, the weights of the full sieves and bottom

dustpan were again recorded (Samuelsen et al., 2021). Durability (%) was calculated according to the following formula:

$$Durability\ (\%) = \frac{Weight\ of\ full\ sieve\ (2.5mm) - Weight\ of\ the\ empty\ sieve\ (2.5mm)}{Initial\ weight\ of\ pellets} \times 100$$

*Figure 10: DORIS Pellet Tester shaker*

*Figure 11: Retsch Vibratory Sieve*

### 3.5.2 Pellet length, width, hardness and expansion

Thirty pellets were randomly selected from each diet and were placed in descending order by length. Amongst these thirty pellets, only the middle fifteen were selected to measure length, width, and hardness. Length and width were measured using electronic calipers. Expansion (%) was calculated using the following formula:

$$Expansion(\%) = \frac{Pellet\ width - die\ diameter}{die\ diameter} \times 100$$

The hardness of the same pellets selected for the determination of length and width was measured using a hand-held Kahl device. The force needed to break the pellet was recorded in kilograms (kg).

### 3.5.3 Bulk Density

The bulk density of the pellets was measured in triplicate using a bulk-density cup. Initially, the bulk density cup was tared on the analytical balance. Afterward, the bulk density cup was filled with pellets and the weight of the pellets was recorded. Bulk density was expressed in g/L.

### 3.5.4 Water Activity:

Water activity was measured in triplicate using the Rotronic Water Activity Measurement Device. Pellets were partially ground using a coffee grinder. Then they were placed in the holders and covered with probes (Figure 12). Water activity was recorded after fifteen minutes.

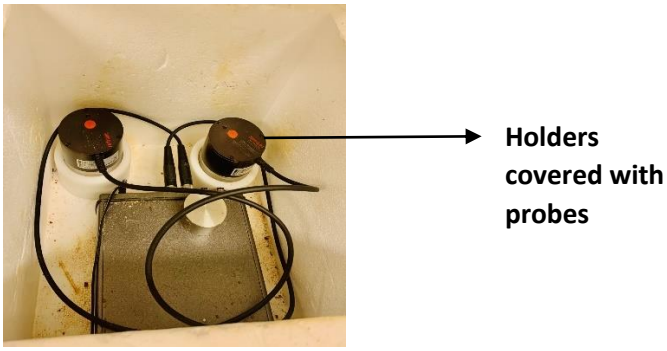


*Figure 12: Rotronic Water Activity Measurement Device*

### 3.5.5 Sinking Velocity

Sinking velocity was measured by filling a 1.2 m long plastic tube (12 cm in diameter) with drinkable tap water to the 1 m mark (Figure 13). One pellet was dropped into the tube at a time. Time was recorded in seconds for each pellet to travel 1m. Thirty-five pellets per Diet were recorded to get an average sinking velocity of the particular diet.

*Figure 13: One-meter water column for measurement of sinking velocity*

### 3.5.6 Water Stability

The water stability of the pellets was measured using a Julabo SW22 Shaking water bath (Figure 14) according to Baeverfjord et al. (2006), but with some modifications. Baskets were weighed before and after the addition of 20 g of samples. The baskets along with beakers containing Milli-Q water (300 mL) were then placed in the water bath at 23°C and shaking was set at 120 rpm. Measurements were recorded in triplicates for each diet sample at four incubation times (i.e., 30 min, 60 min, 120 min and 240 min). Samples were dried for 20 hours at 100-104°C. After oven drying, the weight of the baskets along with the plates were recorded. Dry matter retained gave an estimation of water stability of experimental diets.

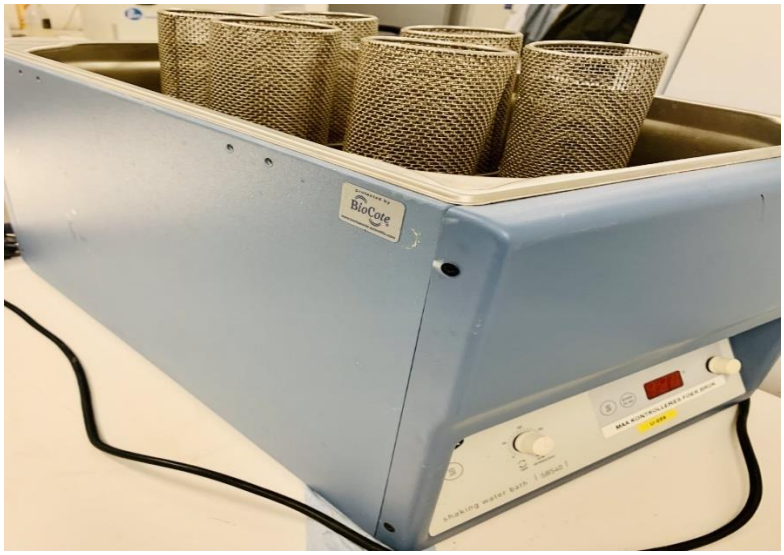


*Figure 14: Shaking water bath with baskets containing the diet samples.*

## 3.6 Chemical Analysis

The feed samples and freeze-dried fecal samples underwent grinding until they were finely and homogeneously consistent. Whole fish samples were partially thawed, ground, freeze-dried, and re-ground before being analyzed. Dry matter (DM) content was determined through oven drying at 104°C until a constant weight was achieved. Ash content was determined through combustion at 550°C. Nitrogen (N) content of the feed, feces, and fish was measured using the Kjeldahl method in accordance with Commission Regulation (EC) No. 152/2009, and crude protein (CP) content was determined as N × 6.25. The Soxtec™ 8000 extraction unit (FOSS Analytical, Hillerød, Denmark) was utilized to extract crude lipid with petroleum ether as a solvent. Gross energy was assessed through a Parr™ 6400 Automatic Isoperibol Bomb Calorimeter (Parr Instrument Company, Moline, IL, USA) based on ISO 9831. Amino acid analysis, excluding tryptophan, was conducted in line with Commission Regulation (EC) No. 152/2009 on a Biochrom 30+ Amino Acid Analyzer (Biochrom Ltd., Cambridge, UK). Tryptophan content was evaluated using an UltiMate™ 3000 UHPLC system equipped with an auto-injector (Thermo Scientific, Massachusetts, USA) and a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan) according to Commission Regulation (EC) No. 152/2009. Yttrium (Y), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), and total phosphorous (P) levels were measured using a Microwave Plasma Atomic Emission Spectrometer (MP-AES 4200, Agilent Technologies, Santa Clara, CA, USA) after undergoing acid decomposition in a microwave digestion system (START D, Milestone Srl, Sorisole, Italy).

## 3.7 Gene expression

The gene expression quantification of the distal intestine samples was done by reverse transcription – quantitative polymerase chain reaction (RT – qPCR).

### 3.7.1 RNA extraction of distal intestinal samples

Total RNA was extracted from DI tissue collected from 48 fish (4 fish per dietary treatment). One 5 mm stainless steel bead was added into each 2.0 mL Micro tube. A piece of distal intestine tissue (approximately between 20-40 mg) was placed in the tube together with 900 µL Qiazol Lysis Reagent (Qiagen, Hilden, Germany). The sample was placed and evenly distributed in TissueLyser (Retsch, Basel, Schweiz) for 2 min at a speed of 20 Hz. The tubes were rearranged in opposite directions and then operated in TissueLyser for another 2 minutes

at 20 Hz. The lysates were carefully pipetted into a new 1.5 mL Eppendorf tube, incubated for 5 min at room temperature and 180 µL chloroform was added. The new mixture was inverted by hand for 15 sec and incubated at room temperature for 2-3 min. The samples were centrifuged at 4 °C for 15 min at a speed of 12,000 rpm. The upper aqueous phase containing the RNA was transferred to a new 1.5 mL Eppendorf tube (approximately 450 µL). An equal volume of 450 µL isopropanol was added. The Eppendorf tubes containing RNA and isopropanol were thoroughly mixed, incubated at room temperature for 10 min and centrifuged at 4 °C for 15 min at a speed of 12,000 rpm. RNA precipitated out as a white pellet. The supernatant was removed without touching the pellet and the tubes were centrifuged at 4 °C for 3 min at a speed of 12,000 rpm. Excess liquid was removed carefully to avoid the Qiazol/chloroform contamination in the sample. Approximately 200 µL of the 70 % EtOH was added in the tubes containing RNA pellets for cleaning purpose and were centrifuged at 4 °C for 10 min at a speed of 12,000 rpm. The supernatant was removed without touching the pellets and the tubes were centrifuged at 4 °C for 3 min at a speed of 12,000 rpm. Excess liquid was removed carefully. The tubes were laid down with an open lid for air drying the RNA pellets for approximately 5-10 mins. When the pellets became completely transparent, they were dissolved in approximately 40µL RNAse-free H2O. Afterwards, tubes containing the RNA were incubated on a heating block at 55°C for 10 mins for dissolution of RNA pellets. The concentration of total RNA was determined using a Nanodrop TM 8000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Total RNA was stored at -80 °C until further analyses.

### 3.7.2 cDNA synthesis

Total RNA was reversely transcribed into cDNA by SuperScript ® IV Reverse Transcriptase according to the protocol provided by Invitrogen. To generate first strand cDNA, 1 µg total RNA was mixed with 0.5 µl dNTP mix (Thermo Fisher Scientific, Wilmington, USA) and 0.5 µL Oligo $d(T)_{20}$ Primer (Thermo Fisher Scientific, Wilmington, USA) and RNase-free water to a volume of 13 µl, incubated at 65 °C for 5 min and then incubated on ice for a minimum of one minute to allow for primer annealing. This was followed by the addition of the following components: 4 µl 5x SSIV Buffer (Thermo Fisher Scientific, Wilmington, USA), 1 µL of 100 mM DTT (Thermo Fisher Scientific, Wilmington, USA), 0.2 µL RNaseOUT™ (Recombinant RNase Inhibitor, Thermo Fisher Scientific, Wilmington, USA), 0.2 µL SuperScript® IV (Reverse Transcriptase, 200 U/µL, Thermo Fisher Scientific, Wilmington, USA) and 1.6 µL

RNase-free water. A negative control reaction with no template was included. Using a GeneAmp® PCR system 9700 (Thermo Fisher Scientific, Wilmington, USA), cDNA synthesis was carried out at 55 °C for 10 min, followed by enzyme inactivation at 80 °C for 10 min. The cDNA was kept at – 80 °C until use.

### 3.7.3 Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

A 1:10 dilution was made with the cDNA from each sample. A 7.5 µL master mix containing 5 µL SYBR Green Master Mix (Roche, Basel, Switzerland), 0.75 µL from each primer (forward and reverse) was made. Reactions were performed in duplicate, the master mix put into wells of a 384 well PCR plate and 2.5 µL of cDNA was added to each well. For each primer pair a negative control reaction with no template was included. The plates were sealed, vortexed and then spun down for 1 min. A CFX Opus 384 Real-Time PCR System thermal cycler (Bio-Rad, California, USA) was programmed as shown in Table 4. At the end of the program, a melting curve analysis was performed for accurate identification of single amplified cDNA sample and to confirm the absence of unspecific products or primer dimers. Relative expression (fold change) was calculated using the delta-delta Ct ($2^{-\Delta\Delta CT}$) method and normalized to a housekeeping gene named elongation factor 1 alpha. The list of the genes analyzed, and their corresponding primers are given in Table 5.

**Table 4: Thermo-cycle for RT-qPCR**

| Step | Duration | Temperature (°C) |
|---|---|---|
| **Activation of DNA-polymerase** | 7min | 95 |
| **Amplification of the target DNA (40x)** | 10sec | 95 |
| **Primer Annealing (40x)** | 15sec | 64 |
| **Elongation (40x)** | 15sec | 72 |
| **Melting Curve** | 5sec<br>1min<br>Variable | 95<br>65<br>97 |
| **Cooling** | 10sec | 40 |

**Table 5:** Genes evaluated by RT-qPCR.

| Gene Names | Forward Primer | Reverse Primer | Melting Temp (°C) |
|---|---|---|---|
| ***ef1a*** **(Elongation factor 1 alpha)** | GCAGTGGCAGTGTGATTTCG | GTAGATCAGATGGCCGGTGG | 64 |
| ***il1b*** **(Interleukin 1 beta)** | AGGACAAGGACCTGCTCAACT | CCGACTCCAACTCCAACACTA | 64 |
| ***tgfb*** **(Transforming growth factor beta)** | AGTTGCCTTGTGATTGTGGGA | CTCTTCAGTAGTGGTTTGTCG | 64 |
| ***inos*** **(Inducible nitric oxide synthase)** | AGGTGCTGAATGTGTTGCAC | GTATTCTCCTGCCTGGGTGA | 64 |
| ***il10*** **(Interleukin 10)** | ACAACAGAACGCAGAACAACC | GCATAGGACGATCTCTTTCTTCAG | 64 |
| ***Infγ*** ***(Interferon gamma)*** | CCGGAGCCACAGTGGAGATA | AAATAGATGGCCAGTTGAGGCA | 64 |
| ***ecad1*** **(E-cadherin 1)** | CTCTTTCACTCCGAGGATTCAC | TGGACTGGGTAGAGACATAGAC | 64 |

## 3.8 Calculations

- Specific growth rate (SGR, % body weight day$^{-1}$) was calculated as [(ln (FBW) – ln (IBW))/experimental period (days)] × 100%, where: FBW = final body weight in g fish$^{-1}$; IBW = initial body weight in g fish$^{-1}$ (Brett and Groves, 1979).

- Thermal-unit growth coefficient (TGC) was calculated as 100 × [(FBW$^{1/3}$ – IBW$^{1/3}$) × (sum T × D)$^{-1}$], where: FBW = final body weight in g fish$^{-1}$; IBW = initial body weight in g fish$^{-1}$; sum T × D = sum degrees Celsius × days (Iwama and Tautz, 1981; Cho, 1992).

- Feed intake (g DM fish$^{-1}$) was calculated as total feed intake (g DM tank$^{-1}$)/number of fish per tank (Hooft et al., 2019).

- FCR was calculated as feed intake (g DM $fish^{-1}$)/ (FBW – IBW), where: FBW = final body weight in g $fish^{-1}$; IBW = initial body weight in g $fish^{-1}$ (Agboola et al., 2022).

- Apparent digestibility coefficients (ADC, %) of the nutrients in the experimental diets were calculated as ADC = 1 – (F/D × Di/Fi), where: D = % nutrient (or kJ/g gross energy) of the diet; F = % nutrient (or kJ/g gross energy) of the feces; Di = % digestion indicator (yttrium) of the diet; Fi = % digestion indicator (yttrium) of the feces (Cho et al., 1982).

- Retained nitrogen (RN, g $fish^{-1}$) and retained energy (RE, kJ $fish^{-1}$) were calculated as: RN = (FBW × N $content_{final}$) – (IBW × N $content_{initial}$) and RE = (FBW × GE $content_{final}$) – (IBW × GE $content_{intial}$), respectively, where: FBW = final body weight in g $fish^{-1}$; IBW = initial body weight in g $fish^{-1}$; N $content_{final}$ = nitrogen content (%) of the final carcass sample; N $content_{initial}$ = nitrogen content (%) of the initial carcass sample; GE $content_{final}$ = gross energy (kJ $g^{-1}$) content of the final carcass sample; and GE $content_{initial}$ = gross energy (kJ $g^{-1}$) content of the initial carcass sample (Hooft et al., 2019).

- Nitrogen retention efficiency (NRE) and energy retention efficiency (ERE) werecalculated for each tank as a percentage of ingested nitrogen (IN, g $fish^{-1}$) and ingested energy (IE, kJ $fish^{-1}$), respectively: NRE (% IN) = [[(FBW × N $content_{final}$) – (IBW × N $content_{initial}$)]/IN] × 100 and ERE (% IE) = [[(FBW × GE $content_{final}$) – (IBW × GE $content_{initial}$)]/IE] × 100, where: FBW = final body weight in g $fish^{-1}$; IBW = initial body weight in g $fish^{-1}$; N $content_{final}$ = nitrogen content (%) of the final carcass sample; N $content_{initial}$ = nitrogen content (%) of the initial carcass sample; GE $content_{final}$ = gross energy (kJ $g^{-1}$) content of the final carcass sample; and GE $content_{initial}$ = gross energy (kJ $g^{-1}$) content of the initial carcass sample (Hooft et al., 2019).

- Nitrogen retention efficiency (NRE) and energy retention efficiency (ERE) were calculated for each tank as a percentage of digested nitrogen (DN, g $fish^{-1}$) and digested energy (DE, kJ $fish^{-1}$), respectively: NRE (% DN) = [[(FBW × N $content_{final}$) – (IBW × N $content_{initial}$)]/DN] × 100 and ERE (% DE) = [[(FBW × GE $content_{final}$) – (IBW × GE $content_{initial}$)]/DE] × 100, where: FBW = final body weight in g $fish^{-1}$; IBW = initial body weight in g $fish^{-1}$; N $content_{final}$ = nitrogen content (%) of the final carcass sample;

N content$_{initial}$ = nitrogen content (%) of the initial carcass sample; GE content$_{final}$ = gross energy (kJ g$^{-1}$) content of the final carcass sample; and GE content$_{initial}$ = gross energy (kJ g$^{-1}$) content of the initial carcass sample (Øverland et al., 2013).

## 3.9 Data Analysis

All growth performance, nutrient digestibility, and nutrient utilization data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS OnDemand for Academics, SAS Institute Inc., Cary, NC, USA). Tank was considered the experimental unit. The Shapiro-Wilk test in PROC UNIVARIATE of SAS was used to assess normality, and the Brown and Forsythe test in PROC GLM of SAS was used to test for homogeneity of variances for all dependent variables prior to other statistical analysis. A one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was conducted to determine differences between treatment means. Linear and quadratic orthogonal polynomial contrasts across dietary inclusion level of *P. variotii* (i.e., crude protein replacement level) were performed for all dependent variables (Kuehl, R.O., 2000). Contrast coefficients were generated using the PROC IML procedure of SAS. Significance was declared at $p < 0.05$.

GraphPad Prism 8.0.2 was used for statistical analysis of the gene expression data, as well as graphical presentation and calculation of means and standard deviation (SD). Gene expression was calculated according to the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001; Rao et al., 2013). Natural log of the delta delta ct values was taken. Furthermore, to ensure normality and homogeneity of the data, the Shapiro-Wilk test was performed on natural log values and outliers were removed (Teixeira et al., 2022). To assess the differences in gene expression levels of six biomarkers (*ifnγ, inos, tgfb, il10, il1b*, *ecad1*) between the control diet and other experimental diets, a statistical analysis was performed. A parametric t-test was used for the genes *ifnγ, inos, il10, il1b,* and *ecad1*, as their natural log-transformed values exhibited normal distribution. Conversely, the *tgfb* gene didn't exhibit normal distribution of its natural log-transformed values therefore, a non-parametric t-test (Mann-Whitney test) was used. A Pearson correlation plot was made using GraphPad Prism 8.0.2. Correlation was considered significant at $p<0.05$.

# Chapter 4: Results

## 4.1 Extrusion Parameters and Physical Pellet Quality

The extrusion parameters used in the production of the experimental diets are shown in Table 6. The temperature of the second barrel was higher during the production of Diets 2, 3 and 4 (130 °C) compared to the temperature of the same barrel during the production of Diet 1 (110 °C). Conversely, the temperature of the fifth barrel was lower to produce Diets 2, 3 and 4 (100 °C) compared to the temperature of this barrel for Diet 1 (110 °C). Throughput (kg $h^{-1}$) and die pressure (bar) were lower to produce Diet 1 compared to Diets 2, 3 and 4. Moreover, knife speed (rpm) was increased during the production of Diets 2, 3 and 4 compared to the control diet (Diet 1).

**Table 6***:* Extruder parameters during the production of the experimental diets containing increasing inclusion of *P. variotii.*

| Extruder parameter | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| **Throughput (kg $h^{-1}$)** | 4.5 | 5.4 | 5.4 | 5.4 |
| **Barrel 1 (°C)** | 100 | 100 | 100 | 100 |
| **Barrel 2 (°C)** | 110 | 130 | 130 | 130 |
| **Barrel 3 (°C)** | 130 | 130 | 130 | 130 |
| **Barrel 4 (°C)** | 130 | 110 | 110 | 110 |
| **Barrel 5 (°C)** | 110 | 100 | 100 | 100 |
| **Screw speed (rpm)** | 240 | 240 | 240 | 240 |
| **Die pressure (bar)** | 18 | 23 | 23 | 23 |
| **Nozzle diameter (mm)** | 2 | 2 | 2 | 2 |
| **Knife speed (rpm)** | 610 | 750 | 750 | 750 |
| **Pellet moisture at the die (%)** | 30.6 | 30.8 | 31.6 | 31.3 |

The results of different physical pellet quality measurements are shown in Table 7. Significant linear and quadratic decreases in pellet length and width were associated with increasing inclusion of *P. variotii* in the diets. In contrast, significant linear ($p<0.0001$) and quadratic ($p<0.0001$) increases in water activity and bulk density were observed with increasing inclusion of *P. variotii* in the diets. Durability decreased linearly ($p<0.0001$) with an increasing level of *P. variotii* in the experimental diets. Finally, significant linear ($p<0.0001$) and quadratic ($p<0.01$) decreases in expansion were also associated with increasing dietary inclusion of *P. variotii*, whereas sinking velocity increased linearly ($p<0.05$) as the inclusion level of *P. variotii* in the diets increased.

Water stability index (WSI, %) of the experimental diets at four different incubation times (i.e., 30, 60, 120, and 240 min) are shown in Table 8. Significant linear ($p<0.0001$) and quadratic ($p<0.05$) improvements in WSI were associated with the increasing inclusion of *P. variotii* in the experimental diets at all incubation times investigated.

**Table 7.** Physical pellet quality of the experimental diets containing increasing inclusion level of *P.variotii.*

| Pellet quality parameter | Diet 1 | Diet 2 | Diet 3 | Diet 4 | SEM[1] | $P_{ANOVA}$[2] | $P_{linear}$[3] | $P_{quadratic}$[3] |
|---|---|---|---|---|---|---|---|---|
| Pellet length (mm) | 4.42$^{a}$ | 4.14$^{b}$ | 3.82$^{c}$ | 4.14$^{b}$ | 0.03 | <0.0001 | <0.0001 | <0.0001 |
| Pellet width (mm) | 2.85$^{a}$ | 2.66$^{b}$ | 2.59$^{b}$ | 2.57$^{b}$ | 0.04 | <0.0001 | <0.0001 | 0.0020 |
| Hardness (kg) | 4.53 | 4.15 | 4.23 | 5.07 | 0.31 | 0.1628 | 0.1320 | 0.0924 |
| Water activity ($a_w$) | 0.352$^{a}$ | 0.502$^{b}$ | 0.496$^{b}$ | 0.548$^{c}$ | 0.00 | <0.0001 | <0.0001 | <0.0001 |
| Bulk density (g/L) | 591.2$^{a}$ | 624.8$^{b}$ | 624.3$^{b}$ | 643.4$^{c}$ | 1.56 | <0.0001 | <0.0001 | <0.0001 |
| Durability (%) | 99.1$^{a}$ | 98.7$^{ab}$ | 98.3$^{bc}$ | 97.9$^{c}$ | 0.12 | 0.0005 | <0.0001 | 0.2157 |
| Expansion (%) | 42.4$^{a}$ | 33.0$^{b}$ | 29.3$^{b}$ | 28.5$^{b}$ | 1.82 | <0.0001 | <0.0001 | 0.0020 |
| Sinking velocity (m $S^{-1}$) | 0.037$^{a}$ | 0.045$^{ab}$ | 0.054$^{b}$ | 0.050$^{ab}$ | 0.00 | 0.0355 | 0.0278 | 0.0535 |

[1]Standard error mean.

[2]Significance of the one-way ANOVA. Values in the same row with different superscripts are significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).

[3]Significance of the linear and quadratic orthogonal polynomial contrasts of pellet quality parameters across experimental diets containing increasing inclusion of *P.variotii* (0, 5, 10, and 20% replacement of the crude protein content of the diet).

**Table 8.** Water stability index (%) of the experimental diets containing increasing inclusion level of *P.variotii.*

| Incubation time (minutes) | Water Stability Index (%) | | | | SEM[1] | $P_{ANOVA}$[2] | $P_{linear}$[3] | $P_{quadratic}$[3] |
|---|---|---|---|---|---|---|---|---|
| | Diet 1 | Diet 2 | Diet 3 | Diet 4 | | | | |
| 30 | 90.2$^{a}$ | 91.6$^{b}$ | 92.0$^{bc}$ | 92.7$^{c}$ | 0.17 | <0.0001 | <0.0001 | 0.0065 |
| 60 | 87.5$^{a}$ | 89.2$^{b}$ | 89.1$^{b}$ | 90.2$^{c}$ | 0.13 | <0.0001 | <0.0001 | 0.0045 |
| 120 | 84.3$^{a}$ | 86.9$^{b}$ | 86.7$^{b}$ | 88.3$^{c}$ | 0.26 | <0.0001 | <0.0001 | 0.0222 |
| 240 | 83.1$^{a}$ | 85.2$^{b}$ | 86.3$^{bc}$ | 87.2$^{c}$ | 0.38 | 0.0004 | <0.0001 | 0.0224 |

[1]Standard error mean.

[2]Significance of the one-way ANOVA for each incubation time. Values in the same row with different superscripts are significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).

[3]Significance of the linear and quadratic orthogonal polynomial contrasts of pellet quality parameters across experimental diets containing increasing inclusion of *P.variotii* (0, 5, 10, and 20% replacement of the crude protein content of the diet) for each incubation time.

## 4.2 Growth Performance

The growth performance data is presented in Table 9. No significant differences ($p>0.05$) in final body weight, weight gain, specific growth rate (SGR), thermal-unit growth coefficient (TGC), and feed intake of the fish fed the experimental diets were observed. A significant linear decrease ($p<0.05$) in feed conversion ratio (FCR) was associated with the increasing inclusion of *P. variotii* in the diets. No mortalities were observed during the experimental period.

**Table 9**: Growth performance parameters of fish fed the experimental diets with increasing inclusion level of *P. variotti.*

| Parameter | Diet 1 | Diet 2 | Diet 3 | Diet 4 | SEM[1] | $P_{ANOVA}$[2] | $P_{linear}$[3] | $P_{quadratic}$[3] |
|---|---|---|---|---|---|---|---|---|
| Initial body weight (g fish$^{-1}$) | 24.0 | 24.0 | 24.0 | 24.0 | 0.01 | 0.3300 | 0.3016 | 0.7506 |
| Final body weight (g fish$^{-1}$) | 109.3 | 111.2 | 108.9 | 115.5 | 3.53 | 0.5530 | 0.2631 | 0.5583 |
| Weight gain (g fish$^{-1}$) | 85.3 | 87.2 | 84.9 | 91.6 | 3.53 | 0.5497 | 0.2599 | 0.5551 |
| SGR (% day$^{-1}$) | 2.37 | 2.40 | 2.36 | 2.46 | 0.05 | 0.5358 | 0.2495 | 0.5743 |
| TGC [$g^{1/3}$(°C × day)$^{-1}$] | 0.202 | 0.205 | 0.201 | 0.211 | 0.01 | 0.5577 | 0.2724 | 0.5517 |
| Feed intake (g fish$^{-1}$) | 64.0 | 64.9 | 63.1 | 66.7 | 2.38 | 0.7436 | 0.4750 | 0.5822 |
| Feed conversion ratio | 0.75 | 0.74 | 0.74 | 0.73 | 0.00 | 0.0855 | 0.0173 | 0.7410 |

[1]Standard error mean.
[2]Significance of the one-way ANOVA.
[3]Significance of the linear and quadratic orthogonal polynomial contrasts across experimental diets containing increasing inclusion of *P. variotii* (0, 5, 10, and 20% replacement of the crude protein content of the diet).

## 4.3 Digestibility

The apparent digestibility coefficients (ADCs) for the experimental diets are shown in Table 10. There were no significant linear or quadratic differences ($p>0.05$) in the ADC values of dry matter and ash between fish fed the experimental diets and the control diet. According to Tukey's HSD test there was no significant difference ($p>0.05$) in the digestibility of Crude protein (CP) or total amino acids (TAAs) in fish fed Diets 1, 2, and 3. Nonetheless, Diet 4 showed significant linear ($p<0.001$) decreases in the ADCs of CP and TAA as compared to control Diet (Diet 1). There has been significant linear ($p<0.01$) decrease in gross energy (GE) with increased dietary inclusion of *P.variotii* in the experimental diets. In general, significant linear and in some cases quadratic decreases in the ADCs of the essential and non-essential amino acids were associated with increasing inclusion of *P. variotii* in the diets.

## 4.4 Nutrient utilization

Table 11 provides data on the nutrient retention and nutrient retention efficiency of Atlantic salmon fed experimental diets containing increasing levels of *P.variotii* (0, 5, 10, and 20% replacement of the CP content of the diet). No significant differences ($p>0.05$) in retained nitrogen, energy and lipid were observed between fish fed the experimental diets and the control diet (Diet 1). However, as the dietary level of *P. variotii* increased, there was a significant linear ($p<0.05$) increase in retained phosphorus, potassium and magnesium values.

Additionally, there was a significant linear increase in nutrient retention efficiency (% ingested) of nitrogen ($p<0.05$), lipid ($p<0.05$) and minerals (i.e., phosphorus ($p<0.001$) magnesium ($p<0.0001$) and potassium ($p<0.001$)) with increasing inclusion of *P. variotii* in the diets. Moreover, an increase in the inclusion of *P. variotii* resulted in a significant linear increase in nutrient retention efficiency (% digested) of nitrogen ($p<0.001$) and minerals (i.e., magnesium ($p<0.0001$) and potassium ($p<0.0001$)).

Furthermore, there was a significant linear increase in protein efficiency ratio (PER) ($p< 0.01$), protein productive value (PPV) ($p<0.01$) and a significant decrease in lipid efficiency ratio (LER) ($p<0.01$) with increasing inclusion of *P. variotii* in the diets.

## 4.5 Gene expression of the immune-related biomarkers in the distal intestine

Gene expression of the immune related biomarkers in the distal intestine of Atlantic salmon are shown in Figures 15 and 16. Fish fed Diet 2 (5% replacement of the CP content of diet with *P. variotii*) showed an up-regulation of *il10* ($p<0.05$), *tgfb* ($p<0.05$) and *inos* ($p<0.05$) as compared to the control Diet (Diet 1). No other significant differences were detected within expression of other genes (*ifnγ, il1b, e-cad1*) of fish fed Diet 2 as compared to control Diet 1.

Fish fed Diet 3 (10% replacement of the CP content of diet with *P. variotii)* showed significant up-regulation of *ifnγ* ($p<0.05$) and *tgfb* ($p<0.05$) compared to fish fed the control Diet (Diet 1). No other significant differences were detected within expression of other genes (*il10, inos, il1b, e-cad1*) of fish fed Diet 3 as compared to control Diet 1.

Fish fed Diet 4 (20% replacement of CP content of diet with *P. variotii*) showed significant up regulation of *ifnγ* ($p<0.01$), *il10* ($p<0.05$), *tgfb* ($p<0.05$) and *inos* ($p<0.05$) as compared to control Diet (D1). No other significant differences were detected within expression of other genes (*il1b, e-cad1*) of fish fed Diet 4 as compared to control Diet 1.

**Table 10.** Apparent digestibility coefficients (ADCs) of nutrients including essential and non-essential amino acids for Atlantic salmon fed the experimental diets containing increasing inclusion level of *P.variotii.*

| Apparent digestibility coefficient (%) | Diet 1 | Diet 2 | Diet 3 | Diet 4 | SEM[1] | $P_{ANOVA}$[2] | $P_{linear}$[3] | $P_{quadratic}$[3] |
|---|---|---|---|---|---|---|---|---|
| Dry matter | 75.1 | 76.7 | 75.4 | 74.2 | 0.62 | 0.1085 | 0.1166 | 0.1157 |
| Ash | 19.6 | 24.6 | 23.6 | 20.4 | 2.24 | 0.3798 | 0.8973 | 0.1237 |
| Crude protein | 89.2$^{a}$ | 89.8$^{a}$ | 88.7$^{a}$ | 86.8$^{b}$ | 0.37 | 0.0028 | 0.0007 | 0.0612 |
| Gross energy | 84.4$^{ab}$ | 85.1$^{a}$ | 84.0$^{ab}$ | 82.4$^{b}$ | 0.44 | 0.0134 | 0.0046 | 0.0966 |
| **Essential amino acids** | | | | | | | | |
| Arginine | 93.6$^{a}$ | 94.1$^{a}$ | 93.3$^{a}$ | 89.1$^{b}$ | 0.33 | <0.0001 | <0.0001 | 0.0006 |
| Histidine | 91.7$^{a}$ | 91.6$^{a}$ | 90.1$^{b}$ | 89.7$^{b}$ | 0.27 | 0.0015 | 0.0003 | 0.3599 |
| Isoleucine | 92.7$^{a}$ | 92.7$^{a}$ | 91.5$^{a}$ | 89.1$^{b}$ | 0.29 | <0.0001 | <0.0001 | 0.0560 |
| Leucine | 93.9$^{a}$ | 93.8$^{ab}$ | 92.7$^{b}$ | 90.4$^{c}$ | 0.23 | <0.0001 | <0.0001 | 0.0309 |
| Lysine | 92.3$^{a}$ | 92.7$^{a}$ | 91.7$^{a}$ | 90.0$^{b}$ | 0.29 | 0.0010 | 0.0002 | 0.0506 |
| Methionine | 95.5$^{a}$ | 95.5$^{a}$ | 94.9$^{a}$ | 91.8$^{b}$ | 0.41 | 0.0006 | 0.0001 | 0.0288 |
| Phenylalanine | 93.6$^{a}$ | 93.5$^{a}$ | 92.3$^{a}$ | 89.2$^{b}$ | 0.31 | <0.0001 | <0.0001 | 0.0264 |
| Threonine | 90.5$^{a}$ | 91.5$^{b}$ | 90.1$^{a}$ | 88.2$^{c}$ | 0.18 | <0.0001 | <0.0001 | 0.0005 |
| Valine | 91.1$^{a}$ | 91.3$^{a}$ | 89.9$^{a}$ | 87.0$^{b}$ | 0.37 | 0.0001 | <0.0001 | 0.0329 |
| Tryptophan | 88.4$^{a}$ | 89.5$^{a}$ | 87.8$^{a}$ | 84.2$^{b}$ | 0.54 | 0.0007 | 0.0002 | 0.0156 |
| **Non-essential amino acids** | | | | | | | | |
| Alanine | 92.1$^{a}$ | 92.2$^{a}$ | 91.2$^{a}$ | 89.1$^{b}$ | 0.32 | 0.0004 | <0.0001 | 0.0882 |
| Aspartic acid | 82.8$^{a}$ | 84.6$^{b}$ | 83.0$^{ab}$ | 81.0$^{c}$ | 0.35 | 0.0007 | 0.0010 | 0.0033 |
| Glycine | 87.5$^{ab}$ | 88.6$^{b}$ | 87.4$^{ab}$ | 86.2$^{a}$ | 0.43 | 0.0265 | 0.0160 | 0.0917 |
| Glutamic acid | 95.2$^{a}$ | 95.4$^{a}$ | 94.6$^{a}$ | 93.4$^{b}$ | 0.22 | 0.0008 | 0.0002 | 0.0974 |
| Cysteine | 81.0 | 81.8 | 80.4 | 79.2 | 0.56 | 0.0545 | 0.0208 | 0.2684 |
| Tyrosine | 90.2$^{a}$ | 86.4$^{b}$ | 83.4$^{c}$ | 74.5$^{d}$ | 0.41 | <0.0001 | <0.0001 | 0.1226 |
| Proline | 94.0 | 93.8 | 93.5 | 92.9 | 0.33 | 0.1515 | 0.0301 | 0.8633 |
| Serine | 91.0$^{a}$ | 91.9$^{a}$ | 89.4$^{b}$ | 87.8$^{c}$ | 0.25 | <0.0001 | <0.0001 | 0.0655 |
| **Total amino acids** | 92.0$^{a}$ | 92.3$^{a}$ | 91.2$^{a}$ | 89.0$^{b}$ | 0.25 | <0.0001 | <0.0001 | 0.0171 |

[1]Standard error mean.

[2]Significance of the one-way ANOVA. Values in the same row with different superscripts are significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).
[3]Significance of the linear and quadratic orthogonal polynomial contrasts across experimental diets containing increasing inclusion of *P.variotii* (0, 5, 10, and 20% replacement of the crude protein content of the diet).

**Table 11.** Nutrient retention (g fish$^{-1}$ or kJ fish$^{-1}$), nutrient retention efficiency (% ingested or % digested), protein efficiency ratio (PER), protein productive value (PPV), and lipid efficiency ratio (LER) of Atlantic salmon fed the experimental diets containing increasing inclusion level of *P.variotii.*

| Parameter | Diet 1 | Diet 2 | Diet 3 | Diet 4 | SEM[1] | $P_{ANOVA}$[2] | $P_{linear}$[3] | $P_{quadratic}$[2] |
|---|---|---|---|---|---|---|---|---|
| **Nutrient retention (g fish$^{-1}$ or kJ fish$^{-1}$)** | | | | | | | | |
| Retained nitrogen (g fish$^{-1}$) | 2.45 | 2.44 | 2.48 | 2.69 | 0.097 | 0.2926 | 0.0893 | 0.4295 |
| Retained energy (kJ fish$^{-1}$) | 896.4 | 917.5 | 887.9 | 940.6 | 39.3 | 0.7843 | 0.5031 | 0.6867 |
| Retained lipid (g fish$^{-1}$) | 13.2 | 13.8 | 13.0 | 13.4 | 0.683 | 0.8632 | 0.9293 | 0.9946 |
| Retained phosphorous (g fish$^{-1}$) | 0.254$^{a}$ | 0.286$^{a}$ | 0.309$^{a}$ | 0.368$^{b}$ | 0.013 | 0.0013 | 0.0002 | 0.9963 |
| Retained magnesium (g fish$^{-1}$) | 0.023$^{a}$ | 0.024$^{a}$ | 0.025$^{a}$ | 0.030$^{b}$ | 0.001 | 0.0059 | 0.0009 | 0.3208 |
| Retained potassium (g fish$^{-1}$) | 0.295 | 0.294 | 0.305 | 0.339 | 0.012 | 0.0813 | 0.0182 | 0.3558 |
| **Nutrient retention efficiency (% ingested)** | | | | | | | | |
| Nitrogen | 50.4$^{a}$ | 49.9$^{a}$ | 53.0$^{ab}$ | 54.8$^{b}$ | 0.76 | 0.0058 | 0.0013 | 0.6993 |
| Energy | 63.0 | 63.8 | 63.6 | 64.5 | 0.78 | 0.5909 | 0.2274 | 0.9825 |
| Lipid | 126.0$^{ab}$ | 131.4$^{b}$ | 129.5$^{b}$ | 116.2$^{a}$ | 2.47 | 0.0104 | 0.0087 | 0.0124 |
| Phosphorous | 35.6$^{a}$ | 40.9$^{ab}$ | 42.3$^{bc}$ | 46.5$^{c}$ | 1.20 | 0.0014 | 0.0002 | 0.2109 |
| Magnesium | 19.4$^{a}$ | 21.6$^{ab}$ | 23.8$^{b}$ | 30.4$^{c}$ | 0.57 | <0.0001 | <0.0001 | 0.1604 |
| Potassium | 42.4$^{a}$ | 45.7$^{a}$ | 49.4$^{a}$ | 61.4$^{b}$ | 2.02 | 0.0008 | 0.0001 | 0.3314 |
| **Nutrient retention efficiency (% digested)** | | | | | | | | |
| Nitrogen | 56.6$^{ab}$ | 55.6$^{a}$ | 59.7$^{bc}$ | 63.1$^{c}$ | 0.85 | 0.0009 | 0.0002 | 0.3139 |
| Energy | 74.7 | 74.9 | 75.7 | 78.3 | 0.93 | 0.0825 | 0.0170 | 0.4462 |
| Phosphorous | 112.8 | 121.4 | 105.8 | 114.2 | 3.39 | 0.0673 | 0.6348 | 0.5215 |
| Magnesium | 39.4$^{a}$ | 43.2$^{ab}$ | 46.5$^{b}$ | 56.4$^{c}$ | 1.10 | <0.0001 | <0.0001 | 0.3718 |
| Potassium | 44.5$^{a}$ | 47.7$^{a}$ | 51.9$^{a}$ | 64.6$^{b}$ | 2.11 | 0.0007 | <0.0001 | 0.3075 |
| **Protein efficiency ratio (PER)** | 2.64$^{a}$ | 2.65$^{a}$ | 2.69$^{ab}$ | 2.74$^{b}$ | 0.02 | 0.0154 | 0.0025 | 0.7514 |
| **Protein productive value (PPV)** | 0.48$^{ab}$ | 0.46$^{a}$ | 0.49$^{ab}$ | 0.50$^{b}$ | 0.01 | 0.0176 | 0.0064 | 0.5599 |
| **Lipid efficiency ratio (LER)** | 7.66$^{a}$ | 7.71$^{a}$ | 7.84$^{a}$ | 7.33$^{b}$ | 0.05 | 0.0005 | 0.0011 | 0.0006 |

[1]Standard error mean.

[2]Significance of the one-way ANOVA. Values in the same row with different superscripts are significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).

[3]Significance of the linear and quadratic orthogonal polynomial contrasts across experimental diets containing increasing inclusion of *P.variotii* (0, 5, 10, and 20% replacement of the crude protein content of the diet).

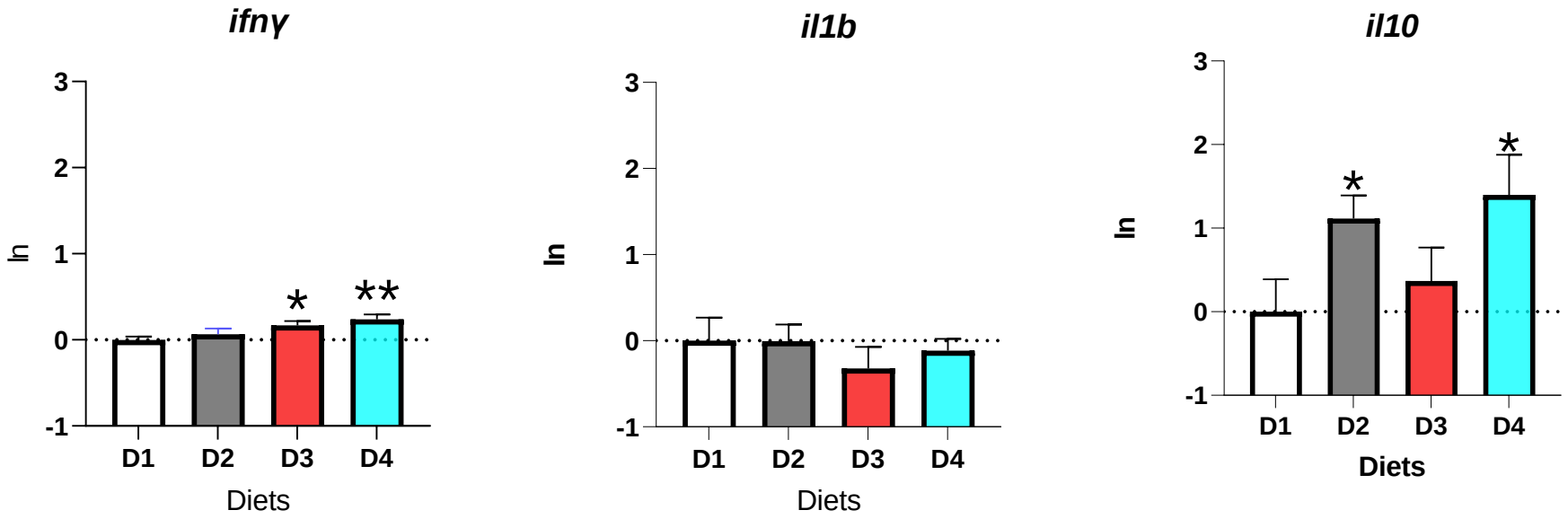


*Figure 15: Graphical Representation of the gene expressions of ifnγ (interferon gamma), il1b (interleukin 1 beta) and il10 (interleukin 10). The prefix "D" refers to Diet.* ***Indicates that p<0.05,*** ** *indicates that p<0.01.*

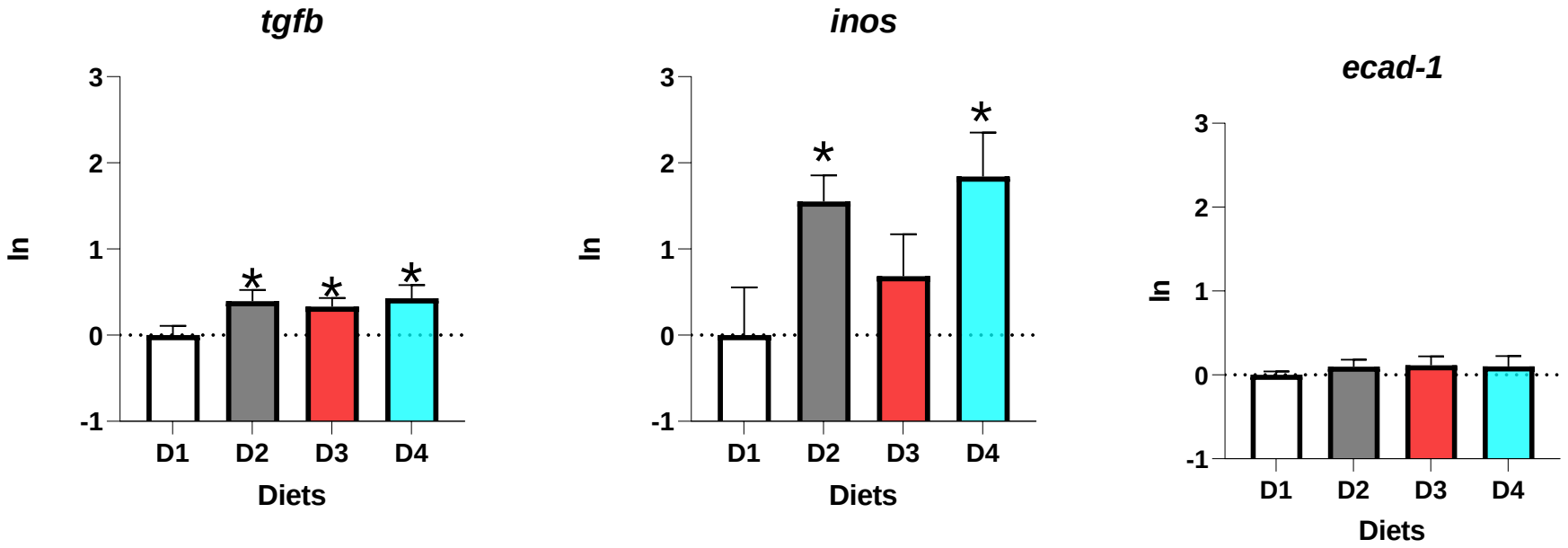


*Figure 16: Graphical Representation of the gene expressions of tgfb (Transforming growth factor beta), inos (inducible nitric oxide synthase) and ecad 1(E-cadherin 1). The prefix "D" refers to Diet.* ***** *Indicates that p<0.05.*

## 4.6 Correlation Plot

The correlation plot between the experimental diets and the control diet is shown in Figure 17. Statistical analysis showed that Diet 2 was significantly positively correlated ($p<0.05$) to Diet 3 and Diet 4. Diet 3 was significantly positively correlated ($p<0.05$) with Diet 2 and Diet 4. Diet 4 was significantly positively correlated ($p<0.05$) with Diet 2 and Diet 3. Moreover, Diets 2, 3 and 4 didn't show any significant correlation ($p>0.05$) with the control diet (Diet 1).

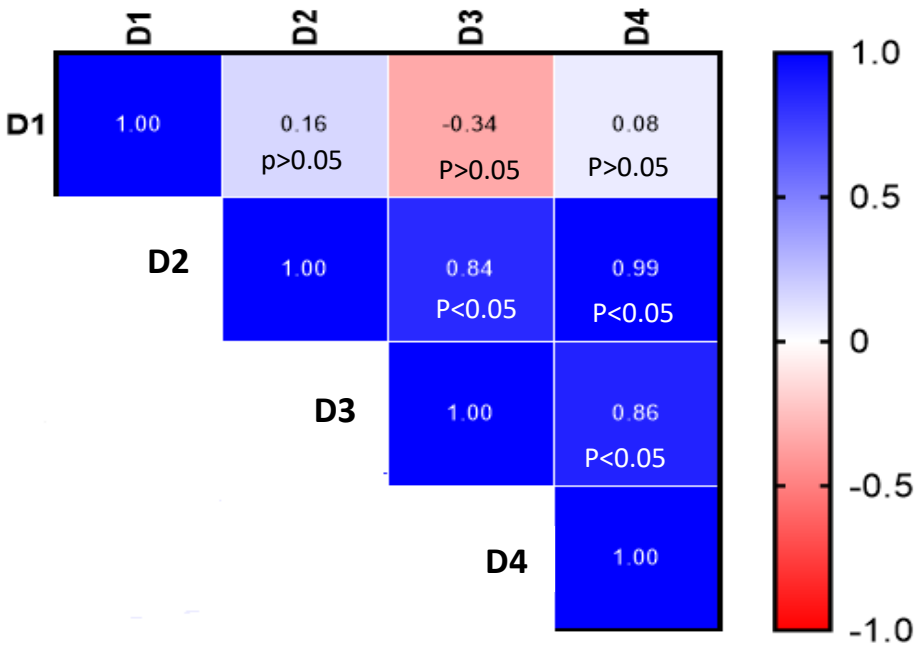


*Figure 17: Correlation analysis among dietary groups using the gene expression data from target immune-related biomarkers. The prefix 'D' refers to Diet. All the parameters that are significantly positively correlated (p-value < 0.05) are in blue. Red represents a negative correlation. Degrees of freedom= 4.*

# Chapter 5: Discussion and conclusion

## 5.1 Pellet Analysis

To sustain intensive salmonid aquaculture, it is essential to use high-quality feeds that meet technical standards. The ideal feed should consist of durable pellets that sink slowly (Øverland et al., 2007) to ensure efficient feeding and minimal wastage. In today's aquaculture feed production, extrusion is a common method to produce pellets of high physical quality to avoid problems related to transport and storage (Aas et al., 2011a; Aarseth et al., 2006).

Extruders have different sections which perform different functions. The temperature profile in the extruder is carefully controlled and depends on a variety of factors, including the type of material being processed and the desired properties of the final product. The first barrel is responsible for heating and compressing the material, while the subsequent barrels are used to melt the material and mix it with any additives or components present. The temperature profile is designed to ensure that the material is melted and mixed thoroughly without overheating or degrading the material (Hosokawa and Nakamura, 2011).

In this study, temperature profile, knife speed and die pressure of the extruder for the control diet and experimental diets were different. The temperature of Barrel 1 was 100°C for all experimental diets, while the temperature of Barrel 2 increased from 110°C for the control diet to 130°C for the *P. variotii*-based diets. This temperature increase may be necessary to ensure that the material is melted and mixed thoroughly, or to achieve the desired texture or other properties in the final product.

The optimal knife speed and barrel temperature for extruding fish diets with filamentous fungi will depend on several factors, including the specific type of filamentous fungi being used, the composition of the diet, and the desired properties of the final product.

According to Guy (2001) adding filamentous fungi to the fish diet can increase the viscosity of the extrudate, as they are fibrous in nature, which can affect the extrusion process and the quality of the final product. To compensate for the increased viscosity, it may be necessary to increase the barrel temperature and die pressure to ensure that the material is properly melted, mixed and have the proper shape.

In this study, pellets containing *P. variotii* showed a significant reduction in length and width compared to the control diet, which is contrary to what Øverland et al. (2007) found with the inclusion of bacterial meal extruded at 122°C (moderate processing). A negative correlation between pellet expansion and bulk density, as well as between bulk density and sinking rate, was also observed. These results suggest that increased dietary inclusion of *P. variotii* decreased pellet expansion, resulting in increased pellet density and higher sinking velocity. Similarly, D'Mello and Acamovic (1976) found that the inclusion of spray dried methanol-grown bacteria in unpelleted diets for young chicks led to increased bulkiness. In contrast, Øverland et al. (2007) reported increased expansion and reduced density after the addition of bacterial protein meal in dog diets extruded at 122°C.

FF, like yeast, also contain β-glucan, which can have a noteworthy influence on both extrusion parameters and the quality of the product. Fibers, which act as dispersed phase fillers, typically exhibit inadequate functionality during extrusion, leading to reduced expansion of the final product (Guy, 2001). Furthermore, fibrous components possess hydration properties and can act as binder in feed which tend to augment the bulk density and water activity of the products, which is in line with this study (D'Mello and Acamovic, 1976; Plavnik et al., 1981; Kilburn-Kappeler and Aldrich, 2023). As a result, the inclusion of fiber often necessitates distinct extruder arrangements and processing circumstances to accomplish the desired end product (Rokey, 2006).

The durability of *P. variotii*-based diets decreased significantly in comparison to the control diet and a similar result was noted by Øverland et al. (2007) in their research involving the inclusion of bacterial protein sources with an extrusion temperature of 122°C. Aarseth et al. (2006) also reported that pellets extruded at a high temperature of 140°C were less durable compared to those extruded at 100°C. However, Morken et al. (2011) observed an improvement in the durability of gluten-based meal pellets with an increase in extrusion temperature from 110 to 141°C. Hence, it can be concluded that the type of material or composition of the diet plays a crucial role in determining the durability of pellets.

Fish feeds need to be water stable to stay intact, so they are available to fish until they are ingested and to reduce the amount of nutrients that leach into the water. In this study, the water stability of the pellets increased linearly with increasing inclusion of *P. variotii* in the diets.

According to Houlihan et al. (2008), Baeverfjord et al. (2006) and Aas et al. (2011a), water stability index of the pellets can affect growth rate and feed intake as higher water-stable diets

lead to increased gastric retention time which ultimately results in lower feed intake. It has been shown that the water stability of the feed did not affect feed intake in rainbow trout significantly (Baeverfjord et al. 2006). While in another study, the feed intake was more than 20% higher in rainbow trout fed a diet with a low water stability compared to a diet with high water stability (Aas et al., 2011b).

There is limited knowledge available about how the pellet quality affects feed utilisation in fish, and the existing data are somewhat conflicting (reviewed by Sørensen, 2012). However, there was no significant effect of the experimental diets on feed intake as compared to the control diet (Diet 1). Therefore, it is evident that water stability didn't have an impact on either feed intake or growth rate.

In this study, digestibility of CP and TAA for Diet 4 was significantly lower as compared to control diet (Diet 1), however it is difficult to conclude that extrusion temperature can be the reason behind it. According to Sørensen et al. (2002), rainbow trout fed a fish meal-based diet did not experience any changes in protein or amino acid digestibility, despite being exposed to extrusion temperatures ranging from 100 to 150°C. Similarly, Barrows et al. (2007) found no effects of extrusion at 93°C on protein digestibility in rainbow trout fed a plant-based diet. However, Ljøkjel et al. (2004) discovered that mink fed a fish meal-based diet experienced a decrease in protein and total amino acid digestibility when extruder temperatures increased from 100 to 150°C. Some of the research has demonstrated that excessive heat treatment can lead to a decline in protein and amino acid digestibility, particularly cysteine, due to the development of disulphide bonds (Opstvedt et al. 1984; Ljøkjel et al. 2000, 2004). Therefore, based on given conflicting research it is unlikely that the extrusion temperatures used to manufacture these diets affected digestibility.

According to this study, replacement of CP of diets (upto 20%) with *P. variotti* has affected physical pellet quality in terms of increased water activity, bulk density, sinking velocity, water stability index and decreased pellet length, width, expansion and durability.

## 5.2 Growth performance

During the entire experiment, no mortality was observed among the fish, indicating their overall healthiness. This can be attributed in part to the fact that the Center for Sustainable Aquaculture at NMBU only uses healthy fish stocks that have been screened for infectious diseases.

The replacement of CP content of the experimental diets with *P. variotii* (upto 20%) did not affect palatability, as demonstrated by the similar feed intake of fish fed all experimental diets compared to the control diet. Sahlmann et al. (2019) conducted a study to assess the impact of adding 25% yeast (*C. jandinii*) to diets for Atlantic salmon. The study demonstrated that the fish that were fed the yeast diet exhibited greater feed intake and growth rate in both freshwater and saltwater than the control group.

Additionally, the specific growth rate (SGR) and thermal-unit growth coefficient (TGC) of fish fed the experimental diets were comparable to those fed the control diet. According to Melberg and Davidrajuh (2009), it is commonly observed that Atlantic salmon reared in freshwater at 14-16°C, with a final weight of 60-100g, typically exhibit a Specific Growth Rate (SGR) of 2.4. This SGR value serves as a standard benchmark for growth in such conditions. In this study, the SGR values for all diets were approximately 2.4, which aligns with the expected growth rate for Atlantic salmon under the specified temperature range and size range.

The study found no significant difference in final body weight and weight gain between fish fed the experimental diets and those fed the control diet. The results also showed that increased dietary inclusion of *P. variotii* in the experimental diets led to lower feed conversion ratio (FCR) as compared to control diet. This might be because of improved nitrogen utilization with increased dietary inclusion of *P.variotii* and the presence of β-glucans which can improve gut health and modulate the immune system, potentially leading to a lower FCR. Similar results were seen with the inclusion of 36% bacterial protein meal (BPM) in Atlantic salmon (i.e., lower FCR as compared to control diet) (Aas et al., 2006).

During the 1970s and 1980s, various studies were conducted to assess the viability of using *P. variotii* as a protein source in feeds for terrestrial animals such as pigs, cows, and hens. Alaviuhkola et al. (1975) and Barber et al. (1977), found that replacing skimmed milk protein and fish meal with *P. variotii*, respectively, did not negatively impact the growth performance of growing pigs. Similarly, Järvinen et al. (1980) observed that replacing 15% of fish meal and soybean meal with *P. variotii* did not affect the feed intake, weight gain, feed efficiency, or reproductive capacity of breeding cows. In addition, hens fed *P. variotii*-based diets had a significantly higher feed intake compared to those fed fish meal and soybean meal, as reported by Näsi (1982). In a study conducted by Alriksson et al. (2014), similar findings were observed regarding the growth performance of Nile Tilapia (*Oreochromis niloticus*) when *P. variotii* was included in the diets. Interestingly, the nutritional value of *P. variotii* used in that study was lower compared to the present study. Specifically, the *P. variotii* used in the previous study had

a crude protein (CP) content of 48g/100g, whereas in this study, the CP content of *P. variotii* was 62.51g/100g. Despite this difference in nutritional value, the inclusion of *P. variotii* did not significantly impact the growth performance of Nile Tilapia in both studies.

In agreement with the current study, other studies have shown that MIs can support good growth performance of a variety of species. Studies have demonstrated that defatted *Desmodesmus* sp. MI and algal meal can be included in salmon feed at up to 20% and 10%, respectively, without any negative effects on growth (Kiron et al., 2016; Kiron et al., 2012). Bacterial MI has also shown promise, as the inclusion of 36% of bacterial protein meal in the diets led to improved growth performance in Atlantic Salmon (Aas et al.,2006). *M. capsulatus* MI was able to constitute up to 52% of the dietary protein in a salmon diet and 38% in a trout diet, without adverse growth effects (Øverland et al., 2010). Interestingly, including *M. capsulatus* MI in a soybean meal-based diet has been found to prevent soybean meal-induced enteritis in salmon, indicating potential additional benefits of MI (Romarheim et al., 2011). Furthermore, KnipBio Meal (*Methylobacterium extorquens*) replaced 55% of fishmeal in salmon diets without any negative effects on growth (Tlusty et al., 2017), and up to 10% of soybean meal in trout diets (Hardy et al., 2018).

Several studies on *S.cerevisiae* have shown that partial replacement of fishmeal or soy protein can be done without negatively impacting the growth performance of aquatic species, such as rainbow trout (Huyben et al,. 2017; Vidakovic et al., 2020), Artic charr (Vidakovic et al., 2016), catfish (Essa et al., 2011; Peterson et al., 2012), goldfish (Gumus et al., 2016), lake trout (Rumsey et al., 1990), Nile tilapia (Abass et al., 2018), sea bass (Oliva-Teles and Gonçalves, 2001), shrimp (Guo et al., 2019) and sea bream (Fronte et al., 2019).

However, a few cases have also reported that the high inclusion of *S. cerevisiae* has negatively affected growth in Atlantic salmon (Øverland et al., 2013), rainbow trout (Hauptman et al., 2014), Nile tilapia (Ozório et al., 2012), Southern African dusky kob (Madibana and Mlambo, 2019) and Mirror carp (Omar et al., 2012) mainly because of reduced digestibility of CP, AA and GE.

Overall, this study supports the hypothesis that growth performance can be improved with increased inclusion of *P. variotii,* as demonstrated by the lower FCR and comparable SGR and weight gain among the experimental diets.

## 5.3 Digestibility

In the present study, inclusion of *P. variotii* had no effect on the apparent digestibility coefficient (ADC) of dry matter (DM) and ash content in Atlantic salmon as compared to the control diet.

The replacement of CP content of the experimental diets (Diet 2 and Diet 3) with *P.variotii* did not significantly alter the apparent digestibility coefficients (ADCs) of crude protein (CP) and total amino acids (TAA) of fish fed on them. The ADC values for Diet 2 were relatively high (89.8% for CP and 92.3% for TAA), while the ADC values for Diet 3 were 88.7% for CP and 91.2% for TAA. These results indicate that both Diet 2 and Diet 3 were highly digestible.

The reduction in ADC of CP and essential and non-essential amino acids observed in fish fed Diet 4 and diets containing *P. variotii*, respectively, can be attributed to the challenge in accessing the rigid cell wall of the fungi, leading to lower nutrient bioavailability (Rumsey et al., 1991a; Øverland et al., 2013). It should be noted that variations in chemical composition can exist between different studies of MIs, which could be attributed to the differences in the specific MI being studied (Nasseri et al., 2011). Moreover, even within the same species of MI, the nutritional quality can vary depending on the growth substrate and environmental conditions. The production process of the feed can also impact digestibility, with differences in factors such as temperature and pressure potentially affecting the outcome. Additionally, the method chosen for fecal collection and the selection of a digestibility marker can have an impact on the ADC values, with some methods being prone to overestimation while others may underestimate the true value (Hajen et al., 1993; Vandenberg and De La Noue, 2001).

Øverland et al. (2013) observed almost similar ADC values of CP (81%-87%) in intact *Candida utilis* and *Kluyveromyces marxianus* in Atlantic salmon. Similarly, high protein digestibility (i.e.,76% and 91%) were observed for intact *S.cerevisiae* in rainbow trout (Vidakovic et al., 2020) and European perch (Langeland et al., 2016) respectively. However, the use of brewer's yeast (*Saccharomyces cerevisiae*) as a substitute for fish meal in sea bass (*Dicentrarchus labrax*), Pacu (*Piaractus mesopotamicus*), and rainbow trout (*Oncorhynchus mykiss*) diets resulted in lower protein digestibility, according to studies conducted by Oliva-Teles and Gonçalves. (2001), Ozório et al. (2010), and Rumsey et al. (1991a), respectively. Skrede et al. (1998) and Storebakken et al. (2004) found reduced digestibility of N with increased inclusion of bacterial protein meal.

Variation in values for digestibility in yeast can be explained by the difference in production methods i.e., type of strain used, difference in fermentation technology (fed-batch or continuous), and drying method (Rumsey et al.,1990a; Øverland et al., 2013; Sharma et al., 2018, Agboola et al., 2022a).

Autolysis, a widely employed technique, involves the breakdown of cellular components by intracellular enzymes. This process leads to the formation of shorter proteins and peptides, decreased levels of nucleotides and free amino acids, and an enhancement in nutrient bioavailability (Babayan and Bezrukov, 1985; Hernawan and Fleet, 1995; Babayan and Latov, 2003). After autolysis, Hansen et al. (2021a) observed an increase of almost 60% in protein digestibility of *S.cerevisiae* while Agboola et al. (2021a) documented an increase of 12% and 9% in *C.jadinii* and *W.anomalus*, respectively. The improved digestibility of proteins attributed to autolysis is because it creates porous cells that allow digestive enzymes, including intracellular β-1,3-glucanases and proteases, to readily access their targets and break down the inner layer of the cell wall and increase the solubility of β-glucans (Middelberg, 1995; Hansen et al.,2020).

In addition to autolysis, cell wall extraction and mechanical disruption (microfluidizer) have also been found to enhance protein and EAA digestibility as demonstrated in the study by Langeland et al. (2016) and Rumsey et al. (1991b), respectively. Langeland et al. (2016) observed that extracted *S.cerevisiae* and *R. Oryzae* exhibited increased protein digestibility (from 71% to 96%) and EAA digestibility compared to their intact counterparts. Therefore, the impact of incorporating autolyzed *P. variotii* could potentially improve its digestibility, but further work is required to investigate this.

Feed intake, digestibility, absorption, utilization, metabolism, and activity level of the fish are all factors that contribute to growth performance (Byerly, 1967; Gjedrem, 2005). Despite the reduced digestibility, there has been no reduction in feed intake with the increased inclusion of *P. variotii*, making it difficult to draw any definitive conclusions about the relationship between feed intake, growth, and feed efficiency in the context of digestibility.

Therefore, the findings of this study support the hypothesis that the 20% replacement of CP content of the experimental diets with *P. variotii* can modulate nutrient digestibility specifically by reducing the digestibility of CP and TAAs.

## 5.4 Nutrient utilization

This study found no significant differences in retention of nitrogen, energy, and lipid between fish fed *P. variotii*-based diets and those fed a control diet. However, there was a significant linear improvement in NRE both as a percentage of ingested and digested nitrogen associated with increasing dietary levels of *P.variotii* despite a reduction in ADC.

Several studies have demonstrated that incorporating MIs into the diet of Atlantic salmon can have significant positive effects on nutrient utilization. Inclusion of 36% of bacterial meal in diets for Atlantic salmon led to higher retention of nitrogen and energy (Aas et al., 2006). This has also been seen with yeasts (*S.cervisiae*) as partial replacement of fishmeal with *S.cervisiae* resulted in improved retention of nitrogen in seabass and pacu (Oliva-Teles and Gonçalves, 2001; Ozório et al., 2010).

Moreover, Rumsey et al. (1991b) observed increased nitrogen retention in rainbow trout fed dietary *S.cervisiae* (500 g/kg nucleic acids). Similarly, Øverland et al. (2013) reported that substitution of 40% of fish meal with *C.utilis* (93 g/kg nucleic acids) grown on lignocellulosic sugar substrate in salmon diets led to significantly improved retention of nitrogen where-as the inclusion of *K.marxianus* (102 g/kg nucleic acids) did not affect the retention of nitrogen, despite a decrease in the ADC of CP. This was likely due to the possibility of a N-sparing effect of nucleic acids as they might be directly incorporated in the body or spare dispensible amino acid through endogenous utilization (Øverland and Skrede, 2017).

The retention of potassium, phosphorus and magnesium increased linearly with the increasing inclusion of *P. variotii* in the diets. This can further be explained by a significant increase in ingested phosphorus retention efficiency with increased inclusion of *P. variotti* and will reduce the output waste. This leads to a positive environmental impact as reduced phosphorus load will cause less eutrophication in fresh water (Guozhi Luo., 2022). Hansen et al. (2021b) found similar results, observing that an increased inclusion of yeast *(C.jadinii*) grown on seaweed hydrolysate resulted in enhanced retention of calcium, phosphorus and magnesium.

Protein efficiency ratio (PER) increased significantly with increased inclusion of *P. variotii*, indicating that EAA are in sufficient amounts and ingested proteins are easily digestible leading to weight gain (DeSilva and Anderson, 1995).

Overall, these findings suggest that incorporating MI in the diet of Atlantic salmon can enhance nitrogen utilization efficiency. Further research is needed to determine the optimal inclusion levels of these ingredients and their effects on other aspects of fish health and performance.

## 5.5 Gene expression

Understanding the relationship between fish nutrition and immune response is critical for improving disease resistance (Buchmann & Secombes, 2022; Kiron, 2012). Like yeast, *P. variotii* also have similar cellular wall constituents such as prebiotic β-glucans (approximately 10-15%) and mannan oligosaccharide (MOS) which have been demonstrated to elicit an immunostimulatory response in fish. By promoting intestinal health, growth, and immune system function and providing protection against infectious agents, these constituents have shown to have a significant impact (Talpur et al., 2014). As a result, β-glucan and MOS are frequently used in the aquaculture industry as immunomodulatory agents (Dawood et al., 2018; Koch et al., 2021). For example, Nile tilapia (*Oreochromis niloticus*) fed a diet enriched with β-glucan exhibited significantly higher levels of intestinal lysozyme activity, indicating improved humoral innate immune responses and enhanced resistance to bacterial diseases such as *Aeromonas sobria* and *Streptococcus agalactiae* (Koch et al., 2021). According to Morales-Lange et al. (2022), the variation in the proportion and concentration of bio-active compounds may cause Atlantic salmon to respond differently to these compounds and alter their physiological response at the intestinal level.

In the present study, the observed increase in the expression of the pro-inflammatory cytokine *ifnγ* and the effector molecule *inos* in fish fed Diet 2 and Diet 4, compared to the control diet (Diet 1), suggests the activation of a type 1 immune response characterized by the involvement of Th1 cells. IFN-γ, primarily produced by T helper 1 (Th1) cells, plays a crucial role in activating macrophages and enhancing cell-mediated immunity against intracellular pathogens during innate and adaptive immune responses. It also triggers the production of iNOS (inducible nitric oxide synthase), an enzyme that regulates immune cell differentiation and function (Buchmann & Secombes, 2022; Xu et al., 2012; Zou & Secombes, 2016). iNOS can be expressed by T-cells, macrophages and dendritic cells and facilitates the conversion of arginine and oxygen into nitric oxide (NO), which participates in the elimination of invading pathogens, such as bacteria and viruses (Panettieri et al., 2019; Xue et al., 2018). However, it is important to note that excessive production of NO by iNOS can lead to tissue damage and contribute to the development of inflammatory diseases. This is primarily due to oxidative stress resulting

from the accumulation of reactive nitrogen species (RNS), including peroxynitrite (Morales-Lange et al., 2018).

The study findings indicate that *P. variotii*-based diets (Diet 2 and Diet 3) upregulated *il10*, while Diets 2, 3, and 4 induced an upregulation of *tgfb* in fish compared to the control diet (Diet 1). These cytokines, IL-10 and TGF-β, are primarily produced by regulatory T cells (Tregs), macrophages, and dendritic cells, and they play key roles in maintaining immune homeostasis by suppressing immune responses. They are also involved in controlling inflammation and maintaining tissue homeostasis by limiting the production of pro-inflammatory cytokines (Buchmann & Secombes, 2022).

Proper regulation of IL-10, an anti-inflammatory cytokine, is essential to prevent an excessive immune response to pathogens, which could increase the risk of autoimmune diseases (Iyer & Cheng, 2012). This aligns with the finding that the experimental diets did not significantly upregulate the pro-inflammatory cytokine *il1b* compared to the control diet. This lack of upregulation may be attributed to the significant upregulation of *tgfb* in all experimental diets (Diets 2, 3, and 4), which exhibits immunosuppressive effects. A study by Zou and Secombes (2016) in grass carp supports this finding, showing that TGF-β suppressed the induction of IL-1β by lipopolysaccharide. However, in contrast to the current study, rainbow trout intestinal epithelial cells exhibited significant expression of *il1b* when exposed to β-glucan-supplemented diets (Schmitt et al., 2015). IL-1β, a pro-inflammatory cytokine, plays a significant role in producing other pro-inflammatory cytokines and activating lymphocytes and phagocytic cells, ultimately enhancing fish resistance to bacterial infections (Sakai et al., 2021). Overall, the findings presented here propose that *P. variotii* has the potential to trigger and regulate the immune response by facilitating a balance between pro-inflammatory and anti-inflammatory responses.

Based on the studies by He et al. (2019) and Osaku et al. (2018), exopolysaccharides (EPS) produced by certain fungal species, such as *Paecilomyces lilacinus* and *P. variotii*, respectively, have immunostimulatory activity and can modulate macrophage activities. He et al. (2019) found that the EPS from *P. lilacinus* activated the TLR4/NF-κB/MAPK signaling pathway in macrophages, leading to the production of pro-inflammatory cytokines such as IL-6 and TNF-α, as well as the anti-inflammatory cytokine IL-10. Meanwhile, Osaku et al. (2018) reported that the β-(1→6)-D-glucan component of the EPS from *P. variotii* was responsible for its immunostimulatory activity, leading to increased phagocytic activity and cytokine production in macrophages.

This study indicates that dietary incorporation of *P. variotii* can result in maintenance of immune homeostasis in the distal intestine, a mucosa-associated lymphoid tissue (MALT) that coordinates local and systemic responses in other immune organs such as gills, spleen, and head kidney, as stated by Morales-Lange et al. (2021).

However, in future studies examining the dietary incorporation of *P. variotii*, it is recommended to subject fish to multiple stressors in unfavourable environmental conditions, as well as relevant pathogenic challenges for Atlantic salmon. This approach will allow for the evaluation of the effectiveness of *P. variotii* in promoting greater fish robustness.

## 5.6 Conclusion

Based on the findings presented, the current study suggests that incorporating *P. variotii* into the diets of Atlantic salmon juveniles reared in freshwater can have multiple benefits. Notably, replacing up to 20% of the CP content of the diet with *P. variotii* improved feed conversion ratio and nutrient utilization efficiency, indicating that *P. variotii* has considerable potential to be used as an alternative protein ingredient in salmon feeds. Furthermore, *P. variotii* showed immunomodulatory effects in the DI, suggesting that it may contribute to the health and well-being of the fish. Taken together, these findings suggest that *P. variotii* is a highly promising ingredient for use in salmon feeds, and further research in this area is warranted.

## References


Aarseth, K. A., Sørensen, M., & Storebakken, T. (2006). Effects of red yeast inclusions in diets for salmonids and extrusion temperature on pellet tensile strength: Weibull analysis. *Animal feed science and technology*, *126*(1-2), 75-91.

Aas, T. S., Åsgård, T., & Ytrestøyl, T. (2022a). Utilization of feed resources in the production of Atlantic salmon *(Salmo salar*) in Norway: An update for 2020. *Aquaculture Reports*, 26, 101316. https://doi.org/10.1016/j.aqrep.2022.101316

Aas, T. S., Åsgård, T., & Ytrestøyl, T. (2022b). Utilization of feed resources in the production of rainbow trout (*Oncorhynchus mykiss*) in Norway in 2020. *Aquaculture Reports*, 26, 101317.

Aas, T. S., Hatlen, B., Grisdale-Helland, B., Terjesen, B. F., Bakke-McKellep, A. M., & Helland, S. J. (2006). Effects of diets containing a bacterial protein meal on growth and feed utilisation in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 261(1), 357-368.

Aas, T. S., Oehme, M., Sørensen, M., He, G., Lygren, I., & Åsgård, T. (2011a). Analysis of pellet degradation of extruded high energy fish feeds with different physical qualities in a pneumatic feeding system. Aquacultural Engineering, 44(1), 25-34.

Aas, T. S., Terjesen, B. F., Sigholt, T., Hillestad, M., Holm, J., Refstie, S., Baeverfjord, G., Rørvik, K.-A., Sørensen, M., Oehme, M., et al. (2011b). Nutritional responses in rainbow trout (*Oncorhynchus mykiss*) fed diets with different physical qualities at stable or variable environmental conditions. *Aquaculture Nutrition*, 17 (6): 657-670. doi: https://doi.org/10.1111/j.1365-2095.2011.00868.x.

Aas, T. S., Ytrestøyl, T., & Åsgård, T. (2019). Utilization of feed resources in the production of Atlantic salmon (*Salmo salar*) in Norway: An update for 2016. *Aquaculture Reports*, 15, 100216. doi: 10.1016/j.aqrep.2019.100216

Abass, D. A., Obirikorang, K. A., Campion, B. B., Edziyie, R. E., & Skov, P. V. (2018). Dietary supplementation of yeast (*Saccharomyces cerevisiae*) improves growth, stress tolerance, and disease resistance in juvenile Nile tilapia (*Oreochromis niloticus*). *Aquaculture International*, *26*, 843-855.

Agboola, J. O. (2022). Can fish grow on trees? Nutritional and functional properties of yeasts in diets for Atlantic salmon (*Salmo salar*) (Doctoral dissertation, Norwegian University of Life Sciences).

Agboola, J. O., Schiavone, M., Øverland, M., Morales-Lange, B., Lagos, L., Arntzen, M. Ø., Lapeña, D., Eijsink, V. G., Horn, S. J. & Mydland, L. T. (2021a). Impact of down-stream processing on functional properties of yeasts and the implications on gut health of Atlantic salmon (*Salmo salar*). *Scientific reports*, 11 (1): 1-14.

Agboola, J.O., Øverland, M., Skrede, A., Hansen, J. Ø. (2021b). Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production. *Reviews in Aquaculture* 13, 949-970.

Alaviuhkola, T., Korhonen, I., Partanen, J., Lampila, M. (1975). Pekilo protein in the nutrition of growing-finishing pigs. *Acta Agriculturae Scandinavica* 254, 301-305.

Albrektsen, S., Kortet, R., Skov, P. V., Ytteborg, E., Gitlesen, S., Kleinegris, D., Mydland, L.-T., Hansen, J. Ø., Lock, E.-J., Mørkøre, T., James, P., Wang, X., Whitaker, R. D., Vang, B., Hatlen, B., Daneshvar, E., Bhatnagar, A., & Jensen, L. B. (2022). Future feed resources in sustainable salmonid production: A review. *Reviews in Aquaculture, 14(4), 1790-1812.* https://doi.org/10.1111/raq.12673

Almås KA, Josefsen KD, Gjøsund SH, et al. (2020). Sustainable Feed for Norwegian Salmon. Norway: *SINTEF*; 01128.

Alriksson B, Hörnberg A, Gudnason AE, Knobloch S, Arnason J, Johansson R. (2014). Fish feed from wood. Nordic Wood Biorefinery Conference, Stockholm

Alvarez-Martinez, L., Kondury, K. P., & Harper, J. M. (1988). A general model for expansion of extruded products. *Journal of Food Science*, *53*(2), 609-615.

Assefa, A. & Abunna, F. (2018). Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary medicine international*, 2018.

Austreng, E. (1978). Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture*, 13 (3): 265-272. doi: https://doi.org/10.1016/0044-8486(78)90008-X.

Austreng, E., Storebakken, T. & Åsgård, T. (1987). Growth rate estimates for cultured Atlantic salmon and rainbow trout. *Aquaculture*, 60 (2): 157-160. doi: https://doi.org/10.1016/0044-8486(87)90307-3.

Asadollahzadeh, M., Ghasemian, A., Saraeian, A., Resalati, H., & Taherzadeh, M. J. (2018). Production of fungal biomass protein by filamentous fungi cultivation on liquid waste streams from pulping process. *BioResources*, 13(3), 5013-5031.

Baeverfjord G, Krogdahl Å. (1996) Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar L*., distal intestine: a comparison with the intestines of fasted fish. *J Fish Dis*, 19:375-387.

Baeverfjord, G., Refstie, S., Krogedal, P. & Åsgård, T. (2006). Low feed pellet water stability and fluctuating water salinity cause separation and accumulation of dietary oil in the stomach of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 261 (4): 1335-1345. doi: https://doi.org/10.1016/j.aquaculture.2006.08.033.

Bajpai, P. (2017). Single cell protein production from lignocellulosic biomass. Singapore, Springer *Briefs in Molecular Science*. 78 pages.

Barber, R.S., Braude, R., Mitchell, K.G. (1977). The value of 'Pekilo protein' for growing pigs. *Animal Feed Science and Technology* 2, 161-169.

Barrows, F.T., Stone, D.A.J., Hardy, R.W. (2007). The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (Oncorhynchus mykiss). Aquaculture 265, 244–252. https://doi.org/10.1016/j.*Aquaculture*.2007.01.017

Bell V, Ferrão J, Chaquisse E, Fernandes T. (2018). Host-microbial gut interactions and mushroom nutrition. *J Food Nutr Res*, 6:576-583.

Bellona. (2022). *Råvarerløftet: Hva skal laksen spise*? Oslo: Miljøstiftelsen Bellona. Available at:

Bhalla TC, Joshi M (1994) Protein enrichment of apple pomace by co-culture of cellulolytic moulds and yeasts. *World J Microbiol Biotechnol* 10(1):116–117

Bieth, J., Spiess, B., Wermuth, C.G., 1974. The synthesis and analytical use of a highly sensitive and convenient substrate of elastase. *Biochemical Medicine* 11, 350–357.

Bjørdal, T., Holte, E.A., Hilmarsen, Ø. & Tusvik, A. (2018) *Analyse av lukka oppdrett av laks – landbasert og i sjø: produksjon, økonomi og risiko* (Sluttrapport FHF prosjekt 901442). NTNU-Ålesund, SINTEF OCEAN og SNF. Available at: landbasert-lakseoppdrett-analyse.pdf (fisk.no)

Bjørgen, H., Li, Y., Kortner, T. M., Krogdahl, Å. & Koppang, E. O. (2020). Anatomy, immunology, digestive physiology and microbiota of the salmonid intestine: Knowns and unknowns under the impact of an expanding industrialized production. *Fish & Shellfish Immunology*, 107: 172-186.

Bjørndal, T. & Tusvik, A. (2017). *Land based farming of salmon: economic analysis*. Available at: https://www.ntnu.no/documents/1265701259/1281473463/WPS+1+2017.pdf/6ee4cd65-e3b0-44a6-aa42-d017cb42d020

Bjørnevik, M., Nilsen, A. (2021, 5. juni). *Dette er noen av problemene som må løses for at lakseoppdretten skal bli mer bærekraftig. Fiskeribladet*. Available from: Dette er noen av problemene som må løses for at lakseoppdretten skal bli mer bærekraftig | Fiskeribladet

Bonaldo, A., Thompson, K., Manfrin, A., Adams, A., Murano, E., Mordenti, A. L., & Gatta, P. P. (2007). The influence of dietary ß-glucans on the adaptive and innate immune responses of European sea bass (*Dicentrarchus labrax*) vaccinated against vibriosis. *Italian Journal of Animal Science*, *6*(2), 151-164.

Brett, J. R., & Groves, T. D. D. (1979). Fish Physiology 6-Physiological Energetics. *Fish Physiology*, 8, 279-352. https://doi.org/10.1016/S1546-5098(08)60029-1

Bridle, A., Carter, C., Morrison, R., Nowak, B., 2005. The effect of β-glucan administration on macrophage respiratory burst activity and Atlantic salmon, *Salmo salar L.*, challenged with amoebic gill disease – evidence of inherent resistance. *Journal of Fish Diseases* 28, 347-356.

Buchmann, K. & Secombes, C. J. (2022). Principles of Fish Immunology.

Byerly, T. C. (1967). Efficiency of Feed Conversion. Science, 157 (3791): 890. doi: 10.1126/science.157.3791.890.

Caballero, B. (2005). Encyclopedia of human nutrition: Elsevier.

Cadillo-Benalcazar, J. J., Giampietro, M., Bukkens, S. G. & Strand, R. (2020). Multi-scale integrated evaluation of the sustainability of large-scale use of alternative feeds in salmon aquaculture. *Journal of Cleaner Production*, 248: 119210.

Cesta, M. F. (2006). Normal structure, function, and histology of mucosa-associated lymphoid tissue. *Toxicologic pathology*, 34 (5): 599-608

Chandra RP, Bura R, Mabee W, Berlin dA, Pan X, Saddler J. (2007). Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics? Biofuels:67-93.

Chen C-L, Chang J-S, Lee D-J. (2015). Dewatering and drying methods for microalgae. *Drying Technol*, 33:443-454.

Chikwati, E.M., Sahlmann, C., Holm, H., Penn, M.H., Krogdahl, Å., Bakke, A.M. (2013). Alterations in digestive enzyme activities during the development of diet-induced enteritis in Atlantic salmon, *Salmo salar L*. *Aquaculture* 402–403, 28–37.

Cho, C. Y. (1992). Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture*, *100*(1-3), 107-123.

Cho, C.Y., Slinger, S.J., Bayley, H.S. (1982). Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. Comp. Biochem. Physiol. Part B Comp. *Biochem*. 73, 25–41. https://doi.org/10.1016/0305-0491(82)90198-5

Dahlberg, A. (2019). Filamentous fungus *Paecilomyces variotii* in feed for Rainbow trout (*Oncorhynchus mykiss*).

Dawood, M. A., Koshio, S. & Esteban, M. Á. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Reviews in Aquaculture*, 10 (4): 950- 974.

De Silva, S. S. and Anderson, T. A. (1995). Fish Nutrition in Aquaculture Chamann and Hall Aquaculture Series U. K. p. 319

Draganovic, V., van der Goot, A. J., Boom, R., & Jonkers, J. (2011). Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. *Animal Feed Science and Technology*, *165*(3-4), 238-250.

Ecotone (u.å.) *Bunnforholdene under SalMars havmerd dokumenteres av Ecotone.* Available from: Bunnforholdene under SalMars havmerd dokumenteres av Ecotone - Ecotone

Eidem, B., & Melås, A. M. (2021). Oversikt over norsk og global akvakultur og akvafôr. RURALIS Report 6/2021. Norwegian. Available at: https://ruralis. no/wp-content/uploads/2021/11/r-6_21-oversikt-over-norsk-og-global-akvakultur-og-akvafor-b–eidemog-a–melas. pdf.

EniferBio. (2022). The Original Mycoprotein. Available at: The Original Mycoprotein | eniferBio

Essa, M. A., Mabrouk, H. A., Mohamed, R. A., & Michael, F. R. (2011). Evaluating different additive levels of yeast, Saccharomyces cerevisiae, on the growth and production performances of a hybrid of two populations of Egyptian African catfish, Clarias gariepinus. *Aquaculture*, *320*(1-2), 137-141.

FAO. 2022. The State of World Fisheries and Aquaculture. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Fronte, B., Abramo, F., Brambilla, F., De Zoysa, M., & Miragliotta, V. (2019). Effect of hydrolysed fish protein and autolysed yeast as alternative nitrogen sources on gilthead sea bream (*Sparus aurata*) growth performances and gut morphology. *Italian Journal of Animal Science*, *18*(1), 799-808.

Føre, H. M., Thorvaldsen, T., Osmundsen, T. C., Asche, F., Tveterås, R., Fagertun, J. T., & Bjelland, H. V. (2022). Technological innovations promoting sustainable salmon (*Salmo salar*) aquaculture in Norway. *Aquaculture Reports*, 24, 101115.

Fry JP, Love DC, MacDonald GK, West PC, Engstrom PM, Nachman KE, Lawrence RS. (2016). Environmental health impacts of feeding crops to farmed fish. *Environ Internation*, 91:201-214.

Fry JP, Mailloux NA, Love DC, Milli MC and Cao L. (2018). Feed conversion efficiency in aquaculture: do we measure it correctly? *Environmental Research Letters*.

Gajardo, K., Jaramillo-Torres, A., Kortner, T. M., Merrifield, D. L., Tinsley, J., Bakke, A. M. & Krogdahl, Å. (2017). Alternative protein sources in the diet modulate microbiota and functionality in the distal intestine of Atlantic salmon (*Salmo salar*). *Applied and environmental microbiology*, 83 (5): e02615-16.

Gjedrem, T. (2005). *Selection and Breeding Programs in Aquaculture*.

Glencross, B. D. (2020). A feed is still only as good as its ingredients: An update on the nutritional research strategies for the optimal evaluation of ingredients for aquaculture feeds. *Aquaculture Nutrition*, 26(4), 813-832. https://doi.org/10.1111/anu.13138.

Glencross, B. D., Booth, M., & Allan, G. L. (2007). A feed is only as good as its ingredients – A review of ingredient evaluation for aquaculture feeds. *Aquaculture Nutrition*, 13, 17–34.

Gumus, E., Aydin, B. A. K. İ., & Kanyilmaz, M. (2016). Growth and feed utilization of goldfish (Carassius auratus) fed graded levels of brewers yeast (*Saccharomyces cerevisiae*). *Iranian Journal of Fisheries Sciences*, *15*(3), 1124-1133.

Guo, J., Qiu, X., Salze, G., & Davis, D. A. (2019). Use of high-protein brewer's yeast products in practical diets for the Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*, *25*(3), 680-690.

Guselle, N., Markham, R., Speare, D.J., 2007. Timing of intraperitoneal administration of β-1, 3/1, 6 glucans to rainbow trout, *Oncorhynchus mykiss* (Walbaum), affects protection against the microsporidian Loma salmonae. *Journal of Fish Diseases* 30, 111-116.

Guy, R. (2001). Extrusion cooking: technologies and applications (Boca Raton, FL: CRC).

H.M. Føre, T. Thorvaldsen, T.C. Osmundsen, F. Asche, R. Tveterås, J.T. Fagertun, H.V. Bjelland

Hahn-Hägerdal B, Karhumaa K, Fonseca C, Spencer-Martins I, Gorwa- Grauslund MF (2007) Towards industrial pentose-fermenting yeast strains. *Appl Microbiol Biotechnol* 74:937–953

Hajen, W.E., Beames, R.M., Higgs, D.A., Dosanjh, B.S., 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytsc*ha) in sea water. 1. Validation of technique. *Aquaculture* 112, 321–332. https://doi.org/10.1016/0044-8486(93)90393-D

Hansen JØ, Lagos L, Lei P, Reveco-Urzua FE, Morales-Lange B, Hansen LD, Schiavone M, Mydland LT, Arntzen MØ, Mercado L, et al., (2021a). Down-stream processing of baker's yeast (*Saccharomyces cerevisiae*) - Effect on nutrient digestibility and immune response in Atlantic salmon (Salmo salar). *Aquaculture*, 530:1-10.

Hansen, J. Ø., Sharma, S., Horn, S. J., Eijsink, V. G. H., Øverland, M., & Mydland, L. T. (2021b). Fecal Excretion and Whole-Body Retention of Macro and Micro Minerals in Atlantic Salmon Fed Torula Yeast Grown on Sugar Kelp Hydrolysate. *Animals*, 11(8), 2409. https://doi.org/10.3390/ani11082409

Hansen, J. Ø., Penn, M., Øverland, M., Shearer, K. D., Krogdahl, Å., Mydland, L. T., & Storebakken, T. (2010). High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (*Salmo salar*). *Aquaculture*, *310*(1-2), 164-172.

Hardy, R. W., Patro, B., Pujol-Baxley, C., Marx, C. J., & Feinberg, L. (2018). Partial replacement of soybean meal with *Methylobacterium extorquens* single-cell protein in feeds for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, *49*(6), 2218-2224.

Hardy, R.W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquac. Res.* 41, 770–776. https://doi.org/10.1111/j.1365-2109.2009.02349.x

Hauptman, B. S., Barrows, F. T., Block, S. S., Gaylord, T. G., Paterson, J. A., Rawles, S. D., & Sealey, W. M. (2014). Evaluation of grain distillers dried yeast as a fish meal substitute in practical-type diets of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *432*, 7-14.

He, C., Lin, H-Y, Wang, C-C, Zhang, M., Lin, Y-Y, Huang, F-Y, Lin, Y-Z, Tan, G-H. (2019). Exopolysaccharide from *Paecilomyces lilacinus* modulates macrophage activities through the TLR4/NF-κβ/MAPK pathway. *Molecular Medicine Reports* 20, 4943-4952.

Helland, S. J., Grisdale-Helland, B. & Nerland, S. (1996). A simple method for the measurement of daily feed intake of groups of fish in tanks. *Aquaculture*, 139 (1): 157-163. doi: https://doi.org/10.1016/0044-8486(95)01145-5.

Hernawan T, Fleet G. Chemical and cytological changes during the autolysis of yeasts. (1995). *J Industr Microbiol*, 14:440-450.

Hoddevik, B. (2020, 27. mai). Lakselus kan gi svært høy dødelighet på utvandrende laks. Havforskningsinstituttet. Available from: Lakselus kan gi svært høy dødelighet på utvandrende laks

Hooft, J. M., Wu, P., Powell, C. D., Lou, Y., Squires, E. J., Cant, J. P., ... & Bureau, D. P. (2019). A comparative investigation of the effects of feed-borne deoxynivalenol (DON) on growth performance, nutrient utilization and metabolism of detoxification in rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*) fed diets containing different levels of digestible carbohydrates. *Aquaculture*, *505*, 306-318.

Hosokawa, M., & Nakamura, S. (2011). Extruder processing technology: Food and non-food biomaterials. *John Wiley & Sons*.

Hunter et al. (2017). Recalibrating Targets for Sustainable Intensification. Bioscience 67, 386–391

Iwama, G. K., & Tautz, A. F. (1981). A simple growth model for salmonids in hatcheries. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*(6), 649-656.

Iyer, S. S. & Cheng, G. H. (2012). Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. *Critical Reviews in Immunology*, 32 (1): 23-63. doi: 10.1615/CritRevImmunol.v32.i1.30.

Järvinen, R., Savonen, R., Ahlström, A. (1980). Physiological effects of Pekilo single cell protein on pigs. *Journal of the Scientific Agricultural Society of Finland* 52, 14-23.

Jones, S. W., Karpol, A., Friedman, S., Maru, B. T., & Tracy, B. P. (2020). Recent advances in single cell protein use as a feed ingredient in aquaculture. *Current Opinion in Biotechnology*, 61, 189-197. doi: 10.1016/j.copbio.2019.12.026.

Jung-Schroers, V., Adamek, M., Harris, S., Syakuri, H., Jung, A., Irnazarow, I. & Steinhagen, D. (2018). Response of the intestinal mucosal barrier of carp (*Cyprinus carpio*) to a bacterial challenge by Aeromonas hydrophila intubation after feeding with β-1, 3/1, 6-glucan. *Journal of Fish Diseases*, 41 (7): 1077-1092.

Karimi, S., Ferreira, J. A., & Taherzadeh, M. J. (2023). Filamentous fungi as animal and fish feed ingredients. *In Current Developments in Biotechnology and Bioengineering*: Filamentous Fungi Biorefinery (pp. 399-433). Elsevier. https://doi.org/10.1016/B978-0-323-91872-5.00002-8

Karimi, S., Soofiani, N. M., Lundh, T., Mahboubi, A., Kiessling, A., & Taherzadeh, M. J. (2019). Evaluation of Filamentous Fungal Biomass Cultivated on Vinasse as an Alternative Nutrient Source of Fish Feed: Protein, Lipid, and Mineral Composition. *Fermentation*, 5(4), 99. https://doi.org/10.3390/fermentation5040099

Karimi, S., Soofiani, N. M., Mahboubi, A., & Taherzadeh, M. J. (2018). Use of Organic Wastes and Industrial By-Products to Produce Filamentous Fungi with Potential as Aqua-Feed Ingredients. *Sustainability*, 10(9), 3296. https://doi.org/10.3390/su10093296

Karimi, S., Soofiani, N. M., Mahboubi, A., Ferreira, J. A., Lundh, T., Kiessling, A., & Taherzadeh, M. J. (2021). Evaluation of Nutritional Composition of Pure Filamentous Fungal Biomass as a Novel Ingredient for Fish Feed. *Fermentation*, 7(3), 152. https://doi.org/10.3390/fermentation7030152

Kasumyan, A. O. & Døving, K. B. (2003). Taste preferences in fishes. *Fish and Fisheries*, 4 (4): 289-347. doi: https://doi.org/10.1046/j.1467-2979.2003.00121.x.

Khansari, A. R., Parra, D., Reyes-López, F. E. & Tort, L. (2017). Cytokine modulation by stress hormones and antagonist specific hormonal inhibition in rainbow trout (*Oncorhynchus mykiss*) and gilthead sea bream (*Sparus aurata*) head kidney primary cell culture. *General and comparative endocrinology*, 250: 122-135.

Kiron, V. (2012). Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology*, 173 (1-2): 111-133.

Koch, J. F. A., de Oliveira, C. A. F. & Zanuzzo, F. S. (2021). Dietary β-glucan (MacroGard®) improves innate immune responses and disease resistance in Nile tilapia regardless of the administration period. *Fish & Shellfish Immunology*, 112: 56-63.

Koivurinta, J., Kurkela, R., Koivistoinen, P. (1979). Uses of Pekilo, a microfungus biomass from *Paecilomyces variotii* in sausage and meat balls. *J. Fd. Technol*. 14, 561-570.

Krogdahl A, Penn M, Thorsen J, Refstie S, Bakke AM. (2010). Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquac Res*, 41:333-344.

Krogdahl, Å., & Bakke-McKellep, A. M. (2005). Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (Salmo salar L.). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *141*(4), 450-460.

Krogdahl, Å., Sundby, A. & Holm, H. (2015). Characteristics of digestive processes in Atlantic salmon (Salmo salar). Enzyme pH optima, chyme pH, and enzyme activities. *FkkaAquaculture*, 449: 27-36

Kuehl, R.O., 2000. Design of Experiments: Statistical Principles of Research and Design Analysis, 2. Duxbury Press, Pacific Grove, CA.

Kumar P, Barret DM, Delwiche MJ, Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res* 48:3713–3729

Langeland M, Vidakovic A, Vielma J, Lindberg J, Kiessling A, Lundh T. (2016). Digestibility of microbial and mussel meal for Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*). *Aquac Nutr*, 22:485-495.

Lekand, O. (2020). Aquaculture engineering. 3rd ed. Ås: NMBU.

Livak, K. J. & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2()(-Delta Delta C T) method. Methods, 25 (4): 402-408. doi: 10.1006/meth.2001.1262.

Ljøkjel, K., Harstad, O. M., & Skrede, A. (2000). Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows. *Animal feed science and technology*, *84*(1-2), 83-95.

Ljøkjel, K., Sørensen, M., Storebakken, T., & Skrede, A. (2004). Digestibility of protein, amino acids and starch in mink (*Mustela vison*) fed diets processed by different extrusion conditions. *Canadian journal of animal science*, *84*(4), 673-680.

Luo, G. (2022). Review of waste phosphorus from aquaculture: Source, removal and recovery. *Reviews in Aquaculture*, e12727. https://doi.org/10.1111/raq.12727.

Madibana, M. J., & Mlambo, V. (2019). Growth performance and hemobiochemical parameters in South African dusky kob (*Argyrosomus japonicus, Sciaenidae*) offered brewer's yeast (*Saccharomyces cerevisiae*) as a feed additive. *Journal of the World Aquaculture Society*, *50*(4), 815-826.

Malherbe S, Cloete TE. (2002). Lignocellulose biodegradation: fundamentals and applications. *Re/Views Environ Sci Bio/Technol* 1:105–114

Maroni, K. (2000). Monitoring and regulation of marine aquaculture in Norway. *Journal of Applied Ichthyology*, 16 (4-5): 192-195.

McCarthy, J. (1983). A review of genetical and physiological effects on selection in meat-type poultry. Animal Breeding Abstracts.

McCleary, B.V., Solah, V., Gibson, T.S., 1994. Quantitative measurement of total starch in cereal flours and products. *J. Cereal Sci*. 20 (1), 51-58.

Meena, D. K., Das, P., Kumar, S., Mandal, S. C., Prusty, A. K., Singh, S. K., Akhtar, M. S., Behera, B. K., Kumar, K., Pal, A. K., et al. (2013). Beta-glucan: an ideal immunostimulant in aquaculture (a review). *Fish Physiology and Biochemistry*, 39 (3): 431-457. doi: 10.1007/s10695-012-9710-5.

Melberg, R. & Davidrajuh, R. (2009). Modeling Atlantic salmon fish farming industry: freshwater sub model simulation. Proceedings of the 2009 Spring Simulation Multiconference.

Meyer, V., Basenko, E. Y., Benz, J. P., Braus, G. H., Caddick, M. X., Csukai, M., ... & Wösten, H. A. (2020). Growing a circular economy with fungal biotechnology: a white paper. *Fungal biology and biotechnology*, *7*(1), 1-23.

Montalto, M., D'onofrio, F., Gallo, A., Cazzato, A. & Gasbarrini, G. (2009). Intestinal microbiota and its functions. *Digestive and Liver Disease Supplements*, 3 (2): 30-34.

Morales-Lange, B., Agboola, J.O., Hansen, J.Ø., Lagos, L., Øyås, O., Mercado, L., Mydland L.T., Øverland, M., 2021. The spleen as a target to characterize immunomodulatory effects of down-stream processed *Cyberlindnera jadinii* yeasts in Atlantic salmon exposed to a dietary soybean meal challenge. *Frontiers in Immunology* 12, 708747. doi: 10.3389/fimmu.2021.708747.

Morales-Lange, B., Djordjevic, B., Gaudhaman, A., Press, C. M., Olson, J., Mydland, L. T., Mercado, L., Imarai, M., Castex, M. & Øverland, M. (2022). Dietary Inclusion of Hydrolyzed *Debaryomyces hansenii* Yeasts Modulates Physiological Responses in

Plasma and Immune Organs of Atlantic salmon (*Salmo salar*) Parr Exposed to Acute Hypoxia Stress. *Frontiers in physiology*: 429.

Morales-Lange, B., González-Aravena, M., Font, A., Guzmán, F. & Mercado, L. (2018). Detection of peroxiredoxin-like protein in Antarctic sea urchin (*Sterechinus neumayeri*) under heat stress and induced with pathogen-associated molecular pattern from *Vibrio anguillarum. Polar Biology*, 41 (10): 2065-2073.

Morken, T., Kraugerud, O. F., Barrows, F. T., Sørensen, M., Storebakken, T., & Øverland, M. (2012). Sodium diformate and extrusion temperature affect nutrient digestibility and physical quality of diets with fish meal and barley protein concentrate for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 338-341, 173-180. https://doi.org/10.1016/j.aquaculture.2012.01.035

Mosier N, Wyman C, Dale B, Elander R, Lee Y, Holtzapple M, Ladisch M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Biores Technol*, 96:673-686.

Näsi, M. (1982). Performance of laying hens on diets containing Eurolysine bacterial protein or Pekilo protein. *Journal of the Scientific Agricultural Society of Finland* 54, 271-278.

Nasseri AT, Rasoul-Amini S, Morowvat MH, Ghasemi Y. (2011). Single cell protein: production and process. *Am J Food Technol* 6(2):103–116

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D. (2009). Feeding aquaculture in an era of finite resources. *Proc. Natl. Acad. Sci*. 106, 15103–15110.

Neu, J. (2008). Neonatology: Questions and Controversies Series: *Gastroenterology and Nutrition*: Elsevier Health Sciences.

Nguyen, D.Q., Mounir, S., Allaf, K. (2015). Functional properties of water holding capacity, oil holding capacity, wettability, and sedimentation of swell-dried soybean powder. *Sch. J. Eng. Tech* 3 (4B), 402-412.

Nitayavardhana S, Issarapayup K, Pavasant P, Khanal SK. (2013). Production of protein-rich fungal biomass in an airlift bioreactor using vinasse as substrate. *Bioresource Technol* 133:301–306

Norwegian Seafood Council. (2022). Record high Norwegian seafood exports in 2021. [Press release]. Retrieved from https://en.seafood.no/news-and-media/news-archive/record-high-norwegian-seafood-exports-in-2021/

NRC (National Research Council), 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.

Ogunniyi, D. S. (2011). Principles of extrusion processing. *International Journal of Food Properties,* 14(3), 494-512. https://doi.org/10.1080/10942910903388545

Oliva-Teles A and Gonçalves P, Partial replacement of fishmeal bybrewers yeast (*Saccharomyces cerevisiae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. (2001). *Aquaculture* 202:269–278

Omar SS, Merrifield DL, Kühlwein H, Williams PEV and Davies SJ. (2012). Biofuel derived yeast protein concentrate (YPC) as a novel feed ingredient in carp diets. A*quaculture*, 330–333:54–62

Opstvedt, J., Miller, R., Hardy, R. W., & Spinelli, J. (1984). Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gairdneri*). *Journal of Agricultural and Food Chemistry*, *32*(4), 929-935.

Osaku, E.F., Menolli, R.A., de M. Costa, C.R.L., Tessaro, F.H.G., de Melo, R.H., do Amaral, A.E., Duarte, P.A.D., de Santana Filho, A.P., Ruthes, A.C., da C. Silva, J.L., Mello, R.G., 2018. β-(1→ 6)-D-glucan secreted during the optimised production of exopolysaccharides by *Paecilomyces variotii* has immunostimulatory activity. *Antonie van Leeuwenhoek* 111, 981-994.

Øverland, M. & Skrede, A. (2017). Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. *Journal of the Science of Food and Agriculture*, 97 (3): 733-742. doi: https://doi.org/10.1002/jsfa.8007.

Øverland, M., Karlsson, A., Mydland, L. T., Romarheim, O. H. & Skrede, A. (2013). Evaluation of *Candida utilis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). *Aquaculture*, 402: 1-7.

Øverland, M., Romarheima, O.H., Ahlstrømb, Ø., Storebakken, T., & Skrede, A. (2007). Technical quality of dog food and salmon feed containing different bacterial protein sources and processed by different extrusion conditions. *Aquaculture Nutrition*, 13(2), 109-119. doi: 10.1111/j.1365-2095.2007.00450.x.

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å. Skrede, A. (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*) – effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. *Aquaculture* 288 (3-4), 305-311.

Øverland, M., Tauson, A-H, Shearer, K., Skrede, A. (2010). Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals. *Archives of Animal Nutrition* 64, 171-189.

Ozório ROA, Turini BGS, Môro GV, Oliveira LST and Portz L, Growth, nitrogen gain and indispensable amino acid retention of pacu (*Piaractus mesopotamicus*) fed different brewer's yeast (*Saccharomyces cerevisiae*) levels. (2010). *Aquaculture Nutrition*16:276–283

Ozório, R. O., Portz, L., Borghesi, R., & Cyrino, J. E. (2012). Effects of dietary yeast (*Saccharomyces cerevisia*) supplementation in practical diets of tilapia (*Oreochromis niloticus*). *Animals*, *2*(1), 16-24.

Pandey A, Soccol CR, Nigam P, Soccol VT. (2000). Biotechnological potential of agro-industrial residues I: sugarcane bagasse. *Bioresour Technol* 74:69–80

Panettieri, R. A., Schaafsma, D., Amrani, Y., Koziol-White, C., Ostrom, R. & Tliba, O. (2019). Non-genomic effects of glucocorticoids: an updated view. *Trends in pharmacological sciences*, 40 (1): 38-49.

PeerJ, 5. (2017). This paper is a portion of the dossier submitted to the FDA for the first (to our knowledge) genetically engineered SCP product to obtain GRAS (generally regarded as safe) recognition by the US Food and Drug Administration. This sets a significant precedent for the advancement of additional, genetically engineered feed ingredients for aquaculture.

Penn, M. H., Bendiksen, E. Å., Campbell, P. & Krogdahl, Å. (2011). High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (*Salmo salar L.*). *Aquaculture*, 310 (3-4): 267-273.

Peterson, B. C., Booth, N. J., & Manning, B. B. (2012). Replacement of fish meal in juvenile channel catfish, *Ictalurus punctatus*, diets using a yeast-derived protein source: the effects on weight gain, food conversion ratio, body composition and survival of catfish challenged with *Edwardsiella ictaluri*. *Aquaculture Nutrition*, *18*(2), 132-137.

Press, C. M. & Evensen, Ø. (1999). The morphology of the immune system in teleost fishes. *Fish & shellfish immunology*, 9 (4): 309-318.

Rao, X., Huang, X., Zhou, Z. & Lin, X. (2013). An improvement of the 2ˆ (–delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostat Bioinforma Biomath*, 3 (3): 71

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B. & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, 433: 50-61.

Reyes-Becerril, M., Salinas, I., Cuesta, A., Meseguer, J., Tovar-Ramirez, D., Ascencio-Valle, F., & Esteban, M. Á. (2008). Oral delivery of live yeast Debaryomyces hansenii modulates the main innate immune parameters and the expression of immune-relevant genes in the gilthead seabream (*Sparus aurata L.*). *Fish & shellfish immunology*, *25*(6), 731-739.

Robertsen, B., Rorstad, G., Engstad, R., Raa, J., 1990. Enhancement of non-specific disease resistance in Atlantic salmon, Salmo salar L., by a glucan from Saccharomyces cerevisiae cell walls. *Journal of Fish Diseases* 13, 391-400.

Robinson T, Nigam P (2003) Bioreactor design for protein enrichment of agricultural residues by solid state fermentation. *Biochem Eng* J 13:197–203

Rokey, G. J. (2006). Pet food production: process description (Pet Food Production). Online article in Animal Feed of Engormix

Romarheim, O. H., Øverland, M., Mydland, L. T., Skrede, A., & Landsverk, T. (2011). Bacteria grown on natural gas prevent soybean meal-induced enteritis in Atlantic salmon. *The Journal of nutrition*, *141*(1), 124-130.

Rumsey, G.L., Hughes, S.G. (1990). Use of Dietary Yeast *Saccharomyces cerevisiae* Nitrogen by Lake Trout. *J. World Aquac.* Soc. 21, 205–209.

Rumsey, G.L., Hughes, S.G., Smith, R.R., Kinsella, J.E., Shetty, K.J. (1991a). Digestibility and energy values of intact, disrupted and extracts from brewer's dried yeast fed to rainbow trout (*Oncorhynchus mykiss*). *Anim. Feed Sci. Technol*. 33, 185–193.

Rumsey, G.L., Kinsella, J.E., Shetty, K.J., Hughes, S.G. (1991b). Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). *Anim. Feed Sci. Technol*. 33, 177–183. https://doi.org/10.1016/0377-8401(91)90058-Z

Rumsey, G.L., Winfree, R.A., Hughes, S.G. (1992). Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 108, 97–110. https://doi.org/10.1016/0044-8486(92)90321-B

Sahlmann, C., Djordjevic, B., Lagos, L., Mydland, L. T., Morales-Lange, B., Øvrum Hansen, J., Ånestad, R., Mercado, L., Bjelanovic, M., Press, C. M., et al. (2019). Yeast as a protein source during smoltification of Atlantic salmon (*Salmo salar*), enhances performance and modulates health. *Aquaculture,* 513: 734396. doi: https://doi.org/10.1016/j.aquaculture.2019.734396.

Sakai, M., Hikima, J.-i. & Kono, T. (2021). Fish cytokines: Current research and applications. *Fisheries Science*, 87 (1): 1-9.

Salinas, I. (2015). The mucosal immune system of teleost fish. *Biology*, 4 (3): 525-539.

Samuelsen, T. A., Hillestad, M., Jacobsen, H. J., Hjertnes, T. J., & Sixten, H. J. (2021). Physical feed quality and starch content causes a biological response in Atlantic salmon (*Salmo salar*). *Aquaculture Reports*, *21*, 100791.

Sarlin, P. A., & Philip, R. (2011). Efficacy of marine yeasts and baker's yeast as immunostimulants in *Fenneropenaeus indicus*: a comparative study. *Aquaculture*, *321*(3-4), 173-178.

Schmid, M., & Krumpholz, P. (2007). Extruder technology in the feed and food industries. In Proceedings of the 20th Annual Conference on Fodder Crops and Amenity Grasses (pp. 105-115).

Schmitt, P., Wacyk, J., Morales-Lange, B., Rojas, V., Guzmán, F., Dixon, B. & Mercado, L. (2015). Immunomodulatory effect of cathelicidins in response to a β-glucan in intestinal epithelial cells from rainbow trout. *Developmental & Comparative Immunology*, 51 (1): 160-169.

Sharma S, Hansen LD, Hansen JØ, Mydland LT, Horn SJ, Øverland M, Eijsink VG, Vuoristo KS. (2018). Microbial protein produced from brown seaweed and spruce wood as a feed ingredient. *J Agric Food Chem*, 66:8328-8335.

Shomorin, G.O, Storebakken, T., Kraugerud, O.F., Øverland, M., Hansen, B.R., Hansen, J.Ø., 2019. Evaluation of wedge wire screen as a new tool for faeces collection in digestibility assessment in fish: The impact of nutrient leaching on apparent digestibility of nitrogen, carbon and sulphur from fish meal, soybean meal and rapeseed meal-based diets in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 504, 81-87.

Singh A, Abidi AB, Darmwal NS, Agrawal AK (1988) Evaluation of chemical pre-treatment for biodegradation of agricultural lignocellulosic wastes by *Aspergillus niger*. *MIRCEN J Appl Microb* 4:473–479

Singh, B., Sharma, C., Sharma, S. (2017) Fundamentals of extrusion processing. In: Novel Food Processing Technologies; Nanda, V. & Sharma, S. New India Publishing Agency, New Delhi.

Siwicki, A.K., Kazuń, K., Glabski, E., Terech-Majewska, E., Baranowski, P., Trapkowska, S., 2004. The effect of beta-1,3/1,6-glucan in diets on the effectiveness of anti-Yersinia ruckeri vaccine – an experimental study in rainbow trout (*Oncorhynchus mykiss*). *Polish Journal of Food and Nutrition Sciences* 13, 59-61.

Skrede, A., Berge, G. M., Storebakken, T., Herstad, O., Aarstad, K. G., & Sundstøl, F. (1998). Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. *Animal Feed Science and Technology*, *76*(1-2), 103-116.

Solberg, B., Moiseyev, A., Hansen, J. Ø., Horn, S. J. & Øverland, M. (2021). Wood for food: Economic impacts of sustainable use of forest biomass for salmon feed production in Norway. *Forest Policy and Economics*, 122: 102337.

Sørensen, M. L. K. S. T., Ljøkjel, K., Storebakken, T., Shearer, K. D., & Skrede, A. (2002). Apparent digestibility of protein, amino acids and energy in rainbow trout (*Oncorhynchus mykiss*) fed a fish meal-based diet extruded at different temperatures. *Aquaculture*, *211*(1-4), 215-225.

Storebakken, T., Baeverfjord, G., Skrede, A., Olli, J. J., & Berge, G. M. (2004). Bacterial protein grown on natural gas in diets for Atlantic salmon, *Salmo salar*, in freshwater. *Aquaculture*, *241*(1-4), 413-425.

Tacon, A. G. J. & Metian, M. (2015). Feed Matters: Satisfying the Feed Demand of Aquaculture. *Reviews in fisheries science & aquaculture*, 23 (1): 1-10. doi: 10.1080/23308249.2014.987209.

Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285, 146–158.

Talpur, A. D., Munir, M. B., Mary, A. & Hashim, R. (2014). Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against Aeromonas hydrophila in snakehead (*Channa striata*) fingerlings. *Aquaculture*, 426: 14-20.

Teixeira, C., Peixoto, D., Hinzmann, M., Santos, P., Ferreira, I., Pereira, G. V., Dias, J. & Costas, B. (2022). Dietary Strategies to Modulate the Health Condition and Immune Responses in Gilthead Seabream (*Sparus aurata*) Juveniles Following Intestinal Inflammation. *Animals*, 12 (21). doi: 10.3390/ani12213019.

Tlusty, M., Rhyne, A., Szczebak, J. T., Bourque, B., Bowen, J. L., Burr, G., ... & Feinberg, L. (2017). A transdisciplinary approach to the initial validation of a single cell protein as an alternative protein source for use in aquafeeds. *PeerJ*, *5*, e3170.

Torrecillas, S., Makol, A., Caballero, M. J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L. & Izquierdo, M. S. (2007). Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. 48 *Fish & Shellfish Immunology*, 23 (5): 969-981. doi: https://doi.org/10.1016/j.fsi.2007.03.007.

Torrecillas, S., Montero, D. & Izquierdo, M. (2014). Improved health and growth of fish fed mannan oligosaccharides: Potential mode of action. *Fish & Shellfish Immunology*, 36 (2): 525-544. doi: https://doi.org/10.1016/j.fsi.2013.12.029.

UIFSA. (2020). A revolution in salmon farming. Available at: https://uifsa.ua/en/news/world-news/a-revolution-in-salmon-farming

UniBio A/S (2014) http://www.unibio.dk

Uran PA, Schrama JW, Jaafari S, Baardsen G, Rombout J, Koppe W, Verreth JAJ. (2009). Variation in commercial sources of soybean meal influences the severity of enteritis in Atlantic salmon (*Salmo salar L.*). *Aquac Nutr*, 15:492-499.

Urán PA, Schrama JW, Rombout J, Obach A, Jensen L, Koppe W, Verreth JAJ. (2008). Soybean meal induced enteritis in Atlantic salmon (*Salmo salar L.*) at different temperatures. *Aquac Nutr*, 14:324-330.

Van Dyk J, Pletschke B. (2012). A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes—factors affecting enzymes, conversion and synergy. *Biotechnol Adv*, 30:1458-1480.

Van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual review of entomology*, *58*, 563-583.

Vandenberg, G.W., De La Noue, J. (2001). Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquac. Nutr*. 7, 237–245. https://doi.org/10.104

Vatlestad, J. (2017, 10. september). Her er den første havmerden til NRS sjøsatt: - Vi nærmer oss nå driftsfasen. Available from: Her er den første havmerden til NRS sjøsatt: – *Vi nærmer oss nå driftsfasen* | IntraFish.no

Vidakovic, A., Huyben, D., Sundh, H., Nyman, A., Vielma, J., Passoth, V., ... & Lundh, T. (2020). Growth performance, nutrient digestibility and intestinal morphology of rainbow trout (*Oncorhynchus mykiss*) fed graded levels of the yeasts *Saccharomyces cerevisiae* and Wickerhamomyces anomalus. *Aquaculture Nutrition*, *26*(2), 275-286.

Vidakovic, A., Langeland, M., Sundh, H., Sundell, K., Olstorpe, M., Vielma, J., ... & Lundh, T. (2016). Evaluation of growth performance and intestinal barrier function in Arctic Charr (*Salvelinus alpinus*) fed yeast (*Saccharomyces cerevisiae*), fungi (*Rhizopus oryzae*) and blue mussel (*Mytilus edulis*). *Aquaculture nutrition*, *22*(6), 1348-1360.

Villa Bôas SG, Esposito E, de Mendonça MM. (2002). Novel lignocellulolytic ability of Candida utilis during solid substrate cultivation on apple pomace. *World J Microbiol Biotechnol* 18:541–545

Volman, J. J., Ramakers, J. D., and Plat, J. (2008). Dietary modulation of immune function by beta-glucans. *Physiol.Behav*. 94, 276–284. doi: 10.1016/j.physbeh.2007.11.045

Wang, J., Kortner, T. M., Chikwati, E. M., Li, Y., Jaramillo-Torres, A., Jakobsen, J. V., Ravndal, J., Brevik, Ø. J., Einen, O. & Krogdahl, Å. (2020). Gut immune functions and health in Atlantic salmon (*Salmo salar*) from late freshwater stage until one year in seawater and effects of functional ingredients: A case study from a commercial sized research site in the Arctic region. *Fish & Shellfish Immunology*, 106: 1106-1119.

Weththasinghe, P., Hansen, J.Ø., Nøkland, D., Lagos, L., Rawski, M., Øverland, M., 2021.Full-fat black solider fly larvae (*Hermetia illucens*) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): Effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performance. *Aquaculture* 530, 735785.

Wikandari, R., Hasniah, N., & Taherzadeh, M. J. (2022). The role of filamentous fungi in advancing the development of a sustainable circular bioeconomy. *Bioresource Technology*, *345*, 126531.

Xu, C., Guo, T.-C., Mutoloki, S., Haugland, Ø. & Evensen, Ø. (2012). Gene expression studies of host response to Salmonid alphavirus subtype 3 experimental infections in Atlantic salmon. *Veterinary research*, 43 (1): 1-10.

Xue, Q., Yan, Y., Zhang, R. & Xiong, H. (2018). Regulation of iNOS on immune cells and its role in diseases. *International journal of molecular sciences*, 19 (12): 3805.

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

Zhang Y, Lin SM, Zhu YJ, Liu CJ, Dong Y, Li FF, Wu GF, Wang HY, Zhang JH (2006) Protoplast fusion between *Geotrichum candidium* and *Phanerochaete chrysosporium* to produce fusants for corn stover fermentation. *Biotechnol Lett* 28:1351–1359

Zou, J. & Secombes, C. J. (2016). The function of fish cytokines. *Biology*, 5 (2): 23.


**Norges miljø- og biovitenskapelige universitet**
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway