UNIVERSITY OF GOTHENBURG

Novel marine ingredients for aquaculture

Fish nutrition, physiology, and intestinal health

Thesis for the degree of Doctor of Philosophy

by

Niklas Warwas

Department of Biological and Environmental Sciences
Faculty of Science
University of Gothenburg
2023

This doctoral thesis in Natural Sciences, specializing in Zoophysiology and Nutrition, is authorized by the Faculty of Science to be publicly defended at 10:00 a.m. on Friday 10th, November 2023 at the Natrium building Medicinaregatan 7b, Room 3401, Korallrevet

The opponent of this thesis is Professor Margareth Øverland, Nutrition and Physiology in Monogastric Animals, Norwegian University of Life Sciences.

NOVEL MARINE INGREDIENTS FOR AQUACULTURE - FISH NUTRITION, PHYSIOLOGY, AND INTESTINAL HEALTH

Niklas Warwas

Department of Biological and Environmental Sciences

University of Gothenburg

Box 463, SE-405-30 Gothenburg

SWEDEN

E-mail: niklas.warwas@bioenv.gu.se

ISBN: 978-91-8069-513-8 (PDF)

ISBN: 978-91-8069-514-5 (Print)

Cover illustration: Terese Hjelmstet

Ale Tryckteam AB

DISSERTATION ABSTRACT

Aquaculture is among the fastest growing food production sectors globally and supplies more fish to the growing human population than capture fisheries. One of the major challenges in salmonid aquaculture is to find renewable and sustainable feed ingredients which also satisfy the nutritional requirements of the fish. Feed production accounts for the majority of both the environmental and economic footprints of modern aquaculture operations. It is therefore clear that sustainable aquaculture can only be achieved using sustainable feed. The overarching aim of this thesis is to expand the pool of alternative feed ingredients by developing and evaluating novel marine ingredients using both a nutritional and a physiological approach. The aspiration is further to carry out this work in the framework of a circular economy approach where side streams and their nutrients are reintroduced into the food production system rather than discarded.

Paper I demonstrates that marine yeast (*C. sake)* can be cultivated on processing water of the fish processing industry. Marine yeast could therefore be used transform side streams into a promising feed ingredient for salmonid fish. *C. sake* contained 55% protein and significant levels of omega-3 fatty acids. Additionally, *C.sake* was highly digestible by rainbow trout and therefore can be used in diet formulations at up to 20% of the overall content without negative effects on animal health and growth. Furthermore, there is potential to use *C. sake* as an immunostimulant due to its complex polysaccharide and nucleotide content.

Paper II assesses the potential of using a marine insect in fish feed. Marine insects contain higher levels of essential nutrients, such as unsaturated fatty acids and amino acids, compared to terrestrial insects. However, they have not been evaluated as feed ingredients for salmonid fish. This thesis shows that the seaweed fly (*Coelopa frigida*) can be cultivated on side streams from an algae farm producing brown seaweed. Furthermore, seaweed fly larvae could substitute 40% of fish meal content without negative effects on growth and intestinal health. Compared to black soldier fly larvae, *C. frigida* inclusion resulted in higher feed intake and growth for rainbow trout.

Papers III & **IV** address the possibility to use fish processing side streams in feed formulations without additional processing. Fish processing side streams are highly nutritious and currently contribute to about 30% of the global fishmeal and fish oil production. However, due to the additional processing costs and the lower price of fishmeal and oil produced from sides streams compared to whole fish fishmeal, large amounts of such side streams remain unused or are converted to lower value commodities. In **paper III**, three different fish processing side streams (fillets and trimming) were included in diets for rainbow trout without separating oil and protein fractions. The results show that whether side streams can be used directly as ingredients, depends on storage condition and handling. The inclusion of 50% fresh sprat trimmings, resulting in high growth rates, high feed intake and good intestinal health, while the inclusion of suboptimal stored marinated herring fillets impaired growth, intestinal health, and appetite. To evaluate if these results are transferable to an industry setting, 500 kg of feed containing moist sprat trimmings (**paper IV**) were extruded using industrial scale equipment. The pellet quality was generally comparable to commercial feed except for a slightly lower boyancy. Compared to a state of the art commercial diet, fish fed the experimental diet displayed slightly lower feed intake and growth. However, the differences in growth and weight gain were largely due to lower lipid deposition in the muscle and intraperitoneal cavity, which would suggest higher product quality of fish fed the experimental diet. Overall, moist side streams can be incorporated in extruded diets which reduce costs and emission.

This thesis demonstrates for the first time, that marine yeast and marine insects are promising alternative marine ingredients for future aquaculture. Additionally, side streams with high nutritional value may be utilized as feed ingredients with no or minimal additional processing. Nonetheless, additional processing has clear benefits regarding storage, transport, and maximum inclusion levels in the feed. The results of this thesis can therefore by applied to both the development of local sustainable aquaculture in Sweden but also to the global aquafeed market, which currently lacks realistic marine alternatives, exhibits increasingly unstable supply chains, and high prices.

SVENSKT SAMANFATTNING

Vattenbruk är en av de snabbast växande livsmedelssektorerna i världen och står idag för mer sjömat för konsumtion till den växande mänskliga befolkningen än fisket. En av de stora utmaningarna för vattenbruk av karnivora fiskar, som laxfiskar, är att hitta förnybara foderingredienser som är hållbara, samtidigt som man ser till att fiskens näringsbehov uppfylls. Eftersom foder står för majoriteten av både det miljömässiga och ekonomiska avtrycket av modernt, hög-intensivt vattenbruksverksamhet, är det mycket tydligt att hållbart vattenbruk endast kan uppnås genom användning av hållbart foder. Det övergripande syftet med denna avhandling är att utöka poolen av alternativa foderingredienser genom att utveckla och utvärdera nya marina ingredienser med fokus på att skapa cirkularitet. Ett av utvärderingens huvudmål är att kombinera näringsmässiga och fysiologiska metoder för bibehållen hälsa och välfärd hos den odlade fisken.

Artikel I visar att den marina jästen, *C. sake*, kan odlas på processvatten från fiskberedningsindustrin. Marin jäst kan därför användas för att omvandla sidoströmmar, som annars slängs eller används för t.ex. biogasproduktion, till en lovande foderingrediens för laxfisk. *C. sake* innehöll så mycket som 55 % protein och betydande halter av omega-3 fettsyror. Dessutom var *C. sake* lättsmält mat för regnbågslaxen och kan därför inkluderas i foder-formuleringar med upp till 20 % av det totala innehållet, utan att negativt påverka fiskarnas hälsa eller tillväxt. Resultaten visade också att *C. sake* hade immunstimulerande egenskaper, troligen på grund av dess komplexa polysackarid- och nukleotidinnehåll, vilket innebär lovande värdehöjning av denna möjliga framtida foderingrediens.

Artikel II undersöker potentialen att använda larver från en marin insekt i fiskfoder. Marina insekter innehåller högre nivåer av essentiella näringsämnen, såsom omättade fettsyror och aminosyror, jämfört med landlevande insekter. De har dock ännu inte utvärderats för användning som foderingredienser för laxfiskar. Denna avhandling visar att tångflugan (*Coelopa frigida*) kan odlas på sidoströmmar (de delar av algerna som ej kan användas till mat eller andra algprodukter) från brunalgsodling – i detta fall odling av sockertång, *Saccharina latissima*a. Dessutom kan tångfluge-larver ersätta upp till 40 % av fiskmjölet i ett kontrollfoder utan några negativa effekter på tillväxt eller tarmhälsa hos regnbågslax. Jämfört med motsvarande inblandning av larvmjöl från landlevande insekter (svart soldatfluga) visade *C. frigida* högre foderintag och bättre tillväxt hos fisken.

Paper III och IV tar upp användningen av direkta sidoströmmar från fiskberedningsindustrin i produktion av fiskfoder. Fiskberedningsindustrins sidoströmmar är mycket näringsrika och bidrar redan idag med cirka 30 % av den globala produktionen av fiskmjöl och fiskolja. Men på grund av de extra kostnader och den ökade energiförbrukningen som tillverkning av fiskmjöl och fiskolja innebär, samt att fiskmjöl och fiskolja från sidoströmmar har lägre näringsvärde än de som producerats från vildfångad hel fisk, är det stora mängder sidoströmmar som idag inte används alls alternativt omvandlas till t.ex. biogas. I papper III inkluderades tre olika sidoströmmar, avskär från filetering och filéer som behandlats på olika sätt, i foder för regnbåge. Sidoströmmarna användes "råa" utan att först separera olje- och proteinfraktioner i syfte att minska både kostnader och miljöavtryck. Studien visar att det är väldigt olika mellan olika sidoströmmar, om de lämpar sig som foderingredienser eller ej. Något som i första hand beror på lagring, tillsatser och hantering. Färskt avskär från filetering av skarpsill resulterade i både hög tillväxt och högt foderintag, medan suboptimala lagrade marinerade sillfiléer gav försämrad tillväxt, tarmhälsa och aptit. För att utvärdera om dessa, för skarpsills avskäret lovande resultaten kan överföras till industriell skala av foderproduktion, tillverkades 500 kg extruderat foder innehållande nästan 6% fuktigt, skarpsillsavskär (papper **IV**) i industriell utrustning och skala. Pelletskvaliteten var jämförbar med kommersiellt foder med undantag för en något lägre flytkraft. Den lägre flytkraften resulterade troligen i ett något lägre foderintag och därmed tillväxt i jämförelse med det kommersiella fodret. Den skillnad i tillväxt och viktökning som observerades, berodde dock också till stor del på lägre fettinlagring i muskeln och bukhålan hos experimentfisken som fick foder med skarpsillsavskär, en egenskap som är positiv för kvaliteten på slutprodukten, den odlade regnbågen.

Denna avhandling visar för första gången att marin jäst och marina insekter, odlade på olika typer av sidoströmmar från sjömatsproduktion, utgör mycket lovande alternativa marina ingredienser för fiskfoderproduktion i framtida vattenbruk. Dessutom visar avhandlingen att sidoströmmar från fiskberedningsindustrin, med höga näringsvärden, kan användas som foderingredienser med ingen eller minimal ytterligare bearbetning. Resultaten från avhandlingen kan användas in i utveckling av lokalt hållbart vattenbruk i Sverige, men även överföras till den globala fiskfoderproduktionen som idag saknar bra och hållbara marina alternativ och där dagens ingredienser ofta har osäker tillgång och leveranskedjor samt höga priser.

LIST OF PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Marine yeast (*Candida sake*) cultured on herring brine side streams is a promising feed ingredient and omega-3 source for rainbow trout (*Oncorhynchus mykiss*). Warwas, N*., Vilg, J. V., Langeland, M., Roques, J. A. C., Hinchcliffe, J., Sundh, H., Undeland, I. & Sundell, K. S. (2023). *Aquaculture*, *571*, 739448.

II. The seaweed fly (*Coelopa frigida*) converts marine algae side streams into a high-quality ingredient and omega-3 source in diets for rainbow trout (*Oncorhynchus mykiss*). Warwas, N*., Berdan, B., Xie, X., Roques, J. A. C., Doyle D., Heden, I., Hinchcliffe J., Pavia, H., Langeland, M., Jönsson, E., & Sundell, K. S. (2023) *Submitted to Aquaculture Nutrition*.

III. Fish processing side streams are promising ingredients in diets for rainbow trout (*Oncorhynchus mykiss*) – Effects on growth physiology, appetite, and intestinal health. Warwas. N*., Langeland. M., Roques. J.A.C., Montjouridès. M., Smeets. J, Sundh. H., Jönssona E. & Sundell. K.S. (2023) *Journal of Fish Biology in press*

IV. Moist fish processing side streams, sprat trimmings, as a feed ingredient in extruded diets for rainbow trout – Pellet quality, intestinal health and product quality. Warwas. N*., Andersson. M., Vidakovic. A., Johansson. E., Roques. J.A.C., Hinchcliffe. J., Jönssona E., Sundell. K.S. & Langeland. M. (2023) *Manuscript*

*Corresponding author

TABLE OF CONTENT

INTRODUCTION

As population growth, resource scarcity, and the impact of global warming intersect, the issue of global food security is increasingly urgent.

Aquaculutre

"We must plant the sea and herd its animals … using the sea as farmers instead of hunters. That is what civilization is all about - farming replacing hunting."

Jacques-Yves Cousteau

The term aquaculture refers to the farming of aquatic organisms, including vertebrates (mainly finfish), invertebrates, and algae. The total annual production of these products is ca. 90 million tons, making it one of the largest food production sectors (FAO, 2022). In addition to being one of the largest, aquaculture is also the fastest growing food production sector globally. Since 1990, the global output of aquaculture has increased by 600% (FAO, 2022). To put this into context, beef production increased by roughly 50% in the same time span (Smith et al., 2018). Due to its rapid expansion, aquaculture has become critical to food security, particularly in developing countries. The rise of aquaculture can be attributed to several factors. Growing populations, rising gross domestic product, and expansion of the middle class in developing countries have led to increased demand for nutritious and sustainably produced seafood (Boyd et al., 2020; Naylor et al., 2021). While the entire seafood sector has increased drastically in both demand and production, this thesis will focus solely on fish production. The increased demand for farmed fish has occurred simultaneously with the gradual decline and stagnation of global capture fisheries (FAO, 2022). As aquaculture is the only alternative to fisheries in terms of fish production, it has been crucial in meeting this increased demand for seafood. As the global population is expected to increase to close to 9.7 billion by 2030, the aquaculture sector is expected to continue to grow (Glencross et al., 2023; UN 2023). Such growth is likely to be critical in ensuring food security for future generations.

Of the many aquatic species that are farmed globally, finfish represent roughly 66%. Carp, catfish, and tilapia, all of which are mainly farmed in China, dominate this category (FAO, 2022). In Europe, aquaculture is primarily centered around the production of salmonids, and this is also the main focus of my thesis. Atlantic salmon and rainbow trout are the two most farmed fish in Europe, with annual production of 1.8 and 0.2 million tons respectively (Aas et al., 2022; EUMOFA, 2022). In the past 30 years, salmonid aquaculture has expanded rapidly, led by technological developments, improvements in husbandry, and the construction of vast coastal farms. However, despite the successes of salmonid aquaculture in Europe, the industry still faces challenges, many of which threaten future sustainable development. One of the major challenges for salmonid aquaculture is to find renewable feed ingredients that are both ecologically and economically sustainable, while ensuring that the nutritional requirements of the fish are met (Eroldoğan et al., 2023).

Nutrition

"*Life is a tragedy of nutrition*"– Arnold Ehret

According to the Oxford dictionary, nutrition is defined as "the process of providing or obtaining the food necessary for health and growth". Nutritionists may add that rather than food items, organisms need to be provided with the nutrients needed for health and growth instead of specific food or, in the case of animal nutrition, feed items. These nutrients can be divided into macronutrients and micronutrients. Macronutrients are the major building blocks and energy sources for an organism and broadly include three types of molecules: proteins, lipids, and carbohydrates. Micronutrients on the other hand are used in smaller quantities and have more subtle effects on physiological and biochemical processes. Deficiencies in either macro or micronutrients lead to malnutrition, which may have adverse effects i.e. compromised growth and development, reduced reproduction, and increased mortality. Fish include the most diverse group of vertebrates including sharks, lungfish, marine and freshwater fish, and this diversity is reflected by their nutritional demands (Hardy & Kaushik, 2020). Salmonid fish for instance are carnivorous, naturally relying on the consumption of other animals, which has important nutritional implications. The digestive tract and physiology of salmonid fish is optimized for feed high in protein and fat. Carbohydrates on the other hand are less efficiently utilized by salmonids (Hardy & Kaushik, 2020; Schrama et al., 2018). It is apparent that rather than just considering the macronutrients of a diet or ingredients, nutritionists need to consider the digestible and metabolizable energy and nutrient (macro and micro) content (Cho et al., 1982).

Macro- and micronutrients

Proteins - Proteins, and more specifically their building blocks the amino acids (AAs), may be the most important components for an animal to maintain homeostasis and growth. The term "protein" was coined in 1838 by the Swedish Chemist Jöns Jacob Berzelius and is derived from the greek word proteios "πρωτεῖος" meaning "primary". While over 300 amino acids occur naturally, only 20 amino acids are used in the formation of animal proteins. Among these amino acids, 10 are essential for fish, meaning that they need to be taken up by an animal through feed (Hua & Bureau, 2019). Ingested proteins are hydrolyzed and broken down into free AAs or small peptides by a variety of proteases, which are then taken up by the enterocytes mainly through active transport processes (Buddington et al., 1997). Animals therefore have no protein requirement per se, but rather demand a well-balanced mixture of AAs (both essential and nonessential). A deficiency in AAs, particularly those that are essential, may lead to reduced physiological function and growth (Harper et al., 1970). However, excess AAs and protein in general will lead to nitrogen loss, as amino acids are metabolized for energy and nitrogen is excreted (Luo, 2022). Protein or amino acid requirements can be divided into maintenance requirements (the level needed to maintain homeostasis) and the requirements for optimal or fastest growth, with the latter being of more interest in aquaculture (Cho et al., 1982). The amino acid requirements of salmonid fish are comparatively well established, leading to a protein content in commercial diets of around 40%.

Lipids - Lipids are a broad group of hydrophobic or amphiphilic organic compounds. There are three subgroups of lipids that are of particular nutritional importance: triglycerides (fats and oils), phospholipids (the building blocks of biological membranes), and sterols (e.g. cholesterol). Fatty acids are building blocks of both triglycerides (esters of 3 fatty acids and glycerol) and phospholipids (2 fatty acids and glycerol). Additionally, triglycerides are used as energy storage and can be metabolized for more ATP/g than either carbohydrates or proteins (Pollard et al., 2022). Therefore, fatty acids have both essential physiological functions and represent the main energy storage of many organisms including fish. In salmonid fish, fatty acids may be the main energy source even though they are also very efficient at metabolizing amino acids. As for the amino acids, several fatty acids are essential and need to be provided in sufficient amounts through the diet to sustain optimal growth. These include especially fatty acids containing one or more double bonds within the carbon chain - so called unsaturated fatty acids (Hardy & Kaushik, 2020). For salmonid fish, polyunsaturated long chained fatty acids (LC-PUFAs) are especially crucial. While many terrestrial plants are high in short chain unsaturated fatty acids, the LC-PUFAs are characteristic of marine organisms. The handling of PUFAs in feed formulations is another challenge for nutritionists and feed producers, as unsaturated fatty acids are more unstable and prone to oxidation. Optimized diets for salmonid fish contain about 30% lipids including 2% LC-PUFAs (Aas et al., 2022).

Carbohydrates - Carbohydrates are bio-molecules consisting of carbon (C), hydrogen (H) and oxygen (O) atoms. In biochemistry and nutrition, the term is synonymous with saccharides including sugars, starch and fiber (except for lignin). While they may contribute significantly to the total energy in diets for omnivorous and herbivorous fish, they are not essential nutrients for carnivorous fish. As a result, there are no nutritional demands for including carbohydrates in diets for salmonid fish. However, the gelatinization and expansion of starch plays an important role for the physical properties of extruded diets (Lin et al., 1997). Therefore, diets for salmonid fish generally contain a minimum of 6% starch (Sørensen, 2012).

Micronutrients - Micronutrients include a variety of minerals and vitamins. Among others, the essential micronutrients include calcium (bone and scale formation and enzyme activity), zinc (enzyme and immune function), selenium (antioxidant defense and thyroid function) phosphorus (bone and tissue formation, energy metabolism, ATP, and pH regulation), iodine (thyroid hormone synthesis) and iron (oxygen transport). Micronutrients are essential but need to be included in the diet only in relatively small quantities (Hardy & Kaushik, 2020). For instance, salmonid diets contain ca. 0.9% phosphorous (Aas et al., 2022). The required levels of the other micronutrients are even much lower.

The nutritional requirements dictate feed formulations and the raw material choices in commercial diets. For ease of formulation, most micronutrients are added as synthetic pre-mixes. However, feed producers also need to consider raw material availability and price. This is especially challenging for salmonid fish, as lipid and protein sources are considerably more expensive commodities compared to carbohydrates on the global market (Shepherd & Jackson, 2013). This tension may result in a compromise of nutritional quality and price which can be illustrated with the composition of salmonid diets. Since the early 1990s, feed formulations for salmonids have undergone dramatic changes, from almost 100% fish meal and oil to a majority of plant-based ingredients (Fig. 1., FAO, 2022, Aas et al., 2022).

The need for feed

Fishmeal and fish oil

Fishmeal and fish oil are regarded as the gold standard of feed ingredients for salmonid fish, as these ingredients are the most consistent with their natural diet. Therefore, these ingredients are highly palatable and digestible (Miles & Chapman, 2005; Tacon & Metian, 2015). In terms of nutrition, fishmeal is high in protein, possesses no complex carbohydrates or fiber, and displays a balanced amino acid profile. Fishmeal also possesses valuable nutrients of marine origin, which are essential for salmonid fish. These include the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA, Glencross, 2020).

In the early 1990s, fishmeal and fish oil made up over 90% of the diet of farmed salmon. However, the inclusion levels of fishmeal and fish oil have since then been gradually reduced to roughly 25% of the diet (Aas et al., 2022). The gradual substitution of fish meal with alternative ingredients occurred for several reasons. First, the utilization of captured fish, the main source of fish meal and oil, to produce farmed fish has been criticized as unsustainable (Naylor et al., 2009). This is because increased fishing pressure leads to overfishing of wild stocks, and because the fish converted to fishmeal could have been directly consumed by humans. As a result, the public perception of aquaculture deteriorated and metrics such as the 'Fish-in-fish-out' (FIFO) ratio were formulated to determine the amount of wild fish needed to produce one farmed fish (Ytrestøyl et al., 2015). Second, wild fish stocks, the largest source of fishmeal and oil, are finite and therefore cannot expand in the same way as aquaculture, especially if quotas for maximum sustainable yield are kept (FAO, 2022). Finally, this constraint in supply, coupled with increasing demand, has led to gradual increases in the price of fishmeal and fish oil (Shepherd & Jackson, 2013). While fishmeal and fish oil are still central ingredients in salmonid feeds, it is generally accepted that future sustainable growth in salmonid aquaculture depends on the identification and procurement of sustainable alternative ingredients (Naylor et al., 2009; Turchini et al., 2019). In relation to this, salmonid feeds have seen gradual increases in terrestrial plant-based ingredients, now comprising roughly 70% (Fig. 1).

Plant based ingredients

"Good, but not the best" – Unknown

The industry's shift from fishmeal and oil to plant-based ingredients in such a short time is remarkable. Similarly astonishing is the capacity of salmonid fish, being carnivorous, to tolerate such a high load of plant-based ingredients. It is unlikely that any other group of farmed animal has undergone such a drastic change in diet in such a short period of time. One of the main drivers of the switch to plant-based feeds was the high availability of ingredients such as soy and wheat. The incorporation of these relatively low-cost ingredients into salmonid feed greatly aided the expansion of intensive aquaculture, while also reducing the price of the final product (FAO, 2022).

However, there are challenges related to plant-based ingredients in salmonid feeds. In most commercial salmon feeds, the largest protein fraction (ca. 20%) comes from soybean concentrate. Soy, which is mostly imported to Europe from Brazil, requires large amounts of arable land and

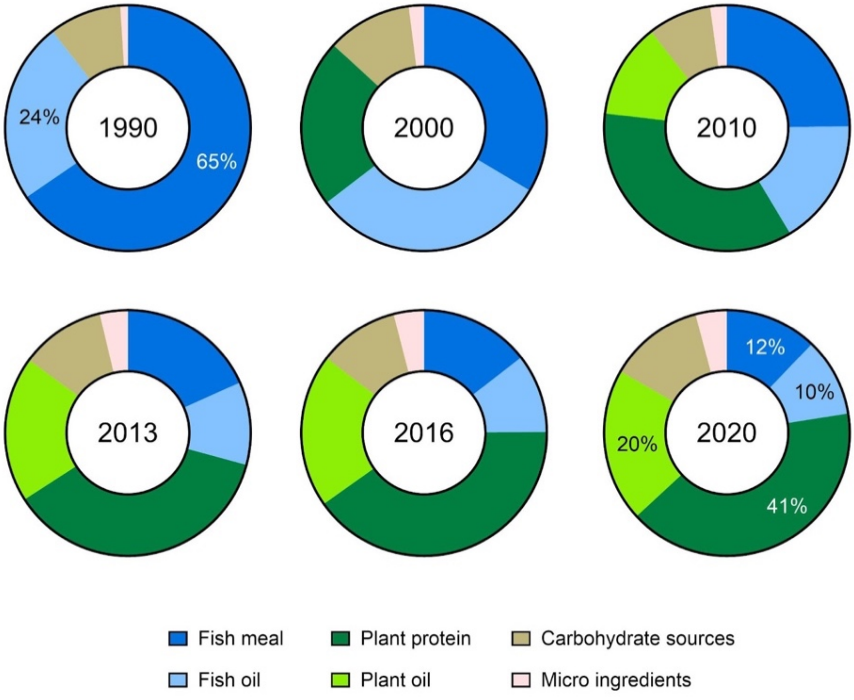

Figure 1: Changes in composition of feed for Atlantic Salmon between 1990-2020. Figure adapted from data in Utilization of feed resources in Norwegian farming of salmon and rainbow trout in 2020 report (Aas et al., 2022; Nofima 2022).

freshwater irrigation, both of which are limiting resources in the face of future population growth and climate change. Additionally, soy production has been linked to deforestation and habitat loss, with adverse knock-on effects for biodiversity. Finally, soy plantations compete directly with other potential land uses for food production, while the soy itself also can be directly consumed by humans. This has led to ethical concerns around the sustainability of soy inclusion in aquafeeds and has highlighted the need to search for alternative ingredients (Albrektsen et al., 2022; Eroldoğan et al., 2023; Turchini et al., 2019).

The inclusion of high levels of plant-based ingredients also has implications for fish health and welfare. Compared to fishmeal, plant-based ingredients are less palatable and less digestible, and they contain anti-nutrients which can cause pathologies such as intestinal inflammation or enteritis (Krogdahl et al., 2010). Additionally, plant-based ingredients generally contain considerably lower levels of lysine, methionine, and LC-PUFAs, which are all essential for salmonids (Gatlin et al., 2007). In modern diets, most of the anti-nutrients are removed through additional separation steps, and salmon are also bred to better handle a diet of plant-based ingredients. However, fishmeal and oil are still essential in feeds for salmonid fish (Turchini et al., 2009).

Sustainability

> "*In today's world, whether aquaculture production should be managed in an environmentally responsible and sustainable fashion is no longer debatable.*"
>
> Claude E. Boyd

Due to the rapid expansion of aquaculture, sustainability, particularly environmental sustainability, has become a growing concern. Perhaps the most common definition of sustainability refers to the wording by the Brundtland commission, "meeting the needs of the present without compromising the ability of future generations to meet their own needs" harmonizing economic, social, and environmental considerations to create a stable and equitable society over the long term (UN, 1987). However, this definition has been criticized as lacking clearly defined goals, which has contributed to the notion of sustainability becoming merely a buzzword and marketing tool for both industry and academic research (Stefanovich 2000, Boyd et al., 2020). In an attempt to unify and structure this multidimensional concept, the United Nations set out in 2015 to define 17 global sustainability goals, with specific targets within each goal (UN, 2015). Aquaculture, one of the major food production sectors clearly has links to most of these goals (Troell et al., 2023). In the framework of this thesis goal 2, zero hunger, goal 12, responsible consumption and production and goal 14, life below water, are especially relevant.

The continued procurement of high-quality feed ingredients represents perhaps the greatest challenge to the sustainable development of salmonid aquaculture. Feed is the backbone of salmonid aquaculture, and global feed production has matched the expansion of the aquaculture sector as a whole. Additionally, feed accounts for both the biggest expense in modern aquaculture operations, as well as the majority of the greenhouse gas emissions and environmental pollution in terms of nutrient leakage (Pelletier et al., 2009). Feed ingredients, especially marine ingredients for European aquaculture have been highly controversial, naturally limited and volatile in price due to the lack of alternatives to fishmeal and oil. The transition from largely fishmeal-based diets is therefore a prime example

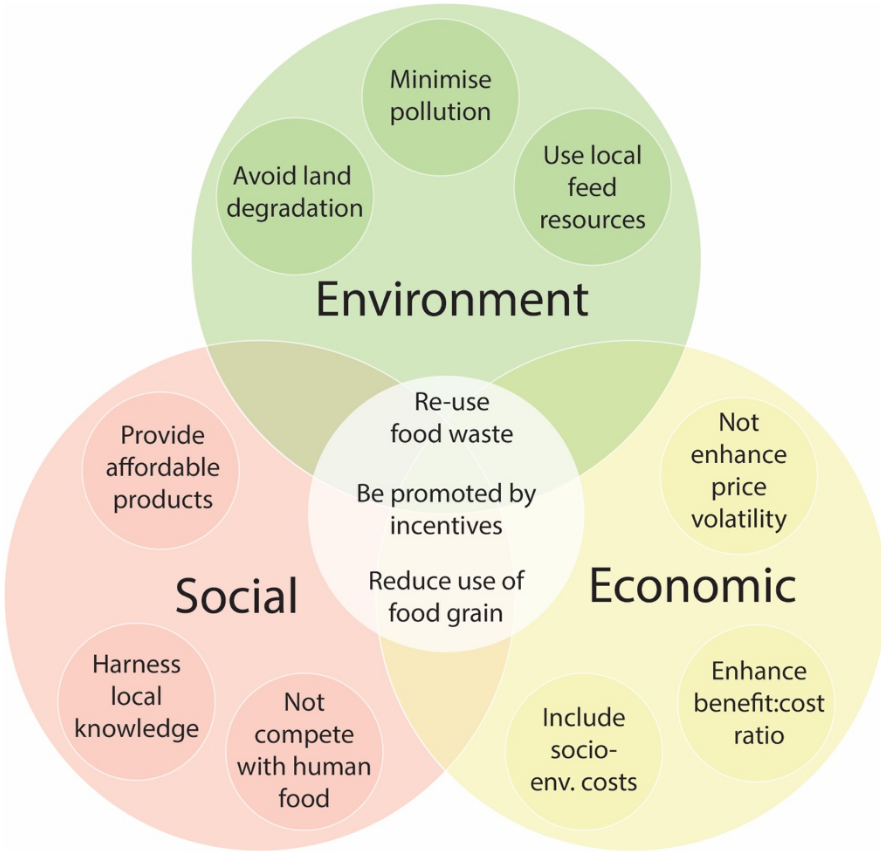

Figure 2: Guidelines for sustainable animal feed characteristics. Adapted from Makkar & Ankers, 2014

of the interplay of the three pillars of sustainability, economic, environmental and societal. As such, it is clear that sustainable aquaculture can only be achieved with sustainable feed (Fig. 2.).

The scope to increase resource efficiency and sustainability in this sector is vast. The food production sector is unique in that food the turnover of food items is days rather than years. As a result, sustainable practices may be implemented quickly due to its relatively decentralized nature, diverse range of products, and ability to adjust production methods to align with sustainability goals. Therefore, the food production sector is much more responsive than for instance the automotive industry as cars sold today will likely be rolling for decades to come.

Alternative feed ingredients for aquaculture

"The food you eat can be either the safest and most powerful form of medicine or the slowest form of poison." – Ann Wigmore

The need for sustainable feed ingredients to support the aquaculture production and expansion in the coming decades is central for global food security. Various alternative feed ingredients have been evaluated with varying success, ranging from the fisheries of mesopelagic fish and the cultivation of tunicates to side streams of the brewery and agriculture industry (Albrektsen et al., 2022; Eroldoğan et al., 2023; B. Glencross et al., 2023). Arguably the most promising groups of alternative ingredients are insects, single cell protein (SCP) and fish processing side streams.

Insects - The farming of insects and their use in aquaculture dates back thousands of years to China, where silkworms were farmed to produce silk and side streams were fed to carp cultures. However, the idea received real traction in 2017 when a change in EU regulations allowed the use of selected insect species as livestock feed. Today, Europe produces ca 5000 tons annually, most of which is used for aquafeed production (Albrektsen et al., 2022). The most promising and most produced insects are black soldier fly larvae (BSFL) and yellow mealworm (Nogales-Mérida et al., 2019). Both species can be farmed efficiently on low value side streams and contain *ca* 40% protein and 30% lipid (depending on substrate composition, Liland et al., 2021)). Their amino acid profile is balanced and both species have been used in diets for salmonid fish without negative effects on growth and feed conversion (English et al., 2021; Melenchón et al., 2021; Renna et al., 2017; St-Hilaire et al., 2007). However, high BSFL inclusion may reduce nutrient digestibility, feed intake and growth, especially when substituting fishmeal (Weththasinghe et al., 2022, Hua., 2021). This may be due to a suboptimal fatty acid profile, containing mainly short chained mono and unsaturated fatty acids, and a relatively high chitin content (Eggink., et al., 2022; Albrektsen et al., 2022). The effect of chitin in diets for salmonid fish is still debated however (Albrektsen et al., 2022; Nogales ‐ Mérida et al., 2019). In mice, chitin has been shown to reduce nutrient absorption (Tanaka et al., 1997). In support of this, krill which also contains chitin has been shown to reduce growth in Atlantic salmon (Hansen et al., 2010; Olsen et al., 2006).

Single Cell Protein - Single cell protein (SCP) in aquaculture refers to the utilization of microorganisms, such as bacteria, fungi (yeast) and algae, as a protein-rich feed source for aquatic animals. The benefits of SCP protein are that they contain high levels of protein, for yeast and bacteria between 50 – 80 %, can be produced on low value organic material, and that humans have a long-lasting knowledge regarding the cultivation of for example yeast in baking and fermentation industries (Agboola et al., 2021; Glencross et al., 2020; Mahnken et al., 1980; Rumsey et al., 1990). The challenges using yeast are related to their digestibility due to a rigid cell wall, optimization of production and substrate conditions, and upscaling (Agboola et al., 2022; Hansen et al., 2021; Rumsey et al., 1991). In salmonid nutrition, the most commonly used yeast species is baker's yeast (*Saccharomyces cerevisiae*) which contains ca. 50% protein and 18% lipids (Agboola et al., 2021).

Fish processing side streams - Fish processing side streams refer to the by-products and residues generated during the processing of fish for human consumption. These can include primary side streams such as heads, tails, bones, skins, viscera, and a host of secondary side streams which are created along the production line due to inferior size, coloration, and texture as well through mismatches in supply and demand leading to overproduction and the loss of consumer grade products (AMEC, 2003; Ghaly et al., 2013; Guerard et al., 2002; Sandström et al., 2022; Stevens et al., 2018).

Fish processing side streams, being fish based, are of high nutritional value for carnivorous fish. Today roughly 30% of the fishmeal produced globally originates from such side streams (FAO, 2022). However, the scope to increase feed production using side streams is great, especially products from further down the production line (Sandström et al., 2022). The challenges for the utilization of these side streams include logistics and processing due to high content of valuable but unstable LC-PUFAs, and their heterogeneous nature. This is especially true for side streams from further down the production line. Such side streams may be mixed with additional ingredients making it less obvious that they could be used as feed.

Alternative marine ingredients

"No water, no life. No blue, no green." – Dr. Sylvia Earle

The aquaculture industry has made great strides in reducing the inclusion rate of marine ingredients (fishmeal and fish oil; Aas et al., 2022). However, most terrestrial (both established plant-based and potentially more sustainable alternative) ingredients contain less optimal nutrient profiles including lower levels of essential amino acids such as lysine and methionine and lower levels of LC-PUFAs (Gatlin et al., 2007; Glencross et al., 2006) Therefore while many lipid and protein sources have been identified to satisfy the need of salmonid fish, certain amino and fatty acids remain bottlenecks. Could novel marine ingredients be the solution?

How to evaluate novel feed ingredients

"*Novelties please less than they impress.*" – Friedrich Nietzsche

Novel ingredients within a compound feed have direct implications for feed production, animal health, and product quality and their evaluation is therefore a comprehensive, interdisciplinary and challenging process. The contemporary nutritionist's approach includes seven general steps: characterization, palatability, digestibility, utilization, immunological properties, processing effects, and product quality influences (Glencross, 2020). Characterization relates to the chemical and nutritional composition of a novel raw material, which is essential to be able to formulate sensible experimental diets. For those nutrients to become available, the novel feed material has to be ingested. Palatability is a property of a feed or ingredient and has direct impact on animal behavior which manifest through visual and chemical cues (flavor and odor, Volkoff et al., 2005). After a feed has been ingested, it has to be digested, broken down into molecules which can be taken up by the intestine. Utilization then refers to the fate of these nutrients and how they are metabolized by the animal. In most feeding trials utilization is evaluated as growth, nutrient retention, and body biometrics. Immunological properties refer to the potential interactions with the immune system impacting the resilience of the fish towards stressors. The last two steps, processing effects, and product quality influences, relate to practical and physical aspects of the feed production and product properties, such pellet stability and sinking speed.

All seven evaluation steps can be divided into three assessment categories: chemicals (properties of the feed), biological/physiological (effects of novel feed ingredients on the animal) and physical (properties affecting handling, production and pellet properties). While the nutrient profile is easily accessible using contemporary analytical methods, the biological interaction of an animal with novel feed items is highly complex and may be harder to understand. Virtually every molecule in an animal's body is made of molecules taken up from the feed (only exceptions being minerals taken up from water, water uptake and gas exchange). While also including chemical and physical assessments, in this thesis special attention is put on possible physiological implications of novel feed ingredients.

Parameters which are arguable missing and could be added to this seven-step evaluation process are: product quality, and a sustainability assessment.

Growth physiology

"Ninety per cent of the diseases known to man are caused by cheap foodstuffs. You are what you eat." – Victor Lindlahr

Animal growth is central for any profitable farming operation. Therefore, it is desirable that a feed, being the largest expense for the farmer, is converted as efficiently as possible to body mass (Aas et al., 2022). This efficiency can be quantified using the feed conversion ratio (FCR), which is defined as the amount of feed consumed divided by the weight gained during the same period. The FCR for salmonid fish varies between 1 to 1.2 meaning that roughly 1 kg of feed is needed to produce 1 kg of fish (Tacon & Metian, 2015). This efficiency is excellent compared to other animal production systems. For reference, it takes roughly 8 kg of feed to produce 1 kg of beef (Fry et al., 2018). Growth can be divided in two types, hyperplasia, the increase in cell number, and hypertrophy, the increase in cell size. While other livestock accumulate muscle tissue exclusively through hypertrophy, fish exhibit both hypertrophy and hyperplasia. Somatic growth includes both hard (skeletal) and soft tissue (fat and organs) growth. While skeletal growth is directly related to the functional size of the fish, soft tissue growth also includes fat accumulation in the peritoneal cavity and gonadal development. As the filet is generally the final product, the allocation of energy into other tissues structures that are not sold is less desirable. Commonly used matrices to get an idea of nutrient allocation are the condition factor, the relationship between length and weight of a fish, the hepatosomatic index, the relation between liver and body weight, and the viscero somatic index, the relation between visceral and body weight (Bell et al., 2010). These body indices are standardized assessments of the body condition of an animal. Additionally, parts such as the filet or the whole body can be analyzed for their chemical composition. Such analysis may be more interesting if the bioavailability or the retention of certain nutrients is of interest.

The endocrine regulation of growth is multifaceted. It depends on external factors such as season and nutrient availability, and internal factors such stress and life stage (Triantaphyllopoulos et al., 2020). These factors are integrated in the hypothalamus where they influence the release of the principal growth promoter, growth hormone (GH). GH is regulated by two neuropeptides, growth hormone releasing factor GHRF and growth hormone-inhibiting factor GHIF (Luo & Mckeownt, 1991). GH may then be released by the pituitary gland in the bloodstream and exert systemic anabolic and catabolic effects by binding to transmembrane growth hormone receptors. In the liver GH also stimulates the transcription and release of insulin-like growth factor-1 (IGF-1), the other major growth stimulating factor (Reindl et al., 2011). Together, GH and IGF-1 form the GH-IGF-1 system. Crucially, IGF-1 promotes cell differentiation, especially in bone and muscle tissue and therefore usually more closely correlated to the growth curve of an animal (Izutsu et al., 2022; Sheridan, 2021; Triantaphyllopoulos et al., 2020).

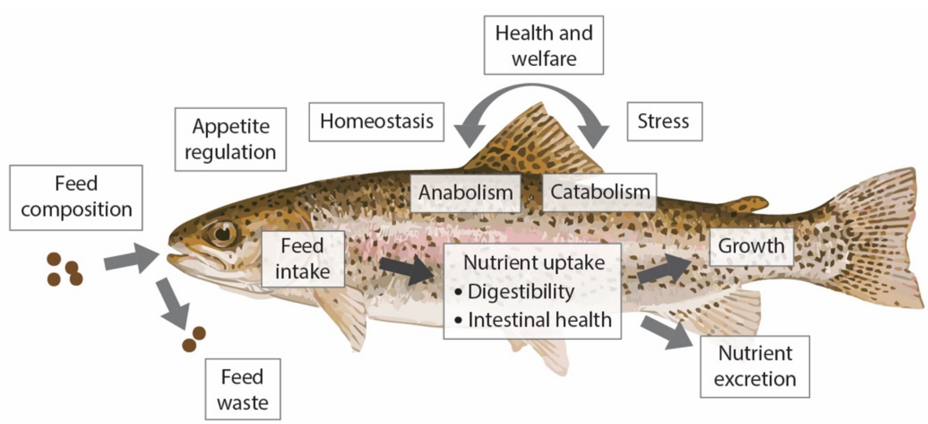

Figure 3. Generalized model for nutrient utilization and the overarching systems; appetite, digestibility, intestinal health, health and welfare, and growth which are assessed in this thesis.

Growth can also be seen as the result of a host of physiological, behavioral and metabolic processes. This cascade starts with the consumption of the feed.

It's all a matter of taste - Appetite, palatability and feed intake

Appetite, the desire to consume food or feed items, is orchestrated by a variety of peripheral and central factors (Perry & Wang, 2012; Volkoff et al., 2005). Peripheral factors include hormones such as leptin, ghrelin and CCK (E. H. Jørgensen et al., 2016; Nakazato et al., 2001). In salmonid fish, ghrelin is produced in the stomach (Kaiya et al., 2011). While clearly orexigenic (feed intake stimulating) in mammals, in salmonid fish and rainbow trout specifically, both orexigenic and anorexigenic (feed intake suppressing) functions have been observed (Rønnestad et al., 2017). In fish leptin may be produced in the liver and, to a lower percentage in adipose tissue (Rønnestad et al., 2010). As for humans, leptin may have a predominantly anorexigenic function. However, long term fasting has been shown to result in an increased leptin expression (Johansson & Björnsson, 2015; Kling et al., 2009). Both leptin and ghrelin signals, as well as a variety of additional peripheral signals including the nutrient sensing systems are integrated in the hypothalamus which involves a variety of both orexigenic and anorexigenic neuropeptides including Npy, Agrp, Cart, Pomc and Crh. Besides the homeostatic feed intake regulation, feed intake in humans is clearly also related to pleasure, anticipation, and reward behavior. These hedonic pathways are related to the palatability a food/feed items and are integrated in the dopamine cannabinoid systems which has been identified in fish (Soengas et al., 2018). While unsaturated lipids have been shown to trigger this pathway, hedonic feed regulation is generally less understood salmonid fish than in humans or medical research models (Díaz-rúa et al., 2020; Soengas et al., 2018). However, in an aquaculture scenario where feed is provided *ad libitum*, the relative importance of these pathways may be significant. Therefore, the integration of fish's appetite and the feed's palatability dictates feed intake.

Digestibility

"Digestion is the chemistry of civilization." – Antoine Lavoisier

A chemical analysis of novel feed ingredients reveals its energy and nutrient content. However, to which extent these nutrients are accessible by the animal cannot be inferred by the chemical composition alone (Figure 3; Cho et al., 1982). For example, the proteins contained in fava bean meal have a digestibility of ca 80 % in salmonid fish, meaning that 20% of the protein available in the feed will not be taken up by the animal (Gomes et al., 1995). Additionally, certain carbohydrates such as fiber may be digestible by herbivorous fish, but not by carnivorous fish such as salmonids. Therefore, fiber does not contribute to the digestible energy in diets for salmonid fish (Kaushik & Hardy, 2020).

Health and Welfare

eudaimonia (n.) lit. "human flourishing"; a contented state of being happy and healthy and prosperous

The nutrients provided by feed are essential for the synthesis of all vital components required for an organism's life, thereby influencing every biological and physiological processes within the animal's body. Introducing new feed materials may have effects on animal health and welfare. Welfare of animals is defined by the world organization for animal health as "the physical and mental state of an animal in relation to the conditions in which it lives and dies" in relation freedoms of hunger and malnutrition, fear and distress, heat stress and physiological discomfort, pain, injury and disease, and normal behavioral patterns (WOAH, 2023). It is no coincidence that the first one of these welfare defining freedoms is related to feed. Apart from not fulfilling the nutritional requirements of the animal, novel feed items may contain pathogens and antinutrients, which directly link to the freedom from pain, injury and disease as well as the freedom to express normal patterns of behavior. Another way of looking at potentially negative effects of feed ingredients is to view them as potential stressors. Any factor leading to malnutrition, disease, injury and physical discomfort will compromise the physiological homeostasis of the fish and thus trigger a stress response. It is therefore essential to monitor health and welfare of the research animals when evaluating novel feed ingredients. In addition, feed ingredients can be utilized to improve health and welfare of a farmed animal. Understanding such physiological implication enables the production of functional feeds which improving both health and welfare in farmed fish. According to EU regulations, functional feed ingredients refer to feed materials used to increase performance related to micronutrition, technological improvements in feed production, sensory improvements related to smell and palatability, and zootechnical, collectively referring to improvements in growth, health, and overall performance of animals in agriculture (Regulation (EC) No 767/2009; FEFANA, 2023). Effects on health and welfare directly relate to all three sustainability pillars. Changes in the health and well-being of livestock can directly impact feed conversion rates, thereby affecting economic sustainability by influencing profits. Moreover, these changes can have implications for environmental sustainability by influencing nutrient emissions and social sustainability by influencing consumer acceptance.

Intestinal health

"Your gut is not Las Vegas. What happens in the gut does not stay in the gut."
Peter Kozlowski

The intestine is arguably the most important organ in the evaluation of novel feed ingredients. It is the organ which directly interacts with the novel materials and any effects on the intestinal tissue are directly translated into alterations in nutrient uptake and therefore downstream processes in the entire organism (Sundell & Rønnestad, 2011). Apart from nutrient uptake, the intestine has important immunological functions and represents a barrier towards pathogens. This barrier includes the mucus, secreted by goblet cells, the enterocytes connected through tight junctions,

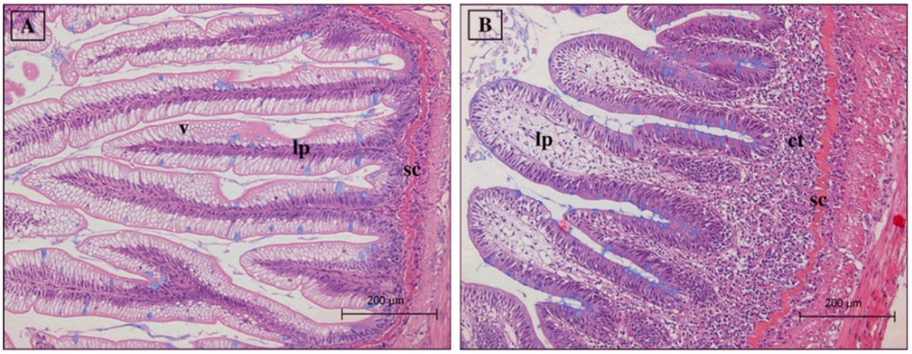

Figure 4. Distal intestine of Atlantic salmon fed A) fish meal based control resulting a heathy morphology, and B) Atlantic salmon fed 10% soybean meal resulting in soy bean induced enteritis characterized by a shortening of the primary and secondary mucosal folds, a wider lamina propria and submucosa, loss of apical vacuolization and increased density of goblet cells. Images adapted from Knudsen et al., 2007

and the gut-associated-lymphoid-tissue comprised of a wide variety of immune cell types including macrophages, dendritic cells, T-cells and B-cells (Yu et al., 2020). Negative effects on this barrier by substances in the feed therefore also directly link to pathogen exposure and disease resistance. The most prominent example may be the inflammatory response in the distal intestine of salmonid fish exposed to antinutrients present in many plant-based ingredients (Figure 4). Soybean meal for instance needs to undergo and additional extraction step to remove antinutrients such as saponins and tannins before it can be included in diets for salmonid fish as soybean concentrate.

AIMS

The overarching aim of this thesis is to expand the pool of alternative feed ingredients by developing and evaluating novel marine ingredients for rainbow trout. As part of this evaluation, this thesis tries to combine a nutritional with a physiological approach. The aspiration is further to carry out this work in the framework of the circular economy approach. The ingredients evaluated in this thesis were either industry side streams, or they were used to convert industry side streams into high quality protein and omega-3 rich biomass suitable to produce salmonid fish. These side streams originated from the seafood production sector and their utilization as feed ingredients for aquaculture therefore closes a loop and potentially improves the total production capacity of this sector. Marine side streams are especially promising due to the bottleneck in global supply of marine ingredients (fishmeal and fish oil). Additionally, the aim was to evaluate potential positive effects of such alternative marine ingredients by linking nutrition and physiology in a sustainability framework.

To meet this overarching aim, specific objectives were formulated:

The first objective (**paper I**) was to evaluate the effect of using the marine yeast, *Candida sake*, cultivated on herring brine, a side stream of the herring processing industry, in rainbow trout feed in terms of growth, digestibility, intestinal health, and immunological properties.

The second objective (**paper II**) was to evaluate the marine insects (*Coelopa frigida*) cultivated on macroalgae, a side stream produced by a local algae farm, as a protein source in diets for rainbow trout in terms of nutrient composition, growth, appetite, and intestinal health.

The third objective (**paper III**) was to screen three side streams from the local fish processing industry as feed ingredients for rainbow trout. The side streams originated from different parts of the production and were incorporated without additional processing which would lower the production cost and reduce the feed's environmental footprint. The evaluation included effects on digestibility, appetite growth and intestinal health.

The fourth objective (**paper IV**) was to evaluate the most promising of the side streams evaluated in **paper III** as part of a commercial-like diet and to compare this diet to a commercial diet with regards to pellet quality, growth, intestinal health and product quality.

METHODOLOGICAL CONSIDERATIONS

This section presents a review and discourse on the methodologies employed in the studies encompassed within this thesis. For a meticulous elucidation of precise procedures and experimental protocols, readers are directed to the respective papers.

The novel ingredients

Marie yeast (*Candida sake*) – The *C. sake* strain was isolated from the surface of sugar kelp (*Saccharina latissima*) at Tjärnö Marine Laboratory at the Swedish west coast and identified as *C. sake* using 26 rRNA sequencing (99.8% sequence identity, National Collection of Yeast Cultures, Norwich, UK). The yeast was then cultivated using a 300 L bioreactor borrowed from the Royal Institute of Technology, Stockholm, Sweden. The cultivation substrate consisted of 50% pre-salting brine from North Sea herring (*Clupea harengus*, Sweden Pelagic AB, Ellös, Sweden) and 10% molasses as carbohydrate source (Lantmännen AB, Stockholm, Sweden). After 22 hours, the growth stagnated and the cells were separated from the culturing medium at 4ºC using a disc stack separator and subsequent centrifugation to reduce the water content. This resulted in a total of 22.7 kg *C. sake* product, which was freeze-dried and stored at -20°C until the feed production.

Seaweed fly larvae (*Coelopa frigida*) – The seaweed fly larvae (SWFL) used in this study were reared at the Department of Marine Science, University of Gothenburg, using a combination of bladder wrack (*Fucus vesiculosus*) and sugar kelp (*Saccharina latissima*) at a ratio of 1:3 as substrate. The seaweed was supplied in kind by Nordic Seafarm AB (Gothenburg, Sweden). The larvae were harvested bi-weekly by hand and stored at -80 ℃ until the larval meal production step. New cultivation media was added weekly. To produce the meal, SWFL were thawed at room temperature, and second and third instar stage larvae were separated from pupae and cleared from residual decomposed feeding substrate. The cleaned larvae were dried in an oven at 50°C overnight and ground to fine powder using a coffee grinder (Andersson Coffee Grinder CEG 1.0). The powder was stored at -80°C before it was sent to the Feed Technology Laboratory (FTL), Swedish University of Agricultural sciences, Uppsala, Sweden, for feed production.

Atlantic herring (*Clupea harengus*) – The herring filets used had been discarded from the production chain of a large processing plant in

Kungshamn, Sweden, due to second grade quality and coloration. The filets were purchased by Renahav Sverige AB and provided in kind to the University of Gothenburg. Upon arrival, the herring filets were washed using tap water to remove brine and drained using a sieve (2 mm mesh). The herring filets were then dried and ground to create a powder which was stored at -20°C until the feed production.

Atlantic mackerel *(Scomber scombrus)* – The canned mackerel filets in tomato sauce which were used in this study had been discarded at the end of the production chain, after canning, due to a mismatch in supply and demand of a large processing plant in Kungshamn, Sweden. The filets were purchased by Renahav Sverige AB and provided in kind to the University of Gothenburg. Six hundred cans were opened manually and the tomato sauce was rinsed off using tap water and a sieve. The filets were then dried, ground and stored as described for the herring filets.

European sprat (*Sprattus sprattus*) – Filleting side streams (trimmings, i.e. heads, frames and viscera) were used that had been produced at a large processing plant in Kungshamn, Sweden. The filets were purchased by RenaHav Sverige and provided in kind to the University of Gothenburg. To preserve the fresh samples, 5% sodium chloride was added. The trimmings were then dried, ground and stored at -20°C until the feed production.

The research animal

"Research animals are the silent heroes of scientific exploration." – Unknown

Rainbow trout (*Oncorhynchus mykiss*) was chosen as the research model in the present thesis for several reasons. First, rainbow trout are the most farmed fish in Sweden as the European Union (EUMOFA, 2022). Second, rainbow trout is a versatile and robust and well studies fish. It can be cultured in a wide range of salinities and temperatures and established model in various field including nutrition, endocrinology and physiology. Thirdly, rainbow trout are related to other intensively farmed species such as Atlantic salmon and Arctic charr and while species specific differences need to be considered, most of the results obtained in this thesis may be considered applicable also to other salmonid species.

Criteria to determine promising feed ingredients

The modern approach of nutritionists involves a seven-step process: characterization, palatability, digestibility, utilization, immunological attributes, processing impacts, and effects on product quality (Glencross, 2020). This approach was broadly followed in this thesis, however, based on the attributes of the novel ingredient in question, different specific aspects were put in focus.

Characterization

To supply the fish with all the essential nutrients needed and to reduce nutrient leakage to the environment due to imbalances in the formulation, knowing the nutrient composition of the ingredients used in the feed is essential. This is especially true for novel ingredients. The resolution of such analyses can vary. The most general analysis encompasses an estimate of the macronutrients (fat, protein and carbohydrates), the energy content and the ash content. For plant-based ingredients, a measurement of the fiber content (long polysaccharides which are largely indigestible for salmonid fish) is of further importance. These fractions of the nutrient composition can be characterized as the proximate analysis, a general assessment of the nutrient composition.

While enabling a screening and a general comparison between ingredients, a proximate analysis should be followed up by more detailed assessments of specific nutrients such as amino and fatty acid as well as mineral profiles. All the ingredients in this thesis were evaluated for their proximate as well as, amino and fatty acid compositions to enable precise feed formulations and digestibility assessments (**papers I-IV**). Furthermore, the mineral composition of the ingredients was evaluated in **papers I, III** & **IV**.

Feed formulation

With hundreds of ingredients and supplements available to a formulator and 40+ nutrients to keep track of, options to compose a feed that satisfies the nutritional demands are challenging and endless. However, modern linear least cost feed formulation software makes this process much easier (Hardy & Kaushik, 2020). For experimental diets however the least cost approach is less relevant and diets can be formulated with the main goal to answer specific research questions. Still, for every formulation there are trade-offs to consider. To evaluate the digestibility of a novel feed ingredient, the ingredient is added to a reference or control feed on a crude inclusion level, often 30% (Cho & Slinger 1979). As a result, the diets are likely different in their nutritional composition. Additionally, the composition of the control diet has big effects. For example, a control diet with 50% fishmeal will likely satisfy the nutritional requirements even after substituting 20% of that diet with a novel ingredient. On the other hand, the inclusion of a novel ingredient in a diet with no excess nutrients likely affects physiological processes and growth negatively. On the contrary, in nutrient-based formulations, experimental diets are balanced on a nutrient basis, and potential shortcomings of novel ingredients may be masked. The following section gives a brief motivation regarding the formulations used in each of **papers I-IV.**

Paper I – The diets were formulated to investigate the digestibility of *C.sake*, possible effects of downstream processing of the yeast, its performance as a source of protein and possible immune stimulating properties. A control diet containing both fish and plant-based ingredients constituted the control diet to which 20% (to evaluate effects on digestibility, growth and intestinal physiology), 20% heat-treated (to evaluate effects of downstream processing) and 3% (to evaluate immune stimulatory properties, replacing 3% soy protein concentrate) *C. sake* was added.

Paper II – The diets were formulated to compare larvae of the seaweed fly (*C. frigida)* to commercial fishmeal and partially defatted meal of black soldier fly larvae (*Hermetia illucens*; BSFL). As terrestrial insects, BSFL were chosen as the promising insect-based feed ingredient for carnivorous fish. To efficiently compare the three protein sources, the diets were iso-caloric, iso-nitrogenous and iso-lipidic. and formulated to substitute 40% of the fishmeal in the control diets on a protein basis with either SWFL or BSFL meal.

Paper III – The aim of this study was to screen three heterogenous fish processing side streams. To efficiently screen the diets and physiologically challenge the fish, and because all side streams were nutrient dense, a high inclusion level of 50% was chosen. The diets were formulated to be iso-caloric, iso-nitrogenous and iso-lipidic. The test ingredients substituted 100% of the control diet's fishmeal and most of the fish oil (depending on lipid content in the ingredient) of the control diet.

Paper IV – In this paper an experimental diet was formulated to contain a realistic amount of the most promising side stream evaluated in **paper III**. The diet formulated to be iso-nitrogenous iso-caloric and iso-lipidc compared to a commercial diet. It was then compared to this diet in a feeding trial.

The diets for paper **I**, **II**, and **III**, were produced at the Feed Technology Laboratory, Swedish University of Agricultural sciences (SLU, Uppsala, Sweden). The diet for paper **IV** was produced at the Norwegian university of life science (NMBU, Ås, Norway). The diets for paper **I**, **II**, and **IV** were produced using a twin-screw extruder and subsequent vacuum coating. The diets used in paper **III** were produced using cold pelleting. All diets were formulated to meet the nutritional requirements of rainbow trout.

Digestibility

The digestibility of a feed or an ingredient can be measured using direct or indirect methods. The most common methods to determine the digestibility are direct measurements and marker measurements. Direct measurements encompass the collection of all fecal matter and comparing the nutrient content with the feed's nutrient content (Glencross et al., 2007). However, this method is difficult to do in aquatic settings. More appropriate the calculation of the apparent digestibility coefficient (ADC) using indigestible marker molecules (Cho & Slinger, 1979). The ratio of markers in feed and feces can then be compared to individual nutrients. In **papers I** and **III,** the digestibility of the experimental diets was evaluated by including the indigestible marker molecule titanium dioxide (Bureau et al., 1999).

Health and Welfare

In the present thesis health and welfare parameters were employed to evaluate possible effects of novels feed ingredients on animal health and welfare. A key tissue included in this evaluation is the intestine, as the central organ in nutrition related welfare.

Intestinal health

The intestine is a multi-functional organ and the first epithelia to be exposed to a novel feed ingredient. The intestinal epithelium may therefore interact with ingested items in various ways. As a result, several complementary methods have been chosen to evaluate the intestinal health at different levels.

Ussing chamber analysis – Introduced in 1959 by the Danish physiologist Hans Ussing to study active transport processes of frog skin, Ussing chambers, also known as diffusion chambers, are extensively employed across various species and tissues to investigate epithelial physiology, including barrier function, ion and nutrient transport, protein and drug absorption and interactions between hosts and pathogens (Clarke, 2009; Jutfelt et al., 2007; Moeser et al., 2007; Santos et al., 2000, 2001; Saunders et al., 2002; K. Sundell et al., 2003; Sundh et al., 2011; Ussing, 1950; Velin et al., 2004). In this thesis Ussing chambers were used to measure electrophysiological parameters related to active epithelial transport and barrier function (Figure 5). These parameters were complemented with tracer studies where radio labeled L-lysine and mannitol were added to the luminal/mucosal side of the tissue to evaluate lysine uptake and mannitol diffusion specifically (**paper I - IV**). With regards to novel feed ingredients, this method is especially suitable as it is sensible to both general stress responses of the animal, and inflammatory reaction associated with feed induced enteritis (Jutfelt et al., 2007; Moeser et al., 2007; Sundh et al., 2011).

Histology – Originating in Italy in the 17th century, histology, the study of tissues at a microscopic level, is a powerful tool for assessing intestinal health in various organisms, including fish and mammals. Visualizing the structure of the intestinal tissue can give indications regarding tissue integrity, mucosal thickness, goblet cell density, immune cell infiltration, and overall inflammatory processes. Histological methods have become a routine tool to evaluate the effects of feed ingredients in intestinal health especially in conjunction with many of the anti-nutrients present in plant-based ingredients (Krogdahl et al., 2010). This routine investigation of intestinal health is based on the scoring of the mucosal morphology in terms of villi length, lamina propria width, submucosa width, goblet cell count and vacuolization (Knudsen et al., 2008). While the scoring method

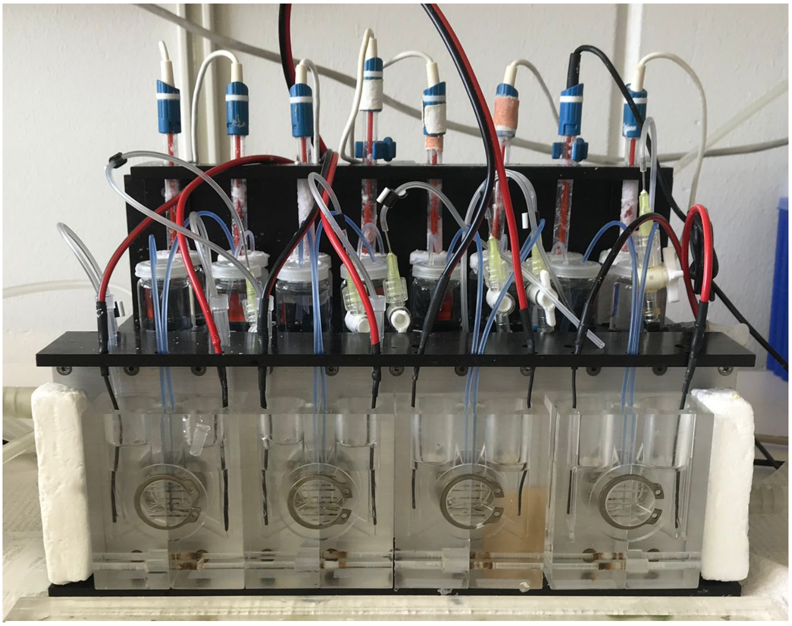

Figure 5. Ussing chamber setup modified for fish intestine

has proven relatively robust, it is a subjective methodology and it may therefore be hard to compare the results from different scorers. In the present thesis measurements of villi length, lamina propria width, submucosa width were carried out by accurately measuring distances using the software ImageJ. This method was used in **papers I – III** to evaluate the intestinal morphology and structure to identify signs of inflammation, malnutrition and general appearance.

Gene expression - In **paper I**, gene expression analysis of pro and anti-inflammatory cytokines was used to trace the immune response after LPS injection and to determine whether or not this response can be modified by *C. sake*. In **papers II** and **III**, it was used to link changes in feed intake to appetite regulating neuropeptides.

RESULTS AND DISCUSSION

Nutritional benefits and challenges

Rather than supplying specific ingredients, feeds need to deliver the optimal proportions of macro and micronutrients to the farmed anima (Turchini et al., 2019). The nutrient composition of the this thesis evaluated novel ingredients is therefore an essential starting point in the development of novel feeds.

Energy – In intensive farming systems, the energy content of feeds needs to be high to facilitate fast growth, which directly translates to shorter production cycles. A high production efficiency is also directly related to energy, feed and land use and therefore the climate footprint and sustainability of a product (Pelletier et al., 2009; Philis et al., 2019). Diets for salmonid fish are energy dense and contain ca. 24 MJ/kg energy (Aas et al., 2022). Naturally, feeds contain ingredients with varying energy levels (oils close to 40 MJ/kg, protein sources around 20 MJ/kg), and ingredients with a lower energy content will reduce the overall energy of the feed. This necessitates the balancing of the final formulation by the incorporation of high energy ingredients such as oils and fats (Hardy & Kaushik 2021). Thus, a too low digestible energy content of a candidate novel ingredient may restrict the ingredient's application in commercial feeds. The ingredients examined in this thesis (**paper I - IV**) all contained above 20 MJ/kg gross energy which is in line with the levels of commercial fishmeal and plant-based protein sources, including soy protein concentrate (Glencross et al., 2007, Glencross, 2020).

Macronutrients – The gross energy content of a raw material may vary drastically from the bioavailable or digestible energy content for a given organism. Carp fish, for example, are omnivorous and can utilize carbohydrates efficiently (ICAR, 2023). Salmonid fish including rainbow trout on the other hand, are carnivorous/omnivorous and therefore efficient at digesting and absorbing lipids and amino acids, but less efficient for carbohydrates (Schrama et al., 2018). Grow-out diets for salmonid fish are therefore high in lipids (ca. 33%) and protein (ca. 36%), and low in carbohydrates (11%; Aas et al., 2022). Ingredients with a high carbohydrate content are therefore less optimal. All seven ingredients evaluated in papers **I-IV**, contained either a high level of protein (54% for *C. sake* and 60% for SWFL) or a high content of both protein and lipid (sprat, 28% & 34%, mackerel, 30% & 35% and herring, 32% & 43% for protein and lipid respectively) making all of them suitable feed ingredients for carnivorous fish, including salmonids.

While the macro nutritional properties (such as high carbohydrate content) of an ingredient may render some ingredients suboptimal, the crude protein and lipid content alone do not determine whether an ingredient is 'good' or not. While partly metabolized for energy in the form of ATP, fatty acids and amino acids are the building blocks of the fish's cells, including proteins, enzymes and the lipid bilayer surrounding each cell (Pollard et al., 2022). Several of these amino and fatty acids are essential for salmonid fish and need to be supplied through the diet. Additionally, a suboptimal balance of AAs and FAs has negative effects on growth, health, and welfare of the fish. This can be illustrated by the fact that in humans, the brain is made of 65% of fat, 50% of which is DHA, a long chained polyunsaturated omega-3 fatty acid (Kaur et al., 2014). These marine PUFAs are therefore essential fatty acids for our brain development, and we need to acquire them from the food, by consuming seafood, such as salmonid fish. While rainbow trout may be able to synthesize DHA, to maintain high growth rates most of the LC-PUFAs need to be provided through the diet (Gregory & James, 2014; Tocher, 2015). Regarding the essential amino acids, they cannot be synthesized by the fish and it is therefore crucial that these AAs are provided in the right amounts through the feed (Hardy & Kaushik, 2020). One of the main benefits of producing alternative marine ingredients, such as the ones included in this thesis, is that they generally contain considerably more of the essential nutrients than those found in most terrestrial ingredients.

Amino acids – The feed ingredients evaluated in this thesis (**paper I-IV**) were either of marine origin and/or cultivated using marine substrates. As a result, their amino acid profile was more comparable to fishmeal than that of many plant-based ingredients (Table 1). With the rising share of plant-based ingredients in the current feed for salmonid fish, lysine and methionine have become the main limiting amino acids in salmonid diets. *C. sake* and *C. frigada* (**paper I and II**) both contained ca. 3.5% lysine on a dry matter basis which is slightly lower than for most fish meals (ca. 5%) but higher than for most plant-based ingredients which commonly contain <3% (Glencross et al., 2007). On a protein basis, the amino acid profiles of all five ingredients mirrored that of fish meal remarkably well. One exception was methionine, which was lower, 1.8 g/100 g protein, in *C. sake* as compared to the roughly 3 g/100g protein commonly seen in fish meal. Methionine may therefore be the first limiting amino acid when substituting fish meal with *C. sake* meal (Figure 6). This is consistent with

Table 1
Nutrient requirments of rainbow trout and nutrient composition of the novel marine ingredients evaluated in this thesis as well as commercial fishmeal and partially defatted black soldier fly larvae meal.

	NRC Req.[4]	*C. Sake*	*C. frigida*	BSFL	Mackerel	Herring	Sprat	Fishmeal
Proximate composition								
Dry matter (%)		90.5	92.7	97.6	88.4	69.6	89.1	91.8
Crude protein[1]	38	54.3	60.0	56.5	29.8	32.1	28.1	67
Sum amino acids		44.5	44.1	46.5	28.8	34.4	21.4	62
Crude lipid[2]		13.3	16.7	17.8	34.9	43	34.8	10.9
Gross energy	16	20.7	22.8	24.4	21.9	22.7	20.1	20.1
Ash		5.4	13.2	7.4	7.8	22.7	29.4	13.9
NDF		NA	7.6	35.3	NA	NA	NA	-
Indispensable amino acids								
Arginine	1.5	3.1	2.5	1.4	1.7	2.2	1.3	3.9
Histidine	0.8	1.1	1.5	0.8	1.3	0.9	0.7	2.5
Isoleucine	1.1	2.0	2.1	1.2	1.2	1.7	1	3
Leucine	1.5	3.0	3.1	2.0	2.2	3	1.7	5
Lysine	2.4	3.3	3.5	1.6	2.5	3.3	1.8	5.3
Methionine	0.7	0.8	1.2	0.5	0.6	0.8	0.6	2
Phenylalanine	0.9	2.1	2.7	1.3	1.1	1.5	1	2.7
Threonine	1.1	2.2	2.2	1.2	1.4	1.7	1	2.5
Thryptophan	0.3	0.6		0.0	0.4	0.5	0.3	
Valine	1.2	2.3	2.7	1.7	1.5	2	1.2	3.7
Dispensible amino acids								
Alanine		3.3	4.2	2.0	1.8	2.1	1.4	4.1
Aspartic acid		4.5	4.3	2.8	3.1	3.7	2.1	5.8
Cysteine		0.5	0.5	0.3	0.2	0.3	0.2	0.9
Glutamic acid		7.4	6.7	3.1	4.6	5.2	2.9	8.3
Glycine		2.1	2.4	1.6	1.8	1.6	1.5	4.3
Proline		1.6	2.5	1.6	1.2	1.2	0.9	2.7
Serine		2.5	1.9	1.2	1.3	1.5	1	2.2
Tyrosine		1.8		2.0	1	1.3	0.8	2.4
Fatty acids (% fatty acids)								
C 18:2 n-6 (Linolsyra)		4.7	3.1		3.4	1.2	1.4	0.6
C 18:3 n-3 (α-Linolensyra)		0.9	1.5		2.2	1.1	1.3	0.5
C 20:5 n-3 (EPA)	0.4-0.5[3]	7.3	3.2		5.7	6.3	8.0	9.5
C 22:5 n-3 (Dokosapentaensyra)		0.8	<0.1		1.0	0.6	0.7	1.4
C 22:6 n-3 (DHA)	0.4-0.5[3]	6.6	0.2		8.9	7.9	12.7	14.6
Summa mättade fettsyror		17.4	26.8		17.5	19.8	25.7	20.2
Summa enkelomättade fettsyror		36.8	47.0		42.6	43.6	32.0	13.5
Summa fleromättade fettsyror		23.7	12.3		27.4	22.0	28.5	30.3
Kvot omega6/omega3 fettsyror		0.4	0.9		0.2	<0.10	<0.10	0.4

[1]According to Kjeldahl (N*6.25)

[2]According to Schmid-Bondzynski-Ratslaff

[3]0.4 - 0.5 % combined

the methionine content found in other yeast species (Agboola et al., 2021). However, due to the high content of plant-based ingredients in contemporary feeds, methionine is routinely supplemented in crystalline form. As the methionine content of *C. sake* was comparable to soy protein concentrate and higher than found in other plant based ingredients such as lupin kernel meal and pea protein, the methionine level of *C. sake* may impose little practical restriction for its commercial application given the supplementation of crystalline methionine (Glencross et al., 2007). *C. frigida* (**paper II**) contained, with 60%, the highest level of protein out of the evaluated ingredients. On a protein basis, the amino acid profile of *C. frigida* was similar to that of fishmeal and with slightly higher levels of phenylalanine and alanine and slightly lower levels of histidine and leucine (**paper II**). Based one the AA profile, there are no restrictions for including SWFL in diets for rainbow trout.

The processing side streams evaluated in paper III and IV were fish based and contained roughly 30% protein, which is low compared to the *C. sake, C. frigida* and commercially available fishmeal (Glencross 2020). On a dry matter basis, they therefore contained lower levels of all amino acids, including lysine and methionine. On a protein basis however, their amino acid profile was highly similar to fishmeal. However, these marine ingredients are not only a protein source but also contain high levels of valuable lipids and fatty acids.

Fatty acids – With ca. 40%, the highest lipid content was found in the fish processing side streams (**paper III & IV**). Being fish based, the sprat, herring and mackerel side streams displayed fatty acid profiles similar to commercial fishmeal and oil. These side streams therefore contained high levels of both essential amino and fatty acids. While highly nutritious, there are practical challenges when dealing with ingredients containing high levels of both lipid and protein. The high content of unsaturated fatty acids is of great nutritional value, but LC-PUFAs also oxidize easily when in contact with air. The resulting lipid oxidation products are related to a plethora of negative effects on the nutritional value, palatability as well as negative effects on digestibility and animal health and welfare (Tacon, 1996, Bell & Koppe, 2010 Furthermore, the oxidation products such as peroxides and aldehydes can react with other molecules in the feed such as amino acids, even further reducing the nutrient content of a diet (Ayala et al., 2014; Viedma-Poyatos et al., 2021). Thus, for ingredients high in PUFAs, adequate storage conditions are essential and the addition of antioxidants is strongly recommended (Wu et al., 2022). Additionally, the high lipid content has implications on the feed production process and pellet quality, particularly as any promising ingredient should function well in the production process of standard commercial feed pellets using extrusion and lipid vacuum coating. Commercial feed for salmonid fish is produced by mixing the meals of the protein and carbohydrate sources to create a mash. Using pressure heat and water vapor, the paste is then pressed through a narrow pipe or 'barrel' using one or more metal screws (Hilton et al., 1981). Excess lipids in the mash can interfere with this process as they act as lubricants, reducing friction and pressure in the extruder barrel, which may negatively affect pellet stability and shape (Hilton et al., 1981; Ilo, 2000). Additionally, excess lipids interfere with the vacuum coating process, which is used to increase the lipid content of commercial diets to about 30%. Vacuum coating relies on the expansion of the pellets by reducing the pressure to near 0 (vacuum) resulting in an expansion of the pores in the pellets (Wang et al., 2021). When the air pressure increases again, added lipid droplets are pressed into the pellet pores. If lipid levels in the extruded pellets are too high, they may interfere with the starch gelatinization and reduce the expansion capacity of the pellet. As a result, the pellets may not be absorbing added lipids efficiently

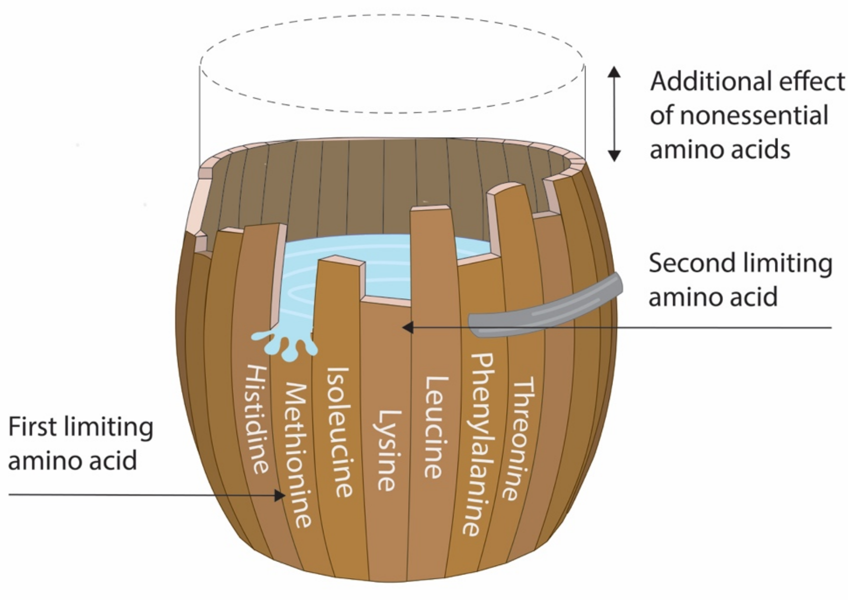

Figure 6. Conceptualisation of the effects of limiting nutrients. In this example methionine levels may restrict overall growth as could be the case with *C. sake* (**paper I**).

(Lin et al., 1997). This suggests that a separation of the lipid and protein fraction of the fish processing side streams, as is done for fishmeal and fish oil, would benefit commercial applications. However, one of the main aims of this thesis is to evaluate if the different marine side streams could be utilized in aquafeeds without additional separation steps, which could lower production costs and greenhouse gas emissions. An inclusion level of 6% sprat trimmings (**paper IV**) was chosen as a compromise to include as much as possible of the unprocessed side stream in the commercially extruded diet without compromising pellet quality. The *C. sake* and the SWFL both contained lipid (13-16% DM) which is only slightly higher than the ca. 11% commonly reported fpr fishmeal (Glencross, 2020). The lipid content of these ingredients may therefore not be a problem for the commercial production of extruded pellets and therefore not limit the dietary inclusion of SWFL and *C. sake*, the lipid profile of *C. sake* was remarkably similar to the lipid profile of commercial fishmeal (Glencross, 2020; Table 1). Of special interest for both animal and human nutrition and health, are LC-PUFAs. All the tested marine ingredients, *C. sake, C. frigida* and the fish processing side streams contained EPA and DHA. While the observed levels were lower than in fishmeal the presence of these LC-PUFAs is an added value compared to various plant ingredients and a highly sought after quality in feed stuffs (Bureau et al., 2002; Turchini et al., 2009).

Minerals and micronutrients – The inorganic fraction of an ingredient is measured through the remaining ash after incineration and includes minerals and trace elements, both essential components of nutrition. However, if the minerals are supplied in excess of the nutritional requirements, they will not add to the metabolizable energy and therefore mostly be excreted. A high ash content may therefore merely dilute the nutrient content of the feed as well as pose an environmental challenge as it may increase the risk of eutrophication due to leakage of minerals to the surrounding water (Amirkolaie, 2011; G. Luo, 2022). Commercial diets contain ca. 9% ash (Aas et al., 2022; Dessen et al., 2017).

The sprat and herring side streams (**paper III & IV**) contained above 20% ash. In case of the sprat trimmings this value is related to the high level of bone and cartilage, which is in line with commercial fishmeal produced from fileting sides streams. As a result, the side stream also contained high levels of phosphorus, an essential micronutrient for the formation of bone and therefore growth (Sugiura et al., 1998). However, phosphorus is also a limiting factor in many ecosystems, especially freshwater (G. Luo, 2022). Considering global phosphorus scarcity and the potential eutrophication effects, the leakage of P to the environment, through excess levels in the feed, should be avoided (Desmidt et al., 2015). The herring side streams on the other hand, contained high ash levels mainly due to the sodium chloride present in the brine, which was added as part of the refining process. While sodium and chloride are also essential micronutrients, the dietary requirements for these minerals in salmonid fish is low, especially when reared in salt or brackish water where they are taken up directly from the environment (Potts et al., 1989; K. S. Sundell & Rønnestad, 2011; K. Sundell & Sundh, 2012) Overall, both the herring and the sprat side streams contain high levels of ash which needs to be considered in feed formulations to avoid nutrient dilution and eutrophication. In case of the sprat trimmings, the essential minerals contained in the ash may be an added value if included in the right concentrations, while in the case of the herring, the ash content may add little in terms of nutritional benefits.

In summary, *C. sake* **(paper I)** and *C. frigida* **(paper II)** display a high protein content, 55 – 60 %, making them a suitable protein source for salmonid fish (Gatlin et al., 2007). The fish processing side streams (**paper III** & **IV**) on the other hand were relatively high in both protein and lipid which needs to be considered in feed formulations and storage to avoid oxidation and suboptimal pellet extrusion. The amino acid profile (% AA) of all the evaluated ingredients is highly promising. The fist limiting amino acid is likely methionine when including high levels of *C. sake* in the diet. All diets contained medium to high levels of DHA and EPA illustrating the benefits of marine ingredients.

Appetite and feed intake

Appetite (the desire to eat) is the precursor and prerequisite for feed intake and therefore growth. Palatability on the other hand is a quality of a food/feed item which is defined as "acceptable in taste or smell" and thus related to biochemical (flavor and odor) and visual cues ingredients (Kasumyan & Doving, 2003). The palatability of a feed can be altered through changes in the ingredients. A reduction in feed intake directly relates to reduced growth and may therefore negate any potentially positive effects a feed may have when ingested (Glencross, 2020). As plant-based ingredients are generally less palatable than marine ingredients for salmonid fish, feed intake and palatability are among the main bottlenecks in the shift towards a less fishery dependent aquaculture sector. This observation was one of the main premises to embark on the journey to identify specifically novel marine feed ingredients. To complicate the assessment, there is and interplay between palatability and appetite. For example, feed items with low palatability may be rejected despite the fish having a high appetite. The molecular and neuronal integration of both palatability and intrinsic appetite are complex and include the neuronal integrations of numerous chemoreceptors (taste and smell), peripheral signals hormones (feeding status, stress) and central neuro peptides (Rønnestad et al., 2017; Soengas et al., 2018). The separation of these factors in an aquaculture context is challenging as many experiments elucidating appetite regulation in fish employed restrictive feeding regimes in some way (deficient diets, feed deprivation and starving, Frøiland et al., 2012; Jönsson et al., 2010; E. H. Jørgensen et al., 2016; McLeese & Moon, 1989). However, in aquaculture settings, feed is supplied constantly in metabolically sufficient amounts. Therefore, it is difficult to extrapolate the results of these mechanistic studies on a feeding trial where feed is provided *ad libitum*. As a result, palatability and hedonistic feed regulation may be more important in aquaculture settings than previously thought.

To evaluate potential effects of the test diets on appetite regulation in an aquaculture scenario where fish are fed *ad libitum*, the gene expression (mRNA levels) of appetite regulating neuropeptides was analyzed in the hypothalamus (**paper II & III**). No difference in mRNA concentration for any of the neuropeptides was observed despite observed differences in feed intake. This suggests that all fish were provided with all necessary nutrients to maintain normal body function and homeostasis (Rønnestad

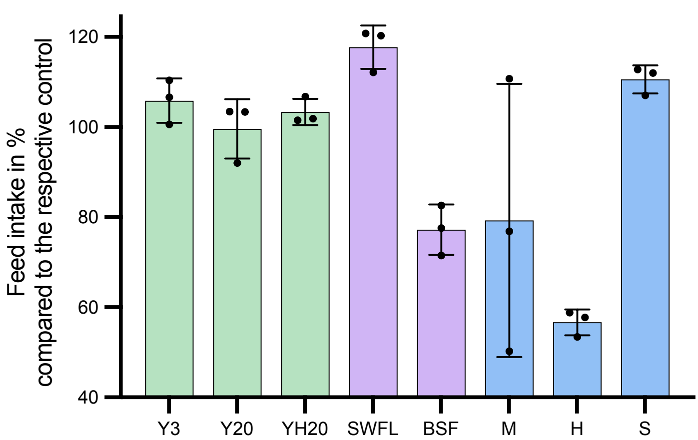

Figure 7. Feed intake of fish fed diets containing 3 % *C. sake* (Y3), 20 % *C. sake* (Y20), 20 % heat treated *C. sake*, 12 % sea weed fly larvae (*C. frigida*, SWFL), black soldier fly larvae (BSF), 50% mackerel in sauce (M), 50 % marinated herring (H) and 50 % sprat trimmings (S) relative to the respective control. Individual data points are replicate tanks. Bars intake mean ± SD.

et al., 2017). Both the appetite of the fish and the palatability of the feed determine the amount of feed an animal will consume. The feed intake may therefore act as a proxy for both appetite and palatability. Large differences in feed intake were observed between the diets in both experiments. In **paper II**, substituting 40% of the fishmeal with insect meal resulted in a higher feed intake by the fish fed the SWFL diet whereas there was a reduction in feed intake by fish fed the BSF diet, compared to the control (Figure 7). This is in line with several previous studies where BSFL resulted in a reduction in feed intake as well as a higher feed conversion ratio, when substituting fishmeal (Belghit et al., 2018; Lock et al., 2016; Sealey et al., 2011; Weththasinghe et al., 2022). SWFL on the other hand appear to be highly palatable as indicated by the high feed intake. Omega-3 fatty acids have been shown to promote feed intake (Roy et al., 2020). Thus, the increased feed intake levels of SWFL compared to the BSFL may be partly explained by a more favorable fatty acid profile. Additionally, compared to the defatted BSFL the SWFL contained lower levels of chitin. Chitin is a polysaccharide suggested to have antinutritional properties in salmonid fish, which may also add to the higher palatability resulting in the high feed intake of fish fed the SWFL (Albrektsen et al., 2022; Eggink et al., 2022). However, the chitin and omega-3 fatty acid content may not explain the higher feed intake of SWFL fed fish compared to control diet which contained similar levels of omega-3 fatty acids and no chitin. This suggests the presence of additional feed stimulating substances in the SWFL. One possible palatability increasing compound may be alanine, a major feed intake stimulating AA in rainbow trout which was considerably higher in the SWFL compared to fishmeal and BSFL (Kasumyan & Doving, 2003; Marui et al., 1983; Yamashita et al., 2006). Similarly, krill hydrolysate, also high in alanine, has been shown to increase feed intake (Kousoulaki et al., 2018). It is therefore likely that a combination of several feed stimulating substances such as amino acids and fatty acids as well as the absence of deterring substances like chitin led to a high palatability of the SWFL.

The inclusion of fish processing side streams in diets for rainbow trout (**paper III** & **IV**) also affected feed intake strongly. The inclusion of 50 % sprat trimmings in diets for rainbow trout resulted in the high feed intake while fish fed the diet containing marinated herring filets displayed a low feed intake compared to both control and sprat fed fish. The mackerel diet resulted in variable, but overall lower feed intake compared to the sprat and control diets. All three side streams were based on pelagic fatty fish, which generally are highly palatable for salmonid fish (Miles & Chapman, 2005). The observed differences in feed intake are therefore likely the results of the varying processing and/or storage conditions of the products creating the side streams. The mackerel side stream was the most processed product and contained tomato sauce (mainly tomato puree and rapeseed oil) which are likely less palatable than fishmeal (Gatlin et al., 2007; Hardy, 1996). However, most of the tomato sauce was removed in the washing steps preceding the drying of the material. Additionally, the processing included the addition of antioxidants which likely kept the protein and lipid quality high and the oxidation low. Nonetheless, compared to the sprat trimmings the feed intake observed for this side stream was lower possibly due to the residues of the tomato sauce which likely also penetrated the fillets. This idea is supported by the higher omega 6 (common in plant oils) to omega-3 ratio of the mackerel in tomato sauce compared to the sprat and the herring side streams (Table 1). The marinated herring filets on the other hand, were stored an extended time before being used in the as a feed ingredient. Even though the herring side stream contained also high amount of salt, close to the levels used to preserve the sprat trimmings, the extended and generally suboptimal storage (above 0 degree and without addition of antioxidants) likely counteracted this and promoted lipid peroxidation. Fatty fish products contain high levels of polyunsaturated fatty acids which are prone to oxidize in the presence of oxygen. The resulting peroxidation products such as aldehydes and peroxides likely reduced the palatability of the herring fillets (Hamre et al., 2001; Lund et al., 2013; Wu et al., 2022). Furthermore, fish fed the herring diet also displayed lower plasma ghrelin levels than fish fed the other diets suggesting an effect on the central appetite regulation. In mammals and several fish species, ghrelin is the only known peripheral orexigenic (feed-stimulating) hormone directly linked to the release of growth hormone and somatic growth (Kojima et al., 1999; Nakazato et al., 2001; Rønnestad et al., 2017). However, the role of ghrelin in rainbow trout is ambiguous as both anorexigenic and orexigenic functions have been observed (Jönsson et al., 2010; Velasco et al., 2016). The present results are in line with the latter, and support an orexigenic function, since rainbow trout fed the herring diet had the lowest ghrelin levels along with a lower feed intake and growth. Alternatively, considering palatability and appetite separately, it is possible that the palatability of the herring diet was so low that despite an increased appetite, the deterring effect of the lipid peroxidation products resulted in a lower feed intake triggering a response similar to fish in a food deprived state i.e., lower ghrelin (Jönsson et al., 2010). As a result, significant improvements in storage and logistics are necessary before this particular side stream can be recommended as an ingredient in diets for rainbow trout. The sprat trimmings were the freshest and least processed of the three side streams tested and were preserved using only salt (NaCl). Therefore, the sprat trimmings likely maintained a more 'natural' taste, resulting in the highest palatability. In summary, fish processing side streams may be highly palatable however the addition of plant based ingredients and suboptimal storage conditions may reduce palatability.

In **paper I**, 20% inclusion of the marine yeast *C. sake* in the diet did not alter feed intake. An inclusion rate of 20% can be considered as high considering that in compound feed single ingredients generally contribute with less than 20% to the complete feed, and these results are therefore highly promising (Aas et al., 2022). As 20% of the control diet was substituted, these results indicate that *C.sake* may be as palatable as the blends of fish and plant based ingredients generally used in aquafeeds.

To summarize the observed feed intake fish fed the *C. sake* (**paper I**), *C. frigida* (**paper II**) and sprat trimmings (**III** & **IV**) was numerically higher than the feed intake of fish fed the respective control diets (Figure 7) which suggests a high palatability. As feed intake is one of the best proxies for animal growth, these results are highly promising. While the mechanisms are largely unknown, marine ingredients may be more palatable than many terrestrial and specifically plant based ingredients. However, due to heterogeneity of marine ingredients (including algae, crustations, fish and yeast) as well as the over representation of fishmeal and oil as marine ingredients, such generalizations may be difficult to make. However, salmonid fish have evolved to identify and locate aquatic fish and arthropods (including crustaceans and notably insects) as their food items (Cada et al., 1987; Gustafsson et al., 2010). It is therefore likely that the chemical cues released by such prey items overlap more with other marine or aquatic ingredients than with terrestrial ingredients (Marui et al., 1983; Sheridan, 2021). Considering that the palatability of novel ingredients including plant-based ingredients is a bottleneck for the application of alternative ingredients in aquafeeds, the potential of marine ingredients should not be understated (Gatlin et al., 2007).

Intestinal health

Novel feed ingredients can alter the intestinal physiology and morphology in various ways. The inclusion of 20% *C. sake* in diets for rainbow trout affected neither the intestinal morphology nor physiology (**paper I**). Thus, both the inclusion of heat treated and untreated yeast resulted in a performance equal to the control which is promising. The replacement of only 3% soy protein concentrate with *C. sake* on the other hand, affected both the intestinal morphology as well as the immune systems as suggested by the increase in lamina propria width as well as an increase in TGF-β expression after in immune stimulation using LPS (lipopolysaccharides; an endotoxin). The yeast's cell wall contains polysaccharides such as β-glucans and mannan oligosaccharides which have immune stimulatory properties and may interact directly with immune cells (Meena et al., 2013; Torrecillas et al., 2014). Additionally, the high content of polysaccharides in the yeast's cell wall may interact and modify the intestinal microbiota which in turn interacts with the immune system (Glencross et al., 2020; Huyben et al., 2018). The increase in lamina propria width may correspond to an increase in lymphocyte infiltration (Baeverfjord & Krogdahl, 1996). At the same time TGF-β is a multifunctional cytokine with anti-inflammatory properties. As no other signs of even a weak enteritis was observed, these changes suggest immune modulating properties of the marine yeasts rather than the development of an intestinal inflammation. However, as these effects where mild long-term studies evaluation possible functional properties of *C. sake* are encouraged. As preparations of yeast derived β-glucans have been shown to increase general health and robustness of salmonid fish, these indications for immune stimulatory properties of *C. sake* may be an added value and applications as functional ingredient are possible (J. B. Jørgensen et al., 1993; Meena et al., 2013; Refstie et al., 2010; Sealey et al., 2008).

The substitution of fishmeal with SWFL did not alter any of the intestinal health related endpoints compared to the control (**paper II**). However, fish fed the SWFL diet displayed lower TEP and lower absolute values of SCC compared to fish fed the BSFL diet, suggesting lower active transport activities in the intestinal epithelium (K. Sundell et al., 2022; Sundh et al., 2010). However, no differences were observed between fish fed the two insect diets, or the control diet, for the specific lysine uptake or the intestinal morphology. This indicates no impairment of nutrient uptake for either the SWFL or the BSFL diet. At the same time these results suggest that terrestrial and marine insects have differing modulatory effects on the intestinal function.

The inclusion of side streams (**paper III** & **IV**) on the other hand, had a strong effect on the intestinal physiology. No difference between the control and mackerel treatment was observed regarding the intestinal function. The lysine uptake in the proximal intestine of fish fed the sprat diet was lower compared to the control. A lower nutrient uptake could be caused by a decrease in the epithelial surface area, which is commonly observed in fish with enteritis (Knudsen et al., 2008; Krogdahl et al., 2010). However, no indication for an enteritis was revealed through the histological analysis suggesting other factors to be involved. Also, the *ex-vivo* Ussing chamber measurements, analyzing a sample of the intestinal area, may not directly reflect the situation at the whole animal level where factors such as chyme transient time, nutrients, minerals, and the intestinal microbiota all contribute to a sufficient nutrient uptake. The herring diet on the other hand, had a clear negative effect on the intestine which manifested in a lower lysine uptake in both proximal and distal intestine which was accompanied by alterations in the electrophysiology of the epithelium. The TEP in the proximal intestine of fish fed the herring diet was lower compared to the control and sprat treatments. The TEP reflects the potential difference generated by the passive diffusion and active transport of charged molecules across the epithelium. Most of the amino acid uptake, including lysine, is sodium dependent as Na^+/amino acid symporters rely on the sodium gradient created by the enterocytes' Na^+K^+-ATPases (Hedén et al., 2022; K. Sundell & Sundh, 2012). The lower serosa positive TEP may therefore be a consequence of a reduction in active Na^+ driven nutrient (including lysine) transport in the proximal intestine. This is further supported by the maintained TER indicating no differences in passive paracellular nutrient uptake in this intestinal region. In the distal intestine on the other hand, amino acid uptake is overall much lower and more reliant on less active processes such as endocytosis and paracellular diffusion (Grosell et al., 2010; Günzel & Yu, 2013; Karasov, 2017). The permeability towards amino acids in this region may therefore be directly related to the higher TER compared to the control fish. The lower lysine uptake in both proximal and distal intestine of fish fed the herring diet is in line with the observed lower low digestibility.

In summary, both yeast and insect ingredients may alter epithelial morphology and/or function. The exoskeleton of insects as well as the cell wall of yeasts contain carbohydrates which may modulate the microbiome of the fish as well as act as antigens for the gut associated immune system.

This suggests the possibility to utilize yeasts and insects as functional ingredients. Additionally, the difference between SWFL and BSFL illustrates that marine and terrestrial insects may have different effects on the intestine. However, additional research is needed to understand the interplay between these factors and the fish. As a source of protein, neither marine yeast nor insects had a negative effect on intestinal health which suggests that they are potential candidates for commercial feeds. The effects of the different fish processing side streams on the intestinal health varied with the individual side stream, clearly illustrating the need to evaluate each side stream individually. Additionally, these results highlight the importance of including an assessment of intestinal health in the

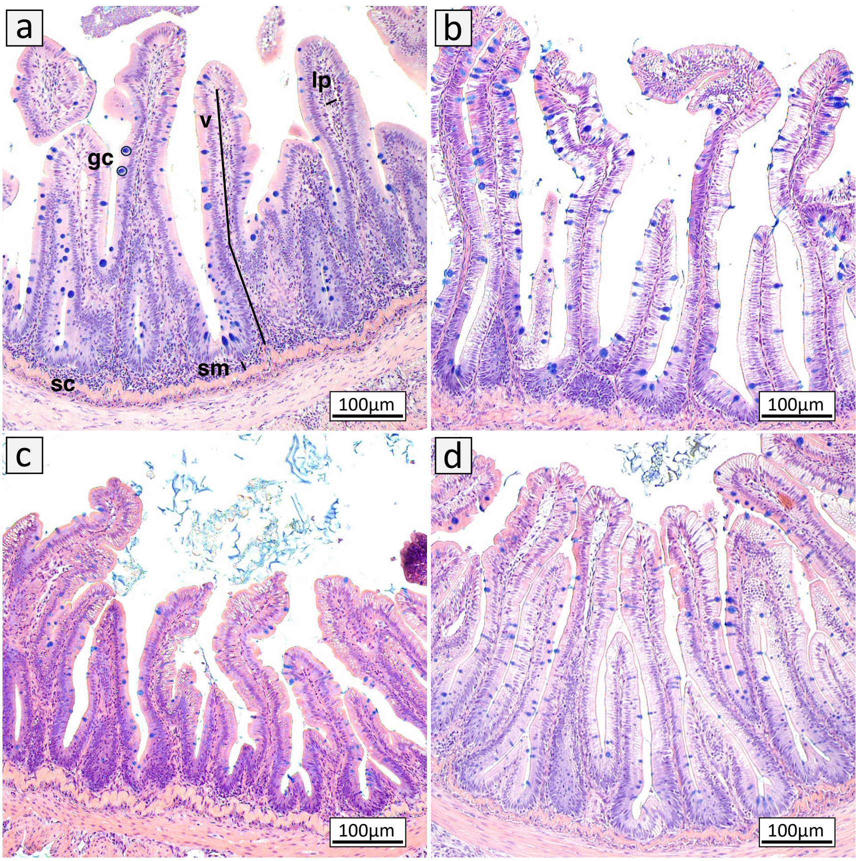

Figure 8. Histological section of the distal intestine of juvenile rainbow trout fed a; the control, b; the mackerel, c; the herring and d; the sprat diet. The control and sprat diets resulted in a slightly increased lamina propria and submucosa. Sections were stained using hematoxylin, erythrosine and alcian blue. Abbreviations indicate lp; lamina propria width, sc; stratum compactum, sm: submucosa width, v; villi length and gc; goblet cell count.

evaluation of novel ingredients, as the observed negative effects in the intestine of fish fed the herring side streams may decrease fish welfare and relate to the lower observed digestibility, feed intake and growth.

Growth and feed utilization

The previously discussed effects on appetite, digestibility, and intestinal health as well as the nutritional properties of the alternative ingredients, ultimately culminate in modified growth. The specific growth rate (SGR) of rainbow trout is further influenced by external factors such as temperature, feeding regime, feed production and size of the animal (Jobling, 2003). In **papers I – IV,** the growth rates of salmonid fish varied between 0.6%, for fish fed the marinated herring, and 1.6% for fish fed the commercial diet. The lowest growth rates were observed in the **paper III** where diets including 50% of the experimental ingredients were cold pellet rather than extruded. Extruded pellets have been shown to improve pellet stability water quality in RAS systems which in turn has positive effects on health and welfare and therefore growth (Hilton et al., 1981; Welker et al., 2018). Additionally, fish fed the herring diet displayed reduced feed intake, intestinal lysine uptake and digestibility which all contribute to the observed low growth rates. The spat diet resulted in the highest growth rates observed in **paper III** likely driven by the high palatability and feed intake. The commercial diet aside, the highest SGR was with 1.4% observed for fish fed the SWFL diet. Considering that the SWFL meal substituted 40% of the fishmeal present in the control diet, the high growth rates are very promising. The increased feed intake observed for fish fed the SWFL diet is likely a main contributor to the high growth rate. These results also support the notion that the observed differences in intestinal electrophysiology are an adaptation to, rather than a negative effect of, the SWFL inclusion. Insect meal has been shown to improve growth rates in a limited number of studies. However, in general, insect meal has a lower bioavailability and digestibility compared to fishmeal (Albrektsen et al., 2022; Hua, 2021; Weththasinghe et al., 2022). This is supported by the results of **paper III** where dietary insect inclusion resulted in an increase in FCR from 0.75 to 0.86 compared to the fishmeal control.

C. sake inclusion did not alter the growth rates of rainbow trout (**paper I**). In this experiment, yeast substituted 20% of a control diet which contained commercial fish and plant-based ingredients. The similar growth rates therefore suggest a high digestibility of the yeast meal which was supported by the ADC evaluation. Especially, the amino acid digestibility of *C. sake* was with *ca* 90% for most amino acids high. While several studies have shown that yeast inclusions of up to 40% do not negatively affect growth in rainbow trout, it is generally agreed that yeast meals may be harder to than many commercial ingredients due to the yeast rigid cell walls (Hansen et al., 2019; Hauptman et al., 2014; Mahnken et al., 1980; Øverland et al., 2013; Vidakovic et al., 2020). While various downstream processing techniques have been shown increase digestibility by disrupting these cell walls, the present study revealed no clear positive effect of the additional heat treatment (Agboola et al., 2022). However, considering the high bioavailability of *C. sake,* additional downstream processing may not be required.

Sprat trimmings (**paper III** & **IV**) were included in diets for rainbow trout at drastically different inclusion levels. The 50% inclusion (**paper III**) of sprat trimmings in cold pelleted diets resulted in an 0.2% higher SGR compared to the more commercial-like diet which included only 6% sprat trimmings (**paper IV**). However, due to the differences in feed production, formulation, production system (fresh compared to salt water) and animal size, direct comparisons between the two experiments may be misleading. Compared to the commercial diet, the commercial-like diet including 6% sprat resulted in a reduction in growth which may be unsurprising considering the high degree of refinement in commercial diets. Nonetheless, the results of **paper IV** show that sprat trimmings can be used without further processing in a commercial-like feed pellet production including extrusion. However additional experiments and balancing of the diets is needed to fully evaluate the potential of including non-processed moist sprat trimmings.

In summary, both *C.sake* and *C. frigida* inclusion resulted in high growth rates compared to the respective controls. *C. frigida* additionally resulted in higher growth rates compared to the diet containing BSFL meal confirming the high potential of this marine insects. Large differences in growth related parameters were observed between the different fish processing side streams. While the herring fillets appear to require additional refinement and/or improvements in storage and processing, the mackerel in tomato sauce and the sprat trimming inclusion resulted in promising growth rates. The sprat trimmings further could be included in a commercial-like pellet production using extrusion. The ability to include such a side stream without additional processing such as drying, grinding and pressing to separate protein and oil fractions, has significant implications for the sustainability of the produced feed.

Sustainability

With sustainability becoming a major concern for the future of aquaculture, efforts have been made to developed matrixes to quantify different aspects of sustainability. To measure environmental sustainability, life cycle assessments (LCAs) are a primary tool. Such LCAs may include and identify impacts such as climate change, eutrophication, land use and emissions (Bohnes & Laurent, 2019). As previously discussed, most of the environmental footprint for salmon production is related to the feed (Pelletier et al., 2009). All experiments included in this thesis were carried out using both novel materials/organisms and on an experimental scale in an academic setting, and no LCA was carried out. However, comparable ingredients such as BSFL, SCP and processing side streams have been evaluated allowing for a general discussion of the tested ingredients. For the ingredients evaluated here, the largest contributor to the global warming potential may be related to energy consumption (Boakye-Yiadom et al., 2022; Maiolo et al., 2020). The environmental footprint of SCP **(paper I)** may depend on the energy source (renewable vs. fossil) due to the energy consumption of large-scale fermenters. This is even true within the "fossil fuel" category. For instance, fishmeal produced using natural gas as an energy source may have a 41% lower impact compared to a production using heavy fuel/oil (Fréon et al., 2017). Large differences can also be expected due to the handling of the ingredients. Wet SCP (*S. cerevisiae*) has been shown to have a 16% lower impact on global warming due to the energy used for drying (Kobayashi et al., 2023). The high energy use for drying was one of the main motivating factors for directly including fish processing side streams in the experimental diets without further processing (**paper III and IV**). Surprisingly, fishmeal produced from side streams may have a higher footprint compared to production from dedicated reduction fisheries, due to a higher efficiency (Pelletier & Tyedmers, 2007). Nonetheless, fishmeal produced from side streams may have less impact on other metrics such as biodiversity. The insect industry is still in its infancy. However, energy costs and the allocation of feed inputs may define the sustainability of intensive farming operations (Guo et al., 2021). Upscaling and technological improvements are therefore central for this industry going forward. However, how side streams are to be handled in LCAs is still debated. Should they be seen as a "free" waste product that exists whether it is utilized or not, and therefore carry no emissions? Or should side streams be treated as "co-products" therefore sharing the environmental impact of the primary product

(Coelho et al., 2022; Ziegler personal communication)? One might argue that a compromise is appropriate, whereby the utilization of side streams offsets the footprint of the main product but does not contribute to the same magnitude. Regardless, compared to conventional ingredients, the benefits of the side streams included in this thesis include very low land and water use compared to plant-based ingredients, and a lower impact on biodiversity and habitat destruction compared to commercial plant and fish-based products. Additionally, marine insects and yeasts may be more suitable to produce salmonid fish compared to their terrestrial counter parts. The observed trends regarding increased feed intake and growth will likely shorten production times and therefore lower the environmental footprint for farmed fish. Overall, feed formulations including alternative ingedients such as insects and meals derived from filter feeding organisms

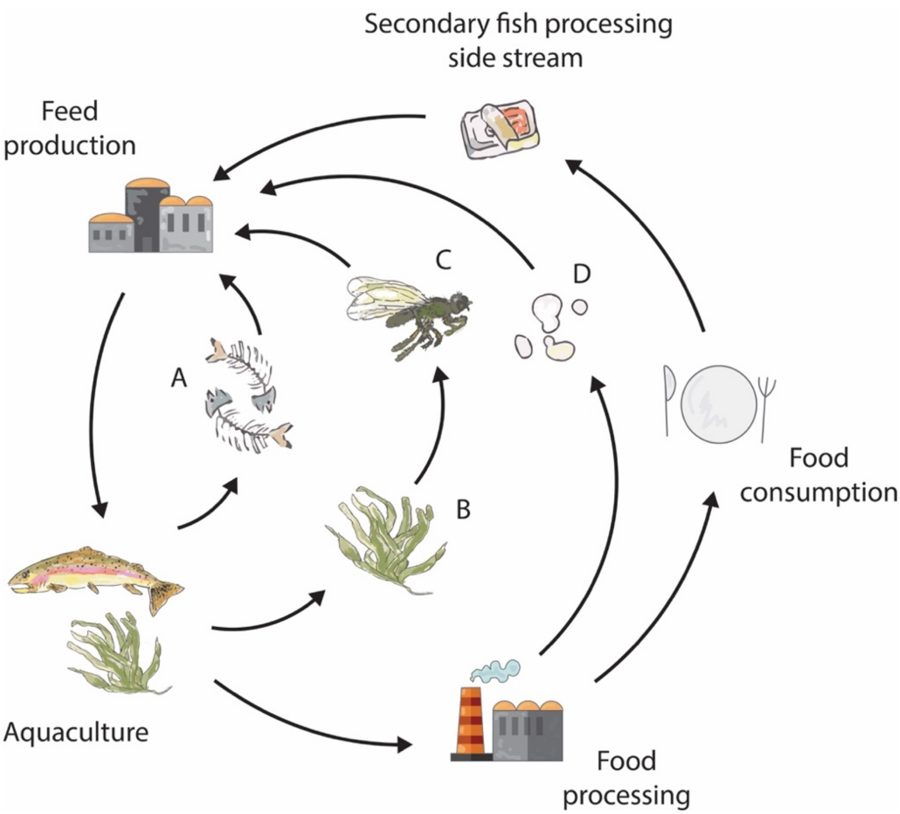

Figure 9. Schematic display of how the different papers contribute to a more circular food production system taking into account the different side streams and processing stages, A: primary sprat filleting side streams (**paper III & IV**), B: brown seaweed side streams and C: seaweed fly *C. frigida* (**paper II**) and D: marine yeast, *C. sake* (**paper I**),

such as mussels and seasquirts have been shown to offesst green house gas emmissions (CO_2) compared to standard feed fromulations by 63 % (Langeland et al., 2023). Considering the early stage of many of these ingredients, the scope to increase the environmental sustainability of feeds for salmonid fish appears vast.

Social and economic sustainability are generally harder to assess. Overall, the use of side streams is as part of a circular economy approach is supported by the public, suggesting a high social sustainability. That the marine side streams here evaluated potentially have a positive effect for animal health further contributes to this notion. Fish processing side streams are already converted to fishmeal and oil, exemplifying economic feasibility. However, considering the additional processing steps, these margins are small. A more direct utilization of the side streams may therefore also enhance the economic sustainability. Economic sustainability hinges at one factor: scalability.

Commercial application

Modern compound feed functions in delivering a complete nutrient package to the animal by combining various ingredients, thereby balancing the different nutrient profiles according to the needs of the farmed animal (Hardy & Kaushik, 2020). This is a strength, as nutritional imbalances of individual ingredients can be accounted and corrected for, but also a challenge in evaluating novel feed ingredients as the experimental design can both enhance and cover-up nutritional properties of an ingredient. For example, substituting either fishmeal or fish oil with a single alternative ingredient likely bears some negative implications for the fish due to their balanced nutrient compositions. This is acknowledged by the industry and therefore the sentiment over the past decades has been to substitute "as much fish-based ingredients as possible" before the negative effects outweigh the, in many cases, monetary benefits of reducing fishmeal/oil inclusion levels. The aim of this thesis was in part to move past this sentiment by identifying novel marine ingredients which may be used to complement fish-based ingredients thereby potentially further lowering the reliance of aquaculture on natural fish stocks without introducing additional stressors for the fish.

The in this thesis evaluated ingredients, marine yeast, marine insects, mackerel in tomato sauce, and the sprat trimmings, are all promising feed ingredients from a nutritional and physiological perspective. However, their commercial success depends largely on two factors, scalability and production costs. The commercial production of yeast has been a central part of global food culture for centuries. The production of *C. sake* is likely scalable using existing industrial reactors and equipment (Buckholz & Gleeson, 1991). The costs of this production are directly linked to the production scale. On a small scale, bio reactors require relatively large amounts of energy to maintain a stable temperature. Similarly, drying and separation processes demand high energy inputs on a small scale (Kobayashi et al., 2023). As the energy efficiency goes up with the production scale, upscaling and optimization of marine yeast production is crucial their commercial viability (Jeevan Kumar & Banerjee, 2019).

Despite being consumed by humans for centuries, commercial insect production in Europe is in its infancy. With the Netherlands and France in the forefront, several large(r) scale production facilities opened in the last decade (Derrien & Boccuni, 2018). While the commercial viability of these enterprises remains to be seen, the scalability of insect production has been demonstrated. Currently, the costs of insects reared in these farms is relatively high compared to fishmeal (Jannathulla et al., 2019). However, insects may also be farmed on a smaller scale in less intense farming situations (Chaalala et al., 2018). This flexibility and low barrier of entry for small scale productions is a big advantage of insect productions. While a commercial production of BSFL for use in fish feed production still depends on further technological developments, SWFL have yet to be produced on any scale outside of the lab. While likely, whether SWFL can be efficiently produced on a large scale remains to be seen.

Fish processing side streams are available on a large scale and may be used in fish feeds with or without minor additional processing. Due to these reasons, around 30% of the globally produced fishmeal is already emanating from seafood side streams. The bottleneck for such products are efficient and stable supply chain and the generally lower price of such meal compared to standard fish meal (Ween et al., 2017).

All novel feed ingredients compete with globally traded commodities such as soybean meal fish meal, corn meal and palm oil, all of which are produced on a large scale (Gatlin et al., 2007; De Maria et al., 2020). Ultimately, the commercial application of all the above-mentioned alternative ingredients depends on the trajectory of humankind. As population growth, resource scarcity, and the impact of global warming intersect, the food production sector we become dependent on alternative raw materials.

CONCLUSIONS

"I think and think for months and years. Ninety-nine times, the conclusion is false. The hundredth time I am right." – Albert Einstein

The landscape of aquafeed ingredients is expected to continue to change. While Sweden is no major player the global game of aquaculture, it is a major fish processing hub due to its proximity to Norway. The results of this thesis may therefore be applied to the development of local sustainable seafood production in Sweden. However, the results are equally applicable to the global aquafeed market where, due to the intersection of population growth, limited natural resources, and the impact of global warming, sustainable marine feed ingredients, and their nutrients, are becoming a bottleneck.

This thesis demonstrated that marine yeast **(paper I)** and marine insects **(paper II)** can convert lower value marine side streams into high quality feed ingredients. Both the processing water and the algae used as cultivation substrate contain relatively low amounts of protein as well as other compounds which make it impossible to used large quantities of them directly in feeds for salmonid fish. The use of both marine yeast and marine insects therefore enables the recovery of otherwise lost nutrients as part of a more circular economy. Both are promising candidates to be used as protein and omega-3 sources in diets for salmonid fish.

Furthermore, this thesis identified possible functional properties of marine ingredients. Apart from resulting in good growth rates, fish fed diets containing *C. frigida* **(paper II)** and *S. sprattus* side streams (**paper III** & **IV**) displayed a high feed intake suggesting a high palatability. Additionally, both *C. sake* **(paper I)** and *C. frigida* **(paper II)** contain complex carbohydrates which may have immune modulatory functions.

To diversify the pool of suitable ingredients for commercial feed production is essential to secure a resilient and sustainable aquaculture development. This is especially true for marine ingredients which contain many of the essential nutrients required for optimal fish growth. Furthermore, the production of ingredients using industry side streams may improve the sustainability of the seafood sectors by, reducing the raw material input (including plant and fish-based sources), and their associated negative environmental effects regarding the use of arable land for monocultures as well as invasive fishing techniques. Ultimately the commercial application of these novel ingredients will depend on their price on the global market which directly related to production scalability.

ACKNOWLEDGMENTS

I am not one for big words or big gestures, but I want to thank all the people who helped me along the way. I am deeply grateful to have gotten to opportunity to meet all of you. This thesis was not the work of me alone.

First of all, I would like to thank my main supervisor, **Snuttan**. It all started almost exactly 9 years ago when I took your course as an exchange student during my bachelor program. It was my first course in Sweden, and I loved it! Thank you for your unbreakable optimism, energy, compassion and support. Your empathy and positivity (and all the smiley emails) are infectious. And you know a thing or two about fish physiology too ☺. Thank you so much for 5 amazing years.

I would like to thank my brilliant co-supervisors. **Lisa**, thank you for your humor and confidence in me. **Markus**, thank you for introducing me to the great world of nutrition and the red telephone. A big thank you also to **Henrik**. Since I worked with you in 2018, I never stopped seeing you as my supervisor.

Thank you to **Margarete Øverland** for agreeing to be my opponent.

Thank you, **Michael**, for being my examiner for 95% of the time and thank you **Lotta** for taking over in the end on such short notice. Thank you both for being so approachable and your valuable input.

Thank you to what is most likely the best research group ever. Thank you **Darragh** (for all those collaborations, and all your help), **Nicklas** (for stealing both my initials), **Ali** (for always making me smile), **Thanh** (for all the late night dinners), **Mica** (for making me dive in Chile), **Raneesha** (for letting me measure my heart rate), **Ida** (for keeping order), **Krisikka**, **Ningping**, **Federico**, **Ebuka**, **Lynne**, **Jonna, Linda** (support in Kristineberg), **James** (for my favorite song), **Ellen** (for all the pep talk) and **Thrandur** (for great talks over a beer). A special thank you also to **Linda**, who never was too angry when I left the lab a mess, and **Malin**, the best student project leader, and of course, **Jon**, for your very special humor and random mystery gifts. It was so great to be part of the FEL group which sometimes feels like one big family.

A special thanks to all the students that for some curious reason decided to join me on my fish feed endeavors. Thank you, **Marie**, **Xintian**, **Jolie** (Würst), **Elin**, **Romana**, **Enya** and **Alba**. It was such a pleasure working with you. A special thank you also to (hallihallo) **Terese** who helped illustrate this thesis.

Thank you also to **Aleks, Pontus** “Pontulus” and **Ashwath** for making me feel welcome at SLU.

Thank you, **Dave** and **Linda**, for taking care of the fish, and **Lilioth** for taking care of the rest.

I feel so fortunate to have met so many great people at this department. Thank you, **Magnus** (my oldest Swedish firend) **Alex** (my twin), **Oscar**, **Mårten**, **Daniel**, , **Lucas**, **Maja**, **Rute**, **Agathe**, **Andreas, Per**, **Andreas**, **Azora**, **Marianne, Charlotte, Eleftherios, Mafalda, Lima** and **Erika** for all the board games, climbing sessions and deep conversations over a beer.

A special thank you also the PhD board, **Kim** (for all the creative and critical input), **Daniella** (for the almonds), **Christina** (for you calming influence).

Dank auch an meine **Familie**. Man kann sich seine Familie nicht aussuchen, aber schlecht habe ich es nicht getroffen. Danke Papa und Mama für eure uneingeschränkte Unterstützung und Liebe. Danke **Sina**, dass du immer unglaublich gut in allem bist. Ich wäre sicher nicht der gleiche, wenn ich du mir nicht gezeigt hättest, wie man es schneller machen kann.

Thank you to **Francesca** for being the happiness to my German grumpiness.

To all of you and everyone that I have missed (likely due to a lack of sleep), Thank you so much from the bottom of my heart.

REFERENCES

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

Agboola, J. O., Lapeña, D., Øverland, M., Arntzen, M. Ø., Mydland, L. T., & Hansen, J. Ø. (2022). Yeast as a novel protein source - Effect of species and autolysis on protein and amino acid digestibility in Atlantic salmon (Salmo salar). *Aquaculture*, *546*. https://doi.org/10.1016/j.aquaculture.2021.737312

Agboola, J. O., Øverland, M., Skrede, A., & Hansen, J. Ø. (2021). Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production. *Reviews in Aquaculture*, *13*(2), 949–970. https://doi.org/10.1111/raq.12507

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., & Øverland, M. (2022). Future feed resources in sustainable salmonid production: A review. *Reviews in Aquaculture*, *14*(4), 1790–1812. https://doi.org/10.1111/RAQ.12673

AMEC, E. & E. L. (2003). Management of Wastes From Atlantic Seafood Processing Operations. In *National programme of action Atlantic regional team Environment Canada Atlantic Region* (Vol. TE23016, Issue December).

Amirkolaie, A. K. (2011). Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. *Reviews in Aquaculture*, *3*(1), 19–26. https://doi.org/10.1111/j.1753-5131.2010.01040.x

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). *Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal.* https://doi.org/10.1155/2014/360438

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. *Journal of Fish Diseases*, *19*(5), 375–387. https://doi.org/10.1111/j.1365-2761.1996.tb00376.x

Belghit, I., Liland, N. S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., Krogdahl, Å., & Lock, E. J. (2018). Potential of insect-based diets for Atlantic salmon (Salmo salar). *Aquaculture*, *491*(November 2017), 72–81. https://doi.org/10.1016/j.aquaculture.2018.03.016

Bell, J. G., Pratoomyot, J., Strachan, F., Henderson, R. J., Fontanillas, R., Hebard, A., Guy, D. R., Hunter, D., & Tocher, D. R. (2010). Growth, flesh adiposity and fatty acid composition of Atlantic salmon (Salmo salar) families with contrasting flesh adiposity: Effects of replacement of dietary fish oil with vegetable oils. *Aquaculture*, *306*(1–4), 225–232. https://doi.org/10.1016/j.aquaculture.2010.05.021

Bell, J. G., & Koppe, W. (2010). Lipids in aquafeeds. Fish oil replacement and alternative lipid sources in aquaculture feeds, 1, 21-59.

Boakye-Yiadom, K. A., Ilari, A., & Duca, D. (2022). Greenhouse Gas Emissions and Life Cycle Assessment on the Black Soldier Fly (Hermetia illucens L.). In *Sustainability (Switzerland)* (Vol. 14, Issue 16). MDPI. https://doi.org/10.3390/su141610456

Bohnes, F. A., & Laurent, A. (2019). LCA of aquaculture systems: methodological issues and potential improvements. In *International Journal of Life Cycle Assessment* (Vol. 24, Issue 2, pp. 324–337). Springer Verlag. https://doi.org/10.1007/s11367-018-1517-x

Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D. C., Juarez, L. M., Lockwood, G. S., McNevin, A. A., Tacon, A. G. J., Teletchea, F., Tomasso, J. R., Tucker, C. S., & Valenti, W. C. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Journal of the World Aquaculture Society*, *51*(3), 578–633. https://doi.org/10.1111/jwas.12714

Buckholz, R. G., & Gleeson, M. A. G. (1991). Yeast Systems for the Commercial Production of Heterologous Proteins. *Bio/Technology*, *9*(11), 1067–1072. https://doi.org/10.1038/nbt1191-1067

Buddington, R. K., Krogdahl, A., & Bakke-Mckellep, A. M. (1997). The intestines of carnivorous fish: structure and functions and the relations with diet. *Acta Physiologica Scandinavica. Supplementum*, *638*, 67–80. http://europepmc.org/abstract/MED/9421581

Bureau, D. P., Gibson, J., & El-mowafi, A. (2002). Review : Use of Animal Fats in Aquaculture Feeds. *VI Simposium Internacional de Nutrición Acuícola*, 487–504.

Bureau, D. P., Harris, A. M., & Cho, C. Y. (1999). Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). *Aquaculture*, *180*(3–4), 345–358. https://doi.org/10.1016/S0044-8486(99)00210-0

Brundtland, G. H. (1987). Report of the World Commission on environment and development:" our common future.". UN.

Cada, G. F., Loar, J. M., & Cox, D. K. (1987). Food and Feeding Preferences of Rainbow and Brown Trout in Southern Appalachian Streams. In *Source: The American Midland Naturalist* (Vol. 117, Issue 2).

Chaalala, S., Leplat, A., & Makkar, H. (2018). Importance of insects for use as animal feed in low-income countries. In *Edible Insects in Sustainable Food Systems* (pp. 303–319). Springer International Publishing. https://doi.org/10.1007/978-3-319-74011-9_18

Cho, C. Y., & Slinger, S. J. (1979). Apparent digestibility measurement in feedstuffs for rainbow trout. In *Finfish Nutrition and Fishfeed Technology, Vol. II*. Heenemann Verlagsgesellschaft. https://doi.org/10.11233/AQUACULTURESCI1953.55.319

Cho, C. Y., Slinger, S. J., & Bayley, H. S. (1982). Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comparative Biochemistry and Physiology -- Part B: Biochemistry And*, *73*(1), 25–41. https://doi.org/10.1016/0305-0491(82)90198-5

Clarke, L. L. (2009). A guide to Ussing chamber studies of mouse intestine. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *296*(6), G1151–G1166. https://doi.org/10.1152/ajpgi.90649.2008

Coelho, C. R. V, Peters, G., Zhang, J., Hong, B., Abdollahi, M., & Undeland, I. (2022). A comparative life cycle assessment of cross-processing herring side streams with fruit pomace or seaweed into a stable food protein ingredient. *Future Foods*, *6*, 100194. https://doi.org/10.1016/j.fufo.2022.100194

Derrien, C., & Boccuni, A. (2018). Current status of the insect producing industry in Europe. In *Edible Insects in Sustainable Food Systems* (pp. 471–479). Springer International Publishing. https://doi.org/10.1007/978-3-319-74011-9_30

Desmidt, E., Ghyselbrecht, K., Zhang, Y., Pinoy, L., Van Der Bruggen, B., Verstraete, W., Rabaey, K., & Meesschaert, B. (2015). Global phosphorus scarcity and full-scale P-recovery techniques: A review. *Critical Reviews in Environmental Science and Technology*, *45*(4), 336–384. https://doi.org/10.1080/10643389.2013.866531

Dessen, J. E., Weihe, R., Hatlen, B., Thomassen, M. S., & Rørvik, K. A. (2017). Different growth performance, lipid deposition, and nutrient utilization in in-season (S1) Atlantic salmon post-smolt fed isoenergetic diets differing in protein-to-lipid ratio. *Aquaculture*, *473*, 345–354. https://doi.org/10.1016/j.aquaculture.2017.02.006

Díaz-rúa, A., Chivite, M., Comesaña, S., Velasco, C., & Valente, L. M. P. (2020). Hormones and Behavior The endocannabinoid system is a ff ected by a high-fat-diet in rainbow trout. *Hormones and Behavior*, *125*(April), 104825. https://doi.org/10.1016/j.yhbeh.2020.104825

Eggink, K. M., Pedersen, P. B., Lund, I., & Dalsgaard, J. (2022). Chitin digestibility and intestinal exochitinase activity in Nile tilapia and rainbow trout fed different black soldier fly larvae meal size fractions. *Aquaculture Research*, *53*(16), 5536–5546. https://doi.org/10.1111/are.16035

English, G., Wanger, G., & Colombo, S. M. (2021). A review of advancements in black soldier fly (Hermetia illucens) production for dietary inclusion in salmonid feeds. *Journal of Agriculture and Food Research*, *5*, 100164. https://doi.org/10.1016/j.jafr.2021.100164

Eroldoğan, O. T., Glencross, B., Novoveska, L., Gaudêncio, S. P., Rinkevich, B., Varese, G. C., de Fátima Carvalho, M., Tasdemir, D., Safarik, I., Nielsen, S. L., Rebours, C., Lada, L. B., Robbens, J., Strode, E., Haznedaroğlu, B. Z., Kotta, J., Evliyaoğlu, E., Oliveira, J., Girão, M., … Rotter, A. (2023). From the sea to aquafeed: A perspective overview. *Reviews in Aquaculture*, *15*(3), 1028–1057. https://doi.org/10.1111/raq.12740

EUMOFA. (2022). *Organic aquaculture in the EU : current situation, drivers, barriers, potential for growth.*

FAO. (2022). *The State of World Fisheries and Aquaculture 2022*. FAO. https://doi.org/10.4060/cc0461en

FEFANA, https://fefana.org/our-industry/product-classification/. Accessed Oct. 2023

Fréon, P., Durand, H., Avadí, A., Huaranca, S., & Orozco Moreyra, R. (2017). Life cycle assessment of three Peruvian fishmeal plants: Toward a cleaner production. *Journal of Cleaner Production*, *145*, 50–63. https://doi.org/10.1016/j.jclepro.2017.01.036

Frøiland, E., Jobling, M., Björnsson, B. T., Kling, P., Ravuri, C. S., & Jørgensen, E. H. (2012). Seasonal appetite regulation in the anadromous Arctic charr: Evidence for a role of adiposity in the regulation of appetite but not for leptin in signalling adiposity. *General and Comparative Endocrinology*, *178*(2), 330–337. https://doi.org/10.1016/j.ygcen.2012.06.017

Fry, J. P., Mailloux, N. A., Love, D. C., Milli, M. C., & Cao, L. (2018). Feed conversion efficiency in aquaculture: do we measure it correctly? *Environmental Research Letters*, *13*(2), 024017. https://doi.org/10.1088/1748-9326/aaa273

Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., J Souza, E., Stone, D., Wilson, R., & Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research*, *38*(6), 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

Ghaly, A. E., Ramakrishnan, V. V., Brooks, M. S., Budge, S. M., & Dave, D. (2013). Fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. *Journal of Microbial and Biochemical Technology*, *5*(4), 107–129. https://doi.org/10.4172/1948-5948.1000110

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*(6), 1871–1883. 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 strategies for aquaculture feeds. *Aquaculture Nutrition*, *13*(1), 17–34. https://doi.org/10.1111/anu.13138

Glencross, B. D., Huyben, D., & Schrama, J. W. (2020). The Application of Single-Cell Ingredients in Aquaculture Feeds—A Review. *Fishes*, *5*(3), 22. https://doi.org/10.3390/fishes5030022

Glencross, B., Evans, D., Rutherford, N., Hawkins, W., McCafferty, P., Dods, K., Jones, B., Harris, D., Morton, L., Sweetingham, M., & Sipsas, S. (2006). The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (Oncorhynchus mykiss) growth, feed utilisation and gastrointestinal histology. *Aquaculture*, *253*(1–4), 512–522. https://doi.org/10.1016/j.aquaculture.2005.07.009

Glencross, B., Fracalossi, D. M., Hua, K., Izquierdo, M., Mai, K., Øverland, M., Robb, D., Roubach, R., Schrama, J., Small, B., Tacon, A., Valente, L. M. P., Viana, M. T., Xie, S., & Yakupityage, A. (2023). Harvesting the benefits of nutritional research to address global challenges in the 21st century. In *Journal of the World Aquaculture Society* (Vol. 54, Issue 2, pp. 343–363). John Wiley and Sons Inc. https://doi.org/10.1111/jwas.12948

Gomes, E. F., Rema, P., & Kaushik, S. J. (1995). Replacement of fish meal by plant proteins in the diet of rainbow trout (Oncorhynchus mykiss): digestibility and growth performance. *Aquaculture*, *130*(2–3), 177–186. https://doi.org/10.1016/0044-8486(94)00211-6

Gregory, M. K., & James, M. J. (2014). *Rainbow trout (Oncorhynchus mykiss) Elovl5 and Elovl2 differ in selectivity for elongation of omega-3 docosapentaenoic acid.* https://doi.org/10.1016/j.bbalip.2014.10.001

Grosell, M., Farrell, A. P., & Colin J. Brauner. (2010). The multifunctional gut of fish. In *The Multifunctional Gut of Fish* (Vol. 30). https://doi.org/10.1016/S1546-5098(10)03014-1

Guerard, F., Guimas, L., & Binet, A. (2002). Production of tuna waste hydrolysates by a commercial neutral protease preparation. *Journal of Molecular Catalysis B: Enzymatic*, *19*(20), 489–498. https://doi.org/10.1016/S1381-1177(02)00203-5

Günzel, D., & Yu, A. S. L. (2013). Claudins and the Modulation of Tight Junction Permeability. *Physiological Reviews*, *93*(2), 525–569. https://doi.org/10.1152/physrev.00019.2012

Guo, H., Jiang, C., Zhang, Z., Lu, W., & Wang, H. (2021). Material flow analysis and life cycle assessment of food waste bioconversion by black soldier fly larvae (Hermetia illucens L.). *Science of the Total Environment*, *750*. https://doi.org/10.1016/j.scitotenv.2020.141656

Gustafsson, P., Bergman, E., & Greenberg, L. A. (2010). Functional response and size-dependent foraging on aquatic and terrestrial prey by brown trout (Salmo trutta L.). *Ecology of Freshwater Fish*, *19*(2), 170–177. https://doi.org/10.1111/j.1600-0633.2009.00401.x

Hamre, K., Kolås, K., Sandnes, K., Julshamn, K., & Kiessling, A. (2001). Feed intake and absorption of lipid oxidation products in Atlantic salmon (Salmo salar) fed diets coated with oxidised fish oil. *Fish Physiology and Biochemistry*, *25*(3), 209–219. https://doi.org/10.1023/A:1022257928437

Hansen, J. Ø., Hofossæter, M., Sahlmann, C., Ånestad, R., Reveco-Urzua, F. E., Press, C. M., Mydland, L. T., & Øverland, M. (2019). Effect of Candida utilis on growth and intestinal health of Atlantic salmon (Salmo salar) parr. *Aquaculture*, *511*(5006), 734239. https://doi.org/10.1016/j.aquaculture.2019.734239

Hansen, J. Ø., Lagos, L., Lei, P., Reveco-Urzua, F. E., Morales-Lange, B., Hansen, L. D., Schiavone, M., Mydland, L. T., Arntzen, M. Ø., Mercado, L., Benicio, R. T., & Øverland, M. (2021). Down-stream processing of baker's yeast (Saccharomyces cerevisiae) – Effect on nutrient digestibility and immune response in Atlantic salmon (Salmo salar). *Aquaculture*, *530*(5006), 735707. https://doi.org/10.1016/j.aquaculture.2020.735707

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. https://doi.org/10.1016/j.aquaculture.2010.10.003

Hardy, R. W. (1996). Alternate protein sources for salmon and trout diets. In *Animal Feed Science Technology* (Vol. 59).

Hardy, R. W., & Kaushik, S. J. (Eds.). (2021). Fish nutrition. Academic press.

Harper, A. E., Benevenga, N. J., & Wohlhueter, R. M. (1970). *Effects of Ingestion of Disproportionate Amounts of Amino Acids* (Vol. 60, Issue 8).

Hauptman, B. S., Barrows, F. T., Block, S. S., Gibson Gaylord, T., Paterson, J. A., Rawles, S. D., & Sealey, Wendy. 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. https://doi.org/10.1016/j.aquaculture.2014.03.026

Hedén, I., Sundell, K., Jönsson, E., & Sundh, H. (2022). The role of environmental salinity on Na+-dependent intestinal amino acid uptake in rainbow trout (Oncorhynchus mykiss). *Scientific Reports*, *12*(1), 22205. https://doi.org/10.1038/s41598-022-26904-6

Hilton, J. W., Cho, C. Y., & Slinger, S. J. (1981). Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (salmo gaird-neri r.). *Aquaculture*, *25*, 185–194.

Hua, K. (2021). A meta-analysis of the effects of replacing fish meals with insect meals on growth performance of fish. *Aquaculture*, *530*, 735732. https://doi.org/10.1016/j.aquaculture.2020.735732

Hua, K., & Bureau, D. P. (2019). *Estimating changes in essential amino acid requirements of rainbow trout and Atlantic salmon as a function of body weight or diet composition using a novel factorial requirement model*. https://doi.org/10.1016/j.aquaculture.2019.734440

Huyben, D., Sun, L., Moccia, R., Kiessling, A., Dicksved, J., & Lundh, T. (2018). Dietary live yeast and increased water temperature influence the gut microbiota of rainbow trout. *Journal of Applied Microbiology*, *124*(6), 1377–1392. https://doi.org/10.1111/jam.13738

ICAR. (2023). *Nutrient Requirement and Feed Formulation of Commercially Important Freshwater Fish Species with Special Reference to Carp*. https://www.researchgate.net/publication/369146604

Ilo, S. , S. R. , & B. E. (2000). Role of lipids in the extrusion cooking processes. *Grasas y Aceites* , *51*.

Izutsu, A., Tadokoro, D., Habara, S., Ugachi, Y., & Shimizu, M. (2022). Evaluation of circulating insulin-like growth factor (IGF)-I and IGF-binding proteins as growth indices in rainbow trout (Oncorhynchus mykiss). *General and Comparative Endocrinology*, *320*. https://doi.org/10.1016/j.ygcen.2022.114008

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., & Dayal, J. S. (2019). Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. In *Aquaculture Research* (Vol. 50, Issue 12, pp. 3493–3506). Blackwell Publishing Ltd. https://doi.org/10.1111/are.14324

Jeevan Kumar, S. P., & Banerjee, R. (2019). Enhanced lipid extraction from oleaginous yeast biomass using ultrasound assisted extraction: A greener and scalable process. *Ultrasonics Sonochemistry*, *52*, 25–32. https://doi.org/10.1016/j.ultsonch.2018.08.003

Jobling, M. (2003). The thermal growth coefficient (TGC) model of fish growth: A cautionary note. *Aquaculture Research*, *34*(7), 581–584. https://doi.org/10.1046/j.1365-2109.2003.00859.x

Johansson, M., & Björnsson, B. T. (2015). Elevated plasma leptin levels of fasted rainbow trout decrease rapidly in response to feed intake. *General and Comparative Endocrinology*, *214*, 24–29. https://doi.org/10.1016/j.ygcen.2015.02.020

Jönsson, E., Kaiya, H., & Björnsson, B. T. (2010). Ghrelin decreases food intake in juvenile rainbow trout (Oncorhynchus mykiss) through the central anorexigenic corticotropin-releasing factor system. *General and Comparative Endocrinology*, *166*(1), 39–46. https://doi.org/10.1016/j.ygcen.2009.11.001

Jørgensen, E. H., Bernier, N. J., Maule, A. G., & Vijayan, M. M. (2016). Effect of long-term fasting and a subsequent meal on mRNA abundances of hypothalamic appetite regulators, central and peripheral leptin expression and plasma leptin levels in rainbow trout. *Peptides*, *86*, 162–170. https://doi.org/10.1016/j.peptides.2015.08.010

Jørgensen, J. B., Sharp, G. J. E., Secombes, C. J., & Robertsen, B. (1993). Effect of a yeast-cell-wall glucan on the bactericidal activity of rainbow trout macrophages. *Fish and Shellfish Immunology*, *3*(4), 267–277. https://doi.org/10.1006/fsim.1993.1026

Jutfelt, F., Olsen, R. E., Björnsson, B. T., & Sundell, K. (2007). Parr-smolt transformation and dietary vegetable lipids affect intestinal nutrient uptake, barrier function and plasma cortisol levels in Atlantic salmon. *Aquaculture*, *273*(2–3), 298–311. https://doi.org/10.1016/J.AQUACULTURE.2007.10.012

Kaiya, H., Miyazato, M., & Kangawa, K. (2011). Recent advances in the phylogenetic study of ghrelin. In *Peptides* (Vol. 32, Issue 11, pp. 2155–2174). https://doi.org/10.1016/j.peptides.2011.04.027

Karasov, W. H. (2017). Integrative physiology of transcellular and paracellular intestinal absorption. *Journal of Experimental Biology*, *220*(14), 2495–2501. https://doi.org/10.1242/jeb.144048

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

Kaur, N., Chugh, V., & Gupta, A. K. (2014). Essential fatty acids as functional components of foods- a review. *Journal of Food Science and Technology*, *51*(10), 2289–2303. https://doi.org/10.1007/s13197-012-0677-0

Kling, P., Rønnestad, I., Stefansson, S. O., Murashita, K., Kurokawa, T., & Björnsson, B. T. (2009). A homologous salmonid leptin radioimmunoassay indicates elevated plasma leptin levels during fasting of rainbow trout. *General and Comparative Endocrinology*, *162*(3), 307–312. https://doi.org/10.1016/j.ygcen.2009.04.003

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., & Frøkiær, H. (2008). Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (Salmo salar L.). *British Journal of Nutrition*, *100*(1), 120–129. https://doi.org/10.1017/S0007114507886338

Kobayashi, Y., El-Wali, M., Guðmundsson, H., Guðmundsdóttir, E. E., Friðjónsson, Ó. H., Karlsson, E. N., Roitto, M., & Tuomisto, H. L. (2023). *Life-cycle assessment of yeast-based single-cell protein production with oat processing side-stream*. https://doi.org/10.1016/j.scitotenv.2023.162318

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*(6762), 656–660. https://doi.org/10.1038/45230

Kousoulaki, K., Rønnestad, I., Rathore, R., Sixten, H. J., Campbell, P., Nordrum, S., Berge, R. K., & Albrektsen, S. (2018). Physiological responses of Atlantic salmon (Salmo salar L.) fed very low (3%) fishmeal diets supplemented with feeding-modulating crystalline amino acid mixes as identified in krill hydrolysate. *Aquaculture*, *486*(May 2017), 184–196. https://doi.org/10.1016/j.aquaculture.2017.12.011

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., & Bakke, A. M. (2010). Important antinutrients in plant feedstuffs for aquaculture: An update on recent findings regarding responses in salmonids. *Aquaculture Research*, *41*(3), 333–344. https://doi.org/10.1111/j.1365-2109.2009.02426.x

Langeland, M., Ziegler, F., & Wocken, Y. (2023). *Greenhouse gas emissions of rainbow trout fed conventional and novel feeds from Baltic region, evaluated using Life Cycle Assessment*.

Liland, N. S., Araujo, P., Xu, X. X., Lock, E. J., Radhakrishnan, G., Prabhu, A. J. P., & Belghit, I. (2021). A meta-analysis on the nutritional value of insects in aquafeeds. *Journal of Insects as Food and Feed*, *7*(5), 743–759. https://doi.org/10.3920/JIFF2020.0147

Lin, S., Hsieh, F., & Huff, H. E. (1997). Effects of Lipids and Processing Conditions on Degree of Starch Gelatinization of Extruded Dry Pet Food. *LWT - Food Science and Technology*, *30*(7), 754–761. https://doi.org/10.1006/fstl.1997.0271

Lock, E. R., Arsiwalla, T., & Waagbø, R. (2016). Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (Salmo salar) postsmolt. *Aquaculture Nutrition*, *22*(6), 1202–1213. https://doi.org/10.1111/anu.12343

Lund, I., Dalsgaard, J., Jacobsen, C., Hansen, J. H., Holm, J., & Jokumsen, A. (2013). Effects of organic plant oils and role of oxidation on nutrient utilization in juvenile rainbow trout (Oncorhynchus mykiss). *Animal*, *7*(3). https://doi.org/10.1017/S1751731112001693

Luo, D., & Mckeownt, B. A. (1991). The effect of thyroid hormone and glucocorticoids on carp growth hormone-releasing factor (grf)-induced growth hormone (gh) release in rainbow trout (*Oncorhynchus mykiss*). in *biochem. physiol* (vol. 99, issue 4).

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

Mahnken, C., Spinelli, J., & Waknitz, F. (1980). Evaluation of an alkane yeast (Candida sp.) as a substitute for fish meal in Ore- gon Moist Pellet: feeding trials with coho salmon (Oncor- hynchus kisutch) and rainbow trout (Salmo gairdneri). *Aquaculture*, *20*, 41–56.

Maiolo, S., Parisi, G., Biondi, N., Lunelli, F., Tibaldi, E., & Pastres, R. (2020). Fishmeal partial substitution within aquafeed formulations: life cycle assessment of four alternative protein sources. *International Journal of Life Cycle Assessment*, *25*(8), 1455–1471. https://doi.org/10.1007/s11367-020-01759-z

Marui, T., Evans, R. E., Zielinski, B., & Hara, T. J. (1983). Gustatory responses of the rainbow trout (Salmo gMrdneri) palate to amino acids and derivatives. In *Journal of Comparative Physiology. A* (Vol. 153). Springer-Verlag.

McLeese, J. M., & Moon, T. W. (1989). Seasonal changes in the intestinal mucosa of winter flounder, Pseudopleuronectes americanus (Walbaum), from Passamaquoddy Bay, New Brunswick. *Journal of Fish Biology*, *35*(3), 381–393. https://doi.org/10.1111/j.1095-8649.1989.tb02990.x

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., & Mukherjee, S. C. (2013). Beta-glucan: An ideal immunostimulant in aquaculture (a review). *Fish Physiology and Biochemistry*, *39*(3), 431–457. https://doi.org/10.1007/s10695-012-9710-5

Melenchón, F., Larrán, A. M., de Mercado, E., Hidalgo, M. C., Cardenete, G., Barroso, F. G., Fabrikov, D., Lourenço, H. M., Pessoa, M. F., & Tomás-Almenar, C. (2021).

Potential use of black soldier fly (Hermetia illucens) and mealworm (Tenebrio molitor) insectmeals in diets for rainbow trout (Oncorhynchus mykiss). *Aquaculture Nutrition*, *27*(2), 491–505. https://doi.org/10.1111/ANU.13201

Miles, R. D., & Chapman, F. (2005). The Benefits of Fish Meal in Aquaculture Diets. *University of Florida IFAS Extension*, *May 2006*, 1–6. http://edis.ifas.ufl.edu/pdffiles/FA/FA12200.pdf

Moeser, A. J., Klok, C. Vander, Ryan, K. A., Wooten, J. G., Little, D., Cook, V. L., & Blikslager, A. T. (2007). Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. *Am J Physiol Gastrointest Liver Physiol*, *292*, 173–181. https://doi.org/10.1152/ajpgi.00197.2006.-Weaning

Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., & Matsukura, S. (2001). A role for ghrelin in the central regulation of feeding. *Nature 2001 409:6817*, *409*(6817), 194–198. https://doi.org/10.1038/35051587

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. *Proceedings of the National Academy of Sciences*, *106*(36), 15103–15110. https://doi.org/10.1073/pnas.0905235106

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(7851), 551–563. https://doi.org/10.1038/s41586-021-03308-6

Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewicz, J., Dudek, K., Rawski, M., Kierończyk, B., & Józefiak, A. (2019). Insect meals in fish nutrition. *Reviews in Aquaculture*, *11*(4), 1080–1103. https://doi.org/10.1111/raq.12281

Olsen, R. E., Suontama, J., Langmyhr, E., Mundheim, H., Ringø, E., Melle, W., Malde, M. K., & Hemre, G. I. (2006). The replacement of fish meal with Antarctic krill, Euphausia superba in diets for Atlantic salmon, Salmo salar. *Aquaculture Nutrition*, *12*(4), 280–290. https://doi.org/10.1111/j.1365-2095.2006.00400.x

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*, *402–403*, 1–7. https://doi.org/10.1016/j.aquaculture.2013.03.016

Pelletier, N., & Tyedmers, P. (2007). Feeding farmed salmon: Is organic better? *Aquaculture*, *272*(1–4), 399–416. https://doi.org/10.1016/j.aquaculture.2007.06.024

Pelletier, N., Tyedmers, P., Sonesson, U., Scholz, A., Ziegler, F., Flysjo, A., Kruse, S., Cancino, B., & Silverman, H. (2009). Not All Salmon Are Created Equal: Life Cycle Assessment (LCA) of Global Salmon Farming Systems. *Environmental Science & Technology*, *43*(23), 8730–8736. https://doi.org/10.1021/es9010114

Perry, B., & Wang, Y. (2012). Appetite regulation and weight control: the role of gut hormones. *Nutrition and Diabetes*, *2*, 26. https://doi.org/10.1038/nutd.2011.21

Philis, G., Ziegler, F., Gansel, L. C., Jansen, M. D., Gracey, E. O., & Stene, A. (2019). Comparing life cycle assessment (LCA) of salmonid aquaculture production systems: Status and perspectives. In *Sustainability (Switzerland)* (Vol. 11, Issue 9). MDPI. https://doi.org/10.3390/su11092517

Pollard, T. D., Earnshaw, W. C., Lippincott-Schwartz, J., & Johnson, G. (2022). Cell biology E-book. Elsevier Health Sciences.

Potts, W., Talbot, C., Eddy, F., Primmett, D., Prunet, P., Williams, M., W Potts, W. T., Eddy, F. B., & Prunett, P. (1989). Sodium balance in adult atlantic salmon (Salmo salar L.) during migration into neutral and acid fresh water. *Biochem. Physiol*, *92*(2), 247–253. https://doi.org/10.1016/0300

Refstie, S., Baeverfjord, G., Seim, R. R., & Elvebø, O. (2010). Effects of dietary yeast cell wall β-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (Salmo salar) fed sunflower and soybean meal. *Aquaculture*, *305*(1–4), 109–116. https://doi.org/10.1016/j.aquaculture.2010.04.005

Reindl, K. M., Kittilson, J. D., Bergan, H. E., & Sheridan, M. A. (2011). Growth hormone-stimulated insulin-like growth factor-1 expression in rainbow trout (oncorhynchus mykiss) hepatocytes is mediated by ERK, PI3K-AKT, and JAK-STAT. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *301*(1), 236–243. https://doi.org/10.1152/AJPREGU.00414.2010

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M. T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I., & Gasco, L. (2017). Evaluation of the suitability of a partially defatted black soldier fly (Hermetia illucens L.) larvae meal as ingredient for rainbow trout (Oncorhynchus mykiss Walbaum) diets. *Journal of Animal Science and Biotechnology*, *8*(1). https://doi.org/10.1186/s40104-017-0191-3

Rønnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jönsson, E., & Volkoff, H. (2017). Appetite-controlling endocrine systems in teleosts. *Frontiers in Endocrinology*, *8*(APR), 1–24. https://doi.org/10.3389/fendo.2017.00073

Roy, J., Mercier, Y., Tonnet, L., Burel, C., Lanuque, A., Surget, A., Larroquet, L., Corraze, G., Terrier, F., Panserat, S., & Skiba, S. (2020). Rainbow trout prefer diets rich in omega-3 long chain polyunsaturated fatty acids DHA and EPA. *Physiology and Behavior*, *213*. https://doi.org/10.1016/j.physbeh.2019.112692

Rumsey, G. L., Hughes, S. G., & Kinsella, J. L. (1990). Use of Dietary Yeast Saccharomyces cerevisiae Nitrogen by Lake Trout. *Journal of the World Aquaculture Society*, *21*(3), 205–209. https://doi.org/10.1111/j.1749-7345.1990.tb01024.x

Rumsey, G. L., Hughes, S. G., Smith, R. R., Kinsella, J. E., & Shetty, K. J. (1991). Digestibility and energy values of intact, disrupted and extracts from brewer's dried yeast fed to rainbow trout (Oncorhynchus mykiss). *Animal Feed Science and Technology*, *33*(3–4), 185–193. https://doi.org/10.1016/0377-8401(91)90059-2

Sandström, V., Chrysafi, A., Lamminen, M., Troell, M., Jalava, M., Piipponen, J., Siebert, S., van Hal, O., Virkki, V., & Kummu, M. (2022). Food system by-products upcycled in livestock and aquaculture feeds can increase global food supply. *Nature Food*, *3*(9), 729–740. https://doi.org/10.1038/s43016-022-00589-6

Santos, J., Benjamin, M., Yang, P.-C., Prior, T., & Perdue, M. H. (2000). *Chronic stress impairs rat growth and jejunal epithelial barrier function: role of mast cells*. http://www.ajpgi.org

Santos, J., Yang, P. C., Söderholm, J. D., Benjamin, M., & Perdue, M. H. (2001). Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. *Gut*, *48*(5), 630–636. https://doi.org/10.1136/gut.48.5.630

Saunders, P. R., Santos, J., Hanssen, N. P. M., Yates, D., Groot, J. A., & Perdue, M. H. (2002). *Physical and Psychological Stress in Rats Enhances Colonic Epithelial Permeability via Peripheral CRH*.

Schrama, J. W., Haidar, M. N., Geurden, I., Heinsbroek, L. T. N., & Kaushik, S. J. (2018). Energy efficiency of digestible protein, fat and carbohydrate utilisation for growth in rainbow trout and Nile tilapia. *British Journal of Nutrition*, *119*, 782–791. https://doi.org/10.1017/S0007114518000259

Sealey, W. M., Barrows, F. T., Hang, A., Johansen, K. A., Overturf, K., LaPatra, S. E., & Hardy, R. W. (2008). Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout Oncorhynchus mykiss. *Animal Feed Science and Technology*, *141*(1–2), 115–128. https://doi.org/10.1016/j.anifeedsci.2007.05.022

Sealey, W. M., Gaylord, T. G., Barrows, F. T., Tomberlin, J. K., McGuire, M. A., Ross, C., & St-Hilaire, S. (2011). Sensory Analysis of Rainbow Trout, Oncorhynchus mykiss, Fed Enriched Black Soldier Fly Prepupae, Hermetia illucens. *Journal of the World Aquaculture Society*, *42*(1), 34–45. https://doi.org/10.1111/j.1749-7345.2010.00441.x

Shepherd, C. J., & Jackson, A. J. (2013). Global fishmeal and fish-oil supply: inputs, outputs and markets a. *Journal of Fish Biology*, *83*, 1046–1066. https://doi.org/10.1111/jfb.12224

Sheridan, M. A. (2021). Coordinate regulation of feeding, metabolism, and growth: Perspectives from studies in fish. *General and Comparative Endocrinology*, *312*. https://doi.org/10.1016/j.ygcen.2021.113873

Smith, S. B., Gotoh, T., & Greenwood, P. L. (2018). Current situation and future prospects for global beef production: Overview of special issue. In *Asian-Australasian Journal of Animal Sciences* (Vol. 31, Issue 7, pp. 927–932). Asian-Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.18.0405

Soengas, J. L., Cerdá-Reverter, J. M., & Delgado, M. J. (2018). Central regulation of food intake in fish: An evolutionary perspective. *Journal of Molecular Endocrinology*, *60*(4), R171–R199. https://doi.org/10.1530/JME-17-0320

Sørensen, M. (2012). A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. In *Aquaculture Nutrition* (Vol. 18, Issue 3, pp. 233–248). https://doi.org/10.1111/j.1365-2095.2011.00924.x

Stefanovic, I. L. (2000). Safeguarding our common future: Rethinking sustainable development. Suny Press.

Stevens, J. R., Newton, R. W., Tlusty, M., & Little, D. C. (2018). The rise of aquaculture by-products: Increasing food production, value, and sustainability through strategic utilisation. *Marine Policy*, *90*, 115–124. https://doi.org/10.1016/j.marpol.2017.12.027

St-Hilaire, S., Sheppard, C., Tomberlin, J. K., Irving, S., Newton, L., McGuire, M. A., Mosley, E. E., Hardy, R. W., & Sealey, W. (2007). Fly Prepupae as a Feedstuff for Rainbow Trout, Oncorhynchus mykiss. *Journal of the World Aquaculture Society*, *38*(1), 59–67. https://doi.org/10.1111/j.1749-7345.2006.00073.x

Sugiura, S. H., Dong, F. M., Rathbone, C. K., & Hardy, R. W. (1998). Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture*, *159*(3–4), 177–202. https://doi.org/10.1016/S0044-8486(97)00177-4

Sundell, K., Berge, G. M., Ruyter, B., & Sundh, H. (2022). Low Omega-3 Levels in the Diet Disturbs Intestinal Barrier and Transporting Functions of Atlantic Salmon Freshwater and Seawater Smolts. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.883621

Sundell, K., Jutfelt, F., Ágústsson, T., Olsen, R.-E., Sandblom, E., Hansen, T., & Björnsson, B. T. (2003). Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr–smolt transformation of Atlantic salmon, Salmo salar. *Aquaculture*, *222*(1–4), 265–285. https://doi.org/10.1016/S0044-8486(03)00127-3

Sundell, K. S., & Rønnestad, I. (2011). Integrated function and control of the gut | intestinal absorption. In *Encyclopedia of Fish Physiology* (Vol. 2, pp. 1311–1321). Elsevier. https://doi.org/10.1016/B978-0-12-374553-8.00072-1

Sundell, K., & Sundh, H. (2012). Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. *Frontiers in Physiology*, *3*(September), 1–13. https://doi.org/10.3389/fphys.2012.00388

Sundh, H., Calabrese, S., Jutfelt, F., Niklasson, L., Olsen, R. E., & Sundell, K. (2011). Translocation of infectious pancreatic necrosis virus across the intestinal epithelium of Atlantic salmon (Salmo salar L.). *Aquaculture*, *321*(1–2), 85–92. https://doi.org/10.1016/j.aquaculture.2011.08.011

Sundh, H., Kvamme, B. O., Fridell, F., Olsen, R. E., Ellis, T., Taranger, G. L., & Sundell, K. (2010). Intestinal barrier function of Atlantic salmon (Salmo salar L.) post smolts is reduced by common sea cage environments and suggested as a possible physiological welfare indicator. *BMC Physiology*, *10*(1). https://doi.org/10.1186/1472-6793-10-22

Tacon, A. G. J. (1996). Lipid nutritional pathology in farmed fish. *Archives of Animal Nutrition*, *49*(1), 33–39. https://doi.org/10.1080/17450399609381861

Tacon, A. G. J., & Metian, M. (2015). Feed matters: Satisfying the feed demand of aquaculture. *Reviews in Fisheries Science and Aquaculture*, *23*(1), 1–10. https://doi.org/10.1080/23308249.2014.987209

Tanaka, Y., Tanioka, S.-I., Tanaka, M., Tanigawa, T., Kitamura+, Y., Minamis, S., Okamoto3, Y., Miyashitas, M., & Nanno, M. (1997). Effects of chitin and on BALB/c mice by parenteral administration chitosan particles oral and. In *Biomoterials* (Vol. 18, Issue 8).

Tocher, D. R. (2015). Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture*, *449*, 94–107. https://doi.org/10.1016/j.aquaculture.2015.01.010

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

Triantaphyllopoulos, K. A., Cartas, D., & Miliou, H. (2020). Factors influencing GH and IGF-I gene expression on growth in teleost fish: how can aquaculture industry benefit? *Reviews in Aquaculture*, *12*(3), 1637–1662. https://doi.org/10.1111/RAQ.12402

Troell, M., Costa-Pierce, B., Stead, S., Cottrell, R. S., Brugere, C., Farmery, A. K., Little, D. C., Strand, Å., Pullin, R., Soto, D., Beveridge, M., Salie, K., Dresdner, J., Moraes-Valenti, P., Blanchard, J., James, P., Yossa, R., Allison, E., Devaney, C., & Barg, U. (2023). Perspectives on aquaculture's contribution to the Sustainable Development Goals for improved human and planetary health. In *Journal of the World Aquaculture Society* (Vol. 54, Issue 2, pp. 251–342). John Wiley and Sons Inc. https://doi.org/10.1111/jwas.12946

Turchini, G. M., Torstensen, B. E., & Ng, W.-K. (2009). Fish oil replacement in finfish nutrition. *Reviews in Aquaculture*, *1*(1), 10–57. https://doi.org/10.1111/j.1753-5131.2008.01001.x

Turchini, G. M., Trushenski, J. T., & Glencross, B. D. (2019). Thoughts for the Future of Aquaculture Nutrition: Realigning Perspectives to Reflect Contemporary Issues Related to Judicious Use of Marine Resources in Aquafeeds. *North American Journal of Aquaculture*, *81*(1), 13–39. https://doi.org/10.1002/naaq.10067

UN, Assembly, G. (2015). Sustainable development goals. SDGs Transform Our World, 2030, 6-28.

Ussing, H. H. (1950). *Active Trnrisport of Sodiaiii as the Source of ' Electric Current in the Short-circuited Isolilted Frog Skin* .

Velasco, C., Librán-Pérez, M., Otero-Rodiño, C., López-Patiño, M. A., Míguez, J. M., Cerdá-Reverter, J. M., & Soengas, J. L. (2016). Ghrelin modulates hypothalamic fatty acid-sensing and control of food intake in rainbow trout. *Journal of Endocrinology*, *228*(1), 25–37. https://doi.org/10.1530/JOE-15-0391

Velin, Å. K., Ericson, A. C., Braaf, Y., Wallon, C., & Söderholm, J. D. (2004). Increased antigen and bacterial uptake in follicle associated epithelium induced by chronic psychological stress in rats. *Gut*, *53*(4), 494–500. https://doi.org/10.1136/gut.2003.028506

Vidakovic, A., Huyben, D., Sundh, H., Nyman, A., Vielma, J., Passoth, V., Kiessling, A., & 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. https://doi.org/10.1111/anu.12988

Viedma-Poyatos, Á., González-Jiménez, P., Langlois, O., Company-Marín, I., Spickett, C. M., & Pérez-Sala, D. (2021). Protein lipoxidation: Basic concepts and emerging roles. In *Antioxidants* (Vol. 10, Issue 2, pp. 1–28). MDPI. https://doi.org/10.3390/antiox10020295

Volkoff, H., Canosa, L. F., Unniappan, S., Cerdá-Reverter, J. M., Bernier, N. J., Kelly, S. P., & Peter, R. E. (2005). Neuropeptides and the control of food intake in fish.

General and Comparative Endocrinology, *142*(1-2 SPEC. ISS.), 3–19. https://doi.org/10.1016/j.ygcen.2004.11.001

Wang, H., Ma, S., Yang, J., Qin, Y., Cheng, H., Xue, M., Li, J., & Li, J. (2021). Optimization of the process parameters for extruded commercial sinking fish feed with mixed plant protein sources. *Journal of Food Process Engineering*, *44*(1). https://doi.org/10.1111/jfpe.13599

Ween, O., Stangeland, J. K., Fylling, T. S., & Aas, G. H. (2017). Nutritional and functional properties of fishmeal produced from fresh by-products of cod (Gadus morhua L.) and saithe (Pollachius virens). *Heliyon*, *3*(7). https://doi.org/10.1016/J.HELIYON.2017.E00343

Welker, T. L., Overturf, K., Snyder, S., Liu, K., Abernathy, J., Frost, J., & Barrows, F. T. (2018). *Effects of feed processing method (extrusion and expansion-compression pelleting) on water quality and growth of rainbow trout in a commercial setting*. https://doi.org/10.1080/10454438.2018.1433095

Weththasinghe, P., Hansen, J. Ø., Mydland, L. T., & Øverland, M. (2022). A systematic meta-analysis based review on black soldier fly (Hermetia illucens) as a novel protein source for salmonids. *Reviews in Aquaculture*, *14*(2), 938–956. https://doi.org/10.1111/RAQ.12635

WOAH. https://www.woah.org/en/home/, accessed Oct. 2023

Wu, H., Forghani, B., Abdollahi, M., & Undeland, I. (2022). Lipid oxidation in sorted herring (Clupea harengus) filleting co-products from two seasons and its relationship to composition. *Food Chemistry*, *373*, 131523. https://doi.org/10.1016/j.foodchem.2021.131523

Yamashita, S., Yamada, T., & D T J Hara, A. N. (2006). Gustatory responses to feeding- and non-feeding-stimulant chemicals, with an emphasis on amino acids, in rainbow trout. *Journal of Fish Biology*, *68*, 783–800. https://doi.org/10.1111/j.1095-8649.2006.00965.x

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. https://doi.org/10.1016/j.aquaculture.2015.06.023

Yu, Y., Wang, Q., Huang, Z., Ding, L., & Xu, Z. (2020). Immunoglobulins, Mucosal Immunity and Vaccination in Teleost Fish. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.567941

UNPUBLISHED ARTICLES NOT INCLUDED IN THIS THESIS

Andersson. M., Stedt, K., Steinhagen, S., Roques, J. A.C., Warwas N. Doyle, D., Hinchcliffe J., Hedén, I., Sundh, H., Pavia, H., Sundell, K. Co-cultivation of rainbow trout and sea lettuce in a recirculating aquaculture system

Andersson. M., Warwas. N., Hinchcliffe. J., Johansson. E., Roques. J.A.C., Doyle. D., Hedén. I., Sundh. H., Pavia. H., Sundell. K. Ulva as protein source in diets for in rainbow trout – effects on digestibility, appetite and immune responses.

Hedén, I., Roques, J.A.C., Andersson, M., Doyle, D., Warwas, N., de Fosenka, R., Hinchcliffe, J., Jönsson, E., Sundell, K., and Sundh, H. Acute stress response in Atlantic wolffish (Anarhichas lupus). Manuscript

Hedén, I., Hinchcliffe, J., Roques, J.A.C., Warwas, N., Langeland, M., Jönsson, E., Sundell, K., and Sundh, H. Intestinal morphology, transport and barrier function, and digestive capacity in Atlantic wolffish (*Anarhichias lupus*). Manuscript

Hinchcliffe. J., Roques. J. A. C., Warwas. N., Heden. I., Langeland. M., Sundh. H., Björnsson B. T., Jönsson. E., and Sundell. K., Effect of mussel meal based diets on growth and health of Atlantic wolffish (*Anarhichas lupus*)

König Kardgar, A., Doyle, D., Warwas, N., Hjelleset, T., Sundh, H., Carney Almroth, B., Microplastics in aquaculture - potential impacts on inflammatory processes in Nile tilapia