Exploring the use of blue mussel silage and fermented sugar kelp as low trophic marine resources in the Atlantic salmon (*Salmo salar* L.) diet

Sahar Sartipiyarahmadi

Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

This PhD project started in January 2021 as a collaboration between the Department of Biology at the University of Bergen (UiB) and the research group feed and nutrition at the Institute of Marine Research in Bergen (IMR). The work for this doctoral thesis was performed under the supervision of Dr. Sofie Charlotte Remø (IMR), Dr. Øivind Strand (IMR), Dr. Martin Wiech (IMR), Dr. Erik-Jan Lock (IMR, UiB), and Dr. Nina Liland (IMR, UiB) at the Institute of Marine Research, Bergen, Norway.

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-Sahar Sartipiyarahmadi-

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Abstract in English

The aquaculture sector is rapidly growing and is expected to provide more food from the ocean to the global food supply. However, sustainable growth in fish farming relies on identifying novel feed ingredients that have the potential for upscaling and cultivation and contribute to minimizing the carbon footprint. At the same time novel ingredients should meet nutritional requirements and ensure the health and welfare of fish. Lower trophic marine species, such as blue mussels (*Mytilus edulis*) and sugar kelp (*Saccharina latissima*) are good candidates with minimal carbon footprint, no reliance on fertilizer or freshwater. The protein content as well as the similarity in amino acid profiles between blue mussels and fish meal makes blue mussel a potential protein source in fish feed. Sugar kelp in turn contains several bioactive compounds with possible functional properties. However, sugar kelp has a high content of complex indigestible carbohydrates, ash and iodine along with limited protein and lipids, which may limit the inclusion levels in feed for Atlantic salmon. Moreover, seasonal harvest, differences in nutritional composition, and short shelf life of the raw materials after harvest necessitate the use of suitable preservation and processing methods to use these as raw materials in fish feed. The main aim of this thesis was to investigate the use of blue mussel silage (BMS) and fermented sugar kelp (FSK) as novel feed ingredients in the diet of post-smolt Atlantic salmon.

In the first feeding trial, different inclusion levels of BMS in the feed were investigated. Atlantic salmon post-smolts were fed three levels of BMS, up to 11% of the feed, as well as one additional diet containing 12% blue mussel meal (BMM). The fish given the diets containing BMS had a dose-dependent decrease in weight gain (WG) and increased feed conversion ratio (FCR), despite similar daily feed intake and nutrient digestibility between experimental groups and the control group. Additionally, a lower iron status was found in fish fed the BMS diets. The fish given BMM had comparable growth and iron status as the fish given the reference feed. Including up to 11% BMS in the diet did not influence the occurrence of production-related disorders or oxidative stress in the fish. These results were followed up in a second feeding study using two new productions of BMS with a lower level of formic acid, with and without

antioxidants. In addition, parts of the first experiment were repeated using one diet containing the same batch of BMS used in experiment 1 as well as one diet including BMM. All diets had an inclusion level of 9% blue mussels. Contrary to the first experiment, the feed utilization and growth rates in the second study were comparable, and the previously seen iron depletion was not present. The differences between the results in the two feeding trials were attributed to the drying methods used before feed production.

A dose-response study was also conducted to investigate the inclusion of FSK in the diet for Atlantic salmon post-smolts. Fish fed diets containing up to 4% FSK had a dose-dependent decrease in growth, however, feed intake, FCR and protein digestibility were comparable between the control and FSK groups. Decreased growth was attributed to dietary energy dilution caused by high inclusion levels of FSK with low DM content. Including up to 3% FSK increased iodine levels in the whole body and fillet of the fish. No production related disorders were observed in fish fed up to 4% FSK in the diet. Minor changes in gut morphology of FSK-fed fish were observed, however, it remained comparable with the control group. No signs of inflammation in the intestine were observed in FSK-fed fish compared to the control. The hepatic antioxidant defense system was modulated by FSK inclusion, increasing glutathione (GSH) levels, GSH/GSSG ratio, and decreasing malondialdehyde (MDA) levels in the liver. Additionally, innate immune responses in fish fed FSK diets were modulated, including changes in lysozyme, bactericidal activity, anti-protease, and peroxidase activities.

In conclusion, BMS can be used in the salmon diet without reducing growth, feed utilization and welfare of fish. However, the methods chosen for processing and preservation, particularly drying techniques to increase DM content, can impact the bioavailability of iron, feed utilization and growth. Inclusion of up to 4% FSK may dilute dietary energy level and lead to reduced growth. However, it does not affect feed utilization or welfare. FSK could be regarded as a natural source of iodine, as well as a natural antioxidant and immunostimulant. However, further research is required to confirm these findings.

The results from this project show the potential of feed raw materials grown in the sea, which may have implications both for the further development of sustainable aquaculture in Norway, and with relevance for the global aquaculture of both feed raw materials and fish.

Abstract in Norwegian

Havbrukssektoren er i rask vekst og er forventa å bidra med meir mat frå havet til den globale matforsyninga. Berekraftig vekst i fiskeoppdrett er avhengig av å finne nye fôringrediensar som har potensiale for oppskalering og dyrking, og som bidreg til å minimere karbonavtrykket. Samtidig skal nye ingrediensar oppfylle ernæringsmessige krav og gje god fiskehelse og velferd. Lågare trofiske marine artar, som blåskjel (Mytilus edulis) og sukkertare (Saccharina latissima) er gode kandidatar som nye fôrråvarer då dei i utgangspunktet har minimalt karbonavtrykk, ikkje er avhengig av gjødsling eller ferskvatn. Innhaldet av protein i blåskjel, i tillegg til likheiter i aminosyreprofilen mellom blåskjel og fiskemjøl, gjer blåskjel til ei mogleg proteinkjelde i fiskefôr. Sukkertare inneheld til gjengjeld fleire bioaktive stoff som kan ha funksjonelle eigenskapar. Sukkertare har i tillegg høgt innhald av komplekse ufordøyelege karbohydrat, oske og jod saman med avgrensa protein- og feittinnhald, noko som kan avgrense kor mykje som kan brukast i fôret til laks. I tillegg må det takast omsyn til at det er sesongmessig hausting, ulik samansetjing av næringsstoff og kort haldbarheit av desse råvarene som gjer at det trengs eigna metoder for konservering og prosessering for at dei skal kunne brukast i fiskefôr. Hovudmålet med denne oppgåva var å undersøke bruken av blåskjelensilasje (BMS) og fermentert sukkertare (FSK) som nye fôringrediensar i fôret til oppdrettslaks.

I det første fôringsforsøket blei ulike nivå av BMS i fôret undersøkt. I dette forsøket blei laks gitt fôr med tre nivå av BMS, opptil 11% av fôret, samt eit ekstra fôr som inneheldt 12% blåskjelmjøl (BMM). Fisken som blei gitt diettane som inneheldt BMS hadde ein doseavhengig reduksjon i vektauke og auka fôrfaktor (FCR), til tross for likt fôropptak og fordøyelegheit av fôret i forsøksgruppene og kontrollgruppa. I tillegg blei det funne at fiskane som hadde fått BMS hadde låg jernstatus. Fisken som blei gitt BMM hadde derimot ein samanliknbar vekst og jernstatus som fisken som blei gitt referansefôret. Bruk av BMS i fôret førte ikkje til utvikling av produksjonsrelaterte lidingar eller oksidativt stress hos fisken. Desse resultata blei fylgt opp i eit nytt fôringsforsøk med to nye produksjonar av BMS med eit lågare nivå av maursyre, med og utan antioksidantar. I tillegg blei delar av det første eksperimentet gjentatt ved å

bruke eit fôr som inneheldt same BMS som blei brukt i det første eksperimentet, samt eit fôr som inneheldt blåskjelmjøl. I dette forsøket blei det brukt 9% blåskjel i alle fôra. I motsetning til det første forsøket var fôrutnyttinga og vekstratane i det andre forsøket like mellom alle forsøksgruppene, og i dette forsøket var det ikkje funne effektar på jernstatus. Skilnadene mellom resultata i dei to fôringsforsøka blei tilskrive tørkemetodene som blei brukt før fôrproduksjonen.

Eit dose-respons studie blei også utført for å undersøke bruk av ulike nivå FSK i fôret til laks. Fisk gitt fôr som inneheldt opptil 4 % FSK hadde ein doseavhengig reduksjon i vekst, medan fôrinntak, FCR og proteinfordøyelegheit var samanliknbare mellom fisken gitt kontroll og for med FSK. Redusert vekst var truleg forårsaka av energifortynning av fôret ved høge nivå av FSK. FSK i fôret førte til ei auke i jodnivå i helikropp og filet. Ingen produksjonsrelaterte lidingar blei observert hos fisk fôra opp til 4 % FSK i dietten. Mindre endringar i tarmmorfologien blei observert i fiskane gitt fôr med FSK, men den var likevel samanliknbar med kontrollgruppa. Det blei ikkje observert teikn til betennelsar i tarmen til fisken gitt FSK i fôret. Antioksidantforsvaret blei påverka av FSK, med auka konsentrasjon av glutation (GSH), GSH/GSSG forhold og lågare malondialdehyd (MDA) i lever. I tillegg blei det funne at bruk av FSK i fôret kunne påverke det medfødde immunforsvaret.

Oppsummert blei det vist at blåskjelensilasje kan brukast i fôr til laks utan å redusere vekst, fôrutnytting og velferd. Men metodane som er valt for prosessering og konservering av blåskjel, særskilt tørketeknikkar, ser ut til å påverke utnytting av næringsstoff og vekst. For fermentert sukkertare blei det vist at bruk av opptil 4 % i fôret kan føre til lågare energinivå og redusert vekst. Det påverkar derimot ikkje fôrutnyttinga eller velferda til fisken. FSK kan betraktast som ei naturleg kjelde til jod, og truleg ein naturleg antioksidant, samt påverke immunresponsen. Det er derimot nødvendig med ytterlegare forsking for å bekrefte desse funna. Resultata frå dette prosjektet viser potensialet i fôrråvarer dyrka i havet, noko som kan ha betydning både for vidare utvikling av berekraftig akvakultur i Norge, og med relevans for global akvakultur av både fôrråvarer og fisk.

List of publications

Paper I

Sartipiyarahmadi, S., Philip, A. J. P., Forshei, A. N., Sveier, H., Steinsund, S., Kleppe. M., Lock, E. J., Madaro, A., Hansen, T. J., Wiech, M., Strand, Ø., Jakobsen., & Remø, S. C. (2024). Blue mussel (*Mytilus edulis*) silage, a possible low trophic marine protein source for Atlantic salmon (*Salmo salar* L.). *Aquaculture, 2024*.

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Paper II

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Paper III

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Manuscript

Abbreviations

ADC	Apparent digestibility coefficient
ANFs	Antinutritional factors
BM	Blue mussel
BMM	Blue mussel meal
BMS	Blue mussel silage
BSF	Black soldier fly meal
CGM	Corn gluten meal
DHA	Docosahexaenoic acid
DM	Dry matter
DRI	Daily iodine intake
EPA	Eicosapentaenoic acid
EAAs	Essential amino acids
FCR	Feed conversion ratio
FM	Fish meal
FO	Fish oil
FPH	Fish protein hydrolysates
FSK	Fermented sugar kelp
GHG	Greenhouse gas emissions
GSH	Reduced glutathione
GSSG	Oxidized glutathione
LAB	Lactic acid bacteria
LC- PUFA	Polyunsaturated long chained fatty acid
LCA	Life cycle assessment
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acid
NCP	Non-cellulose polysaccharide
NDF	Neutral detergent fiber
NSP	Non-starch polysaccharide
PER	Protein efficiency ratio
PUFA	Polyunsaturated fatty acid
SBM	Soybean meal
SDGs	Sustainable development goals
SFA	Saturated fatty acid
SGR	Specific growth rate
SK	Sugar kelp
SPC	Soy protein concentrate
TBARs	Thiobarbituric acid reactive substances
WG	Weight gain
WHO	World health organization
WW	Wet weight

1 Introduction

1.1 General introduction

Aquaculture production has reached record levels globally and is expected to play an increasingly crucial role in meeting food and nutritional needs in the future (FAO, 2022). In 2020, aquaculture accounted for a record 49.2 percent of global aquatic animal production, marking a 3% rise from 2018 (FAO, 2022). Aquaculture growth continued across all regions except Africa in 2020, with notable expansion in Chile, China, and Norway, leading producers in their respective areas (FAO, 2022). Despite the diversity of farmed aquatic species, Atlantic salmon (*Salmo salar* L.) emerged as the predominant marine aquaculture species, with a global production of 2.7 million tons in 2020. Norway is the largest producers of farmed Atlantic salmon, with a production of 1.4 million tons in 2020 (FAO, 2022), requiring a total of 1.8 million tons of feed ingredients (Aas et al., 2022). Future scenarios for Norway predict a production increase of five million metric tons of salmonids by 2050, which would necessitate 6 million metric tons of feed (Olafsen et al., 2012). However, the growth of aquaculture may face limitations due to a scarcity of feed resources (Almås et al., 2020; Tacon & Metian, 2015).

1.1.1 The need for novel feed ingredients

The largest operating costs of aquaculture are related to feed and feeding (Tacon & Metian, 2008), accounting for 46% of production costs in 2020 (NCE, 2022). In recent decades, the formulation of Atlantic salmon feeds has experienced notable adjustments, primarily due to fully or over-exploited fisheries and the increasing cost of marine ingredients such as fish meal (FM) and fish oil (FO) (FAO, 2018; Aas et al., 2022). The utilization of marine ingredients in Atlantic salmon feeds has decreased significantly, from around 90% in 1990 to 22% in 2020 substituted by plant-based alternatives (*Fig.* 1) (Kaushik & Hemre, 2008; Aas et al., 2022). However, challenges arise with plant-based ingredients, including the presence of antinutritional factors (ANFs) like starch (Gillund & Myhr, 2010), and environmental impact and greenhouse gas emissions (GHG) from using crop lands (MacLeod et al., 2020). In response, the Norwegian government aims to enhance the sustainability of aquaculture such as other livestock

sectors by ensuring that all feed produced for the industry originates from sustainable sources by 2030 (Norwegian government's political platform (Regjeringen, 2021)). The most common definition of sustainability refers to the Brundtland commission in 1987 as, 'meeting the needs of the present without compromising the ability of future generations to meet their own needs'. This concept is closely aligned with UN Sustainable Development Goals (SDGs), which aim to address global challenges such as poverty, inequality, climate change, environmental degradation, peace, and justice to ensure a sustainable future for all. Hence, exploring and scaling up novel feed ingredients that meet SDGs while promoting the production of fast-growing and healthy fish is crucial for replacing marine and plant-based components in salmon feed. Novel ingredients like insect meal, single-cell proteins, microalgae, and fermented products represented only 0.4% (8,130 tons) of total ingredients in salmon feed in 2020 (*Fig.1*), indicating the need for further exploration and efficient utilization of available resources (Aas et al., 2022). Furthermore, the composition of feed significantly influences the carbon footprint of salmon production (Pelletier et al., 2009; Winther et al., 2020), emphasizing the importance of selecting ingredients with low GHG emissions and minimizing environmental impacts.

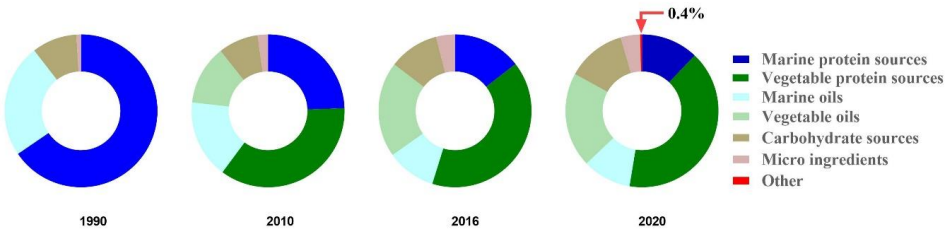

Figure. 1. Sources of feed ingredients (% of feed) in Norwegian salmon feed in 2020 adapted from (Aas et al., 2022) compared to previous years (Ytrestøyl et al., 2015; Aas et al., 2019). Micro ingredients include vitamin- and mineral premixes, phosphorus sources, astaxanthin, crystalline amino acids. 'Other' includes insect meal, single cell protein, fermented products, and microalgae.

1.1.2 The potential for using low trophic resources in fish feed

As part of the effort to achieve sustainable production, low trophic marine aquaculture is a potential pathway to increase food production and biomass derived from the ocean, both directly for human consumption and indirectly as ingredients in feed (Albrektsen et al., 2022; Kousoulaki et al., 2022; SAPEA, 2017). Low trophic species, often referred to as primary producers and primary consumers in the energy trophic pyramid, consist of various organisms such as microalgae, macroalgae, bivalves, and herbivorous fish. Farming of low trophic marine species typically involves cultivating these organisms either in coastal areas or offshore installations (such as floating, submerged structures, longlines, rafts, ropes) (reviewed by Torrissen et al. 2018).

Compared to terrestrial farming practices, farming low trophic marine species has lower environmental impacts, including lower GHG emissions and reduced land and freshwater use (Hilborn et al., 2018; Nijdam et al., 2012). These species can be grown with low energy requirements and zero feed or fertilizer inputs, as they extract nutrients directly from the marine environment while still possessing beneficial nutritional properties (Albrektsen et al., 2022; Kousoulaki et al., 2022; Wright et al., 2018). Furthermore, farming low trophic marine species can contribute to the development of circular nutrient systems by transforming linear nutrient flows from land to sea into a more circular process where nutrients are reused within the marine environment (Filippelli et al., 2020; Petersen et al., 2019)

Blue mussel, *Mytilus edulis* and sugar kelp, *Saccharina latissima* are two possible candidates from the marine low trophic level as novel feed ingredients. These can be cultivated in Norway and have a low climate footprint and are therefore interesting candidates as novel feed ingredients for salmon feed. However, there are several knowledge gaps regarding how these raw materials can be used in all parts of the value chain, from preservation and processing into feed grad raw materials, how to incorporate them into the feeds and at which levels to which inclusion levels can be used in feed and fish performance.

1.1.3 Blue mussel (*Mytilus edulis*)

1.1.3.1 Production and potential

Blue mussels are saltwater mussels, belonging to the Mytilidae family that widely distributed in the Atlantic region (FAO, 2009). Aquaculture production of mussels is conducted in both tidal and subtidal areas employing on-bottom cultivation (culture plot on the sea bottoms) or suspended cultivation (the use of poles, rafts or longlines of ropes) methods (Kamermans & Capelle, 2019). Unlike some aquaculture species, hatchery technology is not utilized in blue mussel cultivation in Europe, relying instead on natural seed sources (Kamermans & Capelle, 2019).

In Norwegian coastal areas several spawning takes place in the period between April and September (Duinker et al., 2008). In most populations, gonad development with an increase of protein, lipid and carbohydrates starts in October /November followed by gametogenesis in winter where the accumulated nutrients are rapidly utilized with gonad maturation occurring in early spring (Zagata et al., 2008).

The growth time from seed to commercial size varies along the Norwegian coastline, ranging from 1 to 3 years (Torrissen et al., 2018). One recent study has proposed distinct harvest times for utilizing blue mussels, whether for feed or food purposes (Gatti et al., 2023). Specifically, it is recommended to harvest after 1 year for feed applications (shell length: 4-5 cm and total wet weight include shell weight 6-8 gr) and after 2 years for food consumption (shell length: 6-7 cm and total wet weight include shell weight 12-20 gr). A shorter production cycle for feed was suggested to be more efficient in exploiting primary production because of a lower maintenance and reproduction costs for young and small mussels compared to a 2-year and larger mussels (Gatti et al., 2023). Additionally, it has been reported that younger mussels (one year old) contain a higher meat yield compared to older ones (two year old) (Duinker et al., 2008). In addition to producing 1-year blue mussels for feed, almost 27% of the total mussel production for food is classified as byproducts that can be utilized for feed (Naik & Hayes, 2019). Byproducts are mussels with specific morphological characteristics such as undersized mussels, mussels fouled with barnacles, and broken shells that are typically discarded or underutilized.

Norwegian fjords, such as the Hardangerfjord, has been suggested as locations for large-scale mussel farming for both food and feed purposes (Gatti et al., 2023).

1.1.3.2 Nutrient composition of blue mussel meat

To produce fish feed, only the meat (flesh) part is used. The optimal harvest time based on the nutritional accumulation in meat part is early spring, before spawning during gonad maturation. Mussel meat typically has high protein content, with carbohydrates such as glycogen being the second most abundant nutrient after protein. Mussel meat contains relatively low levels of lipids, while they are rich in polyunsaturated fatty acids (PUFAs), contributing to a very high omega-3: omega-6 ratio (4:1-11:1) (Naik & Hayes, 2019). However, the meat yields and nutritional composition of blue mussels change seasonally, in relation to water temperature, food availability, reproductive cycle and age of mussels (Gallardi et al., 2017). As an example, the mean percentages of key components in the dry flesh of mussels, along with seasonal ranges in the Conwy estuary, North Wales, are as follows: protein 58.7% of dry matter (DM) (46.4–73.1%), carbohydrates 22.5% of DM (4.1–36.7%), lipids 7.0% of DM (3.9–9.6%), and ash 11.8% of DM (8.8–17.2%) (Dare & Edwards, 1975). The content of DM can be different from 13-25% in the blue mussel (Berge & Austreng, 1989).

1.1.3.3 Blue mussel in fish feed

Blue mussels have been suggested as potential candidate in aquafeed due to similar amino acid profile to FM in addition to a large production potential (Berge & Austreng, 1989; Gatti et al., 2023). However, previous studies have shown that using the whole blue mussel including the shell can have negative effects on growth rate and nutrient digestibility of fish. For example; rainbow trout (*Salmo gairdneri* Richardson) fed diets containing blue mussel meal (BMM) (up to 45% of diet- 90% of FM) had reduced growth and lower digestibility of protein, as well as enlarged liver, while including a lower level in diet (up to 30% of diet- 60% of FM) did not change growth performance, feed utilization and nutrient digestibility (Berge & Austreng, 1989). The presence of shell fractions in the diet resulted in a high ash content (high calcium levels) and carbohydrate levels, which led to a low energy density. These factors contributed to the negative outcomes observed with a high inclusion level of BMM in the diet of rainbow

trout (45% of diet). Therefore, the recommendation was to use deshelled blue mussels in fish feed to overcome these challenges (Berge & Austreng, 1989). The growth performance of Arctic charr (*Salvelinus alpinus*) was unaffected when fed diets containing de-shelled BMM (30% of diet- 60% of FM) (Langeland et al., 2016). Similarly, replacing 10% and 25% of FM (4.5 and 11% of diet, respectively) with deshelled BMM in turbot (*Scophthalmus maximus*) feed did not reduce growth nor did it had a negative effect on liver somatic index (Weiss & Buck, 2017). However, increasing the incorporation level to 50% and 100% of FM (15 and 30% of diet, respectively) resulted in reduced growth due to decreased feed intake and palatability in the same study. However, a low level inclusion of BMM in the diet can act as feed attractant (Nagel et al., 2014). For example; incorporating mussel meal in turbot diets (2, 4 , and 8%) increased daily feed intake as well as specific growth rate (SGR) (Nagel et al., 2014). Collectively, these studies demonstrate the potential of BMM made from de-shelled mussel as an FM replacement (up to 50%) and attractant (in a low level such as 8%) in aquafeed formulations for various fish species.

1.1.3.4 Processing and preservation of blue mussel

Blue mussel silage (BMS)- There are several challenges that need to be addressed to fully utilize blue mussel in aquafeed. The high moisture content (70-80%), neutral pH, and presence of hydrolytic enzymes in fresh mussel meat can lead to rapid degradation, reducing its shelf life (Naik & Hayes, 2019; Ovissipour et al., 2013). As a result, mussel byproduct processing needs to be conducted within 72 hours of harvest to minimize degradation of meat (Naik & Hayes, 2019; Zhou et al., 2019). However, it has been suggested that this timeframe may be even shorter than 72 hours (personal communication, H. Sveier, Ocean Forest). Additionally, seasonal harvest which influence availability of blue mussel throughout the year (early spring as optimal season of harvest) and variations in their chemical composition further complicate the challenge of ensuring a consistent supply of high-quality ingredients.

The production of dry mussel meals offers the advantage of a more standardized, available, and easily manageable product (Nørgaard et al., 2015). However, the process poses challenges due to its cost, energy usage, and environmental footprint.

Additionally, high drying temperatures and processing times can negatively impact the nutritional quality, functionality, and sensory characteristics of the final products (Moses et al., 2014). Utilizing organic acid silage as an alternative processing method instead of meal was suggested as a promising processing technique for mussels (Nørgaard et al., 2015). The silage technique can produce nutritionally stable products while reducing the need for costly and energy-intensive drying methods. This approach also ensures the availability of products throughout the year.

Silage products in aquafeed- Norway has a long history of fish silage and fish protein hydrolysates (FPH) production. Fish silage is produced by adding acid to minced, raw fish or fish offal and hydrolyze it to peptides and free amino acids (reviewed by Raa et al. 1982), while FPH is derived from chemical (e.g. acid and alkaline) or enzymatic (e.g. protease) breakdown of fish proteins (instead of whole fish) into single amino acids, peptides and oligopeptides (Kristinsson & Rasco, 2000).

The use of fish silage in Atlantic salmon diets (20% of dietary protein) did not affect the growth of fish (Espe et al., 1992). Similarly, the use of fish protein concentrate in salmon diets up to 15% resulted in better growth performance compared to diets without fish protein concentrate or with higher inclusion levels (up to 30% of diet) (Espe et al., 1999a). Furthermore, incorporation 5%, 10%, and 15% FPH in Atlantic salmon diets (12, 25, 37% of FM, respectively) led to faster growth and increased feed intake, particularly at the 10% and 15% inclusion levels (Refstie et al., 2004). Post-smolt Atlantic salmon fed diets with 9 and 12% FPH (replaced 18 and 24% of FM) had better weight gain (WG) and a tendency to have higher feed intake compared to those fed diets containing higher inclusion of FPH (15% of diet- 30% of FM) (Hevrøy et al., 2005). In a recent study, the growth of Atlantic salmon fed 80% plant-based diets containing 5% FM and 10% partly hydrolyzed protein was comparable with fish fed control diets with 35% FM or with 80% plant-15% FM (Egerton et al., 2020). Therefore, fish silage or FPH in a moderate inclusion level (5-15%) can improve growth performance in different fish species (reviewed by Siddik et al. 2021).

Knowledge gap: BMS in aquafeed- The use of BMS in aquafeed, in particular in salmon feed, and its impact on salmonid growth performance and feed utilization

remain unknown. One study investigated the addition of BMS in pig diets, which found pigs fed diets containing BMS had higher digestibility of crude protein and amino acids compared to pigs fed diets containing BMM and fish silage (Nørgaard et al., 2015). These findings are likely attributed to protein hydrolysis, resulting in the formation of shorter peptide fractions through the supplementation of formic acid. Therefore, thorough investigation is necessary to fully understand the suitability of incorporating BMS into the fish diet and its potential benefits for aquaculture.

1.1.4 Sugar kelp (*Saccharina latissima*)

1.1.4.1 production and potential

Sugar kelp is a type of brown seaweed, belonging to the Laminariaceae family. It is known as sugar kelp due to the content of the sugar alcohol mannitol. Sugar kelp grows optimally in spring, while the growth stops during the summer months (reviewed by Torrissen et al. 2018). In Norway, the efforts for large-scale cultivation of seaweed biomass have been focusing largely on kelp species, particularly *Saccharina latissima*, like in other parts of Europe (reviewed by Stévent et al. 2017 and Torrissen et al. 2018). This focus is due to the potentially high biomass yield and suitability of them as feed ingredients for livestock (e.g. ruminants, pigs, and poultry) (reviewed by Biancarosa et al. 2018 and Forbord et al. 2020). It should be noted that growth and chemical composition of sugar kelp vary considerably according to the season of collection and different environmental factors (e.g. depth, water salinity, pH, sunlight, water current, waves, nutrient availability) (Forbord et al., 2020).

1.1.4.2 Nutrient composition of brown seaweed- sugar kelp

In general, brown seaweeds contain much water (61 to 94%) and low levels of protein (5-15% of DM) and lipid (0.5-3.4% of DM); however, they contain all essential and non-essential amino acids and a balanced ratio of omega-3 (Eicosapentaenoic acid (EPA) and gamma-linolenic acid) and omega-6 acids (arachidonic acid and linoleic acid) (reviewed by Øverland et al. 2019). Brown seaweeds contain high amounts of varying types of carbohydrates (at least 50% of DM) such as non-starch polysaccharides (NSP), high levels of dietary fibers and lignin (Schiener et al., 2015; Øverland et al., 2019). Main NSPs in *Saccharina latissima* are laminarin, alginate, cellulose, fucoidan,

and the sugar alcohol mannitol (Sharma et al., 2018). During the light seasons, laminarin and mannitol, as the main energy storage compounds, accumulate in the seaweed as the storage carbohydrate, while alginate, fucoidan and cellulose are the main structural components with little annual variation (Schiener et al., 2015; Øverland et al., 2019). They also contain a relatively high ash content which may exceed 40% of DM in spring (Forbord et al., 2020) and are rich in minerals such as iodine, potassium, calcium, magnesium, phosphorus, iron, and zinc (Øverland et al., 2019). In certain species such as sugar kelp the iodine concentration can reach very high levels (up to 7 000 mg kg $^{-1}$ DM) along the coast of Europe (Table 1). Seaweed can also accumulate large amounts of heavy metals (e.g. arsenic, lead, cadmium) (Biancarosa et al., 2018; Øverland et al., 2019). It has been reported that concentrations were mostly below maximum allowed levels set by food and feed legislation in the EU (Biancarosa et al., 2018). Nutrient composition of sugar kelp along the coast of Europe is presented in Table1.

Table1. Nutrient (% DM) and macro-mineral (mg g^{-1} DM) composition of *Saccharina latissima* along the coast of Europe.

Species	Nutrient composition (%)				Mineral composition (mg g^{-1} DM)				
	Protein (% DM)	Lipid (mg g^{-1} DM)	Carbohydrate total (% DM)	Ash	Na	K	Ca	Mg	I (mg kg^{-1} DM)
Saccharina	5-10[a]	1.7-3.9[c]	10-61[d]	22-40[a]	20-39[a]	100[e]	4-23[a]	4-5[a]	4600[e]
latissima	11[b]		42-77[a]	26[h]	24[e]	17-65[a]	17[e]	7.7[e]	420-3965[f]
	6-11[c]		27[b]		36[h]	65[h]			1556-7208[g]
	11[h]		46[h]						4895-6568[h]

[a] (Schiener et al., 2015); [b] (Jard et al., 2013); [c] (Vilg et al., 2015); [d] (Manns et al., 2017); [e] (Biancarosa et al., 2018); [f](Lüning & Mortensen, 2015); [g] (Mæhre et al., 2014); [h] (Stévant, 2019)

1.1.4.3 Seaweeds in fish feed

Numerous studies have investigated the incorporation of various seaweed species at different inclusion levels in fish diets for different fish species. For instance, *Ascophyllum nodosum* (2.5- 5%) in the diet of red seabream (*Pagrus major*) (Nakagawa et al., 1997), *Ulva lactuca* (5, 10, 15%) in the diet of gilthead seabream (*Sparus aurata*) (Wassef et al., 2005), *Ulva rigida, Gracilaria bursa-pastoris* and *Gracilaria cornea* (5, 10%) in the diet of European seabass (*Dicentrarchus labrax*) (Valente et al., 2006), *Porphyra* (5, 10, 15%) in the diet of rainbow trout (*Oncorhynchus mykiss*) (Soler-Vila

et al., 2009), *Gracilaria vermiculophylla, Porphyra dioica*, and *Ulva* spp (10%) in the diet of Nile tilapia (*Oreochromis niloticus*) (Silva et al., 2015), *Gracilaria vermiculophylla* (5, 10%) in the diet of rainbow trout (Araújo et al., 2016; Valente et al., 2016) and *Gracilaria* spp., *Ulva* spp., *or Fucus* spp (2.5, 7.5%) in the diet of European seabass (Peixoto et al., 2016a). These studies showed that partial substitution of dietary FM with seaweed (up to 10%) has no effect or increased growth performance, feed efficiency, physiological activity, carcass quality, disease resistance, and stress response reduction. However, exceeding an inclusion level of 10% can lead to negative effects such as reduced growth performance, protein utilization, protein retention, nutrient digestibility, feed efficiency and survival rate (El-Tawil, 2010; Marinho et al., 2013; Soler-Vila et al., 2009; Valente et al., 2006; Wassef et al., 2005).

Recent studies highlighted the potential benefits of incorporating brown seaweed (Laminaria sp., kelp) into salmonid diets for improved health in addition to growth performance (Ferreira et al., 2020; Granby et al., 2020; Kamunde et al., 2019). However, salmonid species had different responses to various inclusion levels. For instances, including up to 10% AquaArom®, a seaweed meal derived from Laminaria sp., kelp, in the Atlantic salmon diet enhanced food consumption, and promoted growth performance, while hepatosomatic index remained unaffected (Kamunde et al., 2019). Conversely, rainbow trout fed a diet containing 4% sugar kelp had reduced growth and nutrient digestibility, reduced hepatosomatic index, and histomorphological changes in the intestine (reduced tunica muscularis thicknesses) (Granby et al., 2020). However, in the same study, supplementation of rainbow trout diets with 1% or 2% sugar kelp did not affect growth performance or nutrient digestibility. Additionally, it led to improved hepatic antioxidant responses and increased iodine accumulation in the fillet. It has been suggested that brown seaweed species with high iodine content could potentially be used for iodine biofortification of fillets in various fish species (Ribeiro et al., 2015; Schmid et al., 2003; Valente et al., 2015) .

1.1.4.4 Processing and preservation of seaweed

Bacterial fermentation of seaweed- Utilizing macroalgae on a large scale as a feed resource necessitates a continuous supply of biomass. Since harvested wet macroalgae

biomass deteriorates quickly during storage, and the growth and harvesting of macroalgae are typically seasonal, preserving and storing harvested macroalgae for the long term becomes necessary (Øverland et al., 2019). Fermented silage is an ancient and widely used method for the preservation of perishable foods and ingredients without the need for drying (Bruhn et al., 2019; Wan et al., 2019). It has been observed that drying processes can consume up to 60% of the total energy used in seaweed processing (Wang et al., 2019). The high concentration of carbohydrates in sugar kelp makes it particularly suitable for fermentation (Herrmann et al., 2015) rather than acid silage. In fact, a significant portion, around 50-70%, of the carbohydrates present in sugar kelp can be converted into fermentable sugars (Kostas et al., 2016). During fermentation, water-soluble carbohydrates undergo conversion into various organic acids, predominantly lactic acid, acetic acid, propionic acid, or ethanol, through bacterial action in an anaerobic environment (Bruhn et al., 2019; Herrmann et al., 2015). Lactic acid is primarily produced by lactic acid bacteria (LAB) as a key bacterial order that has been used in commercial fermentation processes (Wan et al., 2019).

Fermentation plays a crucial role in simplifying nutrient bonds and degradation of insoluble fibers (Aslamyah & Karim, 2017; Marrion et al., 2003). It has been shown that fermented sugar kelp by LAB had a milder taste, improved visual impression and smell, decreased mannitol level (Bruhn et al., 2019). In terms of mineral and metal content, the fermentation process has shown significant effects. For example, sodium, magnesium, and two harmful trace metals, cadmium and mercury, have been found to decrease significantly in fermented sugar kelp compared to the fresh form (Bruhn et al., 2019). In addition to that, the fermentation technique using LAB is a cost-effective method for the extraction of bioactive molecules (reviewed by Wan et al. 2019). It is important to consider that the quality of fermented seaweed products can vary depending on the type of seaweed and bacteria strain used in the fermentation process (Uchida & Murata, 2002; Uchida et al., 1997).

Fermented seaweed in aquafeed- In aquafeed, LAB-inoculated seaweed is added as a growth promoter, immune enhancer, probiotic, and healthy feed for aquatic organisms (reviewed by Mala et al. 2023). Incorporating fermented *Padina tetrastomatica*,

fermented *Kappaphycus alvarezii*, and fermented *Ulva lactuca* in the diet of freshwater prawn, *Macrobrachium rosenbergii*, led to significant improvements in nutrient ADC, as well as an increase in WG, SGR, feed intake, protein efficiency ratio (PER) and FCR, while the whole-body nutrient composition of freshwater prawn remained consistent (Felix & Brindo, 2014a; Felix & Brindo, 2014b, 2014c). Furthermore, the nutritional body content, PER, and protein retention in Rabbitfish (*Siganus guttatus*) were found to be optimized when consuming a gel diet containing fermented *Kappaphycus alvarezii* (Saade et al., 2020). These findings highlight the potential benefits of incorporating fermented seaweed into aquafeeds.

Knowledge gap: Fermented sugar kelp (FSK) in aquafeed- Using fermented seaweed in fish feed is a recent development. However, the use of FSK in salmon feed, and its impact on salmonid growth performance and feed utilization remain unknown and requires in-depth investigation to fully understand its potential.

1.2 Evaluation of novel feed ingredients in aquaculture feed

Animals require nutrients to sustain life processes, support activity, and facilitate growth and production (NRC, 2011). Commercial salmon feed is energy and nutrient dense and it must cover the nutritional requirements of fish (Aas et al., 2022). In the assessment of novel ingredients for inclusion in aquaculture feeds, it is essential to consider various critical factors including evaluating the nutritional composition of the ingredients, their compatibility with feed pellet formulations, and conducting feeding trials to assess growth performance, feed utilization, nutrient digestibility and retention, and health and welfare of fish fed diets containing new feed ingredients (*Fig. 2*) (Glencross et al., 2007).

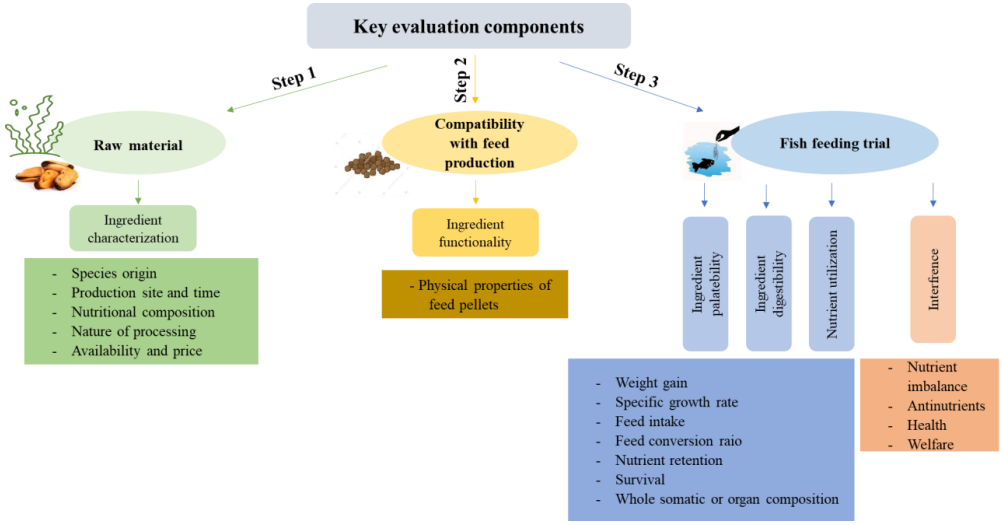

Figure 2: An overview of the key elements involved in evaluating a novel ingredient for use in aquaculture feeds.

Feeding studies- Conducting feeding studies allows for the assessment of how diets affect the physiological responses of fish. These studies consider factors such as feed intake, nutrient digestibility and utilization, growth rate, as well as the overall health and welfare of the fish fed experimental diets (*Fig. 2*). Despite having knowledge or estimates of the dietary requirements for many farmed fish species, nutritional imbalance can still occur due to uncertainties introduced during the diet formulation and manufacturing process (Hardy, 2001). Factors such as grinding, heating, moisture addition, pelleting, and drying can affect the stability and bioavailability of essential nutrients, leading to clinical deficiencies in some cases (Hardy, 2001). Feeding studies can also reveal nutritional challenges such as deficiencies, imbalances, antinutrients, or increased nutrient needs associated with diets containing new feed ingredients (Waagbø, 2008). For example, incorporating plant ingredients in fish feed with high levels of ANFs, such as carbohydrates (NSPs), phytic acid, amylase and trypsin inhibitors, polyphenol compounds (tannins), and lectins, may reduce nutrient digestibility, absorption and growth of fish (Francis et al., 2001).

Production-related disorders- The nutritional challenges induced by new ingredients can also appear as production-related disorders or pathologies such as cataract, bone

deformity, anemia, or changes in gut morphology (reviewed by Waagbø & remø. 2020). For example, soybean meal (SBM) is known to cause intestinal pathology in Atlantic salmon because of the ANFs (Francis et al., 2001; Urán et al., 2008). These led to changes in both the structure and function of the intestines, such as enteritis, shorter mucosal folds, loss of normal vacuoles in the intestinal epithelium, and wider connective tissue (Francis et al., 2001; Urán et al., 2008). Similarly, replacement of dietary FO with vegetable oils caused severe outbreaks of cataract in adult Atlantic salmon (Waagbø et al., 2004), or removing mammalian blood meal from the diets in the late nineties increased cataract in salmon farming due to lack of histidine or histidine-containing compounds (Breck et al., 2003; Wall, 1998). Deficiency of iron, selenium, vitamin C, E, D, K, all B vitamins, and essential fatty acids can also cause anemia (Waagbø 2006).

Oxidative stress- Production-related disorders can also occur due to oxidative stress triggered by nutrient deficiency or toxicity (reviewed by Hamre et al. 2021). Oxidative stress is an imbalance between oxidants and antioxidants that induce the free radicals and lead to reduced immunity and welfare and increased mortalities (Hamre et al., 2021; Sies et al., 2017).

Oxidized glutathione (GSSG) and reduced glutathione (GSH) function together as a redox couple to regulate cellular redox balance. The GSH is transformed to GSSG when cells are under oxidative stress. The relative redox couple concentrations (GSH/GSSG), known as the redox potential, is crucial for maintaining cellular redox homeostasis (Hamre et al., 2010). In healthy fish cells, the ratio of GSH/GSSG remains stable and can serve as an indicator of the intracellular redox state, especially during the growth phase of salmon (Schafer & Buettner, 2001). The primary target of free radicals are cell-membrane PUFAs, which, in turn, lead to damage in the cell structure and function (Floyd, 1990). The decomposition of lipid hydroperoxides produces various end products, such as thiobarbituric acid reactive substances (TBARs) and malondialdehyde (MDA) being recognized markers of lipid peroxidation (reviewed by Hamre et al. 2021).

Immune responses- Nutritional modulation that targets fish immunity has been explored previously (Waagbø, 1994) and being reviewed in several studies (Kiron, 2012; Lall, 2000; Trichet, 2010; Verlhac & Viswanath, 2004). Nutrients like proteins, lipids, carbohydrates, antioxidants, vitamins, and minerals can modulate immune responses in different fish species (reviewed by Trichet 2010). For example, replacing FM with graded level (50, 75, 100%) of plant protein in gilthead sea bream had no effects or improved innate immunity (Sitjà-Bobadilla et al., 2005). However, total replacement of FM by plant proteins in carnivorous diets could lead to amino acid imbalances and caused immune dysfunctions, in another study (Tacon, 1997).

Fish species possess an immune system comprising both innate (non-specific) and adaptive (specific) mechanisms, to effectively defend against stress factors or pathogen invasions (reviewed by Kiron 2012 and Trichet 2010). The innate immune system, including physical barriers, cellular, and humoral components, plays a key role in fish infection processes due to their evolutionary status and poikilothermic nature (Caroll & Prodeus, 1998; Fearon & Locksley, 1996; Magnadóttir, 2006). This innate immune system is particularly influenced by nutritive dietary compounds compared to the adaptive immune system (Oliva-Teles, 2012). Evaluating humoral components like plasma lysozyme activity, from innate immune system, through serum-based assays can offer valuable insights into the animal's dynamic in vivo responses to various challenges (Kiron, 2012).

New feed ingredients with functional properties- New feed ingredients with functional properties are becoming important in aquafeed formulation due to enhancing fish tolerance to stress and resistance against pathogens (reviewed by Waagbø & Remø. 2020). Seaweed as functional ingredients, categorized as a natural antioxidant and immunostimulant, offers promising health benefits beyond basic nutrition (Holdt & Kraan, 2011). Brown seaweeds represent promising sources of bioactive compounds in the formulation of fish feeds and demonstrated a health-promoting effects in an optimal inclusion levels in fish (Øverland et al., 2019). These effects include elevated antioxidant activity, improved stress responses, and enhanced immunological responses such as the increased activity of lysozyme and complement pathway activity (ACH50)

in fish (reviewed by Thepot et al. 2021 and Øverland et al. 2019). For example, Atlantic salmon fed diet containing 10% AquaArom®, meal from Laminaria sp., kelp, had improved antioxidant capacity and mitigated the adverse effects of stressors like temperature likely due to the presence of polyphenols and sulphated polysaccharides (Kamunde et al., 2019). Supplementation of European seabass diets with *Gracilaria* at 7.5% or a mixture of *Gracilaria* spp., *Fucus* spp., and *Ulva* spp. at 7.5% led to alterations in metabolic rate, innate immune response (decreased ACH50), and antioxidant response (increased glutathione S-transferase and glutathione reductase activities), without compromising growth parameters (Peixoto et al., 2016b). Different non-specific immune responses increased in Atlantic salmon fed diets containing alginate extracted from *Ascophyllum nodosum* due to immunostimulant effects of alginate (Gabrielsen & Austreng, 1998). However, it's important to note that the impact of immunostimulants on fish immunity can vary depending on factors such as the type of immunostimulant, fish species and health, delivery method (diet, bath, injection), environmental conditions, and the form of the immunostimulant (whole plant or extract) (reviewed by Thepot et al. 2021).

According to a report from the Norwegian Veterinary Institute, Norway experienced ~15% mortality rate of sea-phase salmon in 2020, primarily attributed to illness and injury as well as disease treatment approaches (Sommerset et al., 2021). Therefore, discovering feed ingredients with functional properties that could potentially enhance the fish immunity and resistance against pathogens and stress can significantly benefit this industry.

2 Thesis aims and outline

The main objective of the PhD work was:

- Investigate the use of blue mussel silage (BMS) and fermented sugar kelp (FSK) as novel feed ingredients in the diet of post-smolt Atlantic salmon.

This included the following sub-objectives:

- **BMS**

- Determine suitable inclusion levels of BMS in the Atlantic salmon diet based on growth performance, feed utilization, nutrient digestibility, and retention **(Paper I)**.
- Compare the growth performance and feed utilization of fish fed diets containing BMS with BMM **(Paper I)**.
- Examine whether using BMS in the diet influence fish welfare and health, including production-related disorders and oxidative stress **(Paper I)**.

- **FSK**

- Determine whether inclusion of FSK in the feed affects growth performance, feed utilization, nutrient digestibility, and retention **(Paper II)**.
- Determine the effect of FSK inclusion on the iodine status of fish **(Paper II)**.
- Examine the effects of up to 4% FSK inclusion on production-related disorders and gut morphology **(Paper II and Paper III)**.
- Determine whether the inclusion of FSK in the feed modulates antioxidant defense system and innate-immune responses **(Paper III)**.

3 Methodological considerations

To address the knowledge gaps associated with the use of BMS and FSK in salmon diets, different stages of the value chain were studied in this work (*Fig. 3*). These stages included assessing the nutritional content of BMS and FSK, determining which inclusion levels were relevant and possible, evaluating biological effects of these new ingredients on the fish in feeding experiments, and finally investigating the health and welfare of the fish fed diets containing BMS and FSK.

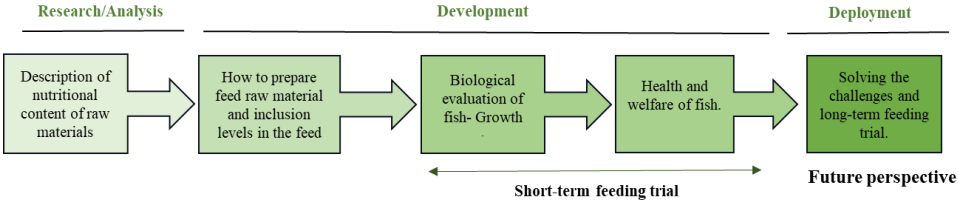

Figure 3: Value chain for incorporating new feed ingredients in the Atlantic salmon diet.

3.1 Raw materials

The raw materials used in the present work, BMS (**Paper I**) and FSK (**Paper II** and **Paper III**), were provided by Lerøy/Ocean Forest AS (Bergen, Norway). Blue mussel meal (BMM) was provided by Triple nine (Esbjerg, Denmark) (**Paper I**). The experimental diets were produced by Cargill (Dirdal, Norway). The preparation steps of ingredients and experimental diets are illustrated in figure 4. Analysis of both BMS and FSK revealed low DM contents, both around 10%.

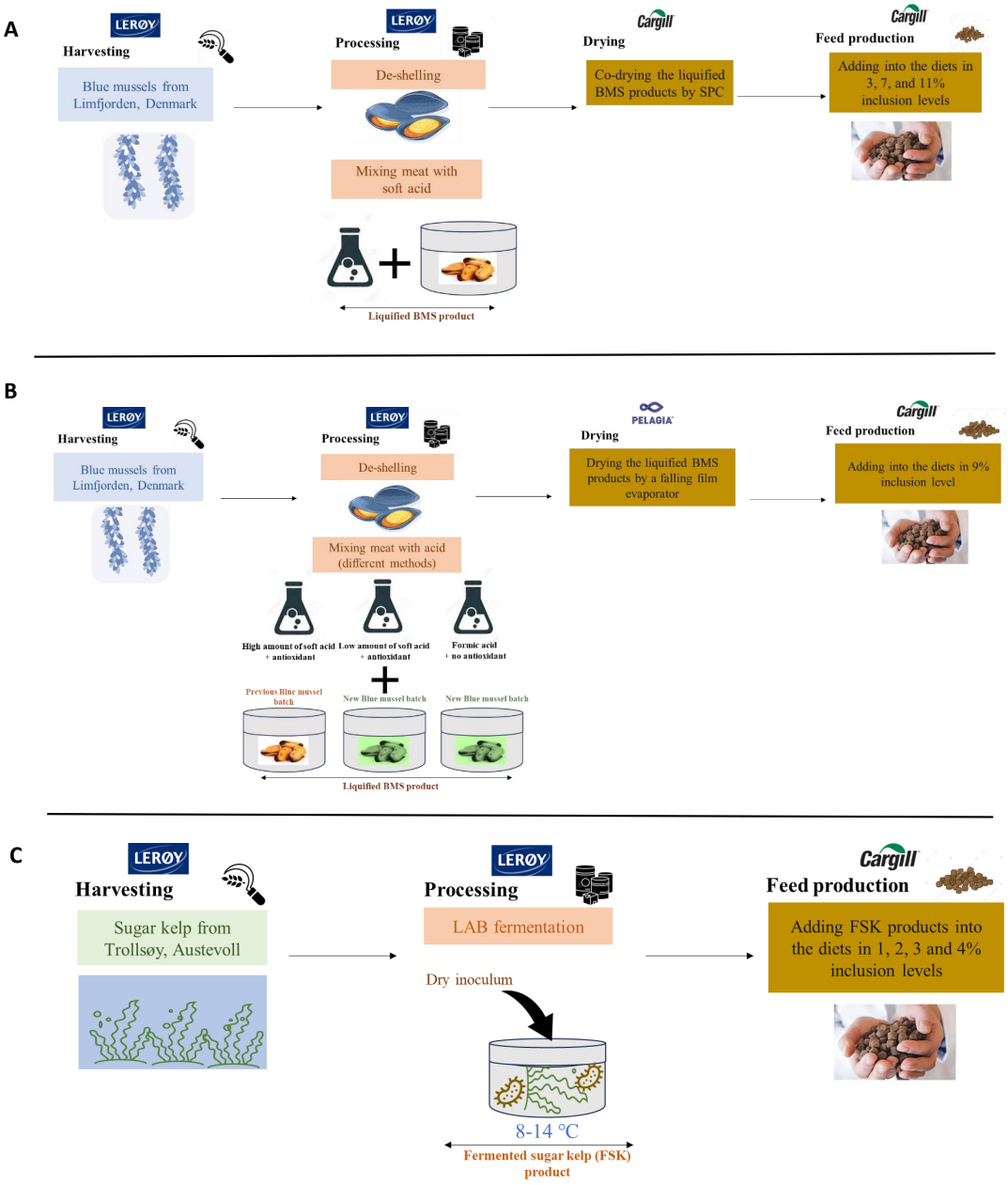

Figure 4. Preparation steps of A) blue mussel silage (BMS) diets used in the first feeding trial, and B) BMS diets used in the second feeding trial (Paper I), and C) fermented sugar kelp (FSK) used in the first trial (Paper II and Paper III). The blue mussel meat shows identical colors (either orange or green) in both figure A and figure B are from the same harvested batch.

3.2 Feed formulation

In both BMS and FSK trials, the reference diets represented a standard commercial diet for Atlantic salmon post-smolt with 42-46% protein and 21-24% lipids.

Choice of inclusion levels- The low DM content of the test ingredients resulted in a limited inclusion level when used "as is", however the range of the inclusion levels were within the relevant levels based on available literature.

BMS- In the first experiment in **Paper I**, the BMS products with 10% DM were mixed with SPC, a dry ingredient, and co-dried before feed production to obtain the target levels of BMS (up to 11%) in the feed. The level of BMS inclusion in the diet (replacing up to 50% of FM) in **Paper I** was relevant with previous studies utilizing silage products like FPH with a 50% DM content (Espe et al., 1999a; Refstie et al., 2004). FPH replaced 50% of FM, constituting 15% of the feed.

In the second experiment (**Paper I**), three different productions of BMS were tested, where one of the feeds contained the previous batch of BMS used in experiment 1 to investigate if the results could be replicated, and the two others were produced to investigate possible effects of acid level and addition of antioxidants. For this experiment, the BMS products were dried to 50% DM by evaporation, allowing direct inclusion in the feed up to 9%. Based on the results from the first experiment this inclusion level was high enough to detect possible effects if present on growth and FCR.

In both experiments, a diet containing blue mussel meal was used with inclusion levels of 12% in the first experiment and 9% BMM in the second experiment for comparison with BMS (**Paper I**).

FSK- Due to the low DM content of the FSK and technical properties of the raw material, it was used "as is" without additional drying before feed production. This limitation restricted the highest inclusion level to 4% for FSK (**Paper II**). Although higher levels would have been desirable in a dose-response study, the available literature indicated that possible effects should be evident at these inclusion levels (Granby et al., 2020; Kamunde et al., 2019).

The choice in reference feed and replacing FM by tested ingredients- The reference diet for both feeding experiments was based on the composition of post-smolt feeds, containing 25% FM. The purpose was to investigate whether replacing FM would maintain comparable growth performance and feed utilization in the fish. To properly evaluate new ingredients, it is essential to have a high-quality reference feed that replaces key ingredients such as FM in the diet. Additionally, the rationale for substituting FM with the tested ingredients comes from the limitation in traditional sources for marine ingredients, such as fisheries and offal, which cannot be expanded. Consequently, there is a need for new marine resources to replace FM for maintaining the marine component of the diet. BMS, with a protein content of approximately 50% of the DM substituted FM, while protein content of the feed remained balanced. FSK replaced FM, albeit with low levels of protein and lipid content, which were compensated for by incorporating other dry ingredients.

3.3 Feeding experiments trials

Two feeding experiments were conducted to evaluate the effects of BMS and one for FSK in the diet of Atlantic salmon post-smolts. To determine appropriate inclusion levels in the diet, a dose-response study was employed as the feeding experimental design in the first BMS trial (**Paper I**) and the FSK trial (**Paper II**). These experiments were performed at the same time with a common reference feed. In the second BMS trial (**Paper I**), designed as a follow-up study, a comparison approach was adopted to evaluate the effects of processing on Fe bioavailability, fish growth, and feed utilization. In all trials, the experimental diets were randomly assigned to triplicate tanks.

The experimental trials were conducted over a short period of time, with the duration selected based on the SGR indicating a doubling of weight for post-smolt fish at 9°C for ~200 g fish in the first trial and 12°C for 120 g fish in the second trial. SGR is used as a standardized metric to estimate fish growth within a defined timeframe and under specific temperature conditions.

To ensure minimal disruption and stress to the fish, sampling was conducted at the beginning and end of both trials. Standard evaluation indicators for growth and feed utilization, such as feed intake, nutrient ADC, WG, SGR, and FCR were employed to

assess the effects of experimental diets, as described by Glencross et al. 2007. Feed intake was estimated as described by Helland et al. 1996. The fish were given 2 meals per day, and uneaten pellets were collected after each meal at consistent times throughout the trial. To assess the ADC of diets, an indirect method based on an inert marker (yttrium oxide) in the feed was utilized. Fecal collection was carried out using the fecal stripping technique, chosen for its conservative approach compared to other fecal collection techniques (Glencross et al., 2007). Fecal collection occurred only once at the end of the trials to prevent any undue stress on the fish with a gentle abdominal pressure approximately over the distal intestine to expel the fecal contents. To ensure the fish had normal growth patterns, SGR was calculated. Biomass was registered at the start and end of the trials for calculation of WG, retention and FCR.

3.4 Analytical methods

To determine the nutritional status of the fish and the retention of nutrients, the concentration of nutrients was determined in whole fish at the start and end of the experiment. Feed and feces were analyzed to determine digestibility of nutrients. Also, specific tissues such as liver, intestine and fillet were analyzed to evaluate oxidative stress/redox status, morphology, and iodine status, respectively. The methods used were established analytical standard methods at IMR (Bergen, Norway), (Table 2), except for carbohydrate analysis, which was conducted by Århus University (**Paper I**).

Due to the observed Fe depletion in fish fed BMS diets in the first BMS trial, posing a risk of anemia, blood parameters such as hematocrit, red blood cells, and hemoglobin were measured in blood samples from the second BMS trial (**Paper I)**. Additionally, to address this issue, the Fe speciation in BMS products and diets containing BMS was determined using a standard method, thiocyanate colorimetry, established by the University of Canterbury (**Paper I**).

Table 2: Analytical methods for the different nutrients in IMR

Nutrient	Principle	Reference
Protein	N × 6.25, protein analyzer	(Hamre & Mangor-Jensen, 2006)
Lipid	Gravimetric after ethyl solven extraction	(Lie et al., 1988)
Ash	Combustion in a muffle furnace	AOAC 2010 method
Energy	Calorimetric after drying	Parr Instrument Co., Moline, IL, USA
Dry matter	Gravimetric after freeze drying	(Hamre & Mangor-Jensen, 2006)
Fatty acids	GLC[1]	(Lie & Lambertsen, 1991)
Aminoacids	UPLC[2]	(Cohen & Michaud, 1993)
Minerals	ICP-MS[3]	(Julshamn et al., 2004)
Iodine	ICP-MS	(Julshamn et al., 2001)
TBARs[4]	Spectrophotometric	(Hamre et al., 2001; Schmedes & Hølmer, 1989)
MDA[5]	HPLC[6]	(Hamre et al., 2001)

Note: Some parts of the table is adapted from (Hamre et al., 2013) and **Paper III**.
[1] Gas–liquid chromatography.
[2] Ultra-performance liquid chromatography.
[3]Inductively coupled plasma mass spectrometry.
[4] Thiobarbituric acid reactive substances.
[5] Malondialdehyde.
[6] High-performance liquid chromatography.

One important aspect of assessing feeds and ingredients in animal diets is their impact on immune responses and overall animal health (Glencross, 2020). Therefore, various welfare indicators, including production-related disorders according to standard scoring systems (Noble et al., 2018; Stien et al., 2013) were used at the sampling to evaluate the status of the fish. Markers for redox regulation and oxidative stress (such as GSH, GSSG, GSH/GSSG, and MDA levels) were evaluated in the liver of fish fed experimental diets. Furthermore, in the case of fish fed FSK diets, known for their high levels of indigestible carbohydrates, concerns arose regarding potential inflammation, adverse impacts on gut morphology, and digestibility. To address these concerns, morphometric analysis, including length and thickness of mucosal folds, thickness of the intestinal wall, size and area covered by mucous cells, were evaluated based on the method described by Moldal et al. 2014. Furthermore, a semi- quantitative scoring system modified from Knudsen et al. 2007 by Hanne Johnsen at Nofima (Johansson, 2014), was employed for histological assessment to evaluate inflammatory signs in the middle intestine.

Considering the bioactive compounds present in FSK and their potential functional effects, innate-immune responses in the plasma of fish were evaluated. This included

assessing plasma lysozyme, bactericidal, antiprotease, and peroxidase activities, as well as IgM levels. These assessments were carried out using in-house methods and kits at IMR (Bergen, Norway) and CIIMAR (Porto, Portugal) (**Paper III**).

4 Summary of results

Paper I

In the first experiment, feed intake and nutrient digestibility were comparable across all dietary groups. However, the WG decreased and FCR increased in BMS fed groups. Fish fed diets containing 12% BMM had similar growth performance and feed utilization compared to the control group and higher than the BMS 11% group. A reduced Fe status was seen with low Fe levels in whole body and liver of the fish fed BMS diets compared to the control group, irrespective of BMS inclusion level. In the second experiment, fish fed BMS diets did not have depleted body Fe levels. Additionally, the growth rate and feed utilization were comparable between BMS fed fish and the control group, regardless of acid level and antioxidant. A comparable growth rate and feed utilization between fish fed 9% BMS and 9% BMM diets were observed in the second trial. None of the production-related disorders such as cataract, bone deformity, gill condition, and skin and fin damages were found across any dietary group in both trials and incorporating up to 11% BMS in the diet did not induce hepatic oxidative stress.

Paper II

Including FSK in the diets resulted in a dose-dependent decrease in WG, however, feed intake and FCR remained similar across all dietary groups. Nutrient digestibility was also comparable between FSK fed fish and the control group, except lipid digestibility, which increased. Nutrient retention decreased with FSK inclusion levels, except for the protein retention, which was comparable between fish groups. Furthermore, dietary iodine levels increased considerably with inclusion of FSK in the diet. The iodine status increased in the whole body and fillet of fish fed diets containing up to 3% FSK. Regulation of high iodine levels in the fish body was evident through increased iodine availability and decreased iodine retention in fish fed FSK diets. Fish fed up to 4% FSK diets had comparable welfare to the control group, with no observed production-related disorders such as cataract, bone deformity, gill condition, or skin and fin damages.

Paper III

Despite minor changes in gut morphology between Atlantic salmon fed diets containing FSK, there were no differences comparable to the control group. Furthermore, there were no signs of inflammation in fish fed up to 4% FSK diets compared to the control group. Hepatic GSH concentration and GSH/GSSG ratio had an increase dose-dependent response, while MDA and redox potential decreased with a higher inclusion of FSK in the diet. The modulatory effects on the hepatic antioxidant defense system were more pronounced with the 4% inclusion level. The findings also suggested potential modulatory effects of FSK inclusion on fish innate-immune responses. These effects varied depending on the inclusion levels. Plasma lysozyme activity increased compared to the control group, while only the fish fed diets containing 1% and 3% FSK only had a significant increase in activity. Plasma bactericidal activity was higher in FSK-fed fish groups, with a significant increase in fish fed 3% FSK diets. Furthermore, an increased dose-response was observed in plasma protease activity in fish fed FSK diets. Plasma peroxidase activity and IgM levels were however decreased by FSK inclusion in the diet.

5 General discussion

5.1 Blue mussel silage (BMS) and meal (BMM)

5.1.1 Nutritional composition- BMS products as a protein and iron source

Finding novel feed raw materials with a high protein content is needed to support further expansion of aquaculture. Carnivorous marine species, such as salmonids, typically necessitate a protein content of 40-55% in their diets, in contrast to most freshwater fish, which require about 25-40% (Hasan et al., 2001). The blue mussel silage investigated in the present project contained around 50% protein on DM basis (**Paper I**), while BMM has been reported to contain protein levels up to 60-70% (Nagel et al., 2014; Nørgaard et al., 2015). However, BMS has a lower protein level compared to other animal and plant protein sources such as FM (60-70%) (NRC, 2011), fish silage (60-77%) (Espe et al., 1992; Nørgaard et al., 2015), SPC (63-80%) (NRC, 2011), and corn gluten meal (CGM- 62-63%) (Liu et al., 2020; NRC, 2011). The protein level in BMS products can be comparable to the protein level in black soldier fly larvae (BSF, 30-63%) (Liland et al., 2017), soy bean meal (SBM-44%) (El-Sayed, 1994), and microalgae (6-71%- reviewed by Nagappan et al. 2021). Therefore, BMS may not be sufficient as a sole protein source for salmonids and needs to be supplemented with other protein sources. The BMS (**Paper I**) and BMM products (Nørgaard et al., 2015) had a complete profile of essential amino acids (EAAs), mirroring that of FM, albeit at a lower level in BMS products (*Fig. 5a-b*). Research indicated that the levels of acid-sensitive amino acids, such as tryptophan, lysine and methionine, may decrease after silage processing in fish silage due to the acidic conditions and pH levels employed in silage processing methods (Espe & Lied, 1999b). Consequently, when incorporating BMS products into salmon diets, consideration of amino acid supplementation or balancing with other sources may be necessary.

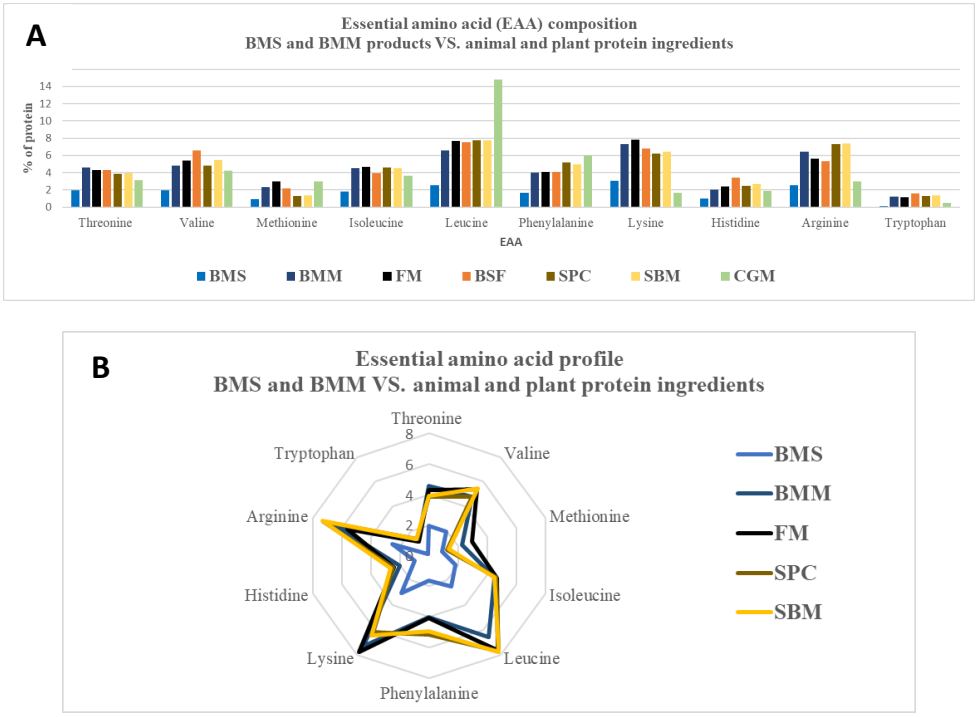

Figure 5: A) Essential amino acid (EAA) comparison between blue mussel silage (BMS) (Paper I), blue mussel meal (BMM) (Nørgaard et al., 2015) and animal and plant protein ingredients B) Essential amino acid profile of BMS and BMM and other animal and plant protein ingredients. The value for fish meal (FM) (international feed number: 5-01-985), soy protein concentrate (SPC), soybean meal (SBM) (international feed number: 5-04-604) and corn gluten meal (CGM) (international feed number: 5-09-318) are presented in NRC, (2011). Data for black solder fly meal (BSF) are from Fisher et al., (2020).

The lipid content of BMM typically ranges from 8-16%, with variability across seasonal harvests (Gallardi et al., 2017; Nagel et al., 2014; Nørgaard et al., 2015). This range is comparable to that of FM (7-10%) (NRC, 2011). In contrast, the BMS products used in the present studies had much lower lipid levels (1.3%), albeit with a well-balanced profile of polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), resulting in a favorable n-6/n-3 ratio such as FM (*Fig. 6*) (**Paper I**). Due to the minimal contribution of BMS as a lipid source in the feed, supplementation with other lipid sources is necessary to fulfill the nutritional requirements of Atlantic salmon.

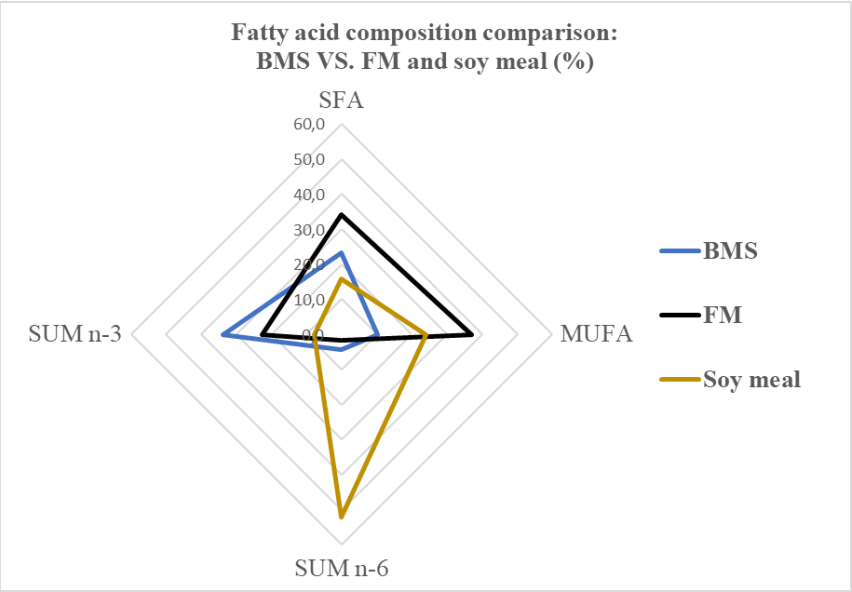

Figure 6: Essential fatty acid comparison between blue mussel silage (BMS) and fish meal (FM- international feed number 5-02-009) (Cho & Kim, 2011) and soy meal (Dubois et al., 2007). SFA- total of saturated fatty acid (sum of C 14:00, C 16:00 and C 18:00); MUFA- monounsaturated fatty acid (sum of 18:1n-7, 18:1-n9, 20:1n9, 22:1n11); SUM n-6 obtained from sum of 18:2n-6 and 20:4n-6; SUM n-3 obtained from sum of 18:3 n-3, 18:4 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3. The sum of MUFA for FM obtained from an average of MUFA in anchovy, sardine and capelin (Sissener et al., 2016).

The high ash content originating from shell fractions in blue mussels can raise concerns when used as a feed ingredient. However, the ash content in deshelled BMM (8-9% of DM) (Nagel et al., 2014; Nørgaard et al., 2015) and BMS products (11-14% of DM) (**Paper I**) are within the reported range for FM, which is typically 11-23% of DM (NRC, 2011). Therefore, proper removal of the shell addressed concerns about elevated ash content. High ash levels have been associated with increased mortality, reduced growth, decreased feed efficiency, and various pathologies such as cataracts and skeletal abnormalities in Atlantic salmon (reviewed by Shearer et al. 1992).

The BMM and BMS product can be considered a source of Fe and Se followed by Mn, Cu, and Zn based on a relative comparison of essential micro-mineral composition in BMS and plant-based ingredients (SBM and SPC), using FM as the baseline at 100% (*Fig. 7*). However, it's important to note that silage processing, particularly the drying step, may influence the bioavailability of Fe in the products, affecting their uptake and

retention in the fish's body (**Paper I**). Therefore, while BMS products may contain high levels of Fe, their value for fish nutrition depends on their availability for uptake and utilization.

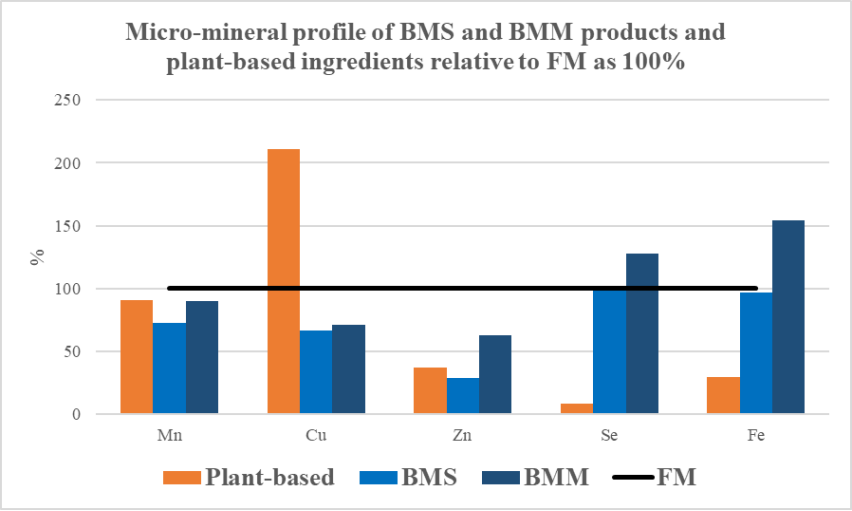

Figure 7: Micro-mineral profile of blue mussel silage (BMS) and blue mussel meal (BMM) products and common plant-based ingredients (soybean meal-5-04-612 and SPC) used in Atlantic salmon feed relative to FM as 100% (black line). The value for plant-based ingredients and FM is from NRC, (2011) and the value for BMS and BMM are from the current work.

5.1.2 Efficient use of BMS in the salmon diet relies on processing techniques

In **Paper I**, different inclusion levels of BMS replaced FM in a commercial diet with 25% FM to determine if fish could perform comparably to FM-based diets.

In the first experiment, fish fed diets containing up to 11% BMS had lower WG compared to the control and BMM groups, despite the BMS diets being readily ingested and digested, comparable to the control groups (**Paper I**). Additionally, the nutrient ADC values in fish fed BMS diets were comparable to those previously reported for salmonids fed diets containing animal and plant protein sources (Table 3, 4). For instance, the range of protein ADC was 76-94%, lipid ADC was 71-99%, and energy ADC was 72-89% in diets. Therefore, incorporating BMS in the salmon diet did not influence digestibility of diets.

Table 3. Apparent nutrient digestibility (ADC) of conventional ingredients used in Atlantic salmon diet.

Feed ingredients	Treatment	Inclusion levels in diet	Fish species	Dietary ADC%				Reference
				DM%	Protein%	Lipid%	Energy%	
Conventional ingredients in salmon feed								
FM	Low temperature (LT 94)	55%	Atlantic salmon (*Salmo salar*)	-	91.5*	90	86	(Storebakken et al., 1998)
FM	Low and high fat level from fish oil or vegetable oil	48-63%	Atlantic salmon (*Salmo salar*)	-	89-92	94-99	-	(Bendiksen et al., 2003)
FM	-	65%	Atlantic salmon (*Salmo salar*)	-	92*	-	85	(Glencross et al., 2004)
FM	LT	50%	Atlantic salmon (*Salmo salar*)	-	85*	84	78	(Refstie et al., 2000)
SBM	Dehulled, extracted (defatted), toasted	30%	Atlantic salmon (*Salmo salar*)	-	84*	71	72	(Refstie et al., 2000)
SBM	contained hulls and was extracted and toasted	31%	Atlantic salmon (*Salmo salar*)	-	91*	82	80	(Storebakken et al., 1998)
SBM	-	30%	Atlantic salmon (*Salmo salar*)	-	91*	-	84	(Glencross et al., 2004)
SPC	-	30%	Atlantic salmon (*Salmo salar*)	-	91*	-	85	(Glencross et al., 2004)
Soy protein isolate	-	30%	Atlantic salmon (*Salmo salar*)	-	94*	-	89	(Glencross et al., 2004)
Lupin kernal meal	-	30%	Atlantic salmon (*Salmo salar*)	-	92*	-	79	(Glencross et al., 2004)
Lupin protein concentrate	-	30%	Atlantic salmon (*Salmo salar*)	-	93*	-	86	(Glencross et al., 2004)
Lupin protein isolate	-	30%	Atlantic salmon (*Salmo salar*)	-	94*	-	89	(Glencross et al., 2004)
Fish silage	Whole minced herring	0-40%	Atlantic salmon (*Salmo salar*)	-	80-85*	90-97	-	(Espe et al., 1999a)
Fish silage	Herring offal	0-30%	Atlantic salmon (*Salmo salar*)	-	76-80*	-	-	(Espe et al., 1999a)

Table 4. Apparent nutrient digestibility (ADC) of blue mussel meal (BMM) and blue mussel silage (BMS) used in fish feed.

Feed ingredients	Treatment	Inclusion levels in diet	Fish species	Dietary ADC%				Reference
				DM%	Protein%	Lipid%	Energy%	
Used blue mussel in animal feed								
BMM	With shell	45%	rainbow trout (*Onchorynchus mykiss*)	80-95	81-84	90-91	80-86	(Berge & Austreng, 1989)
BMM	De-shell	8%	Turbot (*Scophthalmus maximus*)	48-56	76-81	-	-	(Nagel et al., 2014)
BMM	De-shell	30%	Eurasian perch (*Perca fluviatilis*)	90	95	91	-	(Langeland et al., 2016)
BMM	De-shell	30%	Arctic charr (*Salvelinus alpinus*)	86	92	88	-	(Langeland et al., 2016)
BMM	De-shell	40%	Arctic charr (*Salvelinus alpinus*)	73	88	85	-	(Vidakovic et al., 2016)
BMM	De-shell	-	Pig	-	83	-	-	(Nørgaard et al., 2015)
BMM	**De-shell**	**12%**	**Atlantic salmon (*Salmo salar*)**	**66**	**87**	**94**	**79**	**Paper I**
BMS	De-shell-silage	-	pig	-	87	-	-	(Nørgaard et al., 2015)
BMS	**De-shell silage (co-dried by SPC)**	**3-7-11%**	**Atlantic salmon (*Salmo salar*)**	**70**	**88**	**96**	**82**	**Paper I**
BMS	**De-shell silage (falling film evaporator)**	**9%**	**Atlantic salmon (*Salmo salar*)**	**71**	**89**	**94**	**84**	**Paper I**

The reduced growth observed in fish fed BMS diets was attributed to depleted Fe levels in the fish body in the first experiment (**Paper I**). This depletion was likely induced by decreased Fe bioavailability affected by the drying technique used in silage processing (**Paper I**). The low level of DM of BMS products (8-10% DM) made it difficult to incorporate them into feed pellets with 90% DM. In the first BMS trial, BMS broths were co-dried with SPC and simultaneously exposed to controlled heating (**Paper I**), which has been shown to absorb solubilized proteins and some moisture (Dong et al., 1993). Compared to heat drying of whole silage, co-drying with cereals has several advantages including reduced drying times, softer texture of the product after drying, and the possibility to adjust the feed formulation to fish requirements by adding cereal

(Goddard & Al-Yahyai, 2001). However, the findings in **Paper I** suggest also potential drawbacks, such as changes in the chemical form of minerals resulting in altered bioavailability. BMS products co-dried with SPC had a higher ratio of Fe^{3+} to total Fe (**Paper I**). This suggests that the presence of Fe^{3+}, which is considered less bioavailable, likely contributed to Fe depletion in the first trial and subsequently decreased the growth rate (**Paper I**). Although the exact reason for the lower availability of Fe in the first experiment remains unknown, it was hypothesized that high acid levels used in the BMS batch may have led to the formation of insoluble Fe compounds, reducing their availability for absorption. Similarly, antioxidants may be chelated with Fe, forming complexes that are poorly absorbed.

A follow-up study was conducted to investigate the potential impact of processing methods on Fe bioavailability in BMS further. Both the same batch (used in previous trial but dried differently) and new batches of BMS were incorporated in the diet. In addition to lower acid levels and absence/presence of antioxidants in new batches of BMS, as an alternative to co-drying, evaporating was employed to increase the DM content of BMS products to 50% in the second experiment (**Paper I**). The evaporating method, characterized by its short residence time, can produce a concentrated product (heated from 40 to 100°C) with satisfactory nutritional characteristics (Frías-Esquivel et al., 2017). In **Paper I**, in the second experiment, no indications were seen of reduced Fe bioavailability and body Fe depletion in fish fed BMS diets regardless of acid level and used antioxidant, and WG and feed utilization were comparable between fish fed BMS diets and the control and BMM groups. These results suggest that applying an appropriate drying technique potentially reduces issues related to low Fe bioavailability and uptake by fish.

Previous studies have also shown that using diets containing high levels of silage can result in reduced growth. For instance, rainbow trout fed a diet containing fish silage (50% of diet) had reduced growth rates despite its higher digestibility in fish silage compared to FM (Stone et al., 1989). The growth reduction might have been caused by high levels of essential amino acids in free form in the fish silage, which are readily available for immediate absorption. Absorbed too quickly, they may be metabolized

prematurely and become unavailable for protein synthesis (Geiger, 1947). Similar findings were observed in another study with a high replacement of FM by FPH (>15%) in an Atlantic salmon diet (Espe et al., 1999a). Increased levels of solubilized proteins were found in the feed, leading to increased concentration of free amino acids in plasma, however, no effect was found on fish performance (Espe et al., 1999a). To mitigate high levels of free essential amino acids in silage processing, a heating step can be used (Raa & Gildberg, 1976). In heated silage, raw materials undergo a heating process (at 85°C) to deactivate proteolytic and lipolytic enzymes and prevent protein hydrolysis into free amino acids. Conversely, in unheated silage, protein hydrolysis continues until about 90% of proteins break down into free peptides and amino acids (Raa & Gildberg, 1976). In **Paper I**, two different drying techniques were used to increase the DM content. While there is uncertainty regarding whether the heating in both techniques could stop the breakdown of proteins, it can be assumed that the evaporator technique likely stopped protein breakdown to free amino acids earlier than the co-drying technique. Therefore, a high level of free amino acids resulting from protein hydrolysis cannot be dismissed as reason for the lower growth observed in fish fed BMS diets compared to the FM group in the first experiment. This hypothesis, however, requires further investigation.

In conclusion, further work is needed to optimize blue mussel processing methods to enhance their applicability as salmon feed ingredient.

5.1.3 Oxidative stress and production-related disorders in salmon fed BMS diets

Nutrient imbalances in fish diets can lead to disturbances in redox regulation and oxidative stress, increasing the risk of production-related disorders such as anemia, bone deformities, and cataract in fish (Hamre et al., 2021; Waagbø, 2006; Waagbø & Remø, 2020). In **Paper I,** despite increased TBARs levels in BMS diets, the inclusion of up to 11% of BMS in the diet did not induce oxidative stress, and the GSH/GSSG ratio in the liver of fish fed BMS diets remained comparable with the control group. Furthermore, no other pathologies or disorders, such as cataracts, bone deformities, fin erosion, skin disorders, or gill disorders, were observed in fish fed diets containing BMS

(up to 11%), despite the observed Fe depletion in the fish body, which could potentially lead to anemia. Previous research has highlighted the potential health benefits associated with the lipid and protein components of blue mussel meat, including antioxidant, anti-inflammatory, and antimicrobial properties attributed to PUFAs and bioactive peptides, suggesting their potential as functional ingredients (Naik & Hayes, 2019). Hydrolyzation unfolds complex protein structure to produce low molecular weight peptides and amino acids, which improve antioxidant activity of hydrolyzed protein in comparison with the intact protein (Sarmadi & Ismail, 2010), which also might have been the case for the performed trials. Moreover, total antioxidant capacity of fish such as turbot improved with increasing level of small molecular weight FPH (Zheng et al., 2013). Therefore, these findings indicated that incorporation of BMS in the salmon diet did not reduce fish welfare nor did it induce oxidative stress.

5.2 Fermented sugar kelp (FSK)

5.2.1 Nutritional composition- Are high carbohydrate and ash contents in FSK products of concern?

Since the contribution of seaweed and FSK products to dietary protein and lipid is low, it cannot be considered a protein and lipid source. Moreover, the high levels of complex carbohydrates such as NSPs raise concerns regarding their suitability for use in aquafeed. However, due to functional properties and beneficial effects on health and welfare of fish, FSK products are potential candidates for “functional ingredients”.

The composition of cell wall carbohydrates in seaweed differs from that in terrestrial plants and typically contains similar or higher levels of dietary fibers (Øverland et al., 2019). Negative effects of fibers on digestion and absorption of energy and nutrients by fish are primarily induced by soluble NSPs rather than insoluble NSPs such as cellulose (Deng et al., 2021; Glencross, 2009; Glencross et al., 2012; Hansen & Storebakken, 2007). In **Paper II**, more than half of the total fiber in FSK products (15% of DM) was soluble NSPs (8.5% of DM) such as alginate, fucoidan and laminarin (These specific components were not directly measured but were indirectly calculated from the non-cellulosic polysaccharides (NCPs)). However, incorporating up to 4% FSK products into diets did not alter the levels of dietary carbohydrates in the experimental diets

compared to control diet, and even dietary neutral detergent fibers (NDF) and hemicellulose content decreased in diets containing FSK products (**Paper II**). Therefore, concerns about the negative effects of NSPs induced by up to 4% FSK in the diet on fish physiology responses may be unfounded, as will be discussed further in Chapter 4.2.2.

The FSK products had an ash content of 44% DM which is considerably higher than the ash level in FM and plant based ingredients (NRC, 2011). The high ash content may dilute the nutrient content of the feed and reduced feed efficiency (Shearer et al., 1992). However, up to 4% FSK inclusion had small impact on ash content of the diet, remaining comparable to the control diet (**Paper II**). Therefore, like carbohydrates, concerns regarding the negative effects of high ash content resulting from FSK inclusion on feed efficiency may be unfounded at these low levels (Chapter 4.2.2).

FSK products can be considered as a Cu source, while they contained low levels of other essential minerals compared to FM and plant-based ingredients (*Fig. 8*). Furthermore, they contain high iodine levels which were reflected in dietary iodine contents in FSK diets (**Paper II**). The mineral contents in FSK diets were not influenced by FSK inclusion in the diet (**Paper II**).

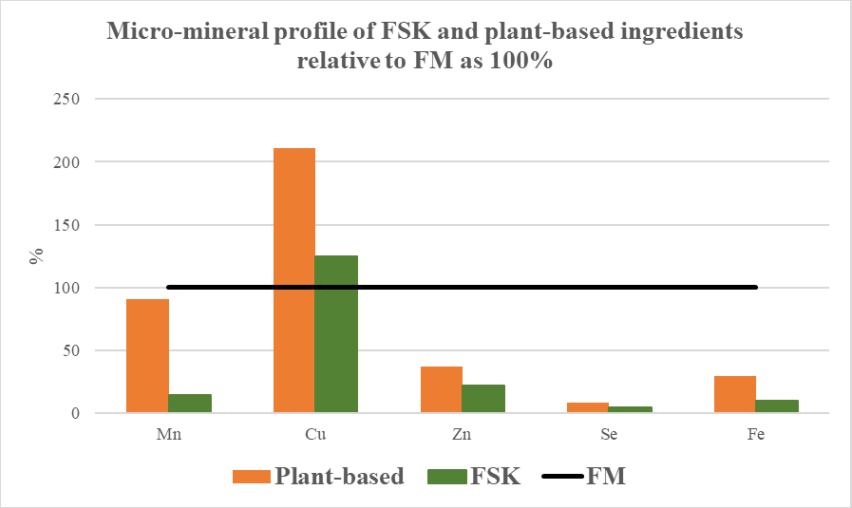

Figure 8: Micro-mineral profile of fermented sugar kelp (FSK) and common plant-based ingredients (soybean meal-5-04-612 and SPC) used in Atlantic salmon feed relative to FM as 100% (black line). The value for plant-based ingredients and FM is from NRC, (2011) and the value for FSK is from the current work.

5.2.2 Dietary energy dilution resulting from incorporating FSK in salmon diet

In **Paper II**, replacing up to 4% FM with up to 4% FSK products caused a slight reduced dose-response in WG of Atlantic salmon due to dietary energy dilution. The low DM content of the FSK product posed challenges for feed production, and likely contributed to the observed dilution of dietary energy in **Paper II**. In previous studies where seaweed was used in feed, the seaweed was typically heat-dried using various techniques and ground into fine powder with high DM content (80-90%), making it easier to be used in feed (Table 5). However, in the fermentation process described in **Paper II**, fresh sugar kelp with a low DM content of 10% was utilized to produce fermented products. These FSK products were directly incorporated into feed pellets without further processing, resulting in a loss of lipid content higher than the inclusion level of sugar kelp. Consequently, this led to a decrease in energy content in diets with higher FSK inclusion levels (4%). The dietary lipid level decreased from 25% in the control diet to 22% and 18% in diets containing 3% and 4% FSK, respectively. These

technical issues with pellet formation may have contributed to a slight reduction in growth observed at higher inclusion levels (**Paper II**).

In a prior study, rainbow trout fed diets containing 4% of non-fermented sugar kelp had a reduced WG, which was attributed to the high fiber content of sugar kelp (Granby et al., 2020). A 2% inclusion level of non-fermented sugar kelp in rainbow trout diets was suggested due to no negative effect on growth at this level by Granby et al., (2020). Comparing findings from the study by Granby et al., (2020) and **Paper I** showed differences in physiological responses of fish fed diets containing 4% non-fermented sugar kelp or 4% FSK. In the study by Granby et al. (2020), reduced growth was associated with reduced protein ADC, hepatosomatic index and morphological changes in the intestine (lower muscularis thickness, vacuolization of enterocytes in the mid intestine). These findings were in accordance with previous studies that showed complex polysaccharides found in algal products coupled with the limited ability of Atlantic salmon to hydrolyze them, can result in decreased protein digestibility (Norambuena et al., 2015). In addition, including red seaweed *Gracilaria vermiculiphylla* (10% of diet) and *Gracilaria pygmaea* (9 and 12% of diet) in the diet of rainbow trout led to reduced intestine diameter, villi height, and length of mucosal folds in the anterior intestine, resulting in reduced nutrient uptake and lower final body weight (Araújo et al., 2016; Sotoudeh & Mardani, 2018). However, in **Paper II**, reduced growth was not associated with any of these observations. Fish fed up to 4% FSK diets had comparable feed intake or protein ADC (**Paper II**). Additionally, the nutrient ADC of FSK diets was comparable with nutrient ADC of other animal or plant-based diets used for Atlantic salmon, and diets containing other seaweed species used in fish feed (Table 3, 5). The average ADC of DM, protein, lipid, and energy was 66%, 86%, 92%, and 82%, respectively, in various fish species fed different seaweed species such as Gracilaria sp., Ulva sp., Sargassum, Porphyra, and sugar kelp (Table 5). Moreover, despite minor changes occurring in gut morphology of fish fed FSK diets, it remained comparable to that of the control group and no inflammatory signs were observed in the mid-intestine of salmon fed FSK diets (**Paper III**). Therefore, the physiological responses of Atlantic salmon to diets containing FSK were better than those observed

in rainbow trout fed diets containing non-fermented sugar kelp at the same inclusion level (4%).

Table 5. Apparent nutrient digestibility (ADC) of different seaweed species used in fish feed.

Feed ingredients	Treatment	Inclusion levels in diet	Fish species	Dietary ADC%				Reference
				DM%	Protein%	Lipid%	Energy%	
Mostly used seaweed species in fish feed								
Gracilaria bursa-pastoris	Sun-dried and grounded	5-10%	European seabass (*Dicentrarchus labrax*)	69	94	99	-	(Valente et al., 2006)
Gracilaria cornea				66	89	96	-	
Ulva rigida				68	92	98	-	
Ulva rigida	Dreid by spray dryer	30 %	European seabass (*Dicentrarchus labrax*)	49	91	89	87	(Batista et al., 2020)
Ulva rigida	Physical process			38	87	88	86	
Ulva rigida	Enzymatic process			42	87	80	86	
G. gracilis	Dreid by spray dryer			60	93	94	86	
G. gracilis	physical process			62	94	92	90	
G. gracilis	Enzymatic process			60	93	92	89	
Porphyra	Oven dried and milled	30 %	Nile tilapia (*Oreochromis niloticus*)	71	78	95	74	(Pereira et al., 2012)
Gracilaria				67	76	91	69	
Ulva				77	80	94	79	
Sargassum				74	81	95	78	
Porphyra			rainbow trout (*Onchorynchus mykiss*)	79	87	96	83	
Gracilaria				77	90	98	81	
Ulva				79	85	94	84	
Sargassum				71	84	89	78	
Porphyra dioica	Dried and milled	5-15- 15%	rainbow trout (*Oncorhynchus mykiss*)	79	-	-	-	(Soler-Vila et al., 2009)
*Verdemin (*derived from *Ulva ohnoi)*	Dry algae meal	2.5-5%	Atlantic salmon (*Salmo salar*)	66	82	87	-	(Norambuena et al., 2015)
Sugar kelp	Dried and milled	1-4%	rainbow trout (*Onchorynchus mykiss*)	78	88	92	-	(Granby et al., 2020)
Sugar kelp	**Fermented**	**1-4%**	**Atlantic salmon *(Salmo salar)***	**68**	**88**	**97**	**80**	**Paper II**

Fermentation using different strains of bacteria can degrade different fibers and improve digestibility of vegetable proteins (Steinkraus, 1996). In a prior study, intestinal morphology in rainbow trout improved when fed dietary fermentable fiber (Vitacel)

(Yarahmadi et al., 2016). The fermentation of dietary carbohydrates can influence gut morphology through the production of short chain fatty acids that can served as an energy source for mucosal and intestinal epithelial cells and improved intestinal morphology (Kihara & Sakata, 1997; Scheppach, 1994). It can be hypothesized that LAB fermentation of FSK potentially contribute to optimize the utilization of sugar kelp in salmonid diets with a positive effect on fiber degradation and maintaining physiology responses such as feed intake, nutrient digestibility, and absorption. However, most importantly, good solutions are needed for incorporating FSK products with low DM content in feed pellets, preventing lipid leaching and consequent energy dilution.

5.2.3 Effect of FSK functional properties on fish welfare and health

Improving the welfare of fish is not only a commercial imperative but also an ethical obligation within the aquaculture industry, aimed at enhancing growth and production outcomes. In **Paper II,** no production-related disorders were detected in fish fed diets containing up to 4% FSK, suggesting that the inclusion of FSK did not lead to nutrient imbalance in the diet and compromise the fish welfare.

The positive impacts of incorporating seaweed as functional ingredients into fish feed have been observed in specific physiological activities, such as enhanced stress response and overall health status (Holdt & Kraan, 2011; Wan et al., 2019). In **Paper III**, dietary inclusion of FSK influenced the antioxidant system, the fish given the diet containing up to 4% FSK had increased hepatic GSH levels and GSH/GSSG ratio, along with reduced lipid peroxidation (MDA levels) and redox potential in a dose-dependent manner. Previous research has shown that oxidative stress marker *gpx1b2* (encodes for glutathione peroxidase) in the liver of rainbow trout fed 2% non-fermented sugar kelp was downregulated, suggesting a reduced need for endogenous antioxidants due to readily available antioxidants from sugar kelp (Ferreira et al., 2020). Similarly, total GSH increased in the liver of Atlantic salmon fed diets containing up to 10% *Laminaria* sp. (Kamunde et al., 2019), and lipid peroxidation decreased in liver of rainbow trout fed diets containing *Gracilaria pygmaea* (9 and 12% of diet) (Sotoudeh & Mardani, 2018). These findings are likely attributed to the presence of carbohydrate compounds

such as fucoidan and phenolic compounds, including phlorotannin (water-soluble) and various tocopherols, in brown seaweed, which have strong antioxidant effects by preventing the formation of free radicals (Holdt & Kraan, 2011). Moreover, fermentation has been observed to facilitate the leaching of antioxidants into the fermentation broth (Gupta et al., 2012), possibly leading to increased antioxidant content in the final products. Moreover, LAB species have been known to produce several ROS-removing enzymes, including glutathione peroxidase (GPx) (Zotta et al., 2017). This was supported by findings in **Paper III**, which demonstrated that the levels of TBARs in the diet containing FSK were lower compared to the control diet. This suggests a possible role for FSK along with LAB species as a natural antioxidant, protecting the feed. Since in aquaculture settings fish experience many types of stress (both physical and environmental), ingredients such as FSK, which not only do not induce oxidative stress but also modulate the antioxidant defense system, making FSK a valuable natural antioxidant in aquaculture.

Disease and stress can impair immune competence, but nutritional interventions offer promise in restoring immune function, thereby enhancing its ability to resist and possibly prevent disease or stress (Trichet, 2010). In addition to the antioxidant effects, seaweed is recognized for its immunostimulant properties. In **Paper III**, inclusion of up to 4% FSK in the diet modulated innate-immune responses, particularly antibacterial effects, in fish, leading to increased plasma lysozyme, anti-protease, and bactericidal activities. These immune responses combat pathogens through various mechanisms, such as directly disrupting cell walls (e.g. lysozyme activity) or producing harmful chemicals like oxidative radicals (e.g. bactericidal activities) (Nayak, 2010). However, the effects were observed to vary at specific inclusion levels (**Paper III**); for instance, Atlantic salmon given diets containing FSK had higher plasma lysozyme activity compared to the control group, with significant increases observed only in the fish fed FSK diets at 1% and 3%. Likewise, plasma bactericidal activity in salmon fed FSK diets was higher compared to the control group, with a significant increase observed only in the 3% FSK-fed fish group compared to other groups (**Paper III**). These findings are in accordance with previous studies that showed increased plasma lysozyme activity in European seabass fed diets containing *Ulva* at 2.5% compared to fish fed control diets

or diets with *Ulva* at 7.5% (Peixoto et al., 2016a). Similarly, rainbow trout fed a diet containing 5% of *Gracilaria vermiculophylla* had the highest plasma lysozyme activities, ACH50, and peroxidase compared to control diets and diets with 10% inclusion (Araújo et al., 2016). Despite the observed increases, the peroxidase activity and IgM levels were found to be lower in all FSK groups compared to the control group **(Paper III)**, which are in contrast with previous literature (Araújo et al., 2016; Nazarudin et al., 2020; Yeganeh & Adel, 2019). The reason for this reduction remained unknown, but it may be linked to prior studies suggesting that immune-modulating responses such as peroxidase activity and IgM levels likely have a specific time window that could be missed during specific sampling times (Giri et al., 2016; Peixoto et al., 2019). Furthermore, it has been shown that effects of seaweed on innate immune responses may be highly influenced by various factors such as fish species, age, weight, rearing water conditions, seaweed composition, and the timing and dosage of seaweed administration (Araújo et al., 2016; Lobo et al., 2018; Vazirzadeh et al., 2020). Therefore, interpreting such findings needs to be done with caution.

Generally, the diverse matrix of bioactive compounds mainly polysaccharides (e.g. fucoidan, alginates, and β-glucans) in seaweed contribute to the immune modulating effects in different fish species (reviewed by Holdt & Kraan. 2011and Wan et al. 2019). Fucoidan shows various biological activities such as immunomodulation (e.g. increased lysozyme and bactericidal activities), antioxidant status (decreased MDA, increased GST), antibacterial activities, and modulation of intestine health in aquaculture (acting as prebiotics) (reviewd by Abdel-Latif et al. 2022). Different processing methods can improve functionality of seaweed compounds and make seaweed derived bioactive compounds more available for fish (reviewed by Wan et al. 2019). Fermentation breakdown complex polysaccharides to oligosaccharides that may aid in their digestion and also enhance the prebiotic properties of indigestible soluble polysaccharides, producing prebiotic saccharides like β-glucan (Gomez-Zavaglia et al., 2019). The β-glucan has been shown to play an immunostimulant role in Atlantic salmon and other fish species (reviewed by Meena et al. 2013). Aside from the seaweed derived prebiotics, certain strains of LAB produce non-specific antimicrobial substances during fermentation, including organic acids like lactic acid, hydrogen peroxide, and toxins

such as bacteriocins, which have immunostimulatory effects (Florou-Paneri et al., 2013). Thus, the presence of these LAB derived antimicrobial agents in FSK diets might also contribute to the observed immune-modulatory effects. Moreover, The beneficial effects of using LAB-based probiotics on immune responses and disease resistance of finfish has been reviewed by Ringø et al. 2018. The presence of inactivated LAB (after undergoing through the extruder) in feed products may still trigger innate immune responses. Vaccines made from inactivated bacteria or viruses, which still retain the ability to stimulate an immune response (Evensen, 2016). Hence, it's plausible that a combination of seaweed functional compounds, inactive bacteria, and antimicrobial agents from LAB play a role in modulating innate immune responses. However, further research is necessary to explore these hypotheses. Challenging trials with pathogens are essential to determine the beneficial effects of these modulating responses on the fish's defense system against pathogens and infections.

5.3 High iodine level in sugar kelp: risk or benefit

Paper II along with previous studies have reported that sugar kelp contains a high iodine level (Biancarosa et al., 2018; Duinker et al., 2016; Mæhre et al., 2014). It raises concerns about its suitability for inclusion in fish diets and potential impacts on both fish and human health.

In **Paper II**, the incorporation of FSK significantly increased the dietary iodine content from 4 mg kg^{-1} WW in the control diet to 138 mg kg^{-1} WW in 4% FSK diet. Fish fed up to 4% FSK diets had a comparable condition factor to the control group (**Paper II**). However, a slight reduction in growth was observed in fish fed high levels of FSK, likely due to the lower dietary energy, rather than the increased dietary iodine level (**Paper II**). Furthermore, the plateau levels for iodine availability and body iodine retention suggested that fish could regulate high dietary iodine levels through absorption and excretion (**Paper II**). These findings are in accordance with prior studies that showed adult Atlantic salmon can tolerate high iodine levels, which are several times (80-fold) higher than their dietary requirement (1.1 mg kg^{-1}) (EFSA, 2005) without compromising their health and growth performance (Granby et al., 2020; Julshamn et al., 2006; Mantovani et al., 2006). For example, feeding adult Atlantic

salmon diets containing up to 86 mg iodine (as potassium iodine, KI) kg^{-1} (Julshamn et al., 2006), and rainbow trout by diets containing 2% non-fermented sugar kelp with an iodine concentration of 117 ± 2 mg kg^{-1} of diet, (Granby et al., 2020) did not induce negative effects on the growth performance and health of these species. Therefore, it is likely that salmon can tolerate an increased dietary iodine content in FSK diets. However, conducting a long-term trial and a more in-depth examination of thyroid hormone status could provide additional insights.

Table 6. Iodine content in muscle tissue of different fish species given diet containing iodine rich seaweeds.

Seaweed species	Fish species	Seaweed inclusion level	Dietary iodine (mg kg^{-1} DM)	Trial duration (month)	Initial iodine level in muscle (mg kg^{-1} WW)	Final Iodine level in muscle (mg kg^{-1} WW)	Iodine availability	References
Saccharina latissima (Sugar kelp)	Atlantic salmon *(Salmo salar)*	4%	157	2	0.07	0.6	88%	**Paper II**
Saccharina latissima (Sugar kelp)	rainbow trout *(Oncorhynchus mykiss)*	4 %	239	3	0.05	1.17 (muscle + skin)	83%	(Granby et al., 2020)
Gracilaria vermiculofylla	rainbow trout *(Oncorhynchus mykiss)*	5%	105	3	0.11	0.22	-	(Valente et al., 2015)
Laminaria digitata	Fresh water char *(Salvelinus* sp.*)*	0.8%	400	9	0.13	0.54 (muscle + skin)	-	(Schmid et al., 2003)
Laminaria digitata	rainbow trout *(Oncorhynchus mykiss)*	0.4%	20	3	0.02	0.12	-	(Ribeiro et al., 2017)
Laminaria digitata	Seabream *(Sparus aurata)*	10%	428	5	0.13	0.84	-	(Ribeiro et al., 2015)

Feeding Atlantic salmon with a diet containing up to 4% FSK resulted in a substantial increase in muscle iodine content, reaching levels 8.5 times higher (0.6 mg kg^{-1} WW)

than those found in the control group (**Paper II**). Similar results have been found earlier highlighting iodine-rich seaweed as a natural source for enriching fish muscles from aquaculture fish with iodine (Table 6). In previous studies muscle iodine content was influenced by factors such as type of seaweed, dietary iodine levels, trial duration, and the fish species. Thus, FSK as a natural source has potential to enrich salmon muscles with iodine.

According to the World Health Organization (WHO, 2007) recommendations, the daily recommended intake (DRI) of iodine is 150 µg day^{-1} for adolescents and adults (above 13 years), with higher levels recommended for pregnant and breastfeeding women (250 µg day^{-1}). In a recent study, adults under 55 years from Mid-Norway were found to have a mild iodine deficiency based on WHO criteria (Abel et al., 2024). Therefore, there is a suggestion to increase the iodine content of the Norwegian diet. As fish contributes 20% of the dietary iodine intake in Norwegian diet (Dahl et al., 2004), the aquaculture sector can enhance the iodine content in fish fillets by including ingredients rich in iodine such as seaweeds. The 100 g fillet of post-smolt salmon fed with FSK4% contained 64 µg of iodine **(Paper II)**, which is significantly higher than the iodine content in a 100 g fillet of conventionally farmed salmon fed with a commercial diet (3.4 µg iodine). This amount is approximately one-third of the iodine content in wild cod (*Fig.*9) (Dahl et al., 2020; Seafooddata, 2023). Consequently, a 200 g portion of salmon fillet fed FSK4% diet would cover about 83% (128 µg I day^{-1}) of the iodine DRI for young and adult individuals (150 µg I day^{-1}) (Russell et al., 2001). This finding is in accordance with a previous study that showed consuming a 160 g fillet of rainbow trout given a 2% sugar kelp (non-fermented) diet also fulfills around 60% of the WHO DRI (90 µg day^{-1}) (Granby et al., 2020). Therefore, incorporating sugar kelp (both fermented and non-fermented) into the diet of salmonids presents a practical approach to improving iodine nutrition through aquaculture practices.

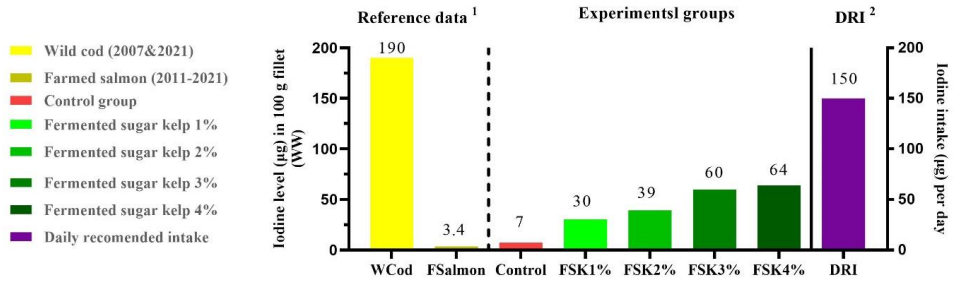

Figure 9: Overview of Iodine level in 100 gr fillet of wild cod, farmed fish and the experimental groups. WW stands for wet weight. [1] (Seafooddata, 2023) [2] (Russell et al., 2001).

5.4 Contribution of BMS and FSK to sustainability

Numerous studies have highlighted the benefits of low trophic aquaculture, particularly seaweed and bivalves, emphasizing their positive contributions to achieving SDGs (*Fig.*10) (SAPEA, 2017). Low trophic marine species, in brief, have low environmental impact, they will grow with low input and may also transform linear nutrient flows from land to the sea into circular systems (Albrektsen et al., 2022; Hilborn et al., 2018; Petersen et al., 2019). However, the integration of these sustainable ingredients into the overall production system, both feed and fish productions, can influence the sustainability of aquaculture by either increasing or decreasing GHG emissions (Winther et al., 2020). Therefore, addressing the contribution of novel ingredients in sustainability of both feed and fish production systems is crucial. The Life Cycle Assessment (LCA) methodology serves as a valuable tool for this purpose, offering a comprehensive evaluation of resource use and environmental impacts throughout the entire value chain. Hempel (2022) conducted an LCA study to address the environmental footprint of production of salmon feeds containing BMS and FSK (as used in **Paper I** and **Paper II**). The findings revealed that substituting FM with up to 11% BMS in the feed led to a reduction of GHG emissions by up to 10% compared to the reference feed. Moreover, using FSK (up to 4% in the diet) resulted in a marginal reduction of GHG emissions of up to 5% compared to the reference feed. The minimal energy consumption for farming and processing BMS and FSK significantly contributed to the observed low GHG emissions in this study (Hempel, 2022). Hence,

it can be assumed that BMS and FSK can make a positive contribution to the sustainability of the feed production system.

Regarding the contribution of novel ingredients to the sustainability of the fish production system, GHG emissions are linked to FCR and the origin of feed components (Hasan & Soto, 2017). A lower FCR signifies reduced undesirable outputs and nutrient losses to the environment, which can lead to environmental issues such as eutrophication and loss of biodiversity (Hasan & Soto, 2017; Waite et al., 2014). Over the years, the FCR in salmon farming decreased significantly, from about 2.8 to approximately 1.2 (Hasan & Soto, 2017). In **Paper I**, FCR increased in fish fed diets containing up to 11% BMS, however, using different processing method resulted in comparable FCR between fish fed diets containing up to 9% BMS and the control diet. In **Paper II**, FSK inclusion in the diet did not influence the FCR. These findings possibly suggest low GHG emissions from the fish production system when using BMS and FSK diets, and no negative impact on sustainability. However, in the LCA study by Hempel (2022), FCR was represented differently, and adjusted to model a net pen. The reference FCR, derived from a real-world scenario covering all fish life stages (FCR = 1.18), served as a benchmark. The observed FCR, obtained from our initial feeding trial (**Paper I-**only the first BMS experiment**)** and (**Paper II**) limited to the smolt stage, was then compared to this reference FCR, creating a relative FCR that represents the change relative to the reference FCR. Hence, fish fed diets containing higher levels of BMS and FSK had increased relative FCR, subsequently elevating the GHG emissions. As emphasized in this study, having FCR results from a comprehensive grow-out study, rather than just a 76-day trial of the smolt stage, would provide more reliable and robust conclusions. However, it should be noted that using FCR from feeding trials, such as those in **Paper I** and **II**, may provide a more accurate representation of the true feed efficiency compared to data from net pen scenarios used as reference FCR. Because in the feeding trials all feed given to the fish is collected and accounted for FCR calculations, a practice not feasible in net pen scenarios.

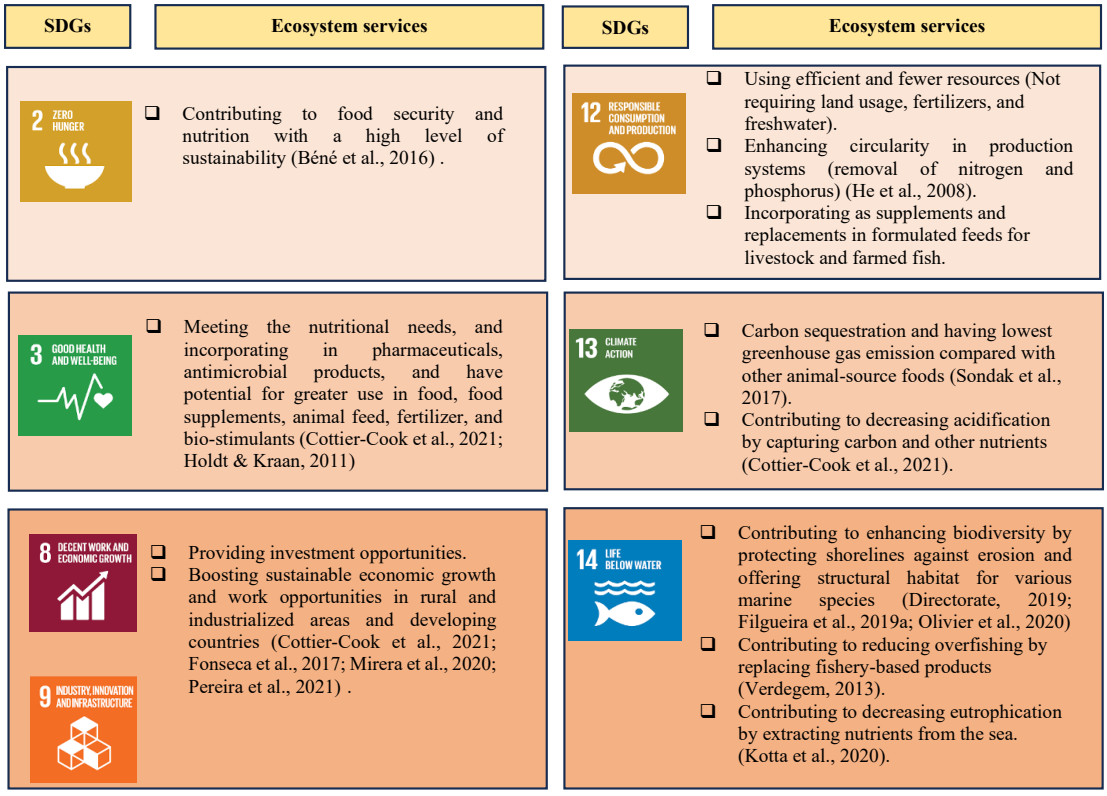

Figure 10: Ecosystem services of low trophic aquaculture such as seaweed and bivalves linked to Sustainable Development Goals (SDGs).

5.5 Is the production of blue mussel and sugar kelp in Norway sufficient to contribute to closing the feed gap?

To meet the growth targets in Norwegian salmon production by 2030, it has been estimated that a total of 2.8 million tons of feed, based on current FCR (1.2 on dry matter basis), will be required (NCE, 2022). This represents an increase of 1 million tons compared to the feed volume in 2020 of 1.8 million tons to support the desired expansion of the salmon industry in Norway. The NCE report (2022) indicated that only 140 000 of the 1 million tons can be met by Norwegian produced ingredients such as blue mussels, land animal by products, microalgae, marine by products, insects and novel marine ingredients (NCE, 2022). This leaves a gap of 860 000 tons that needs to be filled with existing ingredients. Therefore, culturing and up scaling the production

of novel feed ingredients produced in Norway beyond the current level 0.4% (8,130 tons in 2020) could potentially offer a solution to achieve growth and sustainable feed objectives in the future (NCE, 2022).

In Paper I, it was shown that 9% of BMS with 50% DM content could replace 36% of FM in a" commercial" diet with 25% FM for Atlantic Salmon without any negative effects on the performance and welfare of the fish. In 2020, the Norwegian salmon industry utilized 1.8 million tons of feed ingredients to produce nearly 1.5 million tons of salmon (Aas et al., 2022). Based on a hypothetical calculation, adding 10% dry blue mussel to the feed (1.8 million tons), would require approximately 180,000 tons of dry blue mussel to produce BMS or BMM. Considering that approximately 120 kg of dry matter (including shell) can be obtained from 1000 kg of live blue mussels (H. Sveier, Ocean Forest, personal communication), it would necessitate the production of at least 1 500 000 tons of blue mussels to obtain the required amount of dry blue mussel. However, it is essential to note that mussel silage or meal contains only the meat portion of blue mussel. The meat content of blue mussels can vary with region, season, and genetic differences (Bustnes & Erikstad, 1990; Thompson, 1985). Therefore, an even greater amount may be needed to produce enough dry blue mussels. The production of blue mussels for human consumption in Norway in 2022 was 2 612 tons (including shells, after removing all by-products) (Fiskeridirektoratet, 2023a). Considering approximately 30% of total production (3 396 tons) being by-products (Naik & Hayes, 2019), only 784 tons (including shell) would be available for animal feed. This is much lower than the required amount. This estimation is based on the current need to produce 1.8 million tons of salmon diet. However, to meet the projected demand of 2.8 million tons of salmon feed production by 2030 (NCE, 2022), an even larger production of blue mussels as feed ingredients will be necessary.

Norway has a huge potential for blue mussel production which might be utilized (Torrissen et al., 2018). There are several model studies that simulate blue mussel production in Norwegian fjords such as the Lysefjord and the Hardangerfjord (Filgueira et al., 2019b; Gatti et al., 2023; Torrissen et al., 2018). Mussel cultivation potential in the Lysefjord was estimated at 140-200 tons/ km^2 based on empirical information from

mussel farming capacity (Torrissen et al., 2018). It has been shown that food concentration and water flow are the most important factors contributing to blue mussel production potential and the area needed for production in fjords (Rosland et al., 2011; Torrissen et al., 2018). Controlled upwelling of nutrient rich deep water in fjord can increase production and decrease the used area (Aure et al., 2007; Torrissen et al., 2018). For example, a production of 1 million tons using long-line farms would require a total area of 5715 km^2. However, if the mussels are grown in fjords with controlled upwelling, the need for area could be halved or more. Hardangerfjord in western Norway also showed potential to host large-scale mussel farming for both aquafeed and human purpose (Gatti et al., 2023). Simulations of short production cycles of blue mussel for aquafeed (1 year instead of 2 years) were more efficient for exploiting primary production since young and small mussels have lower maintenance and reproduction costs and higher meat yield. In another report it was argued that Hardangerfjord can easily produce 200 000 tons of blue mussel by 2030 (NCE, 2022).

Similarly, to incorporate 1% FSK in the salmon diet (1.8 million tons), approximately 18 000 tons of sugar kelp on DM basis are needed. The production of sugar kelp for food and feed in Norway was 161 tons in 2022, on a small scale but with great growth potential (Fiskeridirektoratet, 2023b). Simulation studies suggested a maximum cultivation potential of 150–200 tons per hectare per year along the Norwegian coast in 6 different regions (Broch et al., 2019). Projections indicate that by 2050, the Norwegian aquaculture industry could produce 20 million tons of kelp with an annual turnover of 4 billion Euros (Olafsen et al., 2012). Based on an average annual production of 170 tons per hectare, this production will require approximately1200 km^2 (Torrissen et al., 2018).

In conclusion, there is a high potential for cultivating blue mussels and sugar kelp in Norway in the future. Natural conditions are in favor of this type of production, but there is a need for building up a completely new industry. Addressing current challenges in the cultivation of these species within a short-term perspective (less than 10 years) while also adapting cultivation methods in response to global warming and ocean

acidification in the long term (more than 10 years) may offer a solution to meet the increasing demand for these novel feed ingredients (Torrissen et al., 2018).

6 Conclusions

Both BMS and FSK can be used in the diet for Atlantic salmon post smolts, however, further work is needed to optimize the processing of these raw materials for use in the salmon feed.

- **BMS**

- Drying BMS to 50% DM by evaporation allowed for an inclusion level of 9% in the Atlantic salmon diet. This level could be used without affecting growth performance, feed utilization, nutrient digestibility, or retention. When co-dried with SPC, feed intake and digestibility were not affected, but there was a dose-dependent decrease in growth performance and feed utilization (**Paper I**).
- The incorporation of BMS in the diet led to comparable growth rates and feed utilization to diets containing similar levels of BMM, depending on the processing method, particularly drying (**Paper I**).
- Using BMS in the feed did not affect the occurrence of production-related disorders or influence the antioxidant defense systems (**Paper I**).

- **FSK**

- Up to the highest tested inclusion level of 4%, FSK can be incorporated into salmon diets without affecting feed utilization and nutrient digestibility. However, a dose-dependent reduction in growth rate and nutrient retention was seen, likely due to dietary energy dilution, which can limit FSK inclusion level in salmon diets (**Paper II**).
- Inclusion of up to 3% FSK increased iodine status in the whole body and fillet of fish by 6 and 8.5-fold compared to the control group (**Paper II**).
- Incorporating up to 4% FSK did not result in occurrence of production-related disorders in FSK-fed fish groups compared to the control group (**Paper II**). Despite minor changes in gut morphology, the gut remained comparable to the control group, with no signs of inflammation observed (**Paper III**).
- The inclusion of FSK modulated the hepatic antioxidant defense systems, showing a dose-dependent increase in GSH and GSH/GSSG ratio, along with a

decrease in MDA levels. Furthermore, FSK may enhance innate immune responses, with modulating effects on lysozyme, anti-protease, bactericidal, and peroxidase activities (**Paper III**).

7 Future perspective

- In this work, the investigation was done on the post-smolt stage of Atlantic salmon under controlled environmental conditions, while it is crucial to consider that certain nutrition-related disorders may become apparent after prolonged feed consumption and in the presence of environmental stressors in farming conditions. Therefore, conducting long-term trials using new feed ingredients across various life stages of fish, ideally in settings closely resembling real farming conditions, can provide a more comprehensive understanding of the suitability of these novel ingredients.
- To achieve the sustainable growth targets set for Norwegian salmon production by 2030, it's crucial after verifying the suitability of blue mussel and sugar kelp in fish feed, addressing strategies for scaling up the production of these new ingredients.
- This study underscored the influence of processing, particularly drying methods, on the suitability of BMS in salmon diets. Additionally, the challenge of low DM content in these products was identified as a limiting factor for higher inclusion levels in feed pellets. Future research should focus on identifying optimal processing methods to improve the quality of silage products, overcome these challenges, and develop novel marine ingredients for aquafeed.
- incorporating iodine-rich seaweed species like *Laminaria* and *Saccharina* can significantly elevate iodine levels in the fillets of salmonids (Table 5). Further investigation, particularly long-term trials involving larger fish at market size, is necessary to assess the impact of FSK on fillet iodine content comprehensively. This would offer valuable insights into addressing iodine deficiency concerns in populations.
- Atlantic salmon fed FSK diets showed modulations in the immune responses, particularly increases in innate immune responses. Conducting a pathogen challenge trial will provide valuable insights into the functional effects of dietary FSK the salmon diet.
- Bioactive compounds like prebiotics derived from carbohydrates have been shown to modulate gut microbiota and immune function (Øverland et al., 2019).

Exploring how dietary FSK influences the microbiota of salmon would be valuable, providing further insights into the functional effects of FSK in aquafeed.

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Paper I

Sahar Sartipiyarahmadi, Antony J. Prabhu Philip, Aksel N. Forshei, Harald Sveier, Silje Steinsund, Malin Kleppe, Erik-Jan Lock, Angelico Madaro, Tom Johnny Hansen, Martin Wiech, Øivind Strand, Jan Vidar Jakobsen, & Sofie C. Remø (2024).

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Blue mussel (*Mytilus edulis*) silage, a possible low trophic marine protein source for Atlantic salmon (*Salmo salar* L.)

Sahar Sartipiyarahmadi [a,b,*], Antony J. Prabhu Philip [a,d], Aksel N. Forshei [c], Harald Sveier [c], Silje Steinsund [c], Malin Kleppe [c], Erik-Jan Lock [a,d], Angelico Madaro [a], Tom Johnny Hansen [a], Øivind Strand [a], Martin Wiech [a], Jan Vidar Jakobsen [e], Sofie C. Remø [a,*]

[a] Institute of marine research (IMR), Bergen, Norway
[b] Department of Biological Sciences, University of Bergen, Bergen, Norway
[c] Lerøy Seafood Group ASA, Bergen, Norway
[d] Nutrition and Feed Technology Group, Nofima, Bergen, Norway
[e] Cargill, Dirdal, Norway

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ABSTRACT

Blue mussel (*Mytilus edulis*) could be a promising marine protein source in fish feeds and is of great interest since it can be cultivated along the Norwegian coastline. However, the use of blue mussels in feeds is dependent on developing suitable preservation and processing methods to produce a feed grade raw material. The present studies were conducted to investigate whether blue mussel silage could be used in the feed for Atlantic salmon post-smolt. Two feeding experiments were conducted using the same reference diet with FM inclusion of 25%, giving a mix of ~59–63% plant-based ingredients vs ~34–36% marine ingredients to simulate a standard grower feed for salmon post-smolts in SW. In experiment 1, fish were fed diets containing three different inclusion levels of blue mussel silage (BMS 3, 7, and 11%), a diet containing blue mussel meal (BMM) (12%) as well as the reference feed. In this experiment, the fish that were fed a diet containing BMS had a decline in both weight gain and condition factor when compared to the fish given the reference and BMM. The daily feed intake was similar in all groups, but the feed conversion ratio (FCR) increased in the fish fed BMS. The inclusion of BMS and BMM did not affect the digestibility of nutrients, but reduced retention of whole-body lipid and protein retention was observed. Salmon given BMS in the diet also had lower iron (Fe) concentrations in liver and whole body, indicating lower Fe uptake, irrespective of inclusion level. These findings were followed up in a second feeding experiment aiming to investigate whether different processing methods of blue mussel silage could influence the bioavailability of iron, as well as feed utilization and growth. The reference feed was formulated similar to the feed in exp. 1. Additionally, fish were fed diets containing BMM (9%) and the same batch of BMS (9%) used in exp. 1 as well as two diets containing new productions of BMS (9%) using either a lower acid level or only formic acid at the same level. In experiment 2, no differences were seen in weight gain, feed intake, FCR, nutrient retention or body composition between fish given BMS and reference diet. The lower Fe status observed in experiment 1 was not seen in the second study. In both experiments, there were no differences in fish welfare indicators between the group of fish fed with BMS, BMM and the reference group.

The present results show that blue mussel silage can be used in the diet for Atlantic salmon, however, the different processing and preservation methods to produce BMS influence the nutritional properties and consequently growth performance and feed utilization of Atlantic salmon post-smolts.

1. Introduction

In 2020, over one million tons of Atlantic salmon, accounting for 53% of the global salmon production, was produced in Norway (Fiskeridirektoratet, 2023a). The annual use of feed ingredients in farming of salmon in Norway is almost 2 mill tons (as is) (Aas et al., 2022). Today >90% of the ingredients used in fish feeds in Norway is imported (Aas et al., 2022), contributing to >70% of the greenhouse gas

* Corresponding authors.
E-mail addresses: Sahar.sartipi.yarahmadi@hi.no (S. Sartipiyarahmadi), Sofie.Remo@hi.no (S.C. Remø).

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emissions from the industry. Thus, new local resources with a low climate footprint are needed.

The past decades, the protein used in aquafeeds has shifted from a high level of fish meal (FM) to plant proteins (Albrektsen et al., 2022; Aas et al., 2019), and today only 12% of the protein and 10% of the lipids in the salmon feed comes from the sea. The traditional sources for marine ingredients, industrially fished species (reduction fisheries) and offal's, cannot be increased, thus, new marine resources will have to come from lower trophic levels. Blue mussels (*Mytilus edulis*) are of high interest as a potential marine protein source (Bellona, 2021; Filgueira et al., 2019; Gjøsund et al., 2020; Kiessling, 2009). The production of blue mussels for use in food in Norway was only 2647 tons in 2022 (Fiskeridirektoratet, 2023b), but the possibilities for increasing the production has recently been simulated (Gatti et al., 2023) which was shown the Hardangerfjord could host large-scale mussel farming for aquafeed and human consumption (Gatti et al., 2023).

The nutritional profile of blue mussels makes them suitable for use in fish feeds (Berge and Austreng, 1989; Kikuchi and Furuta, 2009b; Kikuchi and Sakaguchi, 1997). Several studies have shown that blue mussel processed into a meal can be used in fish feeds (Kikuchi and Furuta, 2009a, 2009b; Langeland et al., 2016; Nagel et al., 2014; Vidakovic et al., 2016; Weiss and Buck, 2017), also improving the palatability of plant protein-diets and growth of fish (Kikuchi and Furuta, 2009a; Nagel et al., 2014). Previous studies have also shown that using the whole shell is challenging due to increased ash level and reduced energy density of the feeds (Berge and Austreng, 1989). Blue mussel meat has a high moisture content (> 95%), neutral pH (6.7–7.1), and hydrolytic enzymes that result in fast degradation, despite using refrigerated storage (Bhunia et al., 2017; Ovissipour et al., 2013; Zhou et al., 2019). Additionally, blue mussels have a large seasonal variation in nutrient composition. For instance, peak carbohydrate accumulation (glycogen) occurs in spring and summer, with subsequent depletion during autumn and winter. Conversely, lipid and protein trends displayed smoother variations and depended mostly on the reproductive cycle of the mussels. It has been shown that the highest nutritional quality accrues before the gametogenesis phase of maturation in mussels which usually is in late spring, while it can vary in different regions (Petes et al., 2008; Fernández et al., 2015). Therefore, efficient preservation methods are also necessary to produce a feed raw material with high nutritional quality throughout the year (Fernández et al., 2015). To minimize the deterioration of fresh by-products like fish offal or meat of blue mussels, preservation by acid silage is a simple and inexpensive alternative (Olsen and Toppe, 2017). Low manufacturing cost, preserving the nutrients with high quality in addition to being an environmentally friendly process (low waste, low carbon footprint) are the main advantages of acid silage (Fagbenro and Jauncey, 1993; Vidotti et al., 2003). Through silage technology using short-chain organic acids, the proteins are hydrolyzed, resulting in the formation of small peptides and free amino acids (Espe et al., 2015). These peptides are quickly digested and absorbed by the gastrointestinal tract, which could impact the overall digestibility of the products (Gilbert et al., 2008) and enhance the availability of nutrients in the feed (Espe et al., 1999). For instance; replacing up to 15% of the FM protein by fish protein hydrolysate (FPH) in Atlantic salmon diet resulted in increased growth, feed utilization, and digestibility (Espe et al., 1999; Refstie et al., 2004). Similarly, the replacement of 18–24% of the FM with FPH in post-smolt salmon diets resulted in increased feed intake, specific growth rate, feed conversion ratio, and protein digestibility (Hevrøy et al., 2005). In the diet of rainbow trout (*Oncorhynchus mykiss*), FM could also be substituted with 20% FPH without adverse effects on growth performance, fatty acid composition and serum biochemical variables (Güllü et al., 2014). However, a high amount of water in the silage products can be a drawback in terms of transportation and commercialization (Barreto-Curiel et al., 2016). It also make it difficult to be used directly in dry or moist feed (Madage et al., 2015). To address this issue, silage can be dried together with dry ingredients, such as soybean-, feather- or poultry by products meals or cereal brans or drum drying have been suggested as a solution (Dong et al., 1993; Goddard and Perret, 2005; Hardy et al., 1984; Madage et al., 2015; Nwanna et al., 2004). Drying techniques that use heat to remove water may affect the nutritional value of the end products (Goddard and Perret, 2005). Therefore, it is important to choose an appropriate drying method that preserves the quality of the final product, while also minimizing the climate footprint of the processing methods.

Blue mussel silage (BMS) has been tested as a dietary ingredient in pigs, resulting in higher ileal crude protein digestibility compared to FM (Nørgaard et al., 2015). However, up to date, there has been no prior study on its use in fish diets, particularly salmonids. It is important to determine the effect of raw material processing as well as the availability of nutrients from the raw material to avoid the occurrence of nutritional deficiencies, imbalances or the effect of potential antinutrients that historically have been related to malnutrition, reduced welfare and occurrence of production-related disorders when introducing new raw materials in the feed (reviewed by Waagbø and Remø, 2020). The present study was conducted to investigate whether blue mussel silage and blue mussel meal can be used in feed for Atlantic salmon post-smolts, based on growth, welfare, nutrient digestibility and retention.

2. Material and method

2.1. Ethical statement

Both feeding experiments were conducted at Matre Research station, Norway, according to the Norwegian regulations on Animal Experimentation (FOTS ID # 25202 for experiment 1).

2.2. Blue mussel silage

The blue mussel silage was provided by Ocean Forest AS (Bergen, Norway). To mitigate the impact of seasonal and geographical variations on the nutritional composition of BM in both experiments, undersized blue mussels were collected from commercial blue mussel farming operated by Blå Biomass A/S in Limfjorden, Denmark in spring season. The first blue mussel silage product was made by adding soft acid (aqua M, produced by Borregaard) to the meat part of blue mussel after a mechanical crushing step and separating the blue mussels into three parts: shell, byssus threads and meat. According to the safety data sheet (revision date 28.12.2022, version 2.4.0), the substance mixture of soft acid is 75–85% formic acid, 15–25% sodium lignosulfonate (lignosulfonic acid, sodium salt, as antioxidant) and liquid.

For experiment 2, a new blue mussel silage batch was produced using a lower soft acid content (aqua M, produced by Borregaard) and one with only formic acid. The acid level was added, and consequently pH level was lower in the second BMS production. The pH level and proximate composition of BMS products are given in Table 1 and Table A and B of supplementary.

2.3. Experimental diets

2.3.1. Experiment 1

The first feeding experiment was designed as a dose-response study using three different inclusion levels of BMS (3, 7, and 11% of diet, substituting 12%, 28%, 44% of the fish meal (FM) in the diet), and in addition one diet contained blue mussel meal (BMM) (12% of diet, substituting 48% of FM). Each experimental diet was tested in triplicate tanks. The reference diet was formulated as a commercially relevant diet for post-smolt in seawater with 25% FM. In this experiment, the BMS used had a dry matter content of 10% and was therefore co-dried with soy protein concentration (SPC) before feed production to obtain the target levels of BMS in the feed by Cargill (Dirdal, Norway). The proximate and amino acids composition of the control and experimental diets of the first experiment are given in Tables 2 and 4, respectively.

Table 1
Macro-nutrient and mineral proximate composition of blue mussel silage (BMS) products.

	BMS High soft acid[1]	BMS Low soft acid	BMS Low formic acid
Macro-nutrients proximate composition (g 100 g^{-1} WW)			
Protein	23	22	21
Lipid	0.6	0.3	0.4
Ash	5.3	6.6	6.4
Dry matter	48	47	45
Macro-mineral composition (mg kg^{-1} WW)			
Ca	2976	4042	4140
Na	11,520	15,040	14,400
K	3024	3337	3015
Mg	1680	2115	1980
P	1968	1692	1395
Micro-mineral composition (mg kg^{-1} WW)			
Mn	14	66	99
Cu	3.7	1.9	1.7
Fe	274	409	387
Se	1.1	0.8	0.7
Zn	22	24	26
TBARS	79	106	73
pH	2.5	3.7	3.5
Histamine	<5	<5	<5

WW refers to wet weight.
[1] BMS High soft acid group is the same product that was used in both experiment1 and 2. A new blue mussel batch and different types and amount of acid was used for producing the other two groups (BMS with lower soft acid and BMS with only formic acid).

2.3.2. Experiment 2

In experiment 2, the fish were given five different diets. The reference diet was formulated to be similar to the diet used in experiment 1, with 25% FM. Four experimental diets were produced, two containing the same blue mussel meal (BMM9) and BMS (BMS9) that was used in the first trial, and two using new productions of BMS, with lower concentration of soft acid and formic acid content (BMSS9 and BMSF9 respectively). All diets were added a similar inclusion level of blue mussel products of 9% of the diet, substituting 36% of the FM. The new blue mussel products were dried to 50% (EPCON technology), and the batch of BMS used in experiment 1 was dried at Hordafôr. The proximate and amino acids composition of the control and experimental diets of the second experiment are given in Tables 3 and 4 respectively.

The diets were produced by Cargill (Dirdal, Norway) and stored at 4 °C until the feeding trial started in both experiments. The BMM used in both experiments were provided by Triple nine (Esbjerg, Denmark). Yttrium oxide (0.02% ≈ 200 mg kg^{-1}) was added as an inert marker to all diets to determine apparent digestibility/availability of nutrients in both experiments.

2.4. Fish and rearing condition

2.4.1. Experiment 1

In experiment 1, a total of 975 Atlantic salmon post-smolts originated from Aqua Gen produced at Matre Research Station were randomly distributed among 15 glass fiber square tanks (1.5 m^3). Each tank had 65 post-smolts consisting of 55 fish (mixed population) that were produced from commercially available eggs obtained from Aqua gen in the fall of 2019 and 10 pit-tagged all-male isogenic salmon from a line originally derived from the Aqua Gen strain in 2011 (Fjelldal et al., 2020; Hansen et al., 2020). The mean weight of the mixed population and all-male population was 200 ± 39 g and 203 ± 34 g (Mean ± SD), respectively. The isogenic fish was added as a standard reference to reduce the effect of genetic variation in the growth evaluation. The average biomass per tank at the start of the experiment was 13 ± 0.7 kg (Mean ± SD). The experiment lasted for 10 weeks. The water temperature ranged between 8.8 and 9.2 °C with a mean of 9 ± 0.07 °C (Mean ± SD) and the fish were kept under continuous light (24:0, L:D period). The acclimatization period was three weeks prior to the experimental start. The fish were fed two times per day by automatic feeders (Arvotec TD 2000), between 9:30 to 11:00 and 12:30 to 14:00. The feeding rate was adjusted according to the increase in biomass as the fish grew.

Table 2
Formulation (g $100g^{-1}$) and proximate composition of the experimental diets containing blue mussel meal (BMM) and different levels of blue mussel silage (BMS) in experiment 1.

Experiment 1					
	Control	BMS3	BMS7	BMS11	BMM12
Fish oil	10.2	10.3	10.4	10.4	10.1
Rapeseed oil	13.9	13.3	12.4	11.6	13.2
Fishmeal LT	25.0	20.3	15.4	10.5	13.0
Soy protein concentrate (SPC)	20	21	18.7	17.5	12.3
Raw wheat	11.0	11.0	10.4	10.5	11.0
Other plant proteins[1]	16.8	17.8	21.2	24.9	24.3
Micro-ingredients	3.17	3.30	3.45	3.62	4.11
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
BMM	–	–	–	–	12
BMS High soft acid	–	3	7	11	–
Analyzed proximate composition (g 100 g^{-1} WW)					
Protein	46	45	43	42	44
Lipid	24	24	21	23	23
Ash	7	7	7	6	6
Gross Energy (MJ kg^{-1} WW)	23	23	22	23	23
Digestible energy (MJ kg^{-1} WW)	19	20	19	19	19
Dry matter	95	94	93	94	95
Vit C (mg kg^{-1} WW)	1100	1100	980	990	1000
Vit E (alfa-tocopherol) (mg kg^{-1} WW)	360	360	380	360	350
TBARs (nmol g^{-1} WW)	14	12	15	19	16
Macro-mineral composition (mg kg^{-1} WW)					
Ca	13,300	11,280	11,160	10,340	11,400
Na	3800	4512	5394	5922	4275
K	10,450	10,340	9300	8272	8170
Mg	2185	2068	2139	2068	1900
P	13,300	11,280	11,160	11,280	13,300
Micro-mineral composition (mg kg^{-1} WW)					
Mn	51	54	50	56	56
Cu	10	9	10	10	10
Fe	190	207	244	291	266
Se	0.8	0.8	0.8	0.8	1.0
Zn	162	150	158	158	181

Notes: Ingredients are listed as percentages of whole feed. WW refers to wet weight basis. The sign "-" means no data is available.
[1] Wheat gluten meal. Pea protein concentrate- and guar meal. BMS refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal.

2.4.2. Experiment 2

The second feeding experiment started with randomly distributing 54 Atlantic salmon post-smolts in each of 15 glass fiber square tanks (1 × 1 m) (810 total fish), with an average weight of 119 ± 2 g (Mean ± SD). The fish originated from SalmoBreed and were obtained as parr from Lerøy Sjøtroll Fitjar and smoltified at Matre Research Station prior to the experiment start. The average tank biomass at the start of the experiment was 6 ± 0.12 kg (Mean ± SD). The experiment lasted for 7 weeks. The water temperature ranged between 11.1 and 12.5 °C with a mean of 12 ± 0.2 °C (Mean ± SD) and the fish were kept under continuous light (24:0, L:D period). The acclimatization period was three weeks prior to the experimental start. The fish were fed two times per day by automatic feeders (Arvotec TD 2000), between 09:00 to

Table 3
Formulation (g 100g^{-1}) and proximate composition of the experimental diets containing blue mussel meal (BMM) and different processed blue mussel silage (BMS) in experiment 2.

Experiment 2					
	Control	BMM9	BMS9[2]	BMSS9[3]	BMSF9[4]
Fish oil	10.4	10.7	10.9	10.9	10.9
Rapeseed oil	14.7	14.6	14.1	14.1	14.2
Fishmeal LT	24.9	16	16	16	16
Soy protein concentrate (SPC)	24.6	19.1	23.5	23.5	23.5
Raw wheat	4.4	4.4	6.5	6.5	6.5
Other plant proteins[1]	17	21	21	21	21
Micro-ingredients	3.6	4.9	3.8	3.8	3.8
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Blue mussel meal	–	9	–	–	–
BMS High soft acid	–	–	9	–	–
BMS Low soft acid	–	–	–	9	–
BMS Low formic acid	–	–	–	–	9
Analyzed proximate composition (g 100 g^{-1} WW)					
Protein	45	45	43	43	41
Lipid	23	23	24	24	24
Ash	7	6	7	8	7
Gross Energy (MJ kg^{-1} WW)	22	22	22	22	21
Digestible energy (MJ kg^{-1} WW)	19	19	20	20	19
Dry matter	93	93	94	93	91
Vit C (mg kg^{-1} WW)	1100	1100	640	670	670
Vit E (alfa-tocopherol) (mg kg^{-1} WW)	210	169	280	330	320
TBARs (nmol g^{-1} WW)	7	14	16	22	16
Macro-mineral composition (mg kg^{-1} WW)					
Ca	13,020	11,160	10,340	10,230	10,010
Na	4185	3813	6486	7626	7280
K	9300	8091	8836	9021	8463
Mg	1953	1860	2162	2352	2184
P	13,020	12,090	11,280	11,160	10,010
Micro-mineral composition (mg kg^{-1} WW)					
Mn	60	66	66	88	100
Cu	12	13	12	12	11
Fe	186	260	282	316	291
Se	0.8	0.8	0.9	0.9	0.8
Zn	158	167	160	158	146

Notes: Ingredients are listed as percentages of whole feed. WW refers to wet weight basis. The sign "-" means no data is available.
[1] Wheat gluten meal. Pea protein concentrate- and guar meal.
[2] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[3] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[4] BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. BMM refers to blue mussel meal.

10:30 and 12:30 to 14:00. The feeding rate was adjusted according to the increase in biomass as the fish grew.

In both experiments, the environmental conditions of temperature and Oxygen were continuously monitored throughout the experimental period. The tanks had a flow-through system and the flow adjusted to maintain the oxygen saturation as the fish grew. To estimate feed intake according to (Helland et al., 1996), the uneaten feed pellets were collected from the tank outlet 15 min after each meal in both experiments.

2.5. Sampling procedure

All sampled fish were euthanized with an overdose of tricaine methane sulfonate (500 mg L^{-1}, FINQUEL MS-222).

2.5.1. Experiment 1

At the start of experiment 1, the weight and length of all pit-tagged fish from the all-male population (n = 150) were registered to determine the individual specific growth rate (SGR). Also, 30 fish from the mixed population and 15 fish from the all-male population were dissected to determine the organ weight (viscera, liver, and heart) and organ nutrient composition. In addition to that, the same number of fish were pooled (n = 10 fish from mixed population per pool and n = 5 fish from all-male population per pool, n = 3 pools per fish group) to determine the whole-body nutrient composition. At the end of the experiment, the weight and length of all fish in each tank were recorded (n = 65). To determine the whole-body and organ nutrient composition, 10 fish from the mixed population and 10 fish from the all-male population from each tank (In total 20 fish per tank) were sampled for determination of nutrient status. Of these 10 fish, 5 fish whole-body were pooled to determine the whole-body nutrient composition (n = 5 fish per tank, n = 3 per diet), while 5 fish were dissected for individual tissue sampling. Blood samples were collected from the caudal vein by heparinized syringes. The plasma samples were obtained after centrifugation (13,200 RPM, 2 min, 4 °C) of the blood samples and kept on dry ice before transfer to −80 °C. The weight of the viscera, liver and heart was recorded in all sampled fish. The individual liver samples were frozen by liquid nitrogen, transferred on dry ice, and stored in −80 °C for determination of antioxidant responses (GSH-GSSG) (n = 5 fish per tank, n = 3 per diet). The whole fillet and liver samples were pooled per tank, kept on dry ice and stored in −20 °C for determination of mineral composition (n = 5 fish per tank, n = 3 pooled per diet). Feces were collected by gently stripping from 55 fish (45 fish from mixed population and 10 fish from all-male population) per tank and stored at −20 °C for determination of nutrient digestibility.

2.5.2. Experiment 2

At the start of experiment 2, the whole-body of 30 fish were sampled and homogenized to determine the whole-body nutrient composition (n = 10 fish per tank, n = 3 pooled). The organs of 30 fish (viscera, liver, and heart) were individually dissected, and weighed. The tank biomass was recorded at the beginning of the trial and weight and length were measured on all fish at the end. At the conclusion of the experiment, 20 fish were sampled per tank, 10 for collection of blood samples and organs, 10 for whole fish. Of 10 fish for blood and organ samples, blood samples were taken from 5 fish (n = 5 fish per tank, n = 15 per diet) and divided in 2 aliquots, one for plasma samples (as described above) and the other for determining hematocrit (HCT) and blood parameters; muscle samples were obtained from 5 fish (n = 5 fish per tank, n = 3 per diet) and liver samples were collected from 10 fish (n = 10 fish per tank, n = 3 per diet) to determine the nutrient composition. The organ weight of 10 fish per tank was measured to determine the somatic indexes (n = 10 fish per tank, n = 3 per diet). The rest 10 whole-body fish per tank were pooled to determine the whole-body nutrient composition (n = 10 fish per tank, n = 3 pooled per diet). Aliquots of heparinized whole blood were transported on ice and kept in the fridge for 24 h before being analyzed for red blood cell count (RBC) and hemoglobin (Hb) concentration. Feces were collected from all fish and stored at −20 °C before freeze-drying for determination of nutrient and yttrium content.

The HCT measurement was done by filling the capillary tubes with heparinized blood, seal the end of the tubes by wax, centrifuge the tube in a hematocrit centrifuge (12,500 RPM, 3 min, room temp), and read the percentage of packed cells directly by using a HCT ruler.

Individual welfare indicators were evaluated, including visual inspection of the eye, jaw wound and deformity, opercula status, spine deformation, gill condition, skin, and fin damage on the sampled fish from both experiments. According to the standard scoring system (SWIM) (Noble et al., 2018; Stien et al., 2013). A total of 20 fish of each tank (in both experiments) were examined for cataract in darkened conditions using a Heine HSL 150 hand-held slit lamp (HEINE Optotechnik GmbH & Co. KG, Herrsching, Germany), where each lens was

Table 4
Amino acid composition of experimental diets.

(mg g $^{-1}$ WW)	Experiment 1					Experiment 2				
	Control	BMS3	BMS7	BMS11	BMM12	Control	BMM9	BMS9[1]	BMSS9[2]	BMSF9[3]
Hydroxy-Proline	1.9	1.6	1.4	1.2	1.7	2.3	2	1.7	1.6	1.7
Histidine	12.5	11.9	10.9	11.4	11.2	12	11.8	11.6	11.3	11.3
Taurine	1.52	2.0	2.3	2.8	2.1	1.09	1.47	3	2.8	2.7
Serine	19.7	18.9	17.4	18.4	20.1	20.9	20.6	20.5	19.9	19.8
Arginine	28.3	27.5	24.9	25.3	28.0	25.9	24.7	23.9	23.3	23.3
Glycine	21.2	20.2	18.1	18.4	20.8	22.8	21.5	21	20.3	20.2
Aspartic acid	40	40.0	36.0	35.0	39.0	41	39	38	38	38
Glutamic acid	74	76.0	71.0	76.0	81.0	85	89	88	86	87
Threonine	15.8	15.3	13.8	14.1	15.8	16.3	15.9	15.5	15.2	15.2
Alanine	19.7	19.1	16.8	16.3	18.7	20.4	19.2	18.4	18.3	18.3
Proline	21.7	21.6	20.6	23.1	24.0	26.2	28	27.3	26.9	27
Lysine	26.8	26.0	22.0	20.6	25.8	29.1	32	24.3	23.3	24.4
Tyrosine	13.9	13.7	12.8	13.5	14.3	14.4	14.4	14.1	13.3	13.3
Methionine	12	11.2	10.4	10.6	12.0	12.4	12.4	11.5	11	11
Valine	18.8	18.9	17.0	17.4	18.5	19.5	18.8	18.2	18.3	18.4
Isoleucine	17.1	17.4	15.7	16.2	17.0	18.1	17.5	17	16.9	17.1
Leucine	31	29.9	27.1	28.3	30.0	32	32	30	29.8	30
Phenylalanine	19.9	19.3	17.8	19.3	19.7	21	20.5	20.6	19.2	19.4
Tryptophan	4.6	4.5	4.1	4.2	4.6	4.4	4.3	3.8	3.9	4

Notes: WW refers to wet weight basis.
BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
[1] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[2] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[3] BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants.

given a score between 0 and 4 according to (Wall and Bjerkas, 1999).

2.6. Analytical methods

Nutrient composition of raw materials, diets, whole-body, plasma, organ (liver and muscle) and feces samples were determined as described below: The crude protein was determined based on the nitrogen content of the samples by a nitrogen analyzer (Vario Macro Cube, Elementar Analysensysteme GmbH, Germany) (AOAC, 1995). Two different analytical methods were used for determination of crude fat in feed/tissue/feces samples and raw material samples. Ethyl acetate was used for extracting fat from feed, plasma, organs, and feces samples. The fat residue was weighted after filtering the solvent (Lie et al., 1988). However, gravimetry after acid hydrolysis was used for determination of crude fat in raw material samples (EU directive 84/41983). Dry matter was measured after drying the samples to constant weight at 105 °C for 24 h (Hamre and Mangor-Jensen, 2006) and a combustion in a muffle furnace at 550 °C for 16–18 h determined ash content. An IKA calorimeter C7000 was used for measuring the energy content of samples after drying the homogenized samples 48 h at 60 °C. The fatty acid composition in feed and raw materials was analyzed by gas-chromatography (GC) as previously described by(Jordal et al., 2007), modified after (Lie and Lambertsen, 1991). The amino acid composition (except cysteine and tryptophan) in feed and raw materials were determined using ultra-performance liquid chromatography (UPLC, Waters Acquity UPLC system) coupled with a UV detector (Cohen and De Antonis, 1994; Cohen and Michaud, 1993; Espe et al., 2014). Tryptophan was determined after basic hydrolysis with barium hydroxide ($Ba(OH)_2$) as described by (Liaset et al., 2003). Histamine in raw materials was determined by high- pressure liquid chromatography (HPLC) as previously describe by (Eerola et al., 1993; Liaset and Espe, 2008).

Inductively coupled plasma mass spectrometry (ICP-MS) as described by (Julshamn et al., 2001; Long and Martin, 1990) was used for determination of micro-minerals and yttrium oxide in raw materials, diets, whole-body, muscle, liver, plasma and feces samples. In brief, after digesting the 0.2 g freeze-dried sample material in a microwave oven (Milstone-MLS-1200) and diluting to 25 mL with Milli-Q Water, ICP-MS (Agilent 7500c) is used to determine the micro-minerals.

To determine the RBCs and Hb, CellDyn 400 (Sequoia-Turner, California, USA) instrument was used. Para 12 control blood (Streck) was used for calibration. After preparation of the diluted samples, the samples were read in the instrument for determining RBC and Hb. The RBC values were expressed as the value obtained $\times 10^{12}$ cells L^{-1} and the Hb measured is expressed as g 100 mL^{-1}.

The vitamin C and E analysis in feed was determined by HPLC as described by (Hamre et al., 2010; Mæland and Waagbø, 1998), respectively. The concentration of oxidation products in feed and raw materials was assessed using a spectrophotometric method by measuring Thiobarbituric Acid Reactive Substances (TBARS) (Hamre et al., 2001; Schmedes and Hølmer, 1989). To analyze the levels of total (tGSH) and oxidized (GSSG) glutathione in the liver samples, a method described by (Skjærven et al., 2013) and (Hamre et al., 2022) was used. The samples were treated with a commercial kit (Prod. No. GT40, Oxford Biomedical Research, Oxford, UK) to obtain supernatants, which were then subjected to analysis for absorbance at 405 nm using a microplate reader (iEMS Reader Ms., Labsystems, Finland).

Iron speciation was done on raw materials (BMS products) and the experimental feed samples from both experiments using the thiocyanate colorimetry method. The Fe^{3+} standard solutions (4,6,8, and 10 × 10–5 mol L^{-1}) and sample solutions were prepared as described in the protocol. The ammonium thiocyanate solution was added to each sample and standard solution tubes to make a stable red colour which is readable in a colorimeter measuring the absorbance at a wavelength of 490 nm for each colored solution.

2.7. Calculations and statistical analysis

The following variables were calculated:

$$Digestible\ energy\left(DE, \frac{MJ}{kg}\right) = Energy\ in\ diet - \left(\frac{yttrium\ in\ diet}{yttrium\ in\ faeces} \times energy\ in\ faeces\right)$$

(Anderson et al., 1991)

$$Weight\ gain\ (WG, g) = final\ mean\ weight\ (g) – initial\ mean\ weight\ (g)$$

$$\text{Specific growth rate } (SGR, \%\text{per day}) = (\text{Ln final biomass} - \text{Ln initial biomass}) \times \frac{100}{t}$$

(Hopkins, 1992)

$$\text{Feed conversion ratio } (FCR) = \frac{\text{Feed intake}}{\text{weight gain}}$$

$$\text{Total feed intake } (TFI, g) = \frac{\left(\frac{A \times ADW}{100}\right) - \left(\frac{W \times WDW}{R}\right)}{\frac{ADW}{100}}$$

$$\text{Recovery } (R, \%) = 100 \times \frac{W \times WDW}{A \times ADW}$$

(Helland et al., 1996)

$$\text{Condition factor } \left(K, \frac{g}{cm3}\right) = 100 \times \frac{\text{body weight } (g)}{\text{body length } (cm3)}$$

$$\text{Survival } (\%) = 100 \times \frac{\text{Final number of fish}}{\text{initial number of fish}}$$

$$\text{Hepatosomatic index } (HSI, \%) = 100 \times \left(\frac{\text{liver weight}}{\text{whole body weight}}\right)$$

$$\text{Cardiosomatic index } (CSI, \%) = 100 \times \left(\frac{\text{Heart weight}}{\text{whole body weight}}\right)$$

$$\text{Viscerosomatic index } (VSI, \%) = 100 \times \left(\frac{\text{viscera weight}}{\text{whole body weight}}\right)$$

$$ADC\ (\%) = 100 - \left(100 \times \frac{\text{yttrium in diet}}{\text{yttrium in faeces}} \times \frac{\text{nutrient in faeces}}{\text{nutrient in diet}}\right)$$

$$AAC\ (\%) = 100 - \left(100 \times \frac{\text{yttrium in diet}}{\text{yttrium in faeces}} \times \frac{\text{Mineral in faeces}}{\text{Mineral in diet}}\right)$$

$$\text{Retention } (\%) = 100 \times \frac{(BM\ f \times \text{nutrient or mineral content } f) - (BM\ i \times \text{nutrient or mineral content I})}{\text{feed intake} \times \text{nutrient or mineral in feed}}$$

Where t is sum of feeding days (70 days in the current study), A is weight of air-dry feed (g), A_{DW} is dry matter content of air-dry feed (%), W is weight of waste feed collected (g), W_{DW} is dry matter content of waste feed (%), and R is recovery of dry matter of waste feed (%), BM f *and* i are standing for final and initial biomass, respectively.

To calculate the daily feed intake per kg biomass (DFI, % biomass), the following equation was used for estimating the daily biomass based on SGR and recorded daily feed intake:

$$lnWdayx = \left(\frac{SGR}{100}\right) \times \left(1 + lnWday(x-1)\right)$$

W_{dayx} is the biomass on a given day (Árnason et al., 2015).

Redox potential (E_h) was calculated by the following equation:

$$Eh = \frac{E0 - RT}{nF\ Ln\ \frac{GSH^2}{GSSG}}$$

Where the GSH and GSSG concentrations are in mol and E_h is in volts. E0 was assumed to be −0.240 V and it is the standard reduction potential at pH 7 and 25 °C. Universal gas constant (R), temperature in Kelvin (T), ion charge (n) (moles of electron), and faraday constant (F) are constant data.

In experiment 1, all data from control and BMS inclusion 3,7 and 11% were analyzed using linear regression (LR) to evaluate dose-dependent responses by determining the best-fit line for each data set. Furthermore, a one-way ANOVA was conducted to determine if there were statistically significant differences among the control group, BMM12 group, and BMS11 group. If a statistically significant difference was found, Tukey's multiple comparisons post-hoc analysis was applied to identify the specific groups with significant differences. In experiment 2, a similar approach was followed. One-way ANOVA was performed to examine the statistical differences between the experimental groups and the control group. Subsequently, Tukey's multiple comparisons post-hoc analysis was utilized to identify any statistically significant differences among the groups.

For all data sets the homogeneity of variance and normality of the data was tested by Bartlett's/ Brown-Forsythe test and Shapiro Wilk's test, respectively. Outliers of the growth dataset were identified with the ROUT test in GraphPad Prism. One of the BMM12 tanks was removed as the outlier in experiment 1. "Tank" was considered as the experimental unit ($n = 3$ for all the experimental diets and $n = 2$ for the BMM12 group) and a significant level of $p < 0.05$ was employed in all cases. The results are expressed as mean ± SEM. All the statistical analysis and the graphs were performed in GraphPad Prism (version 8.4.3 (686) San Diego, California USA).

3. Result

3.1. Fish performance indicators

In experiment 1, the fish given diets containing BMS had a linear decrease in weight gain (WG) and SGR ($p < 0.0001$) (Fig.1a and Table 5). The fish given BMS had a lower growth rate, resulting in up to 46% reduced weight gain (WG), from 275 ± 5 g in the control group to 148 ± 13 g in the fish given the diet containing 11% BMS. Also, the SGR decreased from 1.19 ± 0.01% day^{-1} in the control group to 0.77 ± 0.02% day^{-1} in the fish given the diet containing 11% BMS.

The FCR increased from 0.68 ± 0.01 in the control group to 1.08 ± 0.14 in the BMS11 group ($p < 0.005$) (Fig.1b). Daily feed intake was not influenced by BMS inclusion (Fig.1c). Condition factor decreased with a higher BMS inclusion in the diet ($p < 0.0001$) from 1.25 ± 0.01 to 1.12 ± 0.01 (Fig.1d). No differences were seen in the somatic indices with mean levels of 1.12 ± 0.01 for HSI, 10.05 ± 0.14 for VSI, and 0.13 ± 0.00 for CSI (data not shown). Using BMS in diets did not influence cataract development, combined mean score of all fish of 1.30 ± 0.08, or any of the other welfare assessments (data not shown).

Fish fed BMS11 diet had lower WG ($p = 0.001$), and condition factor ($p = 0.001$) compared to both the BMM12 and control groups (Fig.3a, d). However, Fish given BMM diet performed comparably with the reference group, and no differences were observed in WG (Fig.3a), feed utilization (Fig.3b, c), and condition factor (Fig.3d) between fish fed the BMM12 and the reference group.

The final weight of the all-male population was within the same range as that of the fish from mixed population (Fig. A supplementary). The individual SGR of the all-male population decreased as determined by a segmental linear regression with a broken point in BMS3 (R^2 = 0.69).

In experiment 2, no differences were seen in WG and feed utilization

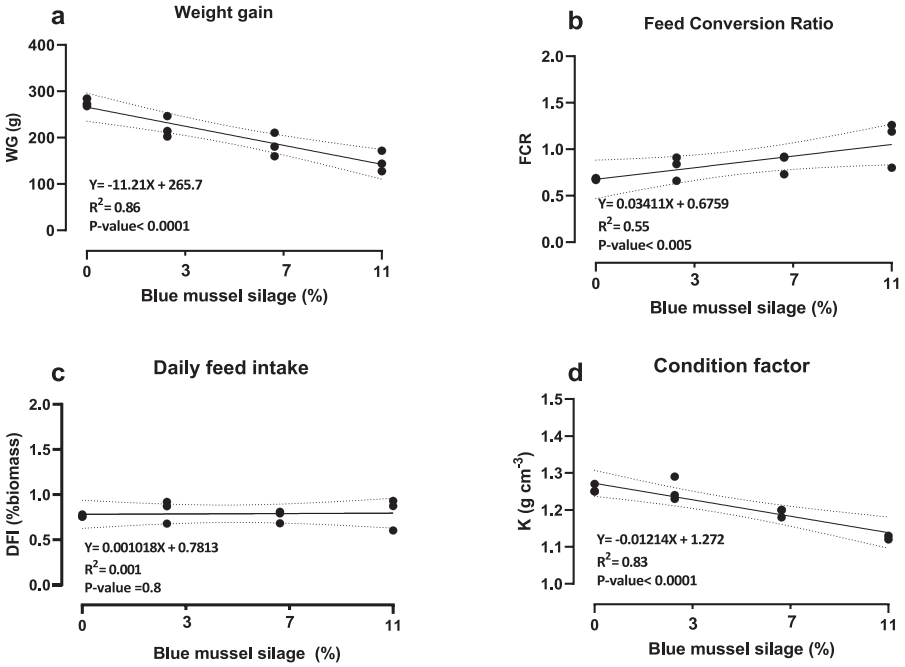

Fig. 1. Growth performance and feed utilization indicators of Atlantic salmon post smolt fed graded inclusion of blue mussel silage (BMS) in experiment 1. The best-fit regression lines for each data set were presented ($n = 15$ fish per diet, each filled circle shows a mean of 5 fish per tank). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig.2a, b, c). The SGR and condition factor was however lower in the fish given the diet containing BMS made with low soft acid (BMSS9) compared to the fish fed the reference feed, but not different from the fish fed the other blue mussel containing feeds (Table 5, Fig.2d).

The HSI was higher in the fish given BMS in the diet compared to fish given the reference feed and BMM9 in the diet, increasing from 1.04 ± 0.03 in the control group and 1.09 ± 0.02 in the BMM9 group to 1.24 ± 0.03 in BMS9, 1.35 ± 0.06 in BMSS9 and 1.25 ± 0.05 in BMSF9 ($p < 0.0001$, data not shown). The CSI and VSI were comparable between experimental groups with a mean of 0.16 ± 0.00 and 8.75 ± 0.11, respectively. The growth performance, feed utilization and somatic indexes were comparable between fish given BMM9 in the diet and fish given the reference feed. No effect was observed on cataract scores with the mean of 2.33 ± 0.07 or other welfare indicators (data not shown).

3.2. Apparent digestibility (ADC), apparent availability (AAC) coefficient

In experiment 1, the inclusion of BMS did not influence the ADC of protein, total fat and energy (Table 6). However, the ADC of dry matter increased from 63.60 ± 1.97% in the control group to 70.13 ± 0.35% in the BMS3 group where the levels appeared to plateau, as determined by a segmental linear regression with a broken point in BMS3 ($R^2 = 0.74$) (Table 6). Additionally, the fish fed BMM12 showed comparable ADC of macro-nutrients with the control and BMS11 groups (Table 6). In experiment 2, the ADC of macro-nutrients was comparable between the fish given control feed, BMM9, and BMS9. Notably, the fish fed BMS with low soft acid (BMSS9) and BMS with only formic acid (BMSF9) had an increase in the ADC of protein, total fat, and energy ($p = 0.006$, $p = 0.01$, $p = 0.005$) compared with the control group. However, ADC of dry matter was not influenced by the experimental diets (Table 6).

In experiment 1, the Fe availability had a linear increase ($p = 0.01$), while the Se availability was expressed by a second-order polynomial equation (quadratic, $R^2 = 0.67$), with increasing from 54.90 ± 1.50% in the control group to 61.93 ± 1.41% in BMS7 and decreased to 60.80 ± 0.66% in the BMS11 group. However, no difference was observed in Fe and Se AAC between the control, BMM12, and BMS11 groups. Moreover, the availability of other micro-minerals was comparable with the control group (Table 6). In experiment 2, The Fe and Se availability were not affected by the experimental diets. However, the Zn availability increased in all experimental groups compared with the control group ($p < 0.0001$). The Zn availability in the fish given the control feed was 19.83 ± 1.08%, while it was higher in the fish fed BMM9 (32.40 ± 1.53%), BMS9 (40.60 ± 1.73%), BMSS9 (46.63 ± 1.78%), and BMSF9 (43.00 ± 0.64%). The availability of Mn and Cu increased in fish fed BMM9, BMSS9 and BMSF9 compared with the fish fed control feed ($p = 0.005$, $p = 0.01$, respectively) (Table 6).

3.3. Whole-body macro-nutrients status

In experiment 1, the fish given diets containing BMS had lower protein ($p = 0.009$), energy ($p = 0.01$), and dry matter ($p = 0.008$) content of whole-body with higher inclusion of BMS in their diets. (Table 7). However, the total lipid level did not change. The whole-body macro-nutrient status of fish fed BMM12 was comparable with fish fed control and BMS11. However, the BMS11 group had a reduction in the levels of energy ($p = 0.01$) and dry matter ($p = 0.03$) compared to the control group (from 9407 ± 104 to 8927 ± 108 j g^{-1} WW in the BMS11 group, and from 33.03 ± 0.40% to 31.35 ± 0.37% in the control group, respectively). In experiment 2, the status of all macronutrients in the whole body was comparable between the experimental groups, and no significant changes were detected (Table 7).

Table 5
Growth performance of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1					
	Control	BMS3	BMS7	BMS11	Regression (0, BMS3, 7, 11)
IBW (g)	210 ± 3	211 ± 5	201 ± 7	204 ± 10	n.s.
FBW (g)	485 ± 8	432 ± 14	385 ± 21	351 ± 23	$R^2 = 0.78$, $p = 0.0001$[1]
SGR (% day $^{-1}$)	1.19 ± 0.01	102 ± 0.04	0.92 ± 0.03	0.77 ± 0.02	$R^2 = 0.90$, $p < 0.0001$[2]
TFI (kg)	11.92 ± 0.19	11.91 ± 0.83	10.21 ± 0.23	10.12 ± 0.82	$R^2 = 0.43$, $p < 0.01$[3]

Experiment 2						
	Control	BMM9	BMS9[4]	BMSS9[5]	BMSF9[6]	ANOVA
IBW (g)	119 ± 2	121 ± 1	120 ± 2	119 ± 1	118 ± 1	n.s.
FBW (g)	232 ± 8^{ab}	254 ± 10^{a}	222 ± 4^{ab}	197 ± 8^{b}	211 ± 10^{b}	$p = 0.007$
SGR (% day $^{-1}$)	1.36 ± 0.08^{a}	1.50 ± 0.07^{a}	1.25 ± 0.01^{ab}	1.03 ± 0.07^{b}	1.28 ± 0.01^{ab}	$p = 0.004$
TFI (kg)	5.68 ± 0.37	5.50 ± 0.55	4.93 ± 0.55	4.63 ± 0.48	5.02 ± 0.35	n.s.

Notes: IBW = initial body weight (g). FBW = final body weight (g). SGR = specific growth rate (% day $^{-1}$). TFI = total feed intake (g).
BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
Data is listed as mean ± SEM. In experiment 1, all diets are triplicate except BMM12%, that is in duplicate. In experiment 2, $n = 3$ tank per diet.
The column labeled "Regression" gives R^2 and p-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "ANOVA" gives a p-value for ANOVA in case of a significant difference between the groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.
[1] Simple linear regression: $Y = -11.99\times + 476.2$.
[2] Simple linear regression: $Y = -0.03624\times + 1.169$.
[3] Simple linear regression: $Y = -193.8\times + 12{,}061$.
[4] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[5] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[6] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

3.4. Body micro-mineral composition and blood parameters

The mineral compositions of whole-body, liver, plasma, and muscle of both experiments are provided in Table 7 and supplementary tables D and E. In experiment 1, the fish given diets containing BMS has low Fe level in whole body expressed by a second-order polynomial equation ($R^2 = 0.89$) compared to the control group (Fig. 4). The whole-body Fe level decreased from 8.36 ± 0.60 mg kg^{-1} WW in the control group to 4.96 ± 0.03 mg kg^{-1} WW in BMS11 (Fig. 4a). Liver and plasma Fe concentrations also decreased and could be expressed by a segmental linear regression with a broken point in BMS3 ($R^2 = 0.98$ and $R^2 = 0.26$, respectively). Fish fed the diet containing BMS11 had a lower concentration of Fe in the liver (18.01 ± 0.54 mg kg^{-1} WW) and in plasma (6.88 ± 1.26 μmol L^{-1} WW) compared to the control group (liver 63.77 ± 2.43 mg kg^{-1} WW and plasma 15.16 ± 2.82 μmol L^{-1} WW) (Fig.4b, c). Similarly, muscle Fe concentration decreased under a second-order polynomial model ($R^2 = 0.62$), from 2.10 ± 0.05 mg kg^{-1} WW in the control group to 1.30 ± 0.17 mg kg^{-1} WW in the fish given BMS7, and then increased to 1.50 ± 0.20 mg kg^{-1} WW in the fish given BMS11 (Fig.4d). The highest level of Fe in the liver and plasma was observed in the fish given BMM12 (82.00 ± 1.00 mg kg^{-1} WW and 27.11 ± 4.89 μmol L^{-1}, respectively) (supplementary table D and E, ANOVA test). Along with Fe, the whole-body Mn and Se concentration had a decreased dose-response which similarly was observed in plasma as well (Table 7 and supplementary table E). In contrast with that, the Cu status in whole-body and liver increased linearly ($R^2 = 0.73$ – $p = 0.0004$, and $R^2 = 0.57$ - $p = 0.004$, respectively), whereas it decreased in muscle ($R^2 = 0.48$, $p = 0.01$). In the control group, whole-body Cu levels were 1.63 ± 0.03 mg kg^{-1} WW, which increased to 2.13 ± 0.08 mg kg^{-1} WW in the BMS11 group (Table 7). The micro-mineral composition in fish fed BMM12 was comparable with the control group, whereas the whole-body Cu and Se was lower ($p = 0.003$) and plasma Mn concentration was higher ($p < 0.0001$) than BMS11.

In experiment 2, the whole-body Fe level increased ($p < 0.0001$) in fish fed diets containing BMS (13.33 ± 0.16 mg kg^{-1} WW) compared to the control group (11.00 ± – mg kg^{-1} WW) (Fig.4e). The liver Fe level in fish fed diets containing BMS also increased ($p = 0.0002$) to 125.60 ± 5.30 mg kg^{-1} WW, while the control group showed lower levels of 71.00 ± 3.21 mg kg^{-1} WW (Fig.4f). Similarly, the muscle Fe status increased ($p = 0.003$) in fish fed with BMS groups (2.85 ± 0.05 mg kg^{-1} WW) compared to the control group (2.36 ± 0.06 mg kg^{-1} WW) (Fig.4f). Along with that, the whole-body Zn level increased in both BMSS9 (39.33 ± 0.88 mg kg^{-1} WW) and BMSF9 (38.00 ± – mg kg^{-1} WW) groups compared with the control group (31.33 ± 0.33 mg kg^{-1} WW) (Table 7). The plasma Zn concentration also increased in fish fed BMS with high soft acid (BMS9) and BMS with only formic acid (BMSF9) compared with the control group (supplementary table E). No changes were seen in the concentration of other micro minerals experiment 2.

No differences were observed in the mean of RBC count 1.32 ± 0.03 × 1012 cells L^{-1}, Hb 9.73 ± 0.07 g 100 mL^{-1}, and HCT 43.20 ± 0.52% in experiment 2 (supplementary table E).

3.5. Nutrient retention

In experiment 1, the retention of all macronutrients decreased linearly in fish fed with a higher inclusion of BMS ($p_{protein} = 0.003$, $p_{total\ fat} = 0.01$, $p_{energy} = 0.004$, $p_{dry\ matter} = 0.004$, and $p_{ash} = 0.04$) (Table 8). The fish fed BMM12 had a comparable retention of macronutrients with control group in their body, while it was lower in the BMS11 ($p_{protein} = 0.04$, $p_{energy} = 0.04$, $p_{dry\ matter} = 0.03$, respectively) (Table 8). In experiment 2, the retention of macronutrients was not affected by the experimental diets, and all were comparable to the control group (Table 8).

The retention of Fe decreased in fish fed with a higher inclusion of BMS, as determined by a segmental linear regression with a broken point in BMS3 ($R^2 = 0.92$) (Table 8). The fish fed BMM12 had a comparable Fe retention with the control group (6.15 ± 0.69% and 4.52 ± 0.40%, respectively), whereas it was found lower ($p < 0.0001$) in the BMS11 group (0.31 ± 0.09%). Moreover, the retention of Zn ($p = 0.003$), Mn ($p = 0.01$), and Se ($p < 0.0001$) decreased linearly. Fish fed with BMS11 had lower levels of Mn ($p = 0.05$), Se ($p = 0.001$) and Zn ($p = 0.02$) compared with the control and BMM12 groups (Table 8). In experiment 2, the retention of microminerals was not influenced by the experimental diets (Table 8).

3.6. Liver antioxidant status

In experiment 1, the fish fed BMS did not have any dose-dependent responses in the levels of GSH and GSSG, or in the ratio of GSH/GSSG in the liver, as well as in the redox potential. The GSH level in liver of the control, BMM12, and BMS groups were 878 ± 138, 1037 ± 89 μmol kg^{-1}, and 943 ± 58 μmol kg^{-1}, respectively. Similarly, the GSSG level in liver of control, BMM12, and BMS groups were 2.52 ± 0.13 μmol kg^{-1}, 2.78 ± 0.21 and 3.1 ± 0.31, respectively. The GSH/GSSG ratio was 304 ± 47 and 346 ± 41 μmol kg^{-1} in the control and BMM12 groups,

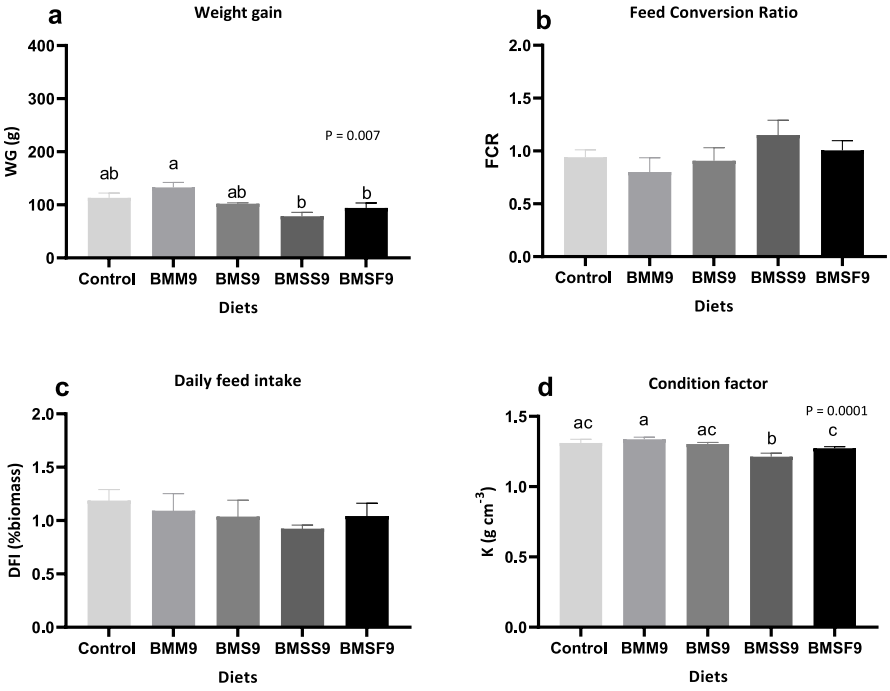

Fig. 2. Growth performance and feed utilization indicators of Atlantic salmon post smolt fed blue mussel silage (BMS) and blue mussel meal (BMM) in experiment 2. Statistically significant differences between the experimental groups were represented with different letters above the bars ($p < 0.05$) under the Tukey HSD test (mean ± SEM, n = 15 fish per diet). BMS9 refers to diets containing blue mussel silage with a high amount of soft acid (pH 2.5) and antioxidants, BMSS9 refers to diets containing blue mussel silage with a lower amount of soft acid (pH 3.7) and antioxidants, and BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

respectively, while it was 380 ± 24 μmol kg^{-1} in BMS groups. The redox potential in all experimental groups was in a similar range with an average of −0.22 ± 0.002 V (data not shown).

3.7. Iron speciation

The BMS product before drying had the lowest Fe^{3+} to total Fe ratio (18% of total Fe), which increased to 39% of total Fe after drying by SPC. However, heat drying increased Fe^{3+} to total Fe ratio almost 2-folds in BMS High soft acid, BMS Low soft acid (35 and 37% of total Fe, respectively) and BMS only formic acid group (43% of total Fe) (Fig.5a).

Furthermore, the inclusion of BMS in the diet resulted in an overall increase in Fe^{3+} to total Fe ratio in both experiments. In experiment 1, the ratio in the BMS diets (3, 7, and 11) was 11, 16, and 24% of total Fe, respectively (Fig.5b). Similarly, the ratio in BMS9, BMSS9, and BMSF9 diets in experiment 2 was 15, 49, and 53% of total Fe), respectively (Fig.5c). The diets contain BMS with low levels of acid (BMSS9 and BMSF9) had the highest ratio (49 and 53% of total Fe, respectively). The BMM group had the lowest ratio in both experiments (6% of total Fe).

4. Discussion

One of the key challenges in salmon production is identifying appropriate alternative feed ingredients for sustainable future salmon production (Albrektsen et al., 2022). Future feed resources are expected to include low-trophic species produced or cultivated in the ocean (Albrektsen et al., 2022). However, the utilization of marine-based organisms as feed materials comes with certain challenges, such as seasonal availability, variation in the nutritional composition and preservation and processing methods, which can limit their use in aquafeed. To address these challenges, two studies were done to investigate the potential use of blue mussel silage (BMS) and blue mussel meal (BMM) as a marine protein ingredient in the Atlantic salmon diet.

In both experiments, no differences were seen in growth or feed conversion ratio between fish given reference feed and BMM. The findings are in line with previous studies that showed Juvenile Ussuri catfish (*Pseudobagrus ussuriensis*) had no negative effects on growth and nutrient utilization when 50% of the FM (28% of control diet – 48% crude protein) was replaced by BMM (Luo et al., 2019). The reference diets used in the present studies were based on a commercially relevant post-smolt diet regarding the protein: lipid ratio, as well as FM inclusion of 25%, giving a mix of ~59–63% plant-based ingredients vs ~34–36% marine ingredients in all the diets. In our study, the experimental feeds contained 9 and 12% BMM, replacing 36 and 48% of the FM, respectively. Therefore, findings from both studies are comparable based on the dietary FM content (25–28% in diet), dietary protein level (45–46%) and the inclusion level of BMM in diet (around 50% of FM). However, it has been shown that replacing 50 or 100% of FM (30% of control diet) led to reduced growth in turbot (Weiss and Buck, 2017).

Contrary to the results shown in the fish given BMM, the fish given BMS in experiment 1 had a dose-dependent reduction in weight gain, SGR and condition factor and an increased FCR. The highest level of BMS was close to the level of blue mussel meal used, however it resulted in a 46% reduction in weight gain, 35% reduction in SGR, 10% reduction in condition factor and 37% increase in FCR. While one study is available on the use of blue mussel silage in animal nutrition (Nørgaard

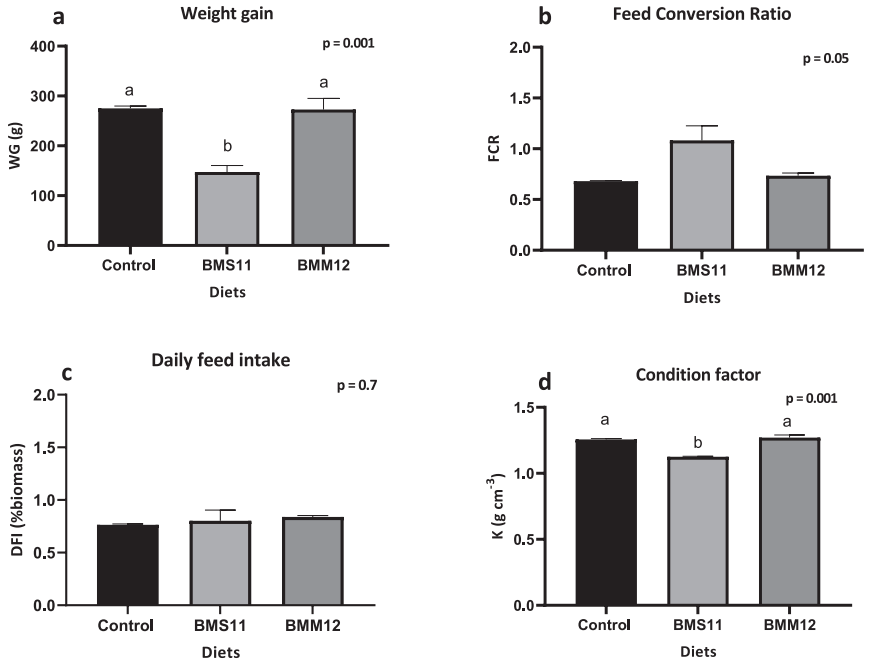

Fig. 3. Comparing growth and feed utilization of Atlantic salmon post-smolt fed control and blue mussel silage 11 (BMS11) versus fed blue mussel meal (BMM12) in experiment 1. Statistically significant differences between the experimental groups were represented with different letters above the bars ($p < 0.05$) under the Tukey HSD test (mean ± SEM, n = 15 fish for control and BMS11, $n = 10$ fish for BMM12). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2015), several studies have shown that fish silage or fish protein concentrates in similar inclusion levels (5–15% of FM) can be used in fish feed such as Atlantic salmon feed (Berge and Storebakken, 1996; Espe et al., 1999; Liang et al., 2006; Olsen and Toppe, 2017; Refstie et al., 2004; Ridwanudin and Sheen, 2014). Early studies on the use of silage-based diets has resulted in either no effect on growth or even marginal enhancements (Heras et al., 1994; Lall, 1991; Parrish et al., 1991), while some have also indicated a significant reduction in growth (Hardy et al., 1984; Stone et al., 1989). For example; replacing whole FM (50% of diet) with 12.5 and 25% fish silage co-dried with soybean meal and feather meal and 50% fish silage dried by vacuum dryer reduced the average weight of rainbow trout, while FCR was comparable between the experimental diets (Hardy et al., 1984). However, similar to the results in experiment 1, it was shown that replacing whole FM (50% of diet) with fish silage made from fresh or frozen ingredients decreased the final mean weight and increased the feed conversion ratio of rainbow trout (Stone et al., 1989).

One reason for the reduced growth was suggested to be a lower availability of lysine and other essential amino acids (Hardy et al., 1984). It has also been shown that the level of acid-sensitive amino acids, especially tryptophan, in fish silage may decrease due to the processing methods (Arason, 1994). The level of acid-sensitive amino acids is influenced more by the acid amount and pH level than the duration of storage (Espe et al., 1999; Gildberg and Raa, 1977; Haaland and Njaa, 1989; Jackson et al., 1984; Mach and Nortvedt, 2009; Nørgaard et al., 2015). Previous studies have also reported reductions in amino acids such as arginine (Haaland and Njaa, 1989; Stone and Hardy, 1986), phenylalanine, glutamic acid (Stone and Hardy, 1986), lysine (Vidotti et al., 2003), tyrosine (Haaland and Njaa, 1989), methionine (Shahidi et al., 1995), leucine, and isoleucine (Vidotti et al., 2003) in fish silage. In the present studies, no effect was seen on tryptophan level in reference vs silage products, while variations were seen in several amino acid levels in BMS products that could also be explained by different blue mussel productions (Table A of the supplementary material), however, the dietary levels were above the amino acid requirements for Atlantic salmon (NRC, 2011). Although no differences were seen in protein digestibility, the fish fed higher BMS had lower protein retention and whole-body protein composition. This may mean that nutrients from diets containing BMS were not efficiently used for growth, despite being easily digested.

Reduced growth might also be attributed to the bitter taste of the feed or the presence of bitter-tasting peptides (Adler-Nissen, 1984; Hevrøy et al., 2005). This bitterness can occur when formic acid, sulfuric acid, or propionic acid is used during the fish silage process, leading to decreased feed intake and growth in fish (Adler-Nissen, 1984; Hevrøy et al., 2005). The presence of rancid lipid compounds in feed can be another factor in the reduced growth and feed utilization (Hevrøy et al., 2005). Lipid rancidity can be a major concern to determine the feed stability and cellular antioxidant homeostasis (Aklakur, 2018). In larger-scale operations, it has been suggested to remove oil from fish silage if it exceeds 4% (Tatterson and Windsor, 1974). In our current study however, the TBARS levels in the diets showed variations, while feed intake remained constant and the redox potential and GSH/GSSG ratio in the liver of fish fed BMS diets remained stable.

The status of almost all the essential micro-minerals in whole-body, liver, and plasma were affected by the lower growth and higher FCR in BMS groups in experiment 1. Notably, lower levels of Fe were observed in the BMS groups which were not dose dependent. Dietary Fe is the primary source of Fe for fish (Bury and Grosell, 2003). It has been reported that dietary Fe deficiency impaired the growth performance of stinging catfish (*Heteropneustes fossilis*) (Zafar and Khan, 2020), bighead carp (*Aristichthys nobilis*) (Feng et al., 2020), and yellow catfish

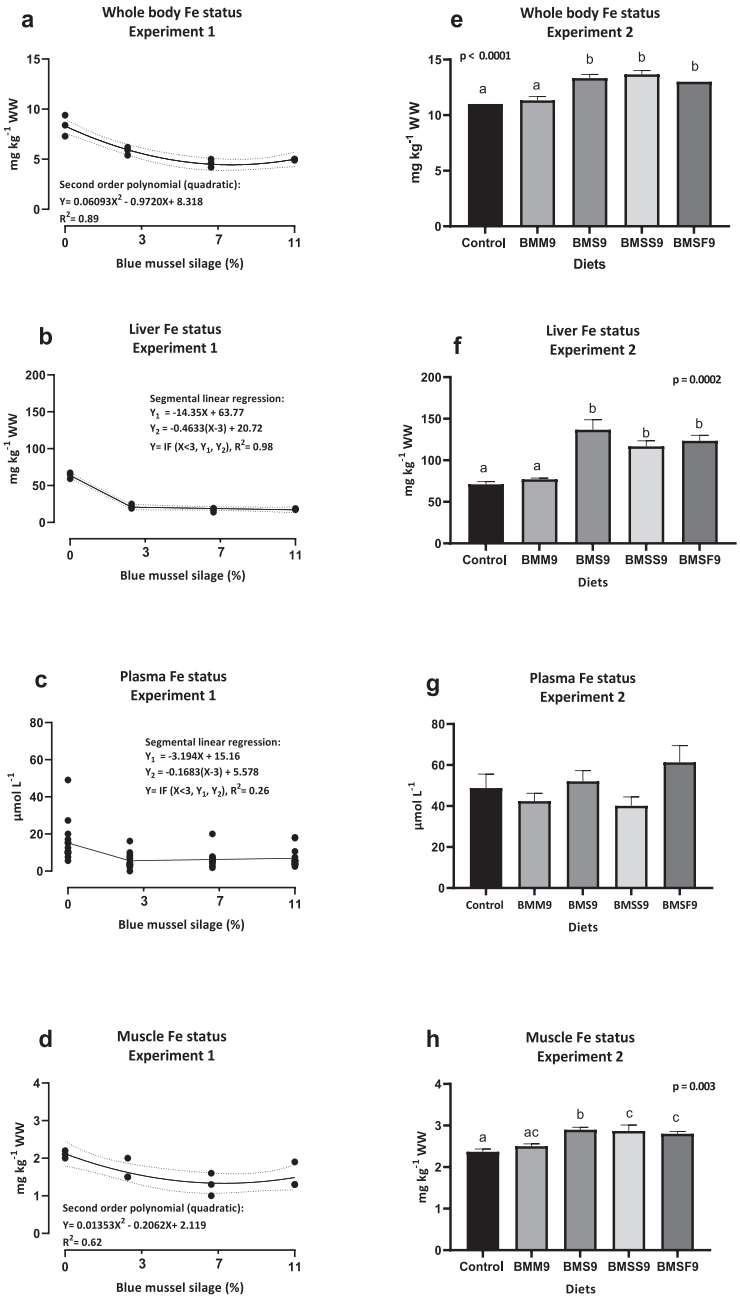

Fig. 4. Whole body, liver, and muscle Fe status of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2. In experiment 1 (a, b, c, and d), the best-fit regression lines for each data set were presented (n = 15 fish per diet, in a, b, and d. Each filled circle shows a mean of 5 fish per tank, while each filled circle is an individual fish in graph c (n = 15 per diet). In experiment 2 (e, f, g and h), statistically significant differences between the experimental groups were represented with different letters above the bars ($p < 0.05$) under the Tukey HSD test (mean ± SEM, n = 15 per diet). BMS9 refers to diets containing blue mussel silage with a high amount of soft acid (pH 2.5) and antioxidants, BMSS9 refers to diets containing blue mussel silage with a lower amount of soft acid (pH 3.7) and antioxidants, and BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

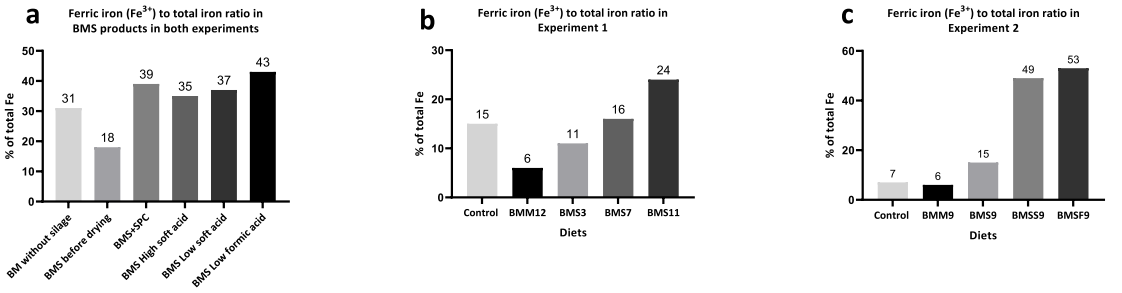

Fig. 5. Ferric iron (Fe^{3+}) to total iron ratio in blue mussel silage (BMS) products and the experimental diets of experiments 1 and 2. In experiment 1, the BMS was mixed with SPC before feed production, and this sample is indicated by the label BMS + SPC in graph (a). In graph (b), BMS 3, 7 and 11 refer to diets containing 3, 7, and 11% blue mussel silage. In graph (c), BMS9 refers to diets containing blue mussel silage with a high amount of soft acid (pH 2.5) and antioxidants, BMSS9 refers to diets containing blue mussel silage with a lower amount of soft acid (pH 3.7) and antioxidants, and BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. BMM refers to blue mussel meal in both experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Apparent digestibility/availability coefficients (ADC/AAC) of nutrients of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1							
	Control	BMS3	BMS7	BMS11	BMM12	Regression (0, BMS3, 7, 11)	Comparison (0, BMS11, BMM12)
Macro-nutrients (%)							
Protein	86.93 ± 0.63	88.93 ± 0.27	88.07 ± 0.53	87.53 ± 0.38	87.25 ± 1.05	n.s.	n.s.
Total fat	94.40 ± 0.35	96.43 ± 0.38	95.93 ± 0.95	95.77 ± 0.49	93.85 ± 1.25	n.s.	n.s.
Energy	77.93 ± 1.15	82.07 ± 0.42	81.50 ± 0.46	80.97 ± 0.43	79.40 ± 1.60	n.s.	n.s.
Dry matter	63.60 ± 1.97	70.13 ± 0.35	70.43 ± 1.10	70.47 ± 0.78	66.20 ± 2.00	$R^2 = 0.74$[1]	n.s
Micro-minerals (%)							
Mn	−29.13 ± 18.52	−9.20 ± 16.83	−19.47 ± 14.68	−25.67 ± 9.73	13.75 ± 11.15	n.s.	n.s.
Cu	42.20 ± 0.96	32.80 ± 2.27	40.07 ± 2.61	40.00 ± 0.90	35.95 ± 5.55	n.s.	n.s.
Fe	−3.66 ± 5.91	3.06 ± 7.37	13.30 ± 2.47	14.00 ± 3.26	−17.15 ± 12.05	$R^2 = 0.45$, $p = 0.01$[2]	n.s.
Se	54.90 ± 1.50	60.17 ± 1.48	61.93 ± 1.41	60.80 ± 0.66	54.30 ± 1.20	$R^2 = 0.67$[3]	n.s
Zn	23.93 ± 3.46	25.47 ± 6.12	28.83 ± 6.15	24.70 ± 3.89	16.50 ± 8.60	n.s.	n.s.

Experiment 2						
	Control	BMM9	BMS9[4]	BMSS9[5]	BMSF9[6]	ANOVA
Macro-nutrients (%)						
Protein	87.77 ± 0.64^{a}	89.90 ± 0.15^{b}	88.97 ± 0.08^{ab}	89.77 ± 0.32^{b}	89.60 ± 0.11^{b}	$p = 0.006$
Total fat	93.07 ± 0.03^{a}	93.87 ± 0.54^{ab}	93.73 ± 0.17^{ab}	94.83 ± 0.18^{b}	94.70 ± 0.37^{b}	$p = 0.01$
Energy	81.30 ± 0.60^{a}	83.50 ± 0.50^{ab}	82.77 ± 0.12^{ab}	84.57 ± 0.68^{b}	84.20 ± 0.20^{b}	$p = 0.005$
Dry matter	67.43 ± 1.88	70.57 ± 0.58	70.50 ± 0.37	71.10 ± 2.04	71.07 ± 0.43	n.s.
Micro-minerals (%)						
Mn	-18.47 ± 4.61^{a}	2.86 ± 0.48^{b}	-1.43 ± 5.99^{ab}	11.33 ± 0.98^{b}	9.70 ± 6.2^{b}	$p = 0.005$
Cu	15.47 ± 2.06^{a}	31.33 ± 1.19^{b}	26.00 ± 2.78^{ab}	27.70 ± 3.95^{b}	25.93 ± 1.80^{ab}	$p = 0.01$
Fe	−16.50 ± 2.89	6.93 ± 12.18	−8.73 ± 3.57	−0.53 ± 2.38	−11.53 ± 1.84	n.s.
Se	52.67 ± 1.44	54.33 ± 1.12	54.60 ± 2.26	57.97 ± 2.55	51.77 ± 1.38	n.s.
Zn	19.83 ± 1.08^{a}	32.40 ± 1.53^{b}	40.60 ± 1.73^{c}	46.63 ± 1.78^{c}	43.00 ± 0.64^{c}	$p < 0.0001$

Notes: Data is listed as mean ± SEM. The mean is from $n = 3$ pooled feces sample per diet. In experiment 1, all diets are triplicate except BMM12, that is in duplicate. In experiment 2, n = 3 tank per diet.
The column labeled "Regression" gives R^2 and p-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "comparison" under experiment 1 gives a p-value for ANOVA in case of a significant difference between control, BMS11 and BMM12. The column labeled "ANOVA" under experiment 2 gives a p-value for ANOVA in case of a significant difference between all experimental groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.

[1] Segmental linear regression: $Y_1 = 2.193\times + 63.60$. $Y_2 = 0.04167$ (X-3) + 70.179. Y=IF (X < 3. Y_1. Y_2). $X_0 = 3$.

[2] Simple linear regression: $Y = 1.690\times - 2.205$.

[3] Second order polynomial (quadratic): $Y = -0.1317X^2 + 1.960\times + 55.04$. BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.

[4] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.

[5] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.

[6] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

Table 7
Whole body nutrient composition of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1							
	Control	BMS3	BMS7	BMS11	BMM12	Regression (0, BMS3, 7, 11)	Comparison (0, BMS11, BMM12)
Whole- body macro-nutrients (g 100 g $^{-1}$ WW)							
Protein	18.33 ± 0.33	17.67 ± 0.33	17.67 ± 0.33	17.00 ± –	17.50 ± 0.50	R^2 = 0.50, p = *0.009*[1]	n.s.
Total fat	13.47 ± 0.42	12.73 ± 0.23	13.13 ± 0.20	12.53 ± 0.33	13.20 ± 0.17	n.s.	n.s.
Energy (J g^{-1} WW)	9407 ± 104^{a}	9190 ± 67	9297 ± 61	8927 ± 108^{b}	9330 ± 35^{ab}	R^2 = 0.47, p = *0.01*[2]	p = *0.9*
Dry matter	33.03 ± 0.40^{a}	32.29 ± 0.20	32.48 ± 0.20	31.35 ± 0.37^{b}	32.89 ± 0.35^{ab}	R^2 = 0.52, p = *0.008*[3]	p = *0.03*
Whole-body micro-minerals (mg kg^{-1} WW)							
Mn	0.99 ± 0.15	0.75 ± 0.07	0.82 ± 0.09	0.61 ± 0.03	1.15 ± 0.15	R^2= 0.36, p = *0.03*[4]	n.s.
Cu	1.63 ± 0.03^{a}	1.76 ± 0.06	1.90 ± 0.10	2.13 ± 0.08^{b}	1.50 ± 0.10^{a}	R^2= 0.73, p = *0.0004*[5]	p = *0.003*
Fe	8.36 ± 0.60^{a}	5.83 ± 0.23	4.60 ± 0.23	4.96 ± 0.03^{b}	9.30 ± 0.30^{a}	R^2= 0.89	P < *0.0001*
Se	0.21 ± 0.01^{a}	0.21 ± –	0.20 ± 0.00	0.18 ± 0.00^{b}	0.23 ± –^{a}	R^2= 0.64, p = *0.002*[6]	p = *0.003*
Zn	26.00 ± 0.57	27.67 ± 1.20	27.67 ± 0.66	28.00 ± 1.52	27.50 ± 0.50	n.s.	n.s.

Experiment 2						
	Control	BMM9	BMS9[7]	BMSS9[8]	BMSF9[9]	ANOVA
Whole-body macro-nutrients (g 100 g $^{-1}$ WW)						
Protein	18 ± –	18 ± –	17 ± –	17 ± –	17 ± –	n.s.
Total fat	11.13 ± 0.22	11.23 ± 0.28	11.63 ± 0.43	11.67 ± 0.08	11.70 ± 0.23	n.s.
Energy (J g^{-1} WW)	8163 ± 67	8280 ± 156	8260 ± 196	8230 ± 112	8250 ± 155	n.s.
Dry matter	29.99 ± 0.30	30.30 ± 0.40	30.23 ± 0.40	30.13 ± 0.29	29.95 ± 0.46	n.s.
Whole-body micro-mineral (mg kg^{-1} WW)						
Mn	1.80 ± 0.05	1.53 ± 0.17	1.50 ± 0.10	1.30 ± 0.11	1.46 ± 0.16	n.s.
Cu	1.23 ± 0.08	1.26 ± 0.03	1.40 ± 0.05	1.26 ± 0.03	1.26 ± 0.03	n.s.
Fe	11.00 ± –^{a}	11.33 ± 0.33^{a}	13.33 ± 0.33^{b}	13.67 ± 0.33^{b}	13.00 ± –^{b}	P < *0.0001*
Se	0.19 ± 0.01	0.19 ± 0.00	0.20 ± 0.00	0.18 ± 0.00	0.18 ± 0.00	n.s.
Zn	31.33 ± 0.33^{a}	33.67 ± 0.66^{a}	35.00 ± 1.52^{ab}	39.33 ± 0.88^{c}	38.00 ± –^{b}	p = *0.0004*

Notes: Data is listed as mean ± SEM. The mean is from n = 3 pooled whole- body sample per diet (n = 5 fish per tank). In experiment 1, all diets are triplicate except BMM12, that is in duplicate. In experiment 2, n = 3 tank per diet.
The column labeled "Regression" gives R^2 and p-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "comparison" under experiment 1 gives a p-value for ANOVA in case of a significant difference between control, BMS11 and BMM12. The column labeled "ANOVA" under experiment 2 gives a p-value for ANOVA in case of a significant difference between all experimental groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.

[1] Simple linear regression: Y = −0.1067× + 18.23.
[2] Simple linear regression: Y = −35.85× + 9393.
[3] Simple linear regression: Y = −0.1303× + 32.97.
[4] Simple linear regression: Y = −0.02823× + 0.9424.
[5] Simple linear regression: Y = 0.04424× + 1.626.
[6] Simple linear regression: Y = −0.002545× + 0.2184. BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
[7] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[8] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[9] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

(*Pelteobagrus fulvidraco*) (Luo et al., 2017). Iron concentration in whole-body, head kidney, and liver in addition to hemoglobin and hematocrit levels are commonly used as indicators of the Fe status (Andersen et al., 1996; Bjørnevik and Maage, 1993; Naser, 2000). In experiment 1, the diets containing BMS had increasing dietary Fe levels, and all diets were above the minimum requirement (60–100 mg Fe kg^{-1}) for Atlantic salmon (Andersen et al., 1996). Furthermore, the same mineral premix containing $FeSO_4$ was added to all experimental diets. Despite this, Fe homeostasis was disrupted in BMS groups, resulting in significantly lower Fe levels in targeted tissues, particularly the liver, which is the main storage site for Fe (Walker and Fromm, 1976), compared to both control and BMM groups. The post-smolt Atlantic salmon normal range of Fe is considered between 10 and 20 mg kg^{-1} WW in whole-body, 96 ± 45 (56–102) mg kg^{-1} WW in liver (Andersen et al., 1996), and 11 ± 0.5 μmol L^{-1} in plasma (Antony Jesu Prabhu et al., 2016). The mean Fe concentrations whole-body, liver and plasma of BMS groups was lower than the mentioned range (Andersen et al., 1996; Antony Jesu Prabhu et al., 2016). It has been shown that it takes at least 22 weeks for fish to develop Fe deficiency when fed a low-Fe diet and uptake Fe from water through the gills (Naser, 2000). Since the gastrointestinal tract is the main site of Fe absorption in fish (Whitehead et al., 1996), the severe reduction of Fe stores after only 10 weeks in the current study may be caused by both weakness in dietary availability and the utilization of Fe. However, the findings from the present study showed the availability of Fe increased with a higher inclusion level of BMS, which can be explained by a relatively increased uptake when the iron status is low (Standal, 1999). It should be mentioned that fecal samples were collected at the end of the experiment when the fish already had low Fe level in the body. Consequently, the enhanced availability and absorption of Fe towards the end of the experiment appear plausible, as the fish's bodily iron status governs the intestinal uptake of iron.

Mineral availability can be affected by various factors, such as the antagonistic interactions between divalent ions like Fe, Mn, and Cu that compete for the same uptake route (Bury and Grosell, 2003; Lorentzen and Maage, 1999; Ogino and Yang, 1980; Prabhu et al., 2019). This finding is consistent with the current study, which revealed lower Mn

Table 8
Macro-nutrients, and mineral retention of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1							
	Control	BMS3	BMS7	BMS11	BMM12	Regression (0, BMS3, 7, 11)	Comparison (0, BMS11, BMM12)
Macro-nutrients (%)							
Protein	54.07 ± 2.06[a]	43.30 ± 512	41.38 ± 4.21	31.10 ± 4.90[b]	48.06 ± 0.43[ab]	R^2= 0.60, *p = 0.003*[1]	*p = 0.04*
Total fat	79.00 ± 4.50	62.00 ± 6.55	66.00 ± 4.93	47.67 ± 10.67	75.50 ± 4.50	R^2= 0.44, *p = 0.01*[2]	n.s.
Energy	57.63 ± 1.20[a]	47.61 ± 5.12	46.28 ± 2.91	34.84 ± 6.49[b]	51.59 ± 1.65[ab]	R^2= 0.57, *p = 0.004*[3]	*p = 0.04*
Dry matter	52.00 ± 1.14[a]	43.81 ± 4.57	41.90 ± 2.59	31.36 ± 5.85[b]	47.74 ± 1.09[ab]	R^2= 0.59, *p = 0.004*[4]	*p = 0.03*
Ash	27.63 ± 2.36	22.88 ± 3.05	22.68 ± 4.60	15.98 ± 4.60	31.26 ± 0.71	R^2= 0.33, *p = 0.04*[5]	n.s.
Micro-minerals (%)							
Mn	2.82 ± 0.73[a]	1.36 ± 0.33	1.60 ± 0.53	0.37 ± 0.08[b]	1.79 ± 0.28[ab]	R^2= 0.50, *p = 0.01*[6]	*p = 0.05*
Cu	23.05 ± 0.90	25.35 ± 4.01	24.26 ± 1.85	25.62 ± 3.74	18.60 ± 2.83	n.s.	n.s.
Fe	6.15 ± 0.69[a]	2.21 ± 0.31	0.51 ± 0.29	0.31 ± 0.09[b]	4.52 ± 0.40[a]	R^2= 0.92[7]	*p < 0.0001*
Se	32.28 ± 2.28[a]	26.00 ± 2.70	22.52 ± 0.93	12.70 ± 2.55[b]	29.75 ± 1.25[a]	R^2= 0.81, *p < 0.0001*[8]	*p = 0.001*
Zn	20.67 ± 0.66[a]	20.67 ± 2.02	18.33 ± 1.20	14.67 ± 0.33[b]	21.50 ± 1.50[a]	R^2= 0.60, *p = 0.003*[9]	*p = 0.02*

Experiment 2						
	Control	BMM9	BMS9[10]	BMSS9[11]	BMSF9[12]	ANOVA
Macro-nutrients (%)						
Protein	37.67 ± 2.33	45.67 ± 6.36	36.67 ± 5.54	26.67 ± 5.54	31.67 ± 3.48	n.s.
Total fat	46.00 ± 5.00	56.33 ± 9.33	50.67 ± 5.69	39.67 ± 7.31	44.33 ± 4.97	n.s.
Energy	35.67 ± 2.84	44.33 ± 7.31	37.67 ± 3.71	28.67 ± 5.78	33.33 ± 4.63	n.s.
Dry matter	34.67 ± 2.84	43.33 ± 6.76	36.67 ± 4.80	27.67 ± 5.92	32.33 ± 2.84	n.s.
Micro-minerals (%)						
Mn	4.17 ± 0.44	358 ± 0.95	3.25 ± 0.72	1.53 ± 0.48	1.68 ± 0.24	n.s.
Cu	9.62 ± 1.88	11.42 ± 2.06	12.91 ± 0.85	8.15 ± 1.90	9.97 ± 1.47	n.s.
Fe	5.72 ± 0.39	5.21 ± 0.86	5.83 ± 0.67	4.37 ± 0.78	4.54 ± 0.37	n.s.
Se	20.46 ± 3.14	22.83 ± 3.76	19.49 ± 2.41	11.10 ± 2.17	15.63 ± 2.83	n.s.
Zn	18.00 ± 1.52	23.33 ± 3.71	24.00 ± 4.93	23.67 ± 2.66	26.00 ± 2.08	n.s.

Notes: Data is listed as mean ± SEM. The mean is from n = 3 pooled whole- body sample per diet (n = 5 fish per tank). In experiment 1, all diets are triplicate except BMM12, that is in duplicate. In experiment 2, n = 3 tank per diet.
The column labeled "Regression" gives R^2 and *p*-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "comparison" under experiment 1 gives a *p*-value for ANOVA in case of a significant difference between control, BMS11 and BMM12. The column labeled "ANOVA" under experiment 2 gives a *p*-value for ANOVA in case of a significant difference between all experimental groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.

[1] Simple linear regression. $Y = -1.892\times + 52.40$.
[2] Simple linear regression. $Y = -2.395\times + 76.24$.
[3] Simple linear regression. $Y = -1.867\times + 56.40$.
[4] Simple linear regression. $Y = -1.715\times + 51.27$.
[5] Simple linear regression. $Y = -0.9447\times + 27.25$.
[6] Simple linear regression. $Y = -0.1883\times + 2.528$.
[7] Segmental linear regression. $Y1 = -1.394\times + 6.150$. $Y2 = -0.2383$ (X-3) +1.968. Y=IF (X < 3. Y1. Y2). X0 = 3.
[8] Simple linear regression. $Y = -1.681\times + 32.20$.
[9] Simple linear regression. $Y = -0.5612\times + 21.53$. BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
[10] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[11] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[12] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

and Fe status alongside a higher Cu status in the whole-body of fish fed BMS. The transport of Fe^{2+} into the absorptive enterocyte of the small intestine requires not only the action of divalent metal protein I (DMT1) but is also dependent on the chemical form of Fe (Fe^{3+} and Fe^{2+}), as previously discussed (Hansen and Spears, 2009). According to a study by (Hansen and Spears, 2009), an acidic environment like silage fermentation may reduce Fe^{3+} to the more soluble Fe^{2+} which is in line with the current result that Fe^{3+} to total Fe ratio in BM raw materials (without acid silage) (31% of total Fe) was 1.7-times higher than BMS product before drying (18% of total Fe) (Fig. 5a). However, co-drying BMS with SPC (BMS + SPC) increased the ratio in the product (39% of total Fe) which was reflected in the diets contained BMS + SPC in experiment 1 (Fig. 5b). Therefore, a high level of dietary Fe^{3+} may be assumed as one reason for impairing the activity of ferric reductase enzyme, in the apical membrane of intestinal epithelial cells, and Fe availability.

Ascorbic acid plays a crucial role in Fe metabolism in animals, including fish, as documented by various studies (Harper et al., 1979; Hilton, 1989; Monsen, 1982; NRC, 1993). It enhances the absorption of Fe from the intestine by converting ferric iron (Fe^{3+}) into a more soluble and absorbable ferrous state (Fe^{2+}) (El-Hawary et al., 1975; Harper et al., 1979; Monsen, 1982). The chemical forms of Fe (Fe^{3+} and Fe^{2+}) are important in transporting the Fe through the epithelium of the cells in the intestinal wall (Bury and Grosell, 2003; Bury et al., 2003). The soluble form of iron is Fe^{2+} which is more absorbable by the intestinal cells in fish than the Fe $^{3+}$ form (Bury and Grosell, 2003; Bury et al., 2003). Ascorbic acid also collaborates with adenosine triphosphate (ATP) in the release and reduction of Fe^{3+} from ferritin. This reduced Fe is then incorporated into Fe-binding proteins, apoferritin, and

transferrin, facilitating its storage in bodily tissues (Harper et al., 1979; Mazur et al., 1960). The dietary vitamin C levels were high and similar in all diets, but the decreased Fe status was only observed for the fish given diets containing BMS in experiment 1, thus it is not likely that the differences are caused by the vitamin C content in the feed.

Based on the results from experiment 1, it was hypothesized that either the amount of acid and thus the low pH in the blue mussel silage used, or the use of antioxidant in the silage could modulate the bioavailability of iron. Thus, experiment 2 was designed to both repeat the reference diet and the blue mussel meal diets as positive control, as well as repeating the same batch of blue mussels used in experiment 1. In addition, two new productions of BMS were tested, using a lower acid level and higher pH (3.5) as well as only formic acid at the same level.

In this study, no differences were seen in feed intake, FCR, and weight gain between the experimental groups. A significant reduction was however seen in SGR and condition factor in fish fed BMSS9 (made with silage containing lower level of soft acid) compared with the control group. A lower daily and total feed intake was observed in this group, although not significantly different from other groups. This could be attributed to the higher TBARs level in this diet compared to other diets, potentially reducing the palatability of the diet and growth performance. Although HSI was significantly higher in the BMS groups compared to the control group, it remained within the normal range for Atlantic salmon (1–2%) (Arnesen and Krogdahl, 1993).

Despite variations in the levels of several amino acids in BMS products, likely caused by variations in seasons and productions, the amino acid composition was balanced in the experimental diets. Further, the inclusion of BMS in the diets did not influence the whole-body composition and retention of macro-nutrients. No sign of Fe depletion was observed despite a higher Fe^{3+} to total Fe ratio in BMS products and experimental diets compared to the control diet in experiment 2 (Fig. 5a and c). The fish fed diets containing BMS also had significantly better Fe status in whole-body and targeted tissues, and the Fe availability was comparable between the experimental groups. The blood parameters such as RBC, Hb, and HCT were not influenced by BMS and were comparable between all experimental groups. No differences were seen in Fe availability or body status in this experiment irrespective of the silage being made with or without antioxidants. In addition to Fe, fish fed both BMSS9 and BMSF9 groups had a higher Zn level in the whole-body which was in line with the Zn availability results that got doubled in these groups. The availability of Mn and Cu also increased significantly in these groups; however, this was not reflected in the body composition. The general welfare of the fish was not compromised, which means the nutrition and environment requirements of fish was fulfilled by the experimental diets (Dawkins, 1990; Noble et al., 2018; Stien et al., 2013).

Different outcomes in experiments 1 and 2 may therefore be due to the difference in the production methodology, particularly in the steps to increase the dry matter content in the feed. This discrepancy is likely due to these differences, rather than variations in pH levels, which are associated with the acid used during production, and the addition of antioxidants. In experiment 1, the BMS with 10% DM was mixed with SPC and dried before being added to the feed to reach the target levels of BMS in the finished extruded feeds. In experiment 2, the same batch of blue mussel silage was used, however dried using a falling film evaporator (heat drying) to reach 50% DM allowing direct inclusion in the feed production. Overall, the findings indicate that the processing method can significantly influence the availability of nutrients and the body composition of fish, as demonstrated by the changes in mineral levels in the different experimental groups.

5. Conclusion

According to the findings from both experiments, Atlantic salmon fed a partial inclusion of blue mussel meal as a FM replacement have comparable growth, feed utilization, digestibility, and retention. In experiment 1, a growth reduction was seen already with the inclusion of 3% BMS, which could be explained by production methodology, the co-drying process with SPC, and interactions that likely caused problems with iron uptake.

In experiment 2, no differences were seen in the iron status as well as growth performance and feed utilization by using different drying method; however, somewhat lower growth was seen in the fish given BMS with lower soft acid compared with other BMS groups.

In conclusion, the limiting step for using blue mussel silage in fish feeds appears to be related to the processing of the raw material, as well as the choice of drying methods to facilitate incorporation into extruded feeds.

The use of blue mussel silage as a marine protein resource should be further elucidated, focusing on optimizing methods with a low carbon footprint, as well as focusing on interactions that may reduce the bioavailability of minerals.

CRediT authorship contribution statement

Sahar Sartipiyarahmadi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization. **Antony J. Prabhu Philip:** Writing – review & editing, Visualization, Validation, Project administration, Investigation, Funding acquisition, Conceptualization. **Aksel N. Forshei:** Writing – review & editing, Visualization, Investigation, Formal analysis, Conceptualization. **Harald Sveier:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Silje Steinsund:** Writing – review & editing, Resources, Investigation. **Malin Kleppe:** Writing – review & editing, Resources, Investigation. **Erik-Jan Lock:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Angelico Madaro:** Writing – review & editing, Investigation. **Tom Johnny Hansen:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Formal analysis. **Øivind Strand:** Writing – review & editing, Investigation. **Martin Wiech:** Writing – review & editing, Investigation. **Jan Vidar Jakobsen:** Writing – review & editing, Resources, Investigation. **Sofie C. Remø:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.740829.

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Paper II

II

Sahar Sartipiyarahmadi, Antony J. Prabhu Philip, Harald Sveier, Silje Steinsund, Erik-Jan Lock, Angelico Madaro, Tom Johnny Hansen, Martin Wiech, Øivind Strand, Jan Vidar Jakobsen, Marleen E. van der Heide, Jan Værum Nørgaard, and Sofie C. Remø, (2023).

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Research Article

Growth Performance, Nutrient Digestibility, and Retention in Atlantic Salmon, *Salmo salar* L., Fed Diets with Fermented Sugar Kelp, *Saccharina latissima*

Sahar Sartipiyarahmadi[1,2] **Antony J. Prabhu Philip**[1,3] **Harald Sveier**[4] **Silje Steinsund**[4] **Erik-Jan Lock**[1,3] **Angelico Madaro**[1] **Tom Johnny Hansen**[1] **Martin Wiech**[1] **Øivind Strand**[1] **Jan Vidar Jakobsen**[5] **Marleen E. van der Heide**[6] **Jan Værum Nørgaard**[6] **and Sofie C. Remø**[1]

[1]*Institute of Marine Research (IMR), Bergen, Norway*
[2]*Department of Biological Sciences, University of Bergen, Bergen, Norway*
[3]*Nutrition and Feed Technology Group, Nofima, Bergen, Norway*
[4]*Lerøy Seafood Group ASA, Bergen, Norway*
[5]*Cargill, Dirdal, Stavanger, Norway*
[6]*Department of Animal and Veterinary Sciences, Aarhus University, Aarhus, Denmark*

Correspondence should be addressed to Sahar Sartipiyarahmadi; sahar.sartipi.yarahmadi@hi.no and Sofie C. Remø; sofie.remo@hi.no

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Using low trophic marine resources such as sugar kelp (*Saccharina latissimi*) is of great interest to increase the circular food production in the ocean. Sugar kelp does, however, contain high levels of carbohydrates and iodine and does not have considerable levels of protein and lipids, which may make it less suitable as a feeding ingredient. A 10-week feeding trial was done to investigate the effect of graded dietary inclusion levels of fermented sugar kelp (FSK), on growth performance, digestibility, retention of nutrients, and mineral composition in postsmolt Atlantic salmon (*Salmo salar* L.). The experimental diets were made to simulate a standard grower feed for salmon postsmolts in SW with ~63% plant-based ingredients vs ~34% marine ingredients and increasing concentrations of FSK between 0% (control feed) and 4% of the diet. During the feeding trial, the weight gain and specific growth rate (SGR) decreased linearly with increasing dietary FSK levels, where the SGR was slightly reduced from 1.2% for the fish given the control feed to 1.1% in the fish given feeds containing 3% and 4% FSK. This resulted in a lower weight gain of up to 9% in the fish given 4% FSK compared to the control. Feed intake and feed conversion ratio were, however, similar in all diet groups, and FSK inclusion did not influence the digestibility of macronutrients or minerals, except for lipid. The reduced growth is likely related to a lower digestible energy level in the diets, and the retention of both lipids and energy was affected by FSK inclusion. Inclusion of FSK also influenced iodine availability and retention, as well as increasing iodine status in whole body and muscle in a dose-dependent manner until reaching a plateau, which corresponds to 124 mg I kg^{-1} WW (135 mg I kg^{-1} DW), at 3% FSK inclusion.

1. Introduction

The aquaculture industry is rapidly expanding and is expected to continue growing worldwide. However, the industry faces a major challenge due to a shortage of available feed resources [1]. Therefore, it is crucial to explore the use of new alternatives for sustainable feed ingredients from underutilized and renewable natural resources that do not compete with human food [2], as well as improving processing technology to produce safe and nutritious aquafeed ingredients [3]. Low trophic species that are produced or cultivated are considered to have potential as future feed sources [3]. The aquaculture industry is increasingly looking toward marine macroalgae (seaweed) as a resource for use in feeds due to their high growth rate,

potential cultivation in salt water, and no requirements for arable land or industrial fertilization [2, 4, 5]. In addition to that, macroalgae contribute to food circularity by taking up dissolved inorganic nutrient wastes from water [6, 7]. Macroalgae are known for their high nutritional quality and are a promising supplement in functional foods or a potential source for extracting compounds [8, 9]. They are a rich source of essential amino acids, beneficial polysaccharides, vitamins, minerals [9, 10], and bioactive substances [11].

Norway aims to produce 5 million metric tons (MT) of salmonids by 2050, which would require 6 million MT of feed [12]. Sugar kelp is one of the most cultivated macroalgae species in Europe and Norway [5, 13–15] with potential economic value as animal feed and food for human consumption [13]. In 2014, the first permission for sea cultivation of macroalgae was launched in Norway, and experience shows the potential in both monoculture and integrated multitrophic aquaculture system [16]. Cultivated macroalgae accounted for 97.1% of the world's annual production of macroalgae (including wild and cultivated) in 2018, which totaled 32.4 million tons [17]. However, the low crude protein (1%–21% of dry matter), low lipid content (0.5%–3.4% of dry matter), and high levels of complex carbohydrates, ash [18], and moisture content (75%–90%) [9, 19] in this species [9, 20, 21] pose challenges for its application in aquafeed.

Previous feeding studies have proven that overall performance of fish on macroalgae in the diet depends on the fish species (species specific) and inclusion level (dose dependent) of macroalgae [22–24]. As shown in Table 1, the incorporation of macroalgae in aquafeed at low levels (<10%) can maintain or enhance growth performance (weight gain, feed utilization, and survival). However, fish growth and feed efficiency might be negatively affected at high inclusion level (≥10%) of macroalgae due to the presence of antinutritional factors such as lectins, protease inhibitors, tannins, phytate, and toxins which are widely distributed in plants and macroalgae [25–27] and low level of energy content [2]. In rainbow trout (*Oncorhynchus mykiss*) including up to 2% dried sugar kelp in the diet did not reduce growth or feed utilization, whereas both weight gain and specific growth rate (SGR) were reduced when including 4%, likely due to decreased protein digestibility [28]. Hence, a critical aspect when developing diets for fish is the evaluation of their capacity to digest novel ingredients and determining the appropriate inclusion level in addition to optimizing feed use. Optimal feed utilization is important to reduce feed costs and environmental impacts such as greenhouse gas emission [29, 30].

Brown macroalgae such as sugar kelp are known as iodine-rich sources containing up to 10,000 mg iodine kg^{-1} dry weight (DW) [31, 32]. However, concerns have been raised about using high levels of sugar kelp in the diet of Atlantic salmon.

To achieve large-scale use of macroalgae as a feed resource, it is crucial to address the challenge of a steady supply of biomass. Seasonal harvesting necessitates proper processing, preservation, and long-term storage [2]. Fermentation is a promising preservation method for brown macroalgae [33] and commonly uses lactic acid bacteria (LAB) [34]. Fermentation is a simple and cheap method for stabilizing a wet biomass that would otherwise rapidly degrade after harvesting [35]. Furthermore, it enhances the shelf life, food safety, and nutritional and sensory properties of the product [34, 36]. Fermentation also affects the nutrient profile and protein digestibility of macroalgae [2]. It lowers crude fiber content and increases protein digestibility, thereby improving its nutritive value as fish feed [2, 37]. Fermentation also reduces the high content of iodine in macroalgae [38]. However, the low levels of DM content of macroalgae species must be considered as a challenge for incorporating them into the diet and pelletizing.

There are few available publications on the inclusion of sugar kelp or other brown macroalgae in feed for fish particularly salmonids as one of the most important groups of aquaculture fish species. Moreover, the generation of novel feed products by fermentation technology has yet only been developed for a few macroalgae species, particularly red algae [38, 39]. Therefore, this study investigated whether including fermented sugar kelp (FSK) (1%, 2%, 3%, and 4%) in Atlantic salmon diet influences growth performance, nutrient digestibility and retention, whole body and muscle composition, and welfare of fish.

2. Materials and Methods

2.1. Ethical Statement. The feeding trial was conducted at Matre Research Station, Norway, according to the Norwegian regulations on animal experimentation. The experimental protocol was approved by the Norwegian Food Safety Authority (FOTS ID # 25202).

2.2. Fermented Sugar Kelp. The FSK was provided by Ocean Forest AS, Lerøy Seafood Group. The sugar kelp was cultivated and harvested by Ocean Forest AS at Trollsøy, Austevoll (Norway; 60° 7.821′, 5° 14.891′) in May 2020. The fermentation process was initiated immediately after harvest on fresh material at ambient temperature (8–14°C) in closed intermediate bulk containers, by adding 10 g of a commercial blend of *Lactobacillus* bacteria (LAB) delivered by European Protein (Pig Stabilizer 600, Version 04.12.2017) per 1,000 kg of finely chopped sugar kelp. The pH dropped to below 4.0 within a span of 3 weeks, which was sustained thereafter. The composition of both fresh sugar kelp, sampled prior to the fermentation process, as well as the FSK is presented in Table 2. Both fresh and FSK contained 1.3 g 100 g^{-1} WW (15% DW) crude protein and less than 1% g 100 g^{-1} WW lipid content, while FSK contained somewhat less carbohydrate than fresh sugar kelp. The cellulose level was similar in both fresh and FSK. Both groups showed the same concentration of manganese (Mn) and zinc (Zn), while copper (Cu), selenium (Se), iron (Fe), and iodine levels were different after fermentation.

2.3. Experimental Diets. The feeding trial was designed as a dose–response study using graded inclusion levels of FSK. The control diet was formulated as a commercially relevant reference feed for postsmolt in seawater. In the experimental diets, FSK was added to reach the target levels of 1%–4% in the finished extruded pellets (Table 3). All diets were formulated to meet the minimum requirements of Atlantic salmon

Table 1: Several macroalgae species used as feed supplement or replacement.

Fish species	Macroalgae species	Tested levels	Best level	Substituted ingredient	Effect	Reference
European sea bass (*Dicentrarchus labrax*)	*Gracilaria bursa-pastoris* *Gracilaria cornea* *Ulva rigida*	5%–10%	10% 5% 10%	Fish protein hydrolysate	No negative consequences on growth performance, nutrient utilization, or body composition 10% inclusion of *Gracilaria cornea* significantly decreased growth performance, dry matter, and lipid ADC and increased FCR	[85]
Gilthead sea bream (*Sparus aurata*)	*Pterocla dia* *Ulva*	5%–15%	10% 5%	Wheat flour	Up to 10% *Pterocladia* and 5% *Ulva* showed the best growth performance, feed utilization, nutrient retention, and survival 15% inclusion reduced the growth and feed utilization	[86]
Red sea bream (*Pagrus major*)	*Ascophyllum nodosum* *Porphyra yezoensis* *Ulva pertusa*	5%	5%	Protein	Increased body weight, feed efficiency, and muscle protein deposition Feeding *Porphyra* showed the most pronounced effects on growth and energy accumulation, followed by *Ascophyllum* and *Ulva*	[87]
Red sea bream (*Pagrus major*)	*Wakame or Ascophyllum*	5%–10%	5%	Protein, rice, and/or wheat bran	The best growth and feed efficiency and higher muscle lipid content in the 5% Wakame diet group	[88]
Rainbow trout *(Oncorhynchus mykiss)*	*Porphyra dioica*	5%–15%	Up to 10%	Fish meal and wheat starch	No significant negative effects on weight gain and growth performance	[89]
Rainbow trout (*Oncorhynchus mykiss*)	*Gracilaria vermiculophylla*	5%–10%	Up to 5%	Fish meal	No effect on growth or FCR, increased iodine and moisture content, and higher color intensity 10% inclusion resulted in significantly smaller fish	[80]
Rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*)	*Porphyra dioica*, *Ulva spp.* *Gracilaria vermiculophylla* *Sargassum muticum*	30%	30%	Mixing 70% basal diet and 30% of each seaweed	Trout digested better Gracilaria, while Nile tilapia does better with Ulva and Sargassum	[24]
Rainbow trout (*Oncorhynchus mykiss*)	*Ulva lactuca* *Enteromorpha linza*	10%	—	Added to the diet	Poor growth and feed utilization	[90]
Rainbow trout (*Oncorhynchus mykiss*)	*Gracilaria vermiculophylla*	5%–10%	Up to 5%	Several dietary protein source	No difference in growth, FCR and protein efficiency ratio, and increased innate immune response 10% inclusion resulted in the lowest final body weight, feed and protein efficiency, and protein retention	[26]
Rainbow trout (*Oncorhynchus mykiss*)	Sugar kelp *(Saccharina latissima)*	1%– 4%	Up to 2%	Wheat meal	No effect on growth performance, increased protein efficiency, and increased iodine level in fillet 4% inclusion resulted in lower growth, lower hepatosomatic indices, and histomorphological change in intestine	[28]
Atlantic salmon (*Salmo salar*)	Laminaria sp., kelp	3%–10%	Up to 10%	Fish meal	Increased food consumption, enhance growth performance, improve antioxidant capacity, and alleviate adverse effects of stressors such as temperaturte	[91]
Atlantic salmon (*Salmo salar*)	Verdemin Rosamin Mixing Verdemin and Rosamin	2.5%–5% 2.5%–5% 2.5% of each	Up to 5% 5% Up to 5%	Added to the diet	No effect on growth and feed efficiency Increased omega3 long-chain polyunsaturated fatty acid in whole body No effect on growth and feed efficiency	[8]

Table 1: Continued.

Fish species	Macroalgae species	Tested levels	Best level	Substituted ingredient	Effect	Reference
Atlantic salmon (*Salmo salar*)	*Palmaria palmata*	5% –15%	Up to 15%	Starch and fish meal	No effect on fish growth, no effect on haematological, immunological, and hepatic function. Five percent inclusion improved body lipid content Alanine transaminase activity significantly decreased in the diet with 5% and 15% *Palmaria palmata*	[92]

Table 2: Macronutrient and mineral proximate composition of fresh and fermented sugar kelp (FSK).

	Fresh sugar kelp	Fermented sugar kelp (after 3 weeks)
Macronutrients proximate composition (g 100 g^{-1} WW)		
Crude protein	1.3	1.3
Fat	<1	<1
Ash	4.1	3.8
Dry matter	8.7	8.6
Carbohydrate composition (% WW)		
T-NCP[1]	1.0	0.8
T-NSP[2]	1.4	1.2
Cellulose	0.4	0.4
Lignin-like substance	0.2	0.1
S-DF[3]	0.8	0.7
I-DF[4]	0.7	0.6
T-DF[5]	1.5	1.3
Micromineral composition (mg kg^{-1} WW)		
Mn	0.5	0.5
Fe	7.0	5.0
Cu	0.2	1.2
Zn	3.0	3.0
Se	<0.008	0.01
I	430	400

Data are given as mean ± SEM ($n = 3$). WW refers to a wet weight basis. [1]T-NCP stands for total noncellulosic polysaccharide that contains soluble and insoluble noncellulosic polysaccharides. Soluble and insoluble noncellulosic polysaccharides are rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, and uronic acid. [2]T-NSP stands for total nonstarch polysaccharides that contain soluble and insoluble nonstarch polysaccharides. Soluble nonstarch polysaccharides are equal to soluble noncellulosic polysaccharides and insoluble nonstarch polysaccharides contains insoluble noncellulosic polysaccharides and cellulose. [3]S-DF stands for soluble dietary fiber that contains soluble noncellulosic polysaccharides. [4]I-DF stands for insoluble dietary fiber that contains insoluble noncellulosic polysaccharide, cellulose, and lignin. [5]T-DF stands for total dietary fiber that contains total nonstarch polysaccharide and lignin.

[40]. In the finished diets, the protein level ranged between 43 and 46 g 100 g^{-1} WW (Table 3). The amino acid profile in the experimental diets was still comparable to the experimental diets (Table 4). The FSK incorporation in the diet resulted in lower lipid (25–18 g 100 g^{-1} WW) and lower digestible energy (DE) (19–18 MJ kg^{-1} WW) in FSK4% diet compared with the control group. Diets with a higher FSK contained lower NDF and hemicellulose content, while the others were comparable between the experimental diets. Some variations were seen in dietary Fe and Se levels and iodine ranged between 4 and 138 mg kg^{-1} WW in the experimental diets. The experimental diets were produced by Cargill (Dirdal, Norway). To determine apparent digestibility/availability of nutrients, yttrium oxide (0.02% ≈ 200 mg/kg) was added as an inert marker to all diets.

2.4. Fish and Rearing Condition. At the start of the experiment, 65 Atlantic salmon (*Salmo salar* L.) postsmolts with an average weight of 204 ± 37 g (mean ± SD) were randomly distributed in 15 quadrangular 1.5 m^3 glass fiber tanks, in total 975 fish, and the five experimental diets were each randomly assigned to triplicate tanks. The postsmolt used in the present study originated from Aqua Gen strain, Agua Gen AS, Trondheim, Norway. In each tank, 55 fish were produced from commercially available eggs obtained in the fall of 2019 (mixed population) and 10 fish were from an isogenic salmon line (all-male population) produced at Matre, also originally made from the Aqua Gen strain in 2011 [41, 42]. The all-male fish were included as a standard reference fish to eliminate the influence of genetic variation on the growth evaluation in the study, and these were pit tagged for determination of individual growth rates. The fish were acclimatized to the tanks for 3 weeks prior to experimental start. The average density of each tank at the start of the experiment was 10.0 ± 0.5 kg m^{-3} (mean ± SD).

During the experiment, the environmental conditions were kept within normal production regimes for Atlantic salmon postsmolt. The fish were kept in seawater with a salinity of 34 ppt that was provided using a flow-through system, and the water flow was adjusted as the fish grew to maintain oxygen saturation in the tanks. The water temperature ranged between 8.8 and 9.2°C with a mean of 9 ± 0.07°C (mean ± SD) during the experimental period, under continuous (24 hr) light.

The fish were given two meals per day (between 9 : 30 to 11 : 00 and 12 : 30 to 14 : 00) for 10 weeks. The fish were fed in excess with automatic feeders (Arvotec TD 2000) to ensure enough feed for all the fish, and the feeding rate was adjusted according to the increase in fish biomass. The uneaten feed pellets were collected 15min after each meal to estimate feed intake according to Helland et al. [43].

2.5. Sampling Procedure. All sampled fish were euthanized with an overdose of tricaine methane sulfonate (500 mg/L, FINQUEL MS-222). At the start of the experiment, 45 fish (30 fish from the mixed population and 15 fish from all-male

Table 3: Formulation (in % of total raw materials) and proximate composition of the experimental diets containing different levels of fermented sugar kelp (FSK).

	Control	FSK1%	FSK2%	FSK3%	FSK4%
Fish oil	10.2	10.3	10.4	10.5	10.6
Rapeseed oil	13.9	13.6	13.4	13.2	12.9
Fishmeal LT	25.0	23.3	21.6	19.9	18.2
Soy protein concentrate (SPC)	20	20	20	20	20
Wheat	11.0	11.0	10.9	10.5	10.0
Other plant proteins[1]	16.8	17.5	18.3	19.4	20.6
Microingredients	3.2	3.3	3.4	3.5	3.6
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Fermented seaweed	-	1	2	3	4
Analyzed proximate composition (g 100 g^{-1} WW)					
Protein	46	45	43	44	46
Lipid	25	25	24	22	18
Ash	7	7	7	7	8
Gross energy (MJ kg^{-1} WW)	23	22	23	22	21
Digestible energy (MJ kg^{-1} WW)	19	18	19	18	18
Dry matter	95	93	94	95	92
Carbohydrate (g 100 g^{-1} WW)					
NDF[2]	16	14	13	13	13
ADF[3]	2	1.9	1.9	2.1	2.1
ADL[4]	0.3	0.3	0.2	0.3	0.3
Hemicellulose	14	12	11	11	11
Cellulose	1.7	1.7	1.7	1.8	1.8
Mineral composition (mg kg^{-1} WW)					
Mn	51	48	51	52	52
Fe	190	186	197	181	193
Cu	10	9	9	10	10
Zn	162	149	150	162	156
Se	0.8	0.8	0.8	0.8	0.7
I (DW)	4 (4)	60 (67)	80 (89)	124 (135)	138 (157)

Notes: Ingredients are listed as percentages of whole feed. WW and DW refer to wet weight and dry weight basis. [1]Wheat gluten meal, pea protein concentrate- and guar-meal. [2]NDF stands for neutral detergent fiber and contains soluble NDF (sugars, pectin, nonprotein *N*, soluble protein) and insoluble NDF (hemicellulose, fiber-bound protein, cellulose, lignin, lignified *N*). [3]ADF stands for acid detergent fiber and contains soluble ADF (hemicellulose, fiber-bound protein) and insoluble ADF (cellulose, lignin, and lignified *N*). [4]ADL stands for acid detergent lignin and contains soluble ADL (cellulose) and insoluble ADL (lignin, cutin).

population) were sampled to register organ weights (viscera, liver, and heart), as well as determination of organ-specific nutrient compositions. The same number of fish ($n = 45$) were sampled to determine the whole-body proximate composition and were divided into three pools each ($n = 30$ fish from the mixed population, $n = 3$ pooled, and $n = 15$ fish from the all-male population, $n = 3$ pooled). At the end of the experiment, weight and length were recorded on all fish. From each tank, 10 fish from mixed population and 10 fish from all-male population were sampled for determination of whole-body and organ-specific nutrient compositions, where five whole fish from each were pooled for determination of whole-body composition ($n = 5$ fish per tank, $n = 3$ per diet, pooled) and five fish were dissected individually for registrations of viscera, liver, and heart to calculate somatic indices ($n = 5$ fish per tank, $n = 15$ per diet). The whole fish, as well as the whole muscle, were frozen in dry ice, homogenized, and stored at −20°C for determination of nutrient composition ($n = 5$ fish per tank, $n = 3$ per diet, pooled).

A visual evaluation was done on the 20 individuals sampled from each tank ($n = 20$ fish per tank, $n = 60$ per diet) prior to dissection to monitor standard welfare indicators and operational indicators, including eye status, jaw wound and deformity, opercula status, spine deformation, gill condition, condition factor, and skin and fin damage according to a standard scoring system (SWIM) [44, 45]. Cataract examination was performed in darkened conditions using a Heine HSL 150 hand-held slit lamp (HEINE Optotechnik GmbH & Co. KG, Herrsching, Germany) [46]. Cataracts were graded 0–4 on each lens, according to the criteria given by Wall and Bjerkas [46].

Feces were collected by stripping (gently expelled using light pressure on the abdomen near the vent) from 55 fish per tank (45 fish from mixed population and 10 fish from all-

Table 4: Amino acids composition of the experimental diets containing different levels of fermented sugar kelp (FSK).

(mg g^{-1} as is)	Control	FSK1%	FSK2%	FSK3%	FSK4%
Hydroxyproline	1.9	1.6	1.6	1.5	1.5
Histidine	12.5	11.8	12.0	12.0	12.6
Taurine	1.5	1.3	1.3	1.2	1.2
Serine	19.7	18.9	18.7	19.4	20.5
Arginine	28.3	26.8	26.8	27.2	29
Glycine	21.2	19.6	19.6	19.6	20.5
Aspartic acid	40.0	39.0	38.0	38.0	41.0
Glutamic acid	74.0	73.0	73.0	75.0	82.0
Threonine	15.8	15.0	14.8	15.0	15.8
Alanine	19.7	18.7	18.4	18.4	19.5
Proline	21.7	21	21.1	21.8	23.6
Lysine	26.8	25.4	24.8	24.5	26.2
Tyrosine	13.9	13.2	13.2	13.8	14.2
Methionine	12.0	11.3	11.1	11.4	11.9
Valine	18.8	17.8	17.9	17.7	19.1
Isoleucine	17.1	16.2	16.4	16.1	17.5
Leucine	31.0	29.1	29.0	29.2	31.0
Phenylalanine	19.9	18.9	19.3	19.6	20.7

Notes: WW refers to wet weight basis.

male population) at the end of the trial, and feces of each population (all-male and mixed) were separately pooled for a composite sample used to determine the apparent digestibility (ADC)/availability coefficient (AAC) of nutrients ($n = 45$ fish from the mixed population per tank, $n = 3$ per diet, pooled and $n = 10$ fish from the all-male population per tank, $n = 3$ per diet, pooled).

2.6. Analytical Methods. DM, crude protein, crude fat, ash, gross energy, and carbohydrate content were determined in the raw materials (fresh and FSK), experimental diets, whole body, and feces samples. Briefly, DM was measured after drying to constant weight at 105°C for 24 hr [47]. Crude protein was analyzed using a protein analyzer (Vario Macro Cube, Elementar Analysen Systeme GmbH, Germany) [48]. Crude fat of the feed, tissue, and feces samples was extracted with ethyl acetate and filtered before the solvent evaporated and the fat residue was weighed. The method is standardized as a Norwegian Standard, NS 9402 [49]. Crude fat of the raw material samples was also measured based on the gravimetry after acid hydrolysis [50]. Combustion in a muffle furnace at 550°C for 16–18 hr determined ash content, and gross energy was measured using an IKA calorimeter C7000 after drying the homogenized diet samples for 48 hr at 60°C. To determine total nonstarch polysaccharides (T-NSP) and their constituent sugars gas–liquid chromatography was used for neutral sugars, and colorimetry was used for uronic acid as modified and described by Englyst, Wiggins et al. [51], Englyst, Quigley et al. [52], Theander, Åman et al. [53], and Knudsen [54]. Total NSP contains cellulose and soluble and insoluble noncellulosic polysaccharides (NCP) based on the analysis of monomeric constituents. Cellulose was determined as the difference of glucose content of NSP when the swelling step with 12 M H_2SO_4 was included ($NSP_{Glucose\ (12\ M\ H2SO4)}$) or omitted ($NSP_{Glucose\ (2\ M\ H2SO4)}$). The sum of glucose, galactose, xylose, arabinose, mannose, rhamnose, fucose, and uronic acids shows T-NCP. Insoluble residue after hydrolysis with 12 M H_2SO_4 determined the lignin-like substances. The fractions in macroalgae that were insoluble in sulfuric acid and consequently indigestible and not fermentable were recognized as lignin. However, it could not be determined whether it is lignin or other acid-insoluble components in macroalgae the fraction will be referred to as the lignin-like substance. The sum of lignin-like substances and T-NSP corresponds to total dietary fiber (T-DF). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose, and cellulose were measured in the feed and feces samples under a carbohydrate analyzer. The list of analyzed polysaccharides in each group is presented in Table 3. Briefly, Ankom technology was used to analyze NDF, ADF, and ADL sequentially using an Ankom 220 Fiber Analyzer. For the determination of NDF, a heat-stable amylase was used as described by Mertens [55]. Afterward, a correction was made for ash using the ash residue obtained after ADL determination. The collected feces samples were freeze dried for 72 hr and homogenized before analysis.

The microminerals, yttrium oxide, and iodine concentrations in diets, and pooled samples of whole body, muscle, and feces were determined by inductively coupled plasma mass spectrometry (ICP-MS), as described by Long and Martin [56] and Julshamn et al. [57]. In brief, for determination of the microminerals, 0.2 g freeze-dried sample material was digested in a microwave oven (Milstone-MLS-1200), diluted to 25 mL with Milli-Q Water, and analyzed using ICP-MS (Agilent 7500c). For the determination of iodine, the sample preparation was a basic extraction with tetramethylammonium hydroxide (TMAH) before ICP-MS analysis.

2.7. Performance Calculations. The following variables were calculated [58]:

$$\text{Digestible energy}\left(\text{DE}, \frac{\text{MJ}}{\text{kg}}\right) = \text{Energy} \in \text{Diet} - \left(\frac{\text{Yttrium} \in \text{Diet}}{\text{Yttrium} \in \text{Faeces}} \times \text{Energy} \in \text{Faeces}\right), \quad (1)$$

$$\text{Weight gain (WG, g)} = \text{Final mean weight (g)} - \text{Initialmeanweight(g)}, \quad (2)$$

$$\text{Specific growth rate (SGR, \% per day)} = (\ln \text{final BW} - \ln\text{initial BW}) \times \frac{100}{t}. \quad (3)$$

As described by Hopkins [59], where ln final BW and ln initial BW are the natural logarithm of final and initial biomass in grams and t is the sum of feeding days (70 days). In the current study, the mean SGR of the fish from mixed population was determined for each tank. In addition, individual SGR was also calculated on the 10 pit-tagged fish from

the all-male population per tank.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Weight gain}}. \quad (4)$$

As described by Helland et al. [43], total feed intake was calculated as an estimate of DM content of the waste feed (obtained in the recovery test):

$$\text{Total feed intake (TFI, g)} = \frac{\left(\frac{A\times \text{ADW}}{100}\right) - \left(\frac{W\times \text{WDW}}{R}\right)}{\frac{\text{ADW}}{100}}, \quad (5)$$

where A is the weight of air-dry feed (g), ADW is the DM content of air-dry feed (%), W is the wet weight of waste feed collected (g), WDW is the DM content of waste feed (%), and R the is recovery of DM of waste feed (%) that was calculated as follows:

$$\text{Recovery } (R, \%) = 100 \times \frac{W \times \text{WDW}}{A \times \text{ADW}}. \quad (6)$$

Average daily feed intake per kg biomass (DFI–% biomass) was calculated from recorded daily feed intake and estimated daily biomass from SGR using the following equation:

$$\ln W \text{ day}x = \left(\frac{\text{SGR}}{100}\right) \times (1 + \ln W \text{ day } (x-1)), \quad (7)$$

where ln W day$x$ is the natural logarithm of biomass on a given day [60].

$$\text{Condition factor}\left(\text{K}, \frac{\text{g}}{\text{cm}^3}\right) = 100 \times \frac{\text{Body weight (g)}}{\text{Body length (cm}^3\text{)}}. \quad (8)$$

The hepatosomatic indexes (HSI), cardio somatic indexes (CSI), and visceral somatic indexes (VSI) were calculated as percentages of the final weight:

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times \left(\frac{\text{Liver weight}}{\text{Whole body weight}}\right), \quad (9)$$

$$\text{Cardiosomatic index (CSI, \%)} = 100 \times \left(\frac{\text{Heart weight}}{\text{Whole body weight}}\right), \quad (10)$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times \left(\frac{\text{Viscera weight}}{\text{Whole body weight}}\right). \quad (11)$$

To understand how much of the ingested feed ingredient is absorbed by the animal and retained in their body, the apparent digestibility/availability coefficient (ADC/AAC), and retention of nutrients were measured as described by Cho [61]:

$$\text{ADC (\%)} = 100 - \left(100 \times \frac{\text{Yttrium in diet}}{\text{Yttrium in faeces}} \times \frac{\text{Nutrient in faeces}}{\text{Nutrient in diet}}\right), \quad (12)$$

$$\text{AAC (\%)} = 100 - \left(100 \times \frac{\text{Yttrium in diet}}{\text{Yttrium in faeces}} \times \frac{\text{Mineral in faeces}}{\text{Mineral in diet}}\right). \quad (13)$$

Nutrient retention (%) was calculated from fish biomass and nutrient content of the fish at the start and end of each growth period and nutrient intake:

$$\text{Retention (\%)} = 100 \times \frac{(\text{BM f} \times \text{Nutrient content f}) - (\text{BM i} \times \text{Nutrient content i})}{\text{Feed intake} \times \text{Nutrient in feed}}, \quad (14)$$

where f and i are the nutrient content in final and initial, respectively.

2.8. Data Analysis. As the trial study was performed in a dose–response design, linear and nonlinear regression analyses were used to evaluate dose-dependent responses by determining the best-fit line for each dataset. In addition, one-way ANOVA was performed to assess statistically significant differences among experimental groups, and if the data were significant different, then followed up with Tukey' s multiple comparison post hoc analysis. For all datasets, Bartlett/Brown–Forsythe's test was used to assess the homogeneity of variance and Shapiro–Wilk's test was used to check the normality residuals. The ROUT test was done for the identification and removal of the outliers of the growth dataset. One of the 4% FSK tanks was removed as outlier. Tank was used as the experimental unit in growth, whole-body proximate, and mineral composition ($n = 3$ for all the experimental diets and $n = 2$ for FSK4% group). Whole body, muscle proximate composition, and mineral status of Atlantic salmon postsmolts from the all-male population were only analyzed for control and high level of FSK4%. All the statistical analyses and the graphs were performed in GraphPad Prism (Version 8.4.3 (686) San

Table 5: Growth performance indicators of Atlantic salmon postsmolts and fed graded inclusion levels of fermented sugar kelp (FSK).

	Control	FSK1%	FSK2%	FSK3%	FSK4%	Regression (P value, R^2)	ANOVA
IBW (g)	210.1 ± 3.5	203.1 ± 1.1	208.8 ± 3.4	203.9 ± 2.3	209.1 ± 0.5	n.s.	n.s.
FBW (g)	485.1 ± 8.0	470.1 ± 6.1	475.8 ± 12.0	451.5 ± 16.1	458.9 ± 20.5	n.s.	n.s.
WG (g)	275.0 ± 4.8	267.0 ± 5.0	267.0 ± 8.5	247.6 ± 13.9	249.8 ± 21.0	$P = 0.04$, $R^2 = 0.30$[1]	n.s.
SGR (% per day)	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	$P = 0.02$, $R^2 = 0.36$[2]	n.s.
TFI (kg)	11.9 ± 0.2	12.6 ± 0.5	12.8 ± 0.1	12.3 ± 0.7	11.9 ± 0.1	n.s.	n.s.
DFI (% of biomass)	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	n.s.	n.s.
FCR	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.1	0.7 ± 0.1	n.s.	n.s.
K	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	n.s.	n.s.
HSI	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	n.s.	n.s.
CSI	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	n.s.	n.s.
VSI	7.2 ± 0.4	6.9 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	7.1 ± 0.4	n.s.	n.s.
Cataract score	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	n.s.	n.s.

Notes: IBW = initial body weight (g), FBW = final body weight (g), WG = weight gain (g), SGR = specific growth rate, K = condition factor, TFI = total feed intake (g), DFI (%) = daily feed intake as percentage of biomass, FCR = feed conversion ratio, HSI = hepatosomatic index, CSI = cardio somatic index, and VSI = visceral somatic index. Data are presented as mean ± SEM. The somatic indices are a mean of 15 fish per diet (five fish per tank), the weight and length data are a mean of all fish ($n = 55$ fish per tank), and the cataract is a mean of 30 fish per diet ($n = 10$ fish per tank). All diets are in triplicate except FSK4%, that is, in duplicate. n.s. stands for not significant. [1]Simple linear regression, $Y = -7.190x + 275.4$. [2]Simple linear regression, $Y = -0.02122x + 1.207$.

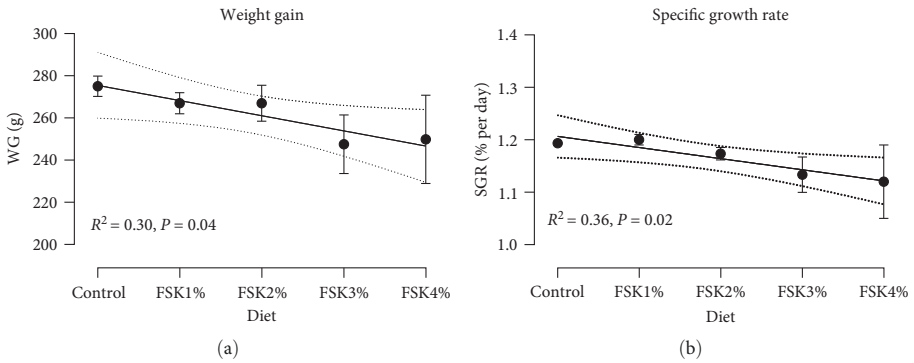

Figure 1: (a) Weight gain and (b) specific growth rate (SGR) of Atlantic salmon postsmolt fed graded inclusion of fermented sugar kelp (FSK). The best-fit regression lines for each dataset were presented (the regression equations are presented in Table 5). Values are presented as mean ± SEM, all diets are in triplicate except FSK4%, that is, in duplicate, $n = 15$ fish per diet.

Diego, California, USA). Significance was set at $P < 0.05$ for all statistical tests, and the value is presented as mean ± SEM.

3. Result

3.1. Fish Performance Indicators. During the experiment, the fish almost doubled the weight in all experimental groups (Table 5). Total feed intake (the mean of all experimental groups, 12.3 ± 0.2 kg) and feed conversion ratio (FCR) (0.7 ± 0.02) were not affected by FSK inclusion levels in experimental diets. However, WG and SGR decreased in a dose–response manner under a simple linear regression ($P = 0.04$ and $P = 0.02$, respectively) with FSK inclusion (Figures 1(a) and 1(b)). In comparison to the control group, the WG of FSK1% and 2% decreased by 3%, FSK3% and 4% decreased by 10% and 9%, respectively. All groups had a similar K of around 1.2 ± 0.0. Furthermore, no dose-dependent responses were seen in morphometric measurements of the somatic indices (HSI, VSI, and CSI) among the experimental groups. At the end of the 10-week trial, the mean cataract score was below 1 (0.7 ± 0.04) for all groups, and no difference was seen among the experimental groups. Moreover, there was no difference in visually assessed welfare indicators between experimental groups (only two fish had the scale loss and short operculum with score 2).

The mean weight of the all-male postsmolts ($n = 10$ per tank, $n = 30$ per diet) was a little higher than the mixed population ($n = 55$ per tank, $n = 165$ per diet) at the end of the experiment, but it was still within the same range for both groups (Supplementary Figure S1A). The SGR of the all-male fish decreased slightly from 1.30 ± 0.03 in control group to 1.26 ± 0.04 in FSK4% group, however not significantly different ($P = 0.2$). This resulted in a 5%–7% lower weight gain, but the reduction was also not significant ($P = 0.15$). The

Table 6: Apparent digestibility coefficients (ADC) of macronutrients and apparent availability coefficient (AAC) of minerals of Atlantic salmon postsmolt fed graded inclusion of fermented sugar kelp.

	Control	FSK1%	FSK2%	FSK3%	FSK4%	Regression (P value, R^2)	ANOVA
Macronutrients ADC (%)							
Crude protein	86.9 ± 0.6	88.0 ± 0.3	89.1 ± 0.3	87.3 ± 0.4	88.5 ± 0.3	n.s.	n.s.
Total fat	94.4 ± 0.4	96.3 ± 0.5	96.5 ± 0.8	96.6 ± 1.1	97.1 ± 0.8	$P = 0.03$, $R^2 = 0.32$[4]	n.s.
Digestible energy	77.9 ± 1.2	79.8 ± 0.2	81.5 ± 0.8	79.2 ± 1.1	79.7 ± 0.2	n.s.	n.s.
Structural carbohydrate ADC (%)							
NDF[1]	70.0 ± 4.6	69.5 ± 4.4	68.4 ± 2.6	62.1 ± 4.7	61.8 ± 9.3	n.s.	n.s.
ADF[2]	−13.5 ± 8.5	−7.5 ± 5.3	−6.8 ± 3.2	−7.2 ± 3.1	−6.7 ± 5.9	n.s.	n.s.
ADL[3]	58.7 ± 1.7	23.4 ± 29.7	33.6 ± 5.3	52.7 ± 8.6	55.5 ± 16.2	n.s.	n.s.
Hemicellulose	82.0 ± 4.1	82.3 ± 2.7	81.3 ± 1.5	76.4 ± 3.8	76.9 ± 6.8	n.s.	n.s.
Cellulose	−24.9 ± 8.5	12.5 ± 2.0	−12.6 ± 4.4	−16.9 ± 2.7	−15.4 ± 3.6	n.s.	n.s.
Micromineral AAC (%)							
Zn	23.9 ± 3.4	26.9 ± 1.5	27.8 ± 1.9	23.1 ± 5.8	26.5 ± 2.7	n.s.	n.s.
Mn	−29.1 ± 18.5	−4.3 ± 3.3	−3.6 ± 6.6	−10.6 ± 13.8	−1.9 ± 7.0	n.s.	n.s.
Cu	42.2 ± 1.0	43.9 ± 2.2	45.3 ± 0.8	35.8 ± 2.4	40.3 ± 0.6	n.s.	n.s.
Fe	−3.7 ± 5.9	9.1 ± 1.5	15.3 ± 1.9	−5.5 ± 8.3	−3.3 ± 1.2	n.s.	n.s.
Se	54.9 ± 1.5[a]	57.6 ± 1.7[ab]	63.4 ± 0.2[b]	57.2 ± 2.1[ab]	54.9 ± 1.2[a]	$R^2 = 0.64$[5]	$P = 0.01$
Iodine	69.9 ± 3.4[a]	84.0 ± 0.7[b]	87.2 ± 0.8[b]	86.2 ± 0.7[b]	88.2 ± 0.0[b]	$R^2 = 0.87$[6]	$P = 0.0008$

Notes: Data are listed as mean ± SEM. The mean is from $n = 3$ pooled feces sample per diet ($n = 65$ fish per tank). All diets are in triplicate except FSK4%, that is, in duplicate. n.s. stands for not significant. [1]NDF stands for neutral detergent fiber and contains soluble NDF (sugars, pectin, nonprotein *N*, and soluble protein) and insoluble NDF (hemicellulose, fiber-bound protein, cellulose, lignin, lignified *N*). [2]ADF stands for acid detergent fiber and contains soluble ADF (hemicellulose, fiber-bound protein) and insoluble ADF (cellulose, lignin, lignified *N*). [3]ADL stands for acid detergent lignin and contains soluble ADL (cellulose) and insoluble ADL (lignin, cutin). [4]Simple linear regression, $Y = 0.5856X + 95.04$. [5]Segmental linear regression, $Y_1 = 3.99x + 54.44$, $Y_2 = 4.148\ (x-2) + 62.42$, $Y = \text{IF}\ (X < 2, Y_1, Y_2)$, $X_0 = 2$ (FSK2%). [6]Segmental linear regression, $Y_1 = 14.73x + 69.92$, $Y_2 = 1.152\ (x-1) + 84.65$, $Y = \text{IF}\ (X < 67, Y_1, Y_2)$, $X_0 = 67$ (iodine in FSK1% diet).

growth performance results for the all-male population are also reported separately as supplementary material (Supplementary Table S1A).

3.1.1. Apparent Digestibility and Apparent Availability Coefficient. No difference was seen in the digestibility of macronutrients except for total fat ADC (Table 6). Total fat ADC increased significantly from 94.4 ± 0.4% in the control group to 97.1 ± 0.8% in the 4% FSK group under a simple linear regression ($P = 0.03$). The protein ADC ranged between 86.9 ± 0.6% in the control group and 88 ± 0.3% in the experimental groups. Gross energy digestibility was 77.9 ± 1.2% and 80.1 ± 0.4% for the control and experimental groups, respectively. The average of the carbohydrate ADC (control and experimental groups together) was calculated to be 66.6 ± 2.0% for NDF, −8.4 ± 2.2% for ADF, 44.0 ± 7.1% for ADL, 79.9 ± 1.5% for hemicellulose, and −16.5 ± 2.2% for cellulose.

Availability of iodine significantly increased with FSK inclusion in the diet under a segmental linear regression response with a broken point in FSK1% (Figure 2(a)). The iodine availability increased from 69.9 ± 3.4% in the control group to 86.0 ± 0.5% in the FSK groups. In addition, Se AAC was significantly increased by FSK diets. The 2% FSK-supplemented group had the highest Se AAC (63.4 ± 0.2%) fitted by a segmental linear regression with a broken point in FSK2% (Table 6). The availability of the other analyzed minerals Zn, Mn, Cu, and Fe was not affected by FSK inclusion in the diet (Table 6).

3.2. Whole Body and Muscle Composition. The total fat, energy, and DM content all showed a dose-dependent response ($P = 0.001$, $P = 0.003$, $P = 0.01$, respectively) and decreased with FSK inclusion in diet (Table 7). The 3% and 4% FSK groups had the lowest amount of total fat, gross energy, and DM in body compared with the other groups. No effect was found on the protein and ash body composition by adding the FSK to the salmon diet.

The concentration of iodine in the fish whole body increased in a dose-dependent manner with increasing iodine level in the feed Figure 3(a). The iodine level was 0.2 ± 0.0 mg kg^{-1} WW in the fish fed the control feed, while it increased around 7.5 times, and reached 1.5 ± 0.1 mg kg^{-1} WW in the whole body of fish fed FSK3%, where the levels appeared to plateau. Whole-body Cu concentration decreased from 1.6 ± 0.0 to 1.3 ± 0.1 mg kg^{-1} WW under a simple linear regression response ($P = 0.03$).

Muscle iodine level increased almost six-fold from 0.1 ± 0.0 mg kg^{-1} WW in the control group to 0.6 ± 0.0 mg kg^{-1} WW in the FSK4% group under a simple linear regression response ($P < 0.0001$, Figure 3(b)). No significant differences were observed in other essential micromineral (Mn, Fe, Se, and Zn) concentrations in whole body and muscle among dietary treatments.

The nutritional status of the fish from the all-male population was determined in both the control and FSK4% groups and is included in Supplementary Table S1B. A similar pattern of nutritional status was seen between both mixed and all-male populations.

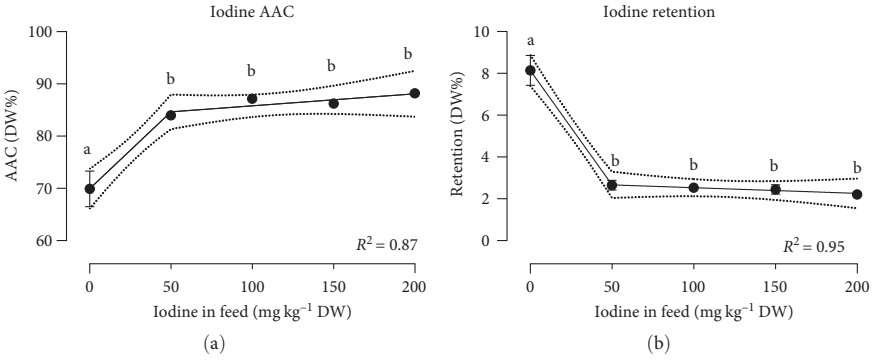

FIGURE 2: (a) Iodine apparent availability coefficient (AAC), (b) iodine retention of Atlantic salmon postsmolt fed graded inclusion of fermented sugar kelp (FSK). The best-fit regression lines for each dataset were presented (the regression equations are presented in Table 6). Statistically significant differences between the experimental groups were represented with different letters above the data points ($P < 0.05$) under the Tukey HSD test. Values are presented as mean ± SEM, all diets are in triplicate except FSK4%, that is, in duplicate.

TABLE 7: Whole body and muscle proximate composition and mineral status of Atlantic salmon postsmolt fed graded inclusion of fermented sugar kelp.

	Control	FSK1%	FSK2%	FSK3%	FSK4%	Regression (P value, R^2)	ANOVA
Macronutrient in whole body (g 100 g^{-1} WW)							
Protein	18.3 ± 0.3	18.0 ± 0.0	17.7 ± 0.3	18.0 ± 0.0	18.0 ± 0.0	n.s.	n.s.
Total fat	13.5 ± 0.4	13.0 ± 0.2	12.9 ± 0.3	12.2 ± 0.3	12.1 ± 0.2	$P = 0.001$, $R^2 = 0.58$[1]	n.s.
Energy (J g^{-1} WW)	9407.0 ± 104.0	9357.0 ± 150.6	9180.0 ± 83.9	8917.0 ± 138.7	8975.0 ± 35.0	$P = 0.003$, $R^2 = 0.52$[2]	n.s.
Ash	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.7 ± 0.0	n.s.	n.s.
Dry matter	33.0 ± 0.4	32.8 ± 0.4	32.4 ± 0.3	31.6 ± 0.4	31.9 ± 0.1	$P = 0.01$, $R^2 = 0.43$[3]	n.s.
Micromineral in whole body (mg kg^{-1} WW)							
Mn	1.0 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	n.s.	n.s.
Cu	1.6 ± 0.0	1.4 ± 0.1	1.5 ± 0.2	1.3 ± 0.0	1.3 ± 0.1	$P = 0.03$, $R^2 = 0.3$[4]	n.s.
Fe	8.4 ± 0.6	9.1 ± 0.4	8.5 ± 0.2	8.5 ± 0.1	8.6 ± 0.2	n.s.	n.s.
Se	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	n.s.	n.s.
Zn	26.0 ± 0.6	25.7 ± 0.3	25.3 ± 0.9	26.3 ± 0.9	25.5 ± 0.5	n.s.	n.s.
Iodine	0.2 ± 0.0[a]	0.9 ± 0.1[b]	1.1 ± 0.0[b]	1.5 ± 0.1[c]	1.6 ± 0.1[c]	$P < 0.0001$, $R^2 = 0.97$[5]	$P < 0.0001$
Micromineral in muscle (mg kg^{-1} WW)							
Mn	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	n.s.	n.s.
Cu	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	n.s.	n.s.
Fe	2.1 ± 0.1	2.1 ± 0.0	2.2 ± 0.0	2.0 ± 0.0	2.2 ± 0.1	n.s.	n.s.
Se	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	n.s.	n.s.
Zn	4.7 ± 0.2	5.1 ± 0.3	5.2 ± 0.3	4.7 ± 0.3	5.8 ± 0.8	n.s.	n.s.
Iodine	0.1 ± 0.0[a]	0.3 ± 0.0[b]	0.4 ± 0.0[c]	0.6 ± 0.0[d]	0.6 ± 0.0[d]	$P < 0.0001$, $R^2 = 0.98$[6]	$P < 0.0001$

Notes: Data are listed as mean ± SEM. The mean is from $n = 3$ pooled whole-body sample per diet ($n = 5$ fish per tank). All diets are in triplicate except FSK4%, that is, in duplicate. n.s. stands for not significant. WW refers to a wet weight basis. [1]Simple linear regression, $Y = -0.3711x + 13.47$. [2]Simple linear regression, $Y = -136.1x + 9433$. [3]Simple linear regression, $Y = -0.3611x + 33.06$. [4]Simple linear regression, $Y = -0.08072x + 1.568$. [5]Simple linear regression, $Y = 0.01010x + 0.2289$. [6]Simple linear regression, $Y = 0.004266x + 0.05323$.

3.3. Retention of Nutrient and Essential Elements. The retention of total fat, gross energy, and DM decreased with a higher FSK inclusion (Table 8). The highest fat retention was seen in the 4% FSK-supplemented group (82.0 ± 8.0%) under a second polynomial model. However, the energy and DM retention decreased with a higher FSK inclusion under a simple linear regression ($P = 0.04$ and $P = 0.03$, respectively). There was no effect of FSK diets on protein and ash retention.

Among the microminerals, Cu and iodine retention decreased with increasing inclusion of FSK into the diet and presented a dose-dependent response (Table 8, Figure 2(b)). Copper retention was reduced from 23.1 ± 0.9% in control group to 12.8 ± 0.0% in 4% FSK-supplemented group under a simple linear regression response ($P = 0.01$). Furthermore, iodine retention decreased significantly by adding 1% of FSK to the diet and followed almost a plateau pattern fitted by a

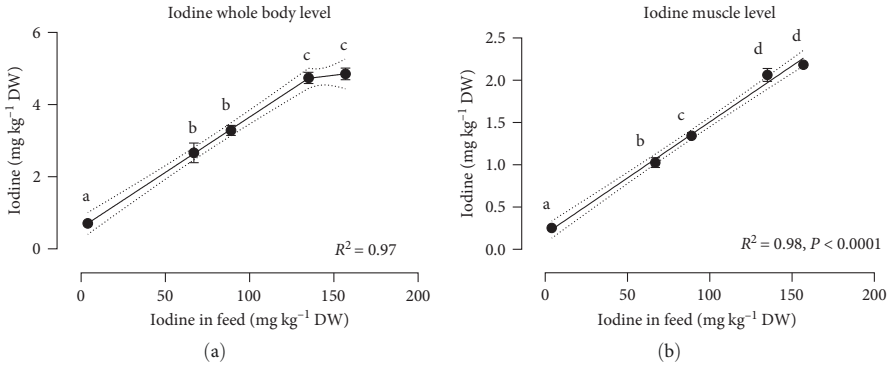

FIGURE 3: (a) Whole body and (b) muscle iodine status of Atlantic salmon postsmolt fed graded inclusion of fermented sugar kelp (FSK). The best-fit regression lines for each dataset were presented (the regression equations are presented in Table 7 in a WW). The whole body and muscle iodine concentrations are presented in DW in the graphs (a) and (b). The regression formula for graph (a) is a segmental linear regression, $Y_1 = 0.03071x + 0.5849$, $Y_2 = 0.005678\ (x-135) + 4.73075$, $Y = \text{IF}\ (X < 135, Y_1, Y_2)$, $X_0 = 135$ and for graph (b) is a simple linear regression, $Y = 0.01326x + 0.1810$. Statistically significant differences between the experimental groups were represented with different letters above the data points ($P < 0.05$) under the Tukey HSD test. Values are presented as mean ± SEM, all diets are in triplicate except FSK4%, that is, in duplicate.

TABLE 8: Macronutrients and mineral retention of Atlantic salmon postsmolt fed graded inclusion of fermented sugarkelp.

	Control	FSK1%	FSK2%	FSK3%	FSK4%	Regression (P value, R^2)	ANOVA
Macronutrients (%)							
Crude protein	54.1 ± 2.1	47.0 ± 2.2	47.8 ± 1.8	48.8 ± 3.2	45.1 ± 3.2	n.s.	n.s.
Total fat	79.0 ± 4.5	67.3 ± 2.3	69.3 ± 2.8	67.3 ± 4.3	82.0 ± 8.0	$R^2 = 0.45$[1]	n.s.
Gross energy	57.6 ± 1.2	53.2 ± 1.9	50.9 ± 1.7	49.2 ± 3.1	51.8 ± 4.1	$P = 0.04$, $R^2 = 0.30$[2]	n.s.
Dry matter	52.0 ± 1.1	48.7 ± 2.2	47.1 ± 1.6	43.4 ± 2.8	47.0 ± 3.6	$P = 0.03$, $R^2 = 0.32$[3]	n.s.
Ash	27.6 ± 2.4	24.7 ± 3.9	25.2 ± 2.3	21.3 ± 1.4	23.6 ± 2.6	n.s.	n.s.
Micromineral (%)							
Zn	20.7 ± 0.7	19.0 ± 1.5	19.0 ± 1.7	19.0 ± 1.7	17.5 ± 0.5	n.s.	n.s.
Mn	2.8 ± 0.7	2.1 ± 0.8	2.0 ± 0.2	1.5 ± 0.4	2.1 ± 0.1	n.s.	n.s.
Cu	23.1 ± 0.9	16.7 ± 1.5	20.1 ± 4.4	14.5 ± 1.3	12.8 ± 0.0	$P = 0.01$, $R^2 = 0.40$[4]	n.s.
Fe	6.2 ± 0.7	6.4 ± 0.6	5.4 ± 0.4	6 ± 0.5	5.5 ± 0.2	n.s.	n.s.
Se	32.3 ± 2.3	32.7 ± 2.8	24.5 ± 0.7	31.4 ± 2.9	31.1 ± 1.0	n.s.	n.s.
Iodine	8.1 ± 0.7[a]	2.7 ± 0.2[b]	2.5 ± 0.1[b]	2.4 ± 0.2[b]	2.2 ± 0.0[b]	$R^2 = 0.95$[5]	$P < 0.0001$

Notes: Data are listed as mean ± SEM. The mean is from $n = 3$ pooled whole-body sample per diet ($n = 5$ fish per tank). All diets are in triplicate except FSK4%, that is, in duplicate. n.s. stands for not significant. WW refers to a wet weight basis. [1]Second polynomial model (quadratic), $Y = 3.419x^2 - 13.16x + 78.72$. [2]Simple linear regression, $Y = -1.769x + 55.87$. [3]Simple linear regression, $Y = -1.724x + 50.90$. [4]Simple linear regression, $Y = -2.264x + 21.95$. [5]Segmental linear regression, $Y_1 = -5.472x + 8.140$, $Y_2 = -0.1356\ (x-1) + 2.668$, $Y = \text{IF}\ (X < 67, Y_1, Y_2)$, $X_0 = 67$ (iodine in FSK1%).

segmental linear regression with a broken point in FSK1% (Figure 2(b)). The retention of the other minerals was not affected by the inclusion of FSK into the diet and dose-dependent responses were not observed (Table 8).

4. Discussion

The present study was conducted to determine the effect of adding increasing levels of FSK in the diet of farmed Atlantic salmon. Since the diets were made to simulate a standard grower feed for salmon postsmolts in SW, the feeds were made with ∼63% plant-based ingredients vs.∼34% marine ingredients. Although sugar kelp is not a considerable source of lipids and proteins for Atlantic salmon, the potential in using this low-trophic marine biomass in aquafeeds is interesting mostly as a source of bioactive compounds, but possibly also as a source of minerals [9, 10]. The high level of indigestible carbohydrates does, however, raise concerns about using it in the feed for salmon, along with the contribution of high levels of iodine. Thus, the present study aimed to investigate how the use of FSK in the diet for Atlantic salmon may modulate growth, welfare, digestibility of nutrients, and retention of nutrients with special emphasis on iodine.

Historically, the use of novel feed ingredients has sometimes resulted in the occurrence of production-related

disorders and welfare issues, that could for instance be related to single nutrient deficiencies or toxicities, as well as lower bioavailability of nutrients in novel resources [62]. In the present study, the fish were fed experimental diets for 10 weeks, and in this period, they more than doubled their weight from around 200 g to the range of 500 g. The inclusion of FSK slightly reduced the SGR from 1.2% to 1.1%, resulting in approximately 3% lower weight gain in the FSK1% and 2% groups, and a weight reduction of 10% and 9% in the FSK3% and 4% groups, respectively, compared to the control group Figures 1(a) and 1(b). A similar response was seen in rainbow trout fed a diet containing 4% sugar kelp, however in that study, including 1% and 2% sugar kelp in the diet did not reduce growth performance [28]. Despite the reduction in weight gain, no differences were observed in daily feed intake and FCR between the experimental groups in the present study, showing that feed utilization was not reduced by including FSK in the diet. The latter is of high importance considering the climate footprint of feed ingredients that is also dependent on the ability of the fish to utilize the feed [29, 30]. Further, the occurrence of production disorders and welfare issues resulting from nutritional deficiencies and possible related pathologies and losses also contribute to the sustainability evaluation of a raw material. At the end of the feeding study, a visual inspection of outer welfare indicators [45] as well as assessment of eye lens health (cataract score) could not identify any nutritionally related pathologies in any of the experimental groups. Therefore, while the inclusion of FSK in the diet resulted in a slight reduction in fish growth, there was no impact on feed utilization and fish welfare.

Previous studies have indicated that using high levels of macroalgae in fish feed influences the digestibility of nutrients, which in turn can be a potential cause of growth impairment [22]. This effect is attributed to the presence of high levels of complex polysaccharides, that can increase the passage of food through the digestive tract and consequently also reduce nutrient absorption [25, 63, 64]. However, in the present study, the dietary carbohydrate composition was similar across all experimental diets. The diets containing FSK had lower concentrations of NDF and hemicellulose, ranging from 13 to 15 g 100 g^{-1} compared to 17 g 100 g^{-1} in the control diet. Due to the contribution of fiber from the plant-based ingredients used in the feed, the inclusion of FSK led to a decrease in the overall carbohydrate content of the feed. Earlier studies have also shown that including various types and different inclusion levels of macroalgae could result in reduced protein digestibility. For instance, this was shown in two previous studies using dry algae meal from different macroalgae (5% Verdemin, 5% Rosamin) in the diet for Atlantic salmon [8] and sugar kelp at similar levels as in the present study (1%, 2%, and 4%) in the diet for rainbow trout [28]. One of the suggested reasons for the decreased protein ADC was the poor ability of fish to digest algae-derived proteins in addition to a limited ability to hydrolyze complex polysaccharides [65], which caused a maximum protein ADC of 45%–56% in fish [66]. It has been shown that including indigestible carbohydrates such as NSP in fish diet, for example tilapia diet, decreased the digestibility of proteins and lipids by impairing the fish's ability to absorb minerals and water and raising the viscosity of the digesta [67, 68]. In contrast to these findings, indigestible carbohydrates did not cause any problem in the current study, and it was found that adding FSK enhanced the fat digestibility (by 3%) while leaving the protein and carbohydrate digestibility unaffected. Since there was no significant increase in dietary carbohydrate or difference in feed consumption, protein, and carbohydrate ADC with increasing levels of FSK, it is unlikely that the observed reduction in growth can be attributed to these factors.

The lower dietary lipid level in the FSK4% diet compared with the other experimental diets (28% lower than the control group), likely resulted in a higher lipid digestibility and a relatively higher retention of total fat in the FSK4% group, while the whole-body fat content in this group was 14% lower than the control group. These changes were not reflected in the somatic indexes, which were contrary to the study by Granby et al. [28] which showed a negative correlation between sugar kelp inclusion in the rainbow trout diet and HSI, the HSI of rainbow trout fed 4% sugar kelp significantly decreased. The excess energy is stored in the liver, and HSI is used as an indirect indicator for measuring the energy status. The inclusion of FSK did, however, result in a lower dietary energy level and DE, and the reduction in growth and weight gain may rather be due to the overall energy dilution caused by incorporating FSK in the diets, which was also reflected in the whole-body composition of fat, energy, and DM. As a challenge of using macroalgae in monogastric animal feed, it has been observed that the high content of polysaccharide components such as alginate and carrageenan resulted in lower nutritionally available energy content of macroalgae and most algae-derived products [2, 69].

Sugar kelp contains a high level of iodine as reported by previous studies (up to 4,600 mg kg^{-1} DW) [32, 70, 71]. This high iodine content is a concern when considering kelp inclusion in fish diets. Notably, the upper tolerance level and no-observable-adverse-effect level (NOAEL) for dietary iodine have not been determined in farmed fish, while it has been proposed that the tolerances are 3–10-fold higher than the requirement [72]. In commercial aquaculture feed iodine is generally derived from fish meal and added as potassium iodide in the mineral premix [73]. Due to the generally lower iodine content of plant-based feeding stuff, incorporating them into fish diets may require an increased need for iodine supplementation [72]. The requirement of salmonids for dietary iodine is relatively low (1.1 mg kg^{-1}) [40, 74], and the maximum recommended level for iodine salt in farmed fish diets is 20 mg iodine kg^{-1} (based on 880 g kg^{-1} DW) [75] which still result in lower tissue concentrations in farmed fish compared with wild marine fish [72]. However, it was shown the maximum tolerable dietary iodine level is higher than 60 mg iodine kg^{-1} in farmed fish [72, 76]. Feed containing up to 86 mg iodine (as potassium iodine, KI) kg^{-1} diet fed to adult Atlantic salmon (*Salmo salar* L.) [77], and 2% kelp diet with an concentration of 117 ± 2 mg iodine kg^{-1} diet fed to rainbow trout (*Oncorhynchus mykiss*) [28] had no adverse

effects on growth performance, and health of these species. In the current study, the addition of sugar kelp increased the dietary iodine content from 4 mg kg^{-1} WW in the control feed up to 138 mg kg^{-1} WW in the 4% FSK feed. Due to the overall low dietary energy that influenced growth performance, it is challenging to determine if the high dietary iodine level caused the growth reduction. Nevertheless, the increase in iodine AAC and improvement in iodine body status until reaching a plateau level (FSK3% containing 124 mg kg^{-1} WW) suggested a possible regulation of iodine uptake and deposition in the fish body. Additionally, exposure to high dietary iodine levels resulted in a decrease in iodine retention. These results indicated that fish have a mechanism to adjust their iodine metabolism in response to high dietary iodine levels. This finding is consistent with previous research which has shown that certain species of fish are capable of efficiently excreting excess metals and maintaining normal levels of concentration in their bodies [78].

Previous studies have shown that the dietary iodine concentrations can be reflected in the muscle iodine level [28, 79–81]. In line with that, in the present study, the muscle iodine level for Atlantic salmon fed FSK1% and 2% (60 and 80 mg iodine kg^{-1} WW, respectively) was around 0.3 ± 0.0 mg kg^{-1} WW (four-fold of control diet) and reached around 0.6 ± 0.0 mg kg^{-1} WW in the muscle of fish fed FSK3% and 4% (124 and 138 mg iodine kg^{-1} WW). However, in the study by Granby et al. [28], the muscle iodine level in rainbow trout exhibited a four-fold increase, rising from 0.3 ± 0.08 mg kg^{-1} WW in fish fed 1% sugar kelp (57 mg iodine kg^{-1} WW) to 1.2 ± 0.45 mg kg^{-1} WW in fish fed 4% sugar kelp (220 mg iodine kg^{-1} WW). It is important to note that Granby et al. [28] included the skin in their muscle samples, whereas the current study did not, which could account for the conflicting results between the two studies. It has been shown that the skin of freshwater Char (*Salvelinus* sp.) displayed a five-fold higher iodine concentration compared to the skinless muscles [79]. Additionally, a higher dietary iodine level in the diet containing 4% sugar kelp was utilized in the study by Granby et al. [28], further contributing to the differences.

The sugar kelp used in this study had a Se concentration of less than 0.008 mg kg^{-1} WW (below detection limit), and just above the detection limit (0.01 mg kg^{-1} WW) after the fermentation, which was consistent with the findings of Bruhn et al. [38]. Although the dietary Se levels met the minimum Se requirement (0.6–0.8 mg kg^{-1} DW) [82], a high level of FSK in the diet led to an 11% and 22% decrease in dietary Se level for FSK3 and 4%, respectively, when compared to the control diet. Brown seaweeds typically have low levels of selenium [70], and it is possible that when included in fish feed, this may dilute the selenium content of the overall diet. The apparent availability of Se was higher in the diet containing 2% FSK; however, this did not translate into increased Se retention or whole-body status. These findings are contrary to the study by Granby et al. [28], which showed decreased Se AAC with the incorporation of sugar kelp (1%, 2%, and 4%) in rainbow trout diets. However, the differences in the results may be related to the overall impact on digestibility and nutrient retention in the study with rainbow trout that was not seen in the present study.

The inclusion of FSK negatively affected the distribution and retention of Cu in the whole body. This may be attributed to the higher level of iodine in the fish, as both iodine deficiency and oversupply can disrupt mineral (e.g., Cu, Mn, Fe, and Zn) homeostasis [83, 84].

5. Conclusion

Overall, the incorporation of FSK in the experimental diets reduced the growth, which may be related to the overall lower energy content in these feeds since feed intake and feed utilization (FCR) were similar. The use of FSK did not influence the digestibility of macronutrients except for lipids. The retention of lipid, energy, and DM was reduced with FSK inclusion in diet, which corresponded with whole-body macronutrient composition. Apparent mineral availability (except iodine and Se) and mineral retention (except iodine and Cu) were not affected by FSK inclusion by up to 4%. The incorporation of FSK in the diets improved iodine availability. Our results indicated that up to 3% FSK supplementation in the Atlantic salmon diet has the potential to improve the muscle iodine concentration. Up to 2% FSK inclusion in the postsmolt salmon diet improved Se availability. FSK inclusion in the diet of Atlantic salmon had no influence on the welfare indices studied.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

Antony J. Prabhu Philip and Erik-Jan Lock (present address): Nutrition and Feed Technology Group, Nofima, Bergen, Norway.

Conflicts of Interest

The equipment, drugs, or supplies were provided by Lerøy Seafood Group ASA and Cargill. Harald Sveier and Silje Steinsund reported a relationship with Lerøy Seafood Group ASA that includes employment and equity or stocks. Jan Vidar Jakobsen reported a relationship with Cargill that includes employment and equity or stocks.

Authors' Contributions

Sahar Sartipiyarahmadi: conceptualization, formal analysis, investigation, supervision, validation, visualization, writing the original draft, review, and editing. Antony Jesy Prabhu Philip: conceptualization, funding acquisition, investigation, project administration, validation, visualization, review, and editing. Harald Sveier: conceptualization, investigation, methodology, resources, review, and editing. Silje Steinsund: investigation, resources, review, and editing. Erik-Jan Lock: conceptualization, funding acquisition, investigation, review, and editing. Angelico Madaro: investigation, review, and editing. Tom J. Hansen: formal analysis, investigation,

methodology, resources, validation, review, and editing. Martin Wiech: investigation, review, and editing. Øivind Strand: investigation, review, and editing. Jan Vidar Jakobsen: investigation, resources, review, and editing. Marleen E. van der Heide: investigation, resources, review, and editing. Jan Værum Nørgaard: investigation, resources, review, and editing. Sofie C. Remø: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, review, and editing.

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Supplementary Materials

The supplementary materials that support the findings of this study can be found online. The growth performance and mineral composition of all-male population of Atlantic salmon postsmolt fed graded inclusion of fermented sugar kelp (FSK) are presented in supplementary materials for future reference in the use of the same fish when testing new raw materials. Figure S1A: comparing the mean and the trendline of final individual weight of all-male population and mixed population of Atlantic salmon postsmolts fed graded inclusion of fermented sugar kelp (FSK). $n = 30$ fish from all-male population, $n = 165$ fish from mixed population per diet, all diets are in triplicate except FSK4%, that is, in duplicate. Table S1A: growth performance indicators of all-male population of Atlantic salmon postsmolts, fed graded inclusion levels of fermented sugar kelp (FSK). Table S1B: whole-body and muscle proximate composition and mineral status of all-male population of Atlantic salmon postsmolt fed graded inclusion of fermented sugar kelp. (*Supplementary Materials*)

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