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Dietary approaches to improve mucosal health of Atlantic salmon (*Salmo salar*)

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FACULTY OF BIOSCIENCES AND AQUACULTURE

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Preface

This thesis is submitted in fulfilment of the requirements of the degree of Philosophiae Doctor (Ph.D.) at the Faculty of Biosciences and Aquaculture (FBA), Nord University, Bodø, Norway. The thesis is based on original feeding experiments with Atlantic salmon. The different studies of the Ph.D. project were mainly funded by Norwegian Research Council (Project No. 267872/E50, Project No. 260190), Slovak Research and Development Agency under contract No. APVV-19-0234, and FBA, Nord University. The project was also part of the project, "Blodanalyser av laks som metode for vurdering av tarmhelse" that was partially sponsored by MABIT (Grant No. AF0082. The project also received extra funding from MABIT project (Project no.: AF0092), Skattefunn (Project no.:306574), the US Department of Energy (Award No.: DE-EE0007091, Project – Marine Algae Industrialisation Consortium), and was part of the COFASP-ERA-NET project, "MARINALGAE4aqua".

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Mette Sørensen, Professor, FBA, Nord University: Main supervisor

Ioannis N. Vatsos, Associate Professor, FBA, Nord University: Co-supervisor

Kiron Viswanath, Professor, FBA, Nord University: Co-supervisor

Nadanasabesan Nimalan
Bodø, Norway
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Dedication

I dedicate this thesis to all women in science who have guided me throughout my research career as well as those who taught me different subjects in the University and schools. To name: Prof. Mette Sørensen, Mrs. Bisa Saraswathy, Dr. Florence Perera, Associate Prof. Melania Borit, Prof. N.D.K. Dayawansa, Mrs. Thayaparan, Mrs. Merry Theresa Yoganathan, Mrs. Pramila Thayaparan, Mrs. Nimaleswari, Mrs. Gnagala, Mrs. Kasipillai, and Mrs. Sivasoruby Sivasingarajah.

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List of abbreviations

AA	Amino acid
AB	Alcian blue
AMPs	Antimicrobial peptides
ANFs	Anti-nutritional factors
ANOVA	Analysis of variance
BBM	Brush-border membrane
BG1	Marine-based diet used in **Paper II**
BG2	SBM-based diet used in **Paper II**
BG5	Plant-based diet used in **Paper II**
cathl1	*Cathelicidin1*
CB	Plant-based diet without saponin with butyrate used in **Paper III**
CF	Condition factor
CFU	Colony forming unit
CO	Plant-based control diet without saponin & butyrate used in **Paper III**
CT	Control diet without microalgae used in **Paper IV**
CT	Control diet without probiotics in **Paper I**
def1	*Defensin1*
def3	*Defensin3*
def4	*Defensin4*
DHA	Docosahexaenoic acid
DI	Distal intestine
DM	Dry matter
DNA	Deoxyribonucleic acid
DX	Diet with microalgae *Desmodesmus* sp. in **Paper IV**
EH	Enterocytes height in **Paper II, III**
EPA	Eicosapentaenoic acid
EU	European Union
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
FDU	Forsøksdyrutvalget
FFDR	Fish dependency ratio
FH	Villi height in **Paper IV**
FIFO	Fish-in-fish-out ratio
FL	Final fork length
FM	Fishmeal
FO	Fish oil
FSBM	Fermented SBM
FW	Final body weight

GALT	Gut-associated lymphoid tissue
GE	Total area of gill epithelium
GIALT	Gills-associated lymphoid tissue
GIT	Gastrointestinal tract
GM	Total area of gill mucous cells
GM2	Gills *muc5ac2*
GM5	Gill *muc5b*
GME	Ratio between gills mucous cells and epithelium
GN	Number of gill mucous cells
GNE	Ratio between number of gill mucous cells and epithelium
GMO	Genetically modified organisms
HB	Plant-based diet with high level of saponin with butyrate used in **Paper III**
HDACs	Histone deacetylases
H&E	Hematoxylin and eosin
HO	Plant-based diet with high level of saponin without butyrate used in **Paper III**
HOE	Height of enterocytes in **Paper I**
HOV	Height of villi in **Paper I**
HSB	Hue, saturation and brightness
HSD	Honestly significant difference
IC1	Intestine *cathelicidin1*
ID3	Intestine *defensin3*
ID4	Intestine *defensin4*
IELs	Intraepithelial lymphocytes
IL	Initial fork length
IM2	Intestine *muc2*
IW	Initial average weight of fish
LAB	Lactic acid bacteria
LAS	Leica Application Suite
LB	Plant-based diet with low level of saponin with butyrate used in **Paper III**
LF	Diet with *Lactobacillus fermentum* used in **Paper I** (This LAB strain has been renamed *Limosilactobacillus fermentum*; the old nomenclature is used in this thesis)
LM	Light microscopy
LO	Plant-based diet with low level of saponin without butyrate used in **Paper III**
LP	Diet with *Lactobacillus plantarum* in **Paper I** (This LAB strain has been renamed *Lactiplantibacillus plantarum*; the old nomenclature is used in this thesis)
LP	Lamina propria width in **Paper III, IV**
LP&LF	Diet with both *L. plantarum* and *L. fermentum* used in **Paper I**
LPS	Lipopolysaccharides
LTA	Lioteichoic acids

MALT	Mucosa associated lymphoid tissue
ML	Muscle layer thickness in **Paper IV**
MODR	Marine oil dependency ratio
MPDR	Marine protein dependency ratio
mRNA	Messenger ribonucleic acid
MRS	Man, Rogosa and Sharpe
NBF	Neutrally buffered formalin
NM	Number of mucous cells (in distal intestine) in **Paper II, III**
NMC	Number of mucous cells (in distal intestine) in **Paper IV**
NOM	Number of mucous cells (in distal intestine) in **Paper I**
NW	Diet with microalgae *Nannochloropsis oceanica* in **Paper IV**
PAS	Periodic acid Schiff
PB	Diet with microalgae *Phaeodactylum tricornutum* (bead-milled) in **Paper IV**
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PPC	Pea protein concentrate
PPI	Pea protein isolate
PW	Diet with microalgae *Phaeodactylum tricornutum* (unprocessed) in **Paper IV**
RBCs	Red blood cells
RVC	Rotating vacuum coater
SA	Serosa thickness in **Paper IV**
SALT	Skin-associated lymphoid tissue
SB	Sodium butyrate
SBM	Soybean meal
SBMIE	SBM-induced enteritis
SC1	Skin *cathelicidin1*
SCFAs	Short chain fatty acids
SD1	Skin *defensin1*
SE	Total area of skin epithelium
SEM	Standard error of mean
SGR	Specific growth rate
SM	Submucosa thickness in **Paper IV**
SM	Total area of skin mucous cells
SM1	Skin *muc5ac1*
SM2	Skin *muc5ac2*
SM5	Skin *muca5b*
SME	Ratio between skin mucous cells area and epithelium
SN	Number of skin mucous cells
SNE	Ratio between number of skin mucous cells and epithelium

SNVs	Supranuclear vacuoles in **Paper I, II, III**
SPC	Soy protein concentrate
SPH	Soy protein hydrolysate
SPI	Soy protein isolate
SSIE	Soyasaponin-induced enteritis
TGC	Thermal growth coefficient
TH	Enterocyte height in **Paper IV**
TJ	Tight junction
Treg	Regulatory T cells
USD	United States Dollar
VH	Villi height in **Paper II, III**
VW	Villi width in **Paper II, III, IV**
WG	Weight gain
WLP	Width of lamina propria in **Paper I**
WOV	Width of villi in **Paper I**

List of authors' abbreviations

AF	Adriána Fečkaninová
AP	Anjana Palihawadana
BS	Bisa Saraswathy
DD	Dalia Dahle
DM	Dagmar Mudroňová
GM	Giulia Micallef
IV	Ioannis Vatsos
JD	Jorge Dias
JK	Jana Koščová
KV	Kiron Viswanath
MS	Mette Sørensen
NN	Nadanasabesan Nimalan
SG	Soňa Gancarčíková
SS	Solveig Sørensen

Declaration on shared publications and their inclusion in other PhD theses at the Faculty of Biosciences and Aquaculture.

The gene expression results of **Papers I** and **II** were provided by Solveig Lysfjord Sørensen. The **Paper II** was equally contributed by both authors and was also included in Solveig's PhD thesis (44/2022). First version of all the chapters (**Papers I**, **II**, **III**, and **IV**) were written by Nadanasabesan Nimalan. All co-authors were involved in editing and improving the chapters.

Abstract

Norway is the largest producer of Atlantic salmon (*Salmo salar*), and national strategies are emphasizing the important role of the Norwegian aquaculture industry to supply the international markets with sustainable food from the ocean. The goal is to increase the aquaculture production in Norway, but such growth must be based on sustainable solutions. Feed is already the biggest cost factor in farming of salmonids, but the current plant-based feed used in commercial farming may not provide the optimal solutions for the future growth of the industry. Novel feed ingredients with low carbon footprint is warranted to improve fish health and address climate change. The skin, gills, and intestine have large surface areas and they have primary defense barriers, which protect the host from pathogens. The additional functions of these organs include sensation, respiration, digestion and absorption, respectively. Maintaining the integrity of the mucosal surfaces is vital for the host health and prevention of diseases. These mucosal organs are investigated in studies addressing preventive fish health. The aim of this Ph.D. project was to investigate if microbial supplements or their metabolites can strengthen the mucosal health of fish.

The first two studies, presented in **Papers I** and **II**, investigated the effect of lactic acid bacteria (LAB) as probiotic feed additives on the histomorphometry of mucosal barrier organs (skin, gills, and intestine) of fish fed marine- or plant-based feeds. The aim was to investigate if single strain or the mix of two strains could prevent soybean meal induced enteritis (SBMIE). In the first study, single LAB species or a mixture of two different LAB species (*Lactobacillus plantarum* and *L. fermentum*) was coated on to feeds containing soybean meal (SBM). In the second study, a blend of two LAB species were coated on to three different basal feeds, marine-, plant- or SBM-based feeds. The purpose was to examine the ability of probiotics to improve gut health and repair the tissues during inflammation. The third study investigated if butyrate can restore the altered histomorphometric features of the intestine of Atlantic salmon fed plant-based

feeds with or without saponin. The fourth study was performed to understand the effect of different microalgae (incorporated at 7.5% inclusion in feeds) on the growth performance as well as gut health of salmon during grow-out phase at sea when the fish grew from 1.80 kg to 4.09 kg.

The main findings of the studies were:

i) acetoacetic, succinic acid, and total short chain fatty acids (SCFAs) were significantly higher in fish fed a mix of probiotics (*L. plantarum* and *L. fermentum*). The gill mucous cell size and number related indices of fish fed the probiotic mix were significantly increased compared to those fed SBM-based feed without probiotics. The probiotic blend added to the SBM-based feed was more effective in preventing SBMIE compared to the single probiotic species.

ii) the mucosal health was better in fish fed marine-based feed than those fed plant-based feeds, based on the observations on increased villi height, reduced lamina propria width, less infiltration of inflammatory cells into lamina propria and submucosa, and large supranuclear vacuoles. On the other hand, fish fed 20% SBM in the diet developed SBMIE; the fish had reduced villi height, increased lamina propria, infiltration of inflammatory cells into lamina propria and submucosa, and fewer supranuclear vacuoles. The fish fed commercial like plant-based feeds had histomorphometric values, in between those of fish fed marine-based and SBM included feeds. Addition of probiotics to SBM included feed improved the mucosal health mainly by increasing villi height, reducing the lamina propria width, and elevating the occurrence of supranuclear vacuoles that likely indicates enhanced endocytosis. The mucin and antimicrobial peptides (AMPs) gene expression as well as SCFA concentration were affected by feed ingredients and probiotics.

iii) soyasaponin added to plant-based feed which was dominated by soy protein concentrate reduced growth and induced enteritis in Atlantic salmon post-smolts. A change in saponin dosage (0.4% or 0.8%) did not have any significant effect on the

growth and micromorphology of the distal intestine of salmon. Butyrate (ButiPEARL®) supplementation improved the growth and mucosal health and countered enteritis, as evidenced by increased villi height, reduced lamina propria and reappearance of supranuclear vacuoles.

iv) microalgae inclusion at 7.5% of feed did not negatively affect growth performance. However, feed conversion ratio was inferior in fish fed the microalgae *Desmodesmus* sp. and *Phaeodactylum tricornutum* in the feed. Histomorphometric indices of distal intestine revealed that the growth and mucosal health of Atlantic salmon reared in sea cages can be improved by novel feed ingredients like microalgae meal (*Nannochloropsis oceanica*) in a plant-based feed.

Sammendrag

Norge er verdens største produsent av atlantisk laks (*Salmo salar*) og nasjonale strategier understreker den viktige rollen norsk havbruksnæring har i å forsyne internasjonale markeder med bærekraftig mat fra havet. Målet er å øke havbruksproduksjonen i Norge, men veksten må være basert på bærekraftige løsninger. Fôr er allerede den største kostnadsfaktoren i oppdrett av laksefisk, men de kommersielle fôrene basert på planteråvarer er kanskje ikke den mest optimale løsningen for fremtidig bærekraftig vekst i næringen. Næringen leter etter nye fôringredienser med lavere karbonavtrykk og som samtidig sikrer god fiskehelse. Huden, gjellene og tarmene representerer de største overflatene på fisken og har også en viktig rolle som førstelinjeforsvar for å beskytte fisken mot patogener. Disse overflatene har også andre hovedfunksjoner hos fisken som sanseorgan, respirasjon, fordøyelse og absorpsjon. Å opprettholde integriteten til slimhinneoverflatene er avgjørende for vertens helse og forebygging av sykdommer, og blir derfor ofte undersøkt i studier som tar for seg forebyggende fiskehelse. Målet med denne studien var å undersøke om bruk av probiotika, mikroalge eller om en mikrobiell metabolitt kan brukes til å styrke slimhinnehelsen til fisk.

To av studiene, presentert i artiklene I og II, undersøkte om tilsetting av probiotiske melkesyrebakterier (LAB) hadde en effekt på slimhinnene som dekker hud, gjeller og tarm hos laks fôret med marine- eller plantebaserte fôr. I forsøk en ble to ulike melkesyrebakterier (*Lactobacillus plantarum* og *L. fermentum*) coatet på fôr som innehold soyamel (SBM), enten enkeltvis eller i blandingen. Målet var å undersøke om melkesyrebakteriene hadde like god effekt om de ble tilsatt enkeltvis eller i blanding til å forebygge soya-indusert enteritt (SBMIE). I forsøk to ble en blanding av to melkesyrebakterier coatet på tre forskjellige basalfôr, basert enten på marine råvarer, planter eller soyamel. Målet med studien var å undersøke om probiotika kunne forbedre tarmhelsen hos fisk fôret marine fôr eller plantebaserte fôr og i tillegg

reparere betennelse hos fisk fôret soyabønner. Eksperiment tre undersøkte om den organiske syren, Ca-butyrat, kan brukes til å forbedre tarmhelse hos fisk fôret plantebasert fôr med eller uten saponin. Målet med eksperiment fire var å undersøke om 7,5% innblanding av mikroalger påvirket vekst så vel som tarmhelse hos laks på sjø som vokste fra 1,80 – 4,09 kg.

Hovedfunnene fra forsøkene med melkesyrebakterier var: i) acetoeddiksyre, ravsyre og totale kortkjedede fettsyrer (SCFAs) var signifikant høyere i tarm hos fisk fôret en blanding av to probiotika. Størrelsen på gjelleslimceller og relaterte histologi indekser hos fisk fôret blandingen av to probiotiske bakterier økte betydelig sammenlignet med fisk som ble fôret med soyamel uten probiotika. Blanding av to melkesyrebakterier tilsatt soyamel fôret ga en mer effektiv forebygging av soyaindusert betennelse enn bruk av melkesyrebakteriene enkeltvis.

ii) Tarmhelsen var bedre hos fisk som ble fôret med marinbasert fôr sammenlignet med fisk som ble fôret med plantebasert fôr. Baktarmen viste økt villi-høyde, redusert bredde på lamina propria, mindre infiltrasjon av inflammatoriske celler i lamina propria og submucosa og tilstedeværelse av store supranukleære vakuoler. Fisk som ble fôret med 20 % soyamel i fôret utviklet betennelse, karakterisert som en reduksjon i villi-høyde, økt lamina propria, infiltrasjon av inflammatoriske celler i lamina propria og submucosa, og supranukleære ble borte. Histologiske parametere hos fisk fôret et kommersielt lignende plantebasert fôrhadde verdier som rangerte seg mellom de som ble fôret med marine fôr og soyamel. Tilsetning av probiotika til fôr med 20% soyamel forbedret slimhinnehelsen hovedsakelig ved å øke villi-høyde, redusere lamina propria bredden og gjennom å øke endocytose vurdert ved tilstedeværelse av supranukleære. Genuttrykket for mucin og antimikrobielle peptider (AMP) samt konsentrasjonen av flyktige fettsyrer SCFA ble påvirket både av fôrråvarene og probiotika.

iii) soyasaponin tilsatt plantebasert fôr (basert på soyaproteinkonsentrat som proteinkilde) førte til redusert vekst og betennelse i tarmen hos post-smolt. Det var

ingen signifikante forskjeller mellom lav (0, 4%) eller høy (0,8%) saponin på vekst og histologi. Tilsetting av Ca-butyrat medførte en signifikant forbedring av tilvekst og histologi. Fisk som fikk ca-butyrat hadde økt villi-høyde, redusert lamina propria og mange supranukleæres.

iv) Det var ingen negative effekter på vekst ved en innblanding av 7,5% mikroalger i fôret. Fôrfaktor var imidlertid litt dårligere hos fisk som ble fôret med mikroalgen *Desmodesmus* sp. Eller *Phaeodactylum tricornutum* i fôret. Histologiske undersøkelser av tarmen viste at tarmhelsen hos atlantisk laks under påvekst på sjø kan forbedres ved å blande inn mikroalgen *Nannochloropsis oceanica* i et plantebasert fôr.

1. Introduction

1.1 Global aquaculture production

Global aquaculture production from the world's fastest growing food production system (FAO, 2022) is crucial to feed the burgeoning world population, which already reached 7.9 billion in April 2022 ("Current World Population," 2022). The growth of capture fisheries has been stagnating since 1990's, but the aquaculture industry has been reporting a steady increase in production. The contribution of world aquaculture to global fish production has grown from 25.7% in 2000 to 46.0% in 2018. The aquaculture production increased from 21.8 million tonnes in 1990 to 87.5 million tonnes in 2020 (FAO, 2022). The world aquaculture production was 122.6 million tonnes in 2020, of which fish and aquatic algae production was 87.5 and 35.1 million tonnes, respectively (FAO, 2022).

In 2020, around 8.3 million tonnes (USD 36.2 billion) and 49.1 million tonnes (USD 109.8 billion) of finfish were farmed through sea and coastal aquaculture and inland aquaculture, respectively, and China was a prominent contributor in farming these fish species (FAO, 2022). Atlantic salmon (*Salmo salar*) production accounts for 4.9% of the total finfish species production (55 million tonnes) (FAO, 2022). In 2020, Norway was the main producer of Atlantic salmon, followed by Chile (FAO, 2022).

1.2 Norwegian aquaculture

Since 1970, aquaculture industry has grown in Norway and now together with capture fisheries generates the largest export value, giving the food producing system a rank after the petroleum industry. In 2021, the aquaculture production was 1.56 million tonnes and 95% of this can be attributed to Atlantic salmon production (Figure 1) (Fiskeridirektoratet, 2022).

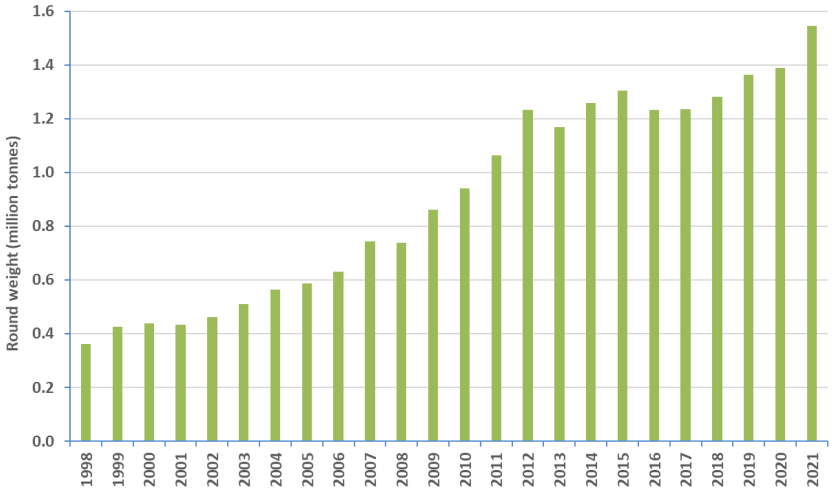

Figure 1: Total production of farmed Atlantic salmon (grow-out) in Norway for the years 1998 to 2021. Excludes juveniles and fish used for research. Source: Directorate of Fisheries, 25.05.2022. The data for 2021 are preliminary figures.

The production of Atlantic salmon increased annually by 10% during the period 1992 - 2012. However, the marine open sea cage facilities faced challenges due to sea lice infestation and production stagnation during 2012-2018. Since 2018, the production volume again started to increase slightly, as shown in Figure 1. Feed cost accounts for the main operational cost of Atlantic salmon aquaculture (Asche and Oglend, 2016).

1.3 Increasing demand for novel feed ingredients and fish feed

Intensive aquaculture production, such as that of salmonids, depends on the use of high-quality feeds. The general trend is to adopt intensive farming for the global aquaculture production (Bartley, 2022; FAO, 2020; Naylor et al., 2021; Rocha et al., 2022). Hence, the demand for commercially produced aquafeeds will increase accordingly. It is estimated that 73.15 million tonnes of aquafeed will be needed in 2025 to meet the demand of feeds for the fed farmed species (Boyd et al., 2020). In 2020, about 2 million tonnes of feeds were used to produce Norwegian Atlantic salmon (Aas et al., 2022). Aquafeeds are usually produced by mixing together a blend of ingredients, by utilizing a least cost feed formulation. The feeds are optimised, at the

lowest possible cost, to meet the nutrient requirements of the farmed species. Atlantic salmon and other carnivorous fish are not physiologically well adapted to utilize high levels of carbohydrates in the feeds. They depend on ingredients with high protein content as well as lipids, and therefore, carbohydrates should be kept at a minimum level.

Feed ingredient composition in the feeds for Atlantic salmon has changed over the last three decades. While in 1990, salmon feeds mainly consisted of fishmeal (FM) and fish oil (FO), in 2020, only 22.4% of the feed contained these marine ingredients (Aas et al., 2022, 2019). A recent survey indicated that the current Norwegian salmon feeds are dominated by plant protein concentrates (40.5%) and plant oil (20.1%), with their FM and FO levels reduced to 12.1% and 10.3%, respectively (Aas et al., 2022). Other ingredients of the feed such as carbohydrates, mainly used for technical purposes, account for 12.5% and the micro ingredient level in salmon feeds is 4.1%. Novel feed ingredients like insect meal, single cell protein or microalgae were only 0.4% of the feed composition (Figure 2).

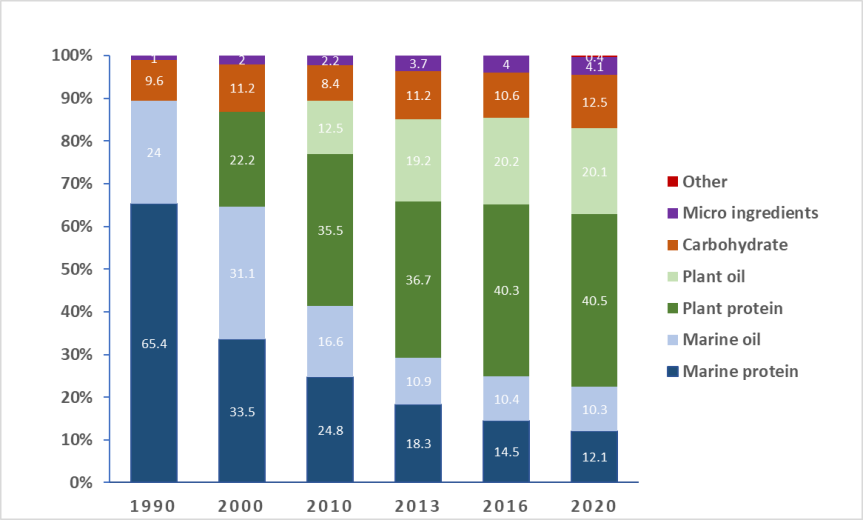

Figure 2: Change in the composition of Norwegian salmon feeds. Other ingredient sources include insect meal, single cell protein, fermented products, and microalgae. Source: Aas et al., 2019, 2022.

The typical plant protein ingredients that are used in salmon feeds are soy protein concentrates (SPC), wheat gluten, corn gluten, pea protein concentrate (PPC) and sunflower. Rapeseed oil is the dominating plant oil, but the feed contains linseed oil and camelina oil also. The carbohydrate sources are wheat and peas (Aas et al., 2022). The micro ingredients are typically premixes of vitamins, minerals, and amino acids (AAs).

Marine ingredients are finite resources and their exploitation to produce carnivorous fish has been debated over the last two decades (Bendiksen et al., 2011; Cottrell et al., 2020; Naylor et al., 2021). Replacing a large proportion of FM and FO with plant ingredients has resulted in a considerable reduction in fish-in-fish-out ratio (FIFO), forage fish dependency ratio (FFDR), marine protein dependency ratio (MPDR) and marine oil dependency ratio (MODR). For example, from 1990 to 2016, the FIFO and FFDR of FM were reduced from 4.4 to 0.8 and 0.6, respectively (Aas et al., 2022). Over the same period, the MPDR and MODR were reduced from 3.8 and 2.8 to 0.6 and 0.5, respectively (Aas et al., 2022).

The aquafeed industry has reduced its dependence on marine ingredients, but now the sustainability of using agricultural land to produce feed ingredients is debated, due to, among others, increased pressure on agricultural land, use of freshwater for irrigation, land erosion due to destruction of rainforest, and use of agrochemicals (Chu and Karr, 2017; Mbow et al., 2019; Newton and Little, 2018; Pelletier et al., 2018). The non-GMO (genetically modified organism) policy of Europe is forcing the feed industry to source SPC from Brazil. This has raised concerns about deforestation of rainforest in Brazil. Geo-political instability, environmental concerns, and the carbon footprint associated with importing the feed ingredients are forcing the aquaculture industry to find more sustainable solutions. The next generation feed ingredients with low environmental footprint are those that can be produced without using agricultural land, are at the lowest trophic level in the food web and are not used directly as human food (Boyd et al., 2020; Farmery et al., 2022; Hansen, 2019; Hua et al., 2019; Vågsholm et

al., 2020). Future aquafeeds should be produced by considering that "the sustainability of diets goes beyond nutrition and environment as to include economic and socio-cultural dimensions" (FAO, 2010).

1.4 Next generation feed ingredients – microbial and low trophic choices

Novel feed ingredients can be obtained from single-celled organisms, and insects (Cottrell et al., 2020). Some of the pros and cons associated with these alternative ingredients are listed in Table 1 (Albrektsen et al., 2022; Ameixa et al., 2020). Microbial ingredients, such as fungi (yeasts), microalgae, and bacteria often have low carbon footprint because they have a rapid growth rate, do not require any agricultural land, use little fresh water, and can be produced from non-food biomass, CO_2 (microalgae) or natural gas (methanotroph bacteria). Overall, microbial ingredients can relieve the pressure on human food resources. Bacterial meal also contains a wide range of bioactive components, such as peptidoglycans, antioxidants and nucleic acids, which may have a positive effect on gastrointestinal health in salmon (Romarheim et al., 2013, 2011).

Table 1: Novel feed ingredient sources - opportunities and challenges[1].

Source	Ingredients	Opportunities	Challenges
Single cell protein	Microalgae Fungi/Yeasts Bacteria	Suitable protein content. Suitable lipid content.	Expensive. Difficult to obtain adequate amount of biomass. Deficient/lacking specific nutrients.
Insects	Insect meal Insect oil Whole insect	Suitable protein content. Suitable vitamin & amino acids. Environmentally suitable. Can use low quality feeds. Low-cost.	Lipid profile not always adequate. Production requires optimization. Legislation inadequate/ non-existent.
Marine low trophic	Antarctic krill Seaweeds Copepods (*Calanus*)	Suitable protein content.	Harvesting would be an issue. Production requires optimization.

[1] Adapted from Albrektsen et al. (2022) and Ameixa et al. (2020).

Microalgae are single-celled aquatic organisms that are usually invisible to the naked eye (Cai et al., 2021). There are approximately 200 000 species, and most of them are autotrophic and can grow in the presence of sunlight, CO_2, and minerals. The heterotrophic species can grow on carbon sources in the presence of O_2. Microalgae can be produced commercially, for example *Chlorella, Dunaliella* are produced in open ponds at relatively low cost, e.g., without exploiting much water and agricultural land. Different photobioreactor technologies are also used for producing the autotrophic microalgae (Acién et al., 2017; Ruiz et al., 2022). The advantage of closed photobioreactor system is that one could have a better control over contamination and environmental parameters, but the disadvantage is higher investment cost. The chemical composition of different strains of microalgae varies a lot and their protein, lipid and carbohydrate are in the ranges 6-71%, 2-40% and 4-64%, respectively. Microalgae are also sources of carotenoids, vitamins, minerals and other organic compounds with health benefits (Koyande et al., 2019).

Microalgae are primary producers of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) in the marine environment (Adarme-Vega et al., 2012; Sukenik, 1991; Yongmanitchai and Ward, 1991). The industry expects to replace FO in feeds with the long-chain polyunsaturated fatty acids from microalgae (Ahmad et al., 2022). Microalgae contain all essential AAs (Brown et al., 1997), even the organic compound taurine is found in some species (Tevatia et al., 2015). Taurine is found in FM but not in terrestrial plant ingredients. Though taurine is not an essential nutrient for fish, it has important biological functions including its role in osmoregulation (Kotzamanis et al., 2020). Some microalgae produce astaxanthin, which can improve fillet pigmentation in fish. The nutritional profile of microalgae may vary depending on species and growing conditions (Schulze et al., 2019; Sevgili et al., 2019; Tibbetts et al., 2015). Microalgae as feed ingredients must therefore be carefully selected based on nutrient profile and bioavailability of nutrients.

Studies have shown that nutrient digestibility and utilization may differ among microalgae (Gong et al., 2018; Kiron et al., 2016; Skrede et al., 2011; Sørensen et al., 2021a). There are also studies that reported negative effect on nutrient digestibility and growth performance with increasing incorporation of microalgae in the feed (Sørensen et al., 2016; Sørensen et al., 2017; Gong et al., 2019). Such an effect is likely due to the rigid cell walls which consist of indigestible carbohydrates (Bernaerts et al., 2018; Gong et al., 2019; Sevgili et al., 2019). Digestibility and utilization of nutrients in microalgae can be improved by mechanical disruption of their cell walls (Batista et al., 2020b; Teuling et al., 2017; Valente et al., 2019). Recently published studies have reported that 10% of microalgae in fish feed did not affect the digestibility and growth performance of the fish (Gong et al., 2019; Knutsen et al., 2019; Sørensen et al., 2017). These observations suggest the potential of microalgae to be next-generation feed ingredients to promote sustainable aquaculture. However, their inclusion must be limited to a level at which their nutrients can be efficiently digested and utilized by farmed fishes.

Insect larvae are promising low trophic sources of protein for future aquafeeds. They convert organic biomass to high value protein and their farming does not need agricultural land or water (Shah et al., 2022). Seven insect species are approved by the EU for use in aquafeeds (*Hermetica illucens*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Musca domestica, Acheta domesticus*, *Gryllodes sigillatus*, *Gryllus assimilis*, and *Bombyx mori*) (European Union, 2017; Lourenço et al., 2022). Due to their limited availability, the suitability of insect meals as feed ingredients are not studied in detail. Although black soldier fly and yellow mealworm are commercially available, they are still produced on a small scale (Bruni et al., 2020; Gasco et al., 2016). Studies with Atlantic salmon have shown that inclusion of black soldier fly meal up to 14.75% (total replacement of FM) in the diet did not affect the growth performance negatively (Belghit et al., 2019). In contrast, feed intake and nutrient digestibility was negatively impacted when insect meals were fed to other species such as European sea bass (25%

Tenebrio molitor (Gasco et al., 2016), turbot (33% *Hermetica illucens*) (Kroeckel et al., 2012), and seabass (50% *Hermetica illucens*) (Reyes et al., 2020). It should be noted that chitin found in exoskeleton of larvae might interfere with the nutrient digestibility (Albrektsen et al., 2022).

For any innovative aquafeed resources to be commercially attractive, they must be produced in large quantities, be available throughout the year, and be competitively priced. Currently, unavailability and economic feasibility are the bottlenecks that limit the use of several novel substitutes (i.e., insect meal) or other sources from low trophic level such as Antarctic krill (*Euphausia superba*), copepod (*Calanus* sp.), polychaetes (*Polychaete* sp.), amphipods (*Gammarus* sp.), tunicates (*Tunicata* sp.), clams and shells, and blue mussels (*Mytilus edulis* L.) (Albrektsen et al., 2022). Conversely macroalgae are available in large quantities but are only used as a functional ingredient in aquaculture feeds (Albrektsen et al., 2022; van Raamsdonk et al., 2017).

1.5 Health impact of different feed ingredients

Feed ingredients provide nutrients to fish. Generally marine-based ingredients do not affect the fish health negatively. The commonly used marine ingredient, FM provide the essential AAs and fatty acids to farmed fishes. Moreover, FM and FO based feeds will have a balanced nutrient profile. Therefore, marine derived feed ingredients are recognized as the “golden standard” ingredients because of the nutrient composition, digestibility, palatability, and content of unique nutrients. Typically, FM contains more than 68% crude protein, 6-10% crude fat, 13-16% ash, and 6-10% moisture (De Santis et al., 2016; Einarsson et al., 2019; Sørensen et al., 2011a; Storebakken et al., 2015). The marine ingredients meet the AA requirement of farmed fishes. In addition, FM contains minerals (P and Ca) and other important nutrients such as EPA, DHA, vitamin B12, and taurine. FO contains the polyunsaturated fatty acids, EPA, DHA and has a well-balanced n-3/n-6 profile, cholesterol and polar lipid contents.

Plant ingredients often lack certain nutrients compared to marine ingredients, and this deficiency must be compensated for with micronutrient supplements. The plant protein concentrates often provide 40-60% protein to the farmed fish. The advantages of these concentrates are that they are attractively priced and easy to ship and store (Gatlin et al., 2007). The lower levels of proteins, high levels of carbohydrates, unfavourable AA profile and mineral contents, and the presence of ANFs are the main challenges that are encountered when FM is replaced with plant protein (Francis et al., 2001; Sahlmann et al., 2013). Synthetic AAs are used to balance the AA profile of feed. Plant oils in fish feeds have changed the fatty acid composition in fish flesh negatively (Bou et al., 2017; Katerina et al., 2020). The fatty acid composition in plant oils is dominated by n-6 fatty acids and they do not contain EPA and DHA. Therefore, EPA and DHA can be provided to aquaculture species via FO or alternative feed ingredient sources like zooplankton, krill, microalgae, some yeast species, and genetically engineered oilseed crops (Betancor et al., 2018; Tocher et al., 2019).

Another concern over some of the plant ingredients is about their antinutritional factors (ANFs). The ANFs (saponins, tannins, phytic acid, lectins, protease inhibitors, amylase inhibitors, antivitamin factors, metal binding ingredients, goitrogens, etc) found in legumes disturb the feed utilization and affect the health condition and production of livestock (Makkar, 1993). These ANFs have different mode of actions but reduce bioavailability of nutrients in aquafeeds and may have adverse effects on fish health (Gatlin et al., 2007). Phytic acid is one ANF that interfere with mineral absorption and protein utilization (Hekmatpour and Torfi Mozanzadeh, 2021; Krogdahl et al., 2010).

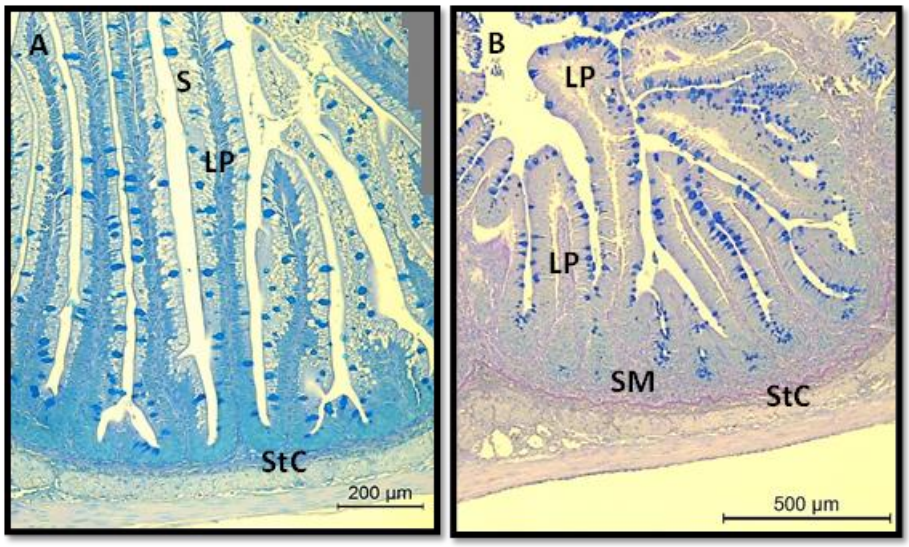

Figure 3: Changes in the micromorphology of the distal intestine when a portion of fishmeal in feed was replaced with terrestrial plant resources. A) Normal morphology of the distal intestine of Atlantic salmon. B) Enteritis (characterized by widening of lamina propria, infiltration of inflammatory cells into submucosa, lamina propria and intra-epithelium, loss of supranuclear vacuoles, and stunt villi) in Atlantic salmon fed soybean meal. (Letters indicate, S - supranuclear vacuoles, LP – lamina propria, SM – connective tissue in submucosa, StC – stratum compactum.

Saponins are another type of ANF that causes histopathological changes in the gastrointestinal tract (GIT) of fish. Many studies have used SBM as a model to study feed induced enteritis in fishes such as Atlantic salmon (Baeverfjord and Krogdahl, 1996), rainbow trout (Rumsey et al., 1994), common carp (Urán et al., 2008b), zebrafish (Hedrera et al., 2013), and chinook salmon (Booman et al., 2018). The SBM-induced enteritis (SBMIE) is characterized by stunt villi, widening of lamina propria and submucosa, infiltration of inflammatory cells, and decrease in the numbers of absorptive vacuoles in the enterocytes (Figure 3) (Baeverfjord and Krogdahl, 1996; Gu et al., 2016; Nimalan et al., 2022; Urán et al., 2008a). Plant protein concentrates that are commonly used in commercial feeds have lower content of ANFs and carbohydrates. For instance, fish fed 19% SPC added to a marine-based diet showed no signs of enteritis (Krogdahl et al., 2020). Therefore, SPC do not usually cause

enteritis (Krogdahl et al., 2020), but PPC at incorporation levels of 35% or higher, may cause enteritis, most likely triggered by saponins in pea (Penn et al., 2011).

Since feeds low in marine ingredients can compromise the gut health and disturb lipid metabolism (Bou et al., 2017; Caballero et al., 2003a; Castro et al., 2019; Sørensen et al., 2021b; Turchini et al., 2009) there is a need to generate more knowledge regarding the effect of new feed ingredients on fish health.

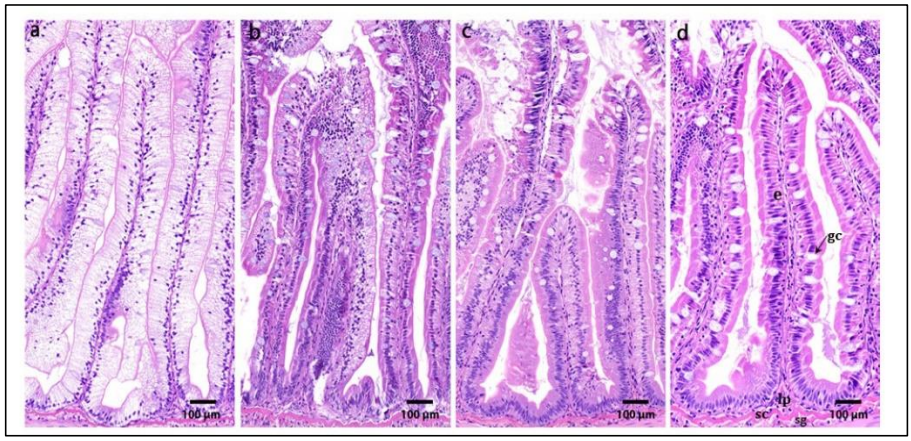

Figure 4: Representative microphotographs showing the severity of enterocyte hyper-vacuolization (steatosis) in pyloric caeca stained with H&E. (a) – severe, (b) moderate, (c) - mild, and (d) normal. Abbreviations in 4d: e, epithelium; gc, goblet cells; lp, lamina propria; sc, stratum compactum; sg, stratum granulosum. Source: (Hansen et al., 2020a). Permission was granted to use the content in dissertation. (https://s100.copyright.com/AppDispatchServlet#formTop).

Plant ingredients may also induce steatosis in salmon fed low FM diets (Hansen et al., 2020). The condition is characterized by hyper-vacuolization of epithelium in pyloric caeca (Figure 4a). Plant oils in feeds might also induce inflammation due to imbalance of the ratio n-6/n-3 fatty acids, low content of EPA and DHA, and lack of choline (Hansen et al., 2020; Krogdahl et al., 2020; Moldal et al., 2014).

Hence, it is necessary to select the right strategy to improve nutrient utilization as well as gut health. Functional feeds are more commonly used by the aquaculture industry to improve feed utilization and animal health. These additives impart

beneficial effects other than that is expected from the nutrients derived from other feed ingredients.

1.6 Feed additives

Feed additives can be defined as non-nutritive ingredients or components that are incorporated in diet to improve physical or nutritional quality of the diet and/or aquatic animals' performance (Encarnação, 2016). Additives that influence feed quality in commercial formulations include pellet binders, preservatives and feeding stimulants (NRC, 2011). Other additives that are intended to directly affect fish performance or product quality include probiotics, prebiotics, acidifiers (organic acids) and plant- or animal-derived extracts (Dawood et al., 2018; Encarnação, 2016; Ng and Koh, 2017).

1.6.1 Probiotics

Fish harbor beneficial microbes on its mucosal barrier surfaces. This complex community of microbes including virus, yeast, and bacteria are termed as microbiota. This community of microbes helps the host in various functions, and its composition is influenced by various factors such as host species, diet, and environment. When there is an imbalance in the microbiota composition, the health of the fish will be compromised, leading to disease outbreaks. Therefore, dietary administration of beneficial microbes or probiotics is intended to support the microbiota and eventually the health of the host. FAO defined the term "probiotics" as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO, 2002; Hill et al., 2014). Probiotics have been incorporated in aquatic feeds to enhance the functions of the host immune system, conserve gastrointestinal morphology, suppress the growth of pathogens by competing for adhesion sites/nutrients or producing natural antimicrobial compounds, thereby improving the host growth and health (Serra et al., 2019). Therefore, use of probiotics can be an effective disease management strategy (Ringø et al., 2018). Among the probiotic bacteria, lactic acid bacteria (LAB) are considered the most promising candidates for

boosting the growth, gut health, immune defense mechanism against pathogenic bacteria, and altering the microbiota profile in host (Gatesoupe, 2008; Gupta et al., 2019; Martínez Cruz et al., 2012).

LAB include *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Enterococococus*. The genus *Lactobacillus* which belongs to the family *Lactobacillaceae* are rod shaped bacteria that contain about 260 recognized species. The taxonomic candidates are characterized based on morphological, biochemical, and physiological characteristics by utilizing phenotypic and genomic methods. Many studies have revealed the effectiveness of dietary incorporation of *Lactobacillus* to improve growth and immune performance of various aquatic organisms (for example: Nile tilapia (Lara-Flores et al., 2003; Villamil et al., 2014; Zhai et al., 2017), Atlantic salmon (Salinas et al., 2008b), Caspian trout (Jami et al., 2019), rainbow trout (Enferadi et al., 2018; Panigrahi et al., 2004; Soltani et al., 2019), common carp (Adeshina, 2018; Kazuń et al., 2018; Soltani et al., 2017), striped catfish (Akter et al., 2019), black swordtail (Hoseinifar et al., 2015), silver pomfret (Gao et al., 2016), African catfish (Butprom et al., 2013), narrow clawed crayfish (Valipour et al., 2019), white shrimp (Kongnum and Hongpattarakere, 2012; Vieira et al., 2010; Wang and Gu, 2010), giant fresh water prawn (Dash et al., 2014), and tiger shrimp (Hoseinifar et al., 2018)). Previous studies have also reported that combination of two or more probiotic bacteria is more effective in enhancing the performance of the host (Alishahi et al., 2018; Wang and Gu, 2010).

Mode of actions of probiotics include production of antimicrobial substances like bacteriocins, secretion of functional proteins, suppression of inflammation-inducing genes and molecules, and immune cells linked to allergies, improvement of overall functions of the immune system (Angahar, 2016; Bermudez-Brito et al., 2012; Hai, 2015; Hemaiswarya et al., 2013; Lazado and Caipang, 2014; Martínez Cruz et al., 2012; Mazziotta et al., 2023; Nayak, 2010; Plaza-Diaz et al., 2019; Ranjan et al., 2022; Ringø et al., 2018; Wuertz et al., 2021; Zorriehzahra et al., 2016). Certain members of the intestine microbiota produce short chain fatty acids (SCFAs) which facilitate the

maintenance of epithelial barrier integrity, stimulate appropriate immune responses and regulate glucose and lipid metabolism (Nogal et al., 2021). Lactobacilli produce exo-polysaccharides which have immunomodulatory (Riaz Rajoka et al., 2020) and antioxidant properties (Xu et al., 2020) and biofilm reduction ability. Symbiotic relationship with host is one of the important characteristics of *Lactobacillus* and under normal circumstances these bacteria produce SCFAs, exopolysaccharides and enzymes that can be utilized by the host (Ranjan et al., 2022). Lactobacilli can produce many vitamins. For example, *L. plantarum* produces folate in the presence of para-aminobenzoic acid and vitamin B12 (Rossi et al., 2011). LAB also produce vitamin B_1 which plays vital role in the development of nervous system (Teran et al., 2021).

Probiotics can be administrated to fish via rearing water or diet. Dietary administration or supplementation to the water is the most common practice in aquaculture. Bacteria need to possess certain characteristics to be classified as probiotics. The lactobacilli candidates, *L. plantarum* and *L. fermentum*, which naturally occur in fish intestine, possess most of the probiotic characteristics (Cingeľová Maruščáková et al., 2021; Fečkaninová et al., 2019). Nevertheless, the health promoting properties of LAB in Atlantic salmon should be further investigated.

1.6.2 Organic acids: the potential of butyrate

Organic carboxylic acids include acetic, butyric, citric, formic, lactic, and propionic acids. These SCFAs are produced in the intestine by bacterial fermentation of carbohydrates (Palma et al., 2022). One of the SCFAs, butyrate is a metabolite of bacteria including *Clostridium butyricum*. Butyrate further undergoes oxidation to produce acetyl Co-A, which facilitates the normal functioning of the epithelial cells. Health and growth performance in various fish have been improved by organic acids (Hoseinifar et al., 2017). Butyrate increases the availability of several essential AAs and nucleotide derivatives, and act as energy providers, signalling molecules, gene expression regulators, inflammation suppressors, and immune cell development regulators. It plays a critical role in maintaining intestinal integrity and health (Koh et

al., 2016; Morrison and Preston, 2016; Richards et al., 2016). Commercial products of butyrate, usually a sodium butyrate (SB), has been shown to improve growth, nutrient utilization, and intestinal health (Abdel-Latif et al., 2020; Palma et al., 2022) of various aquatic animals such as sea bream (Robles et al., 2013), common carp (Liu et al., 2014), tilapia (Ahmed and Sadek, 2015), grass carp (Liu et al., 2017), European sea bass (Abdel-Mohsen et al., 2018), yellow cat fish (Zhao et al., 2021), golden pompano (Zhou et al., 2019), Nile tilapia (Jesus et al., 2019), turbot (Liu et al., 2019), and Asian seabass (Aalamifar et al., 2020).

A recent study on juvenile turbot (*Scophthalmus maximus*) also showed that supplementation of 0.2% dietary SB alleviated soybean-induced enteropathy. SB increased the number of mucous cells and the absorptive surface area of the distal intestine and enhanced the growth performance of turbot. Moreover, butyrate mitigated the infiltration of leukocytes into the lamina propria, downregulated inflammatory genes and upregulated the genes of the tight junction proteins (Liu et al., 2019). In addition, sodium butyrate could alter the intestine bacterial composition. A possible mode of action could be that butyrate improved the intestinal morphology, barrier integrity and established microbial homeostasis. However, the dose and product forms of butyrate e.g. encapsulated or salts (Ca, K, or Na) as well as duration of feeding need to be considered when other species like salmon are fed with butyrate under an inflammatory condition (Palma et al., 2022).

1.7 Mucosal barrier organs (skin, gills, and intestine) of Atlantic salmon

To understand how feed ingredients influence the mucosal health of fish, mucosal tissues should be studied in detail. The skin, gills, and intestine are the main mucosal organs, and they act together to defend against invasive pathogens. The mucosa associated with these organs are collectively responsible for the immunological functions. The mucosa-associated lymphoid tissue is called MALT. The MALT can be

further divided into skin-associated lymphoid tissue (SALT), gills-associated lymphoid tissue (GIALT) and gut-associated lymphoid tissue (GALT) (Esteban, 2012). A

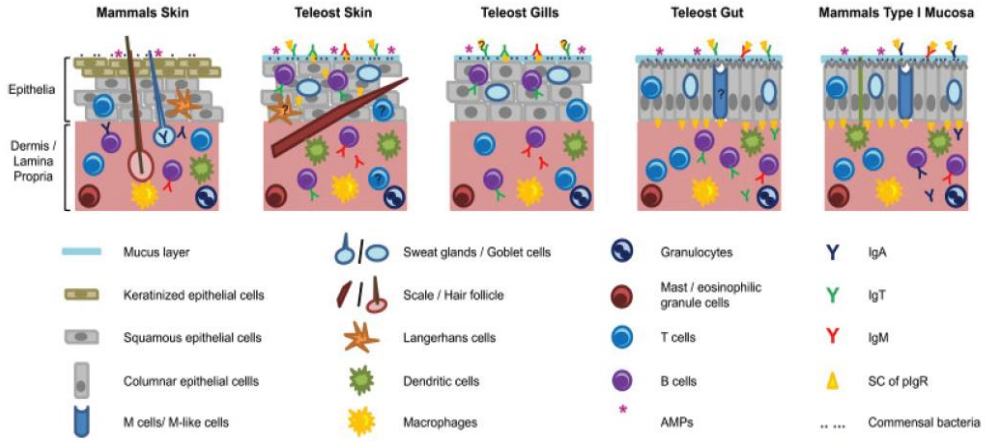

Figure 5: Comparison between teleost fish skin, gills, and gut and mammalian skin and mucosal surfaces. Structural differences between the epithelia and dermis, and similarities in the cellular components of the innate immune system are displayed. Differences in the localization of B and T cells, the isotype of immunoglobulins and presence of the secretory component (SC) of the polymeric immunoglobulin receptor (pIgR) are represented as well. The presence of commensal bacteria and antimicrobial peptides (AMPs) is shown in the outer surface. (Source: Gomez et al. 2013). Permission was granted to use the content in a dissertation.

comparison between similarities and differences in mucosal surfaces of mammals and teleost fish is shown in Figure 5. For instance, fish skin has mucous or goblets cells and scales while mammals' skin has sweat glands and hair follicle (Salinas et al., 2021).

1.7.1 Skin (structure and function)

The outermost cell layer of the skin is called epidermis (Figure 6). It consists of non-keratinized, squamous stratified epithelial cells, also called keratocytes. Thickness of the layer can vary depending on the layer or number of cells (approximately 3 to 20). These cells are capable of mitotic division and are metabolically active. Dead cells are regularly shed from the surface and replaced by new cells. The shape of the epithelial cells in epidermis varies depending on their location. The bottom cells are cuboidal or columnar, whereas the surface cells are mostly flattened, called keratocytes.

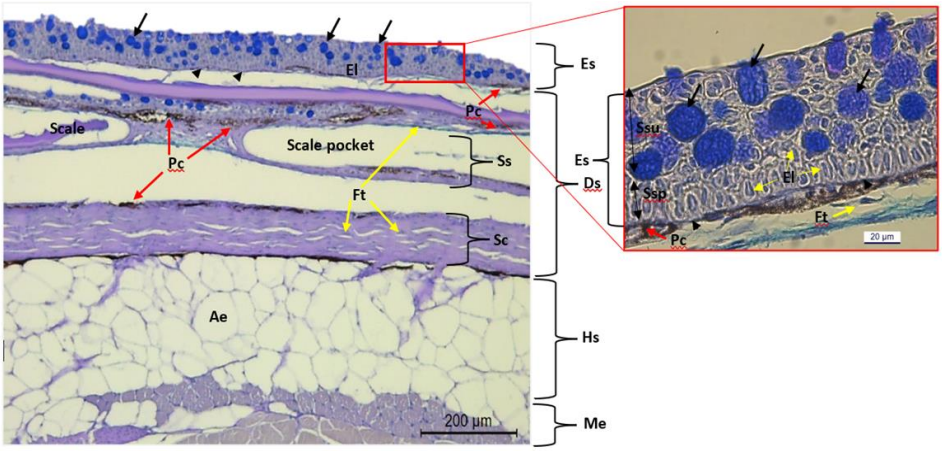

Figure 6: Histo-microphotograph of the skin of Atlantic salmon stained with AB-PAS (transverse section). Arrowhead indicates the basal membrane; black arrows indicate mucous cells. Abbreviation: Es – Epidermis, Ds – Dermis, Hs – Hypodermis, Me – Muscle layer, Ssu – Stratum superficiale, SSp – Stratum spinosum, Ss – Stratum spongiosum, Sc – Stratum compactum, Ft – Fibroblast, El – Epithelial cells, Ae – Adipocyte, Pc – Pigment cells.

The surface cells of the epidermis are termed keratocytes, each of these cells is outlined by a continuous microridge surrounding short-segmented microridges that form fingerprint-like patterns. These microridges hold mucus on the skin surface. These superficial epithelial cells (keratocytes) are able to drift fast, and rapidly cover wounds following physical injury (Bullock et al., 1978; Elliott, 2011, 2000; Ferguson, 1989; Karlsen et al., 2018). Mucus-secreting cells called mucous cells or goblet cells are found in the epidermis. Mucous cells can be neutral (pink colour in AB-PAS staining) or acidic (blue colour in AB-PAS staining) in nature. The shape of these cells differs in different fish species. Some species also have club cells (Pandey et al., 2021) and others have sacciform cells (Fast et al., 2002). In addition to epithelial and mucous cells, there are other type of cells including pigment cells, and different leucocytes, i.e., macrophages and eosinophilic granule cells in teleost epidermis (Kryvi & Poppe, 2016).

The inner cell layer below epidermis is called dermis (Figure 6) and it is divided into two layers. The loose grid of connective tissue in the outermost part, comprising mainly of fibroblasts, collagen fibres, nerves, and pigment cells is called *stratum spongiosum*.

The inner denser layer containing fibroblasts and more orthogonal collagen bands is called *stratum compactum* (Hawkes, 1974). The *stratum compactum* is bounded by a single layer of cells called the dermal endothelium, separating it from a relatively well vascularized layer of loose connective tissue and adipocytes in the hypodermis. The scales originate in dermis covered in scale-pockets. Scales (cycloid scales in salmonids) consist of a plate of collagenous tissue, with a thick mineralized layer. Bundles of collagen fibres anchor the scale in each pocket. The posterior end of the scale is projected into and covered by the epidermis. The epidermis and the dermis are separated and anchored by a basement membrane that assists in controlling the passage of cells and molecules between the dermis and epidermis (Elliott, 2000). The basement membrane plays a role in wound healing, serving as an attachment site for epithelial cells. The skeletal muscle can be found below the hypodermis layer (Figure 6).

The outermost cells of the skin which are in contact with environment (the epithelium in epidermis) are covered by a mucus layer which has a complex composition. The mucus is secreted by mucous cells, which are most frequently recognized in the middle to outer layers of epidermis (*stratum superficiale*). The epithelial cells in the lower layer of the epidermis (*stratum spinosum*) are differentiated into mucous cells, and the differentiating mucous cells migrate towards the surface of the epidermis. Immature mucous cells are rounded but become flattened laterally and develop vesicles which enlarge their size as they approach the outer layers (Harris and Hunt, 1975). At the outer surface of the skin, the mucous cell membrane ruptures at the apical point to release the cell contents (mucus). The number of skin mucous cells vary depending on the location. Usually dorsolateral region harbour more mucous cells than the head region (Pittman et al., 2013).

The features in the fish skin are affected by, among others, rearing water temperature and environmental pathogens. Fish skin has two distinct features that differentiate it from those of other vertebrates. Firstly, it has no keratinized outer layer,

so the viable and active epithelial cells are in direct contact with the environment. Secondly, in most species a large part of the body is covered by scales. The fish skin is an organ that covers a large surface area of its body. It lines all body openings and covers the fins. It is a multifunctional organ, and has roles in protection, communication, sensory perception, locomotion, respiration, ion regulation, excretion, thermal regulation and disease resistance (Alexander and Ingram, 1992; Dash et al., 2018; Elliott, 2011; Esteban, 2012; Pérez-Sánchez et al., 2017; Shephard, 1994; Whitear, 1986).

The mechanical and biochemical properties of skin mucus make it efficient in protection. It acts as the first barrier and prevents mechanical damage and infection by trapping and sloughing off pathogens. Mucus contains a wide range of substances which can have detrimental effects on pathogens. The mucus innate immune components are lectins, pentraxins, lysozymes, proteolytic enzymes, alkaline phosphatase, C-reactive protein, complement, and antimicrobial peptides (AMPs) (Alvarez-Pellitero, 2008; Brinchmann et al., 2018; Dash et al., 2018; Fast et al., 2002; Guardiola et al., 2014; Jones, 2001). Water and mucins are the main components of mucus. Mucins are high molecular weight, heavily glycosylated glycoproteins which provide viscoelastic and rheological properties to the mucus. Each mucin type possesses characteristic repetitive regions rich in threonine, serine and proline (Rose and Voynow, 2006). Integrity of the mucus layer is habitually linked with the nutritional state, for example, in mammals a limited dietary supply of threonine has been shown to impair the synthesis of mucins (Faure et al., 2005). It is now little known that the different diet ingredients have different impacts on mucous cells in fish (Nimalan et al., 2022; Sørensen et al., 2021b). However, not much is known about the impact of diet on skin mucus production in fish but dietary β-glucans did increase the expression of mucins and two β-defensins in carp skin mucus (van der Marel et al., 2012). In Atlantic salmon, seven mucin secreting genes were reported; two *muc2* genes were mainly found in the intestine while five *muc5* were observed in other tissues such as pyloric

caeca, gill or skin (Sveen et al., 2017). In contrast fish skin also has genes that encode for AMPs, among others, cathelicidins and defensins (Chang et al., 2006; Nimalan et al., 2022; Reyes-Becerril et al., 2013; Sørensen et al., 2021b).

The thickness of the mucus layer depends on the rate of secretion and rate of degradation. Several factors including toxic and irritating substances can stimulate mucus secretion and increase the thickness of the mucus layer (Esteban, 2012), as can other stressors and pathogens (Beck and Peatman, 2015; Fast et al., 2002; Iger et al., 1995). Fish physiological conditions such as smoltification (Fagan et al., 2003) and external factors such as season (Roberts and Powell, 2005), salinity (Schrock et al., 2001), stress (Easy and Ross, 2010), disease (Guardiola et al., 2014; Lü et al., 2012), and parasite attack (Easy and Ross, 2009) influence the composition of mucus. In salmon, sea lice infestation changes the mucus composition (Fast et al., 2002). Since skin mucus contains important defence molecules, they can be utilized in aquaculture to develop vaccines to prevent infectious diseases (Baindara and Mandal, 2019). Nevertheless, manipulation of skin mucus production should be sustainable, safe, and cost effective. Therefore, research should focus on and ensure the immunoprophylactic control by priming the mucosal barrier through different feed ingredients.

1.7.2 Gills (structure and function)

The gills are bilaterally positioned on each side of the pharynx and are comprised of a series of pouch-like or arch-like structures that physically support the gill filaments (Wilson and Laurent, 2002). Each arch projects two filaments with a series of lamellae (Haugarvoll et al., 2008). In bony fish, the ends of the bow-shaped gill arch are attached to the dorsal and ventral surfaces of the buccal cavity, with the curved portion projecting posterior-laterally.

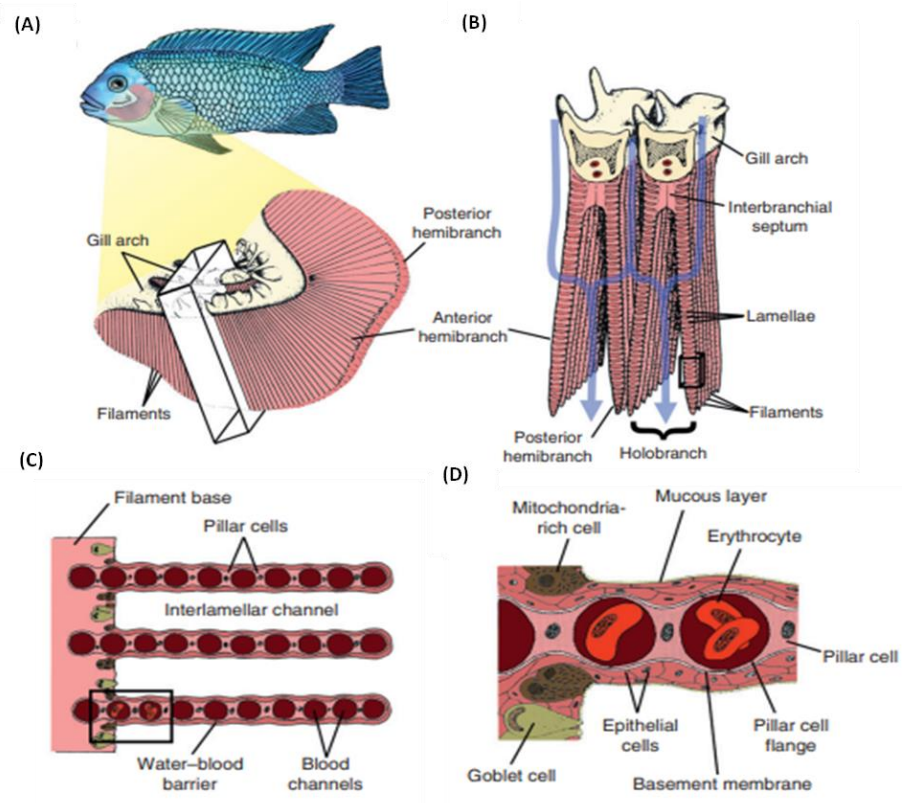

Figure 7: Schematic drawing of teleost gill morphology: (A) anterior two gill arches from the left side of the head; (B) placement and morphology of the two rows of gill filaments extending from each arch; (C) cross section through three adjacent lamellae; (D) enlarged view from (C) showing the detail of the lamellar and filament epithelium. Water flow direction is indicated by blue arrows. Source: Wegner, (2011). Permission was granted by the author.

There are eight gill arches arranged as four pairs on either side of the buccal cavity, which is connected to an extra vestigial gill hemiarch called the pseudo branch, covered by a thick epithelium known to be functionally insignificant, but involved in oxygen transportation into the eye of teleost (Ferguson, 2006; Helfman et al., 2009; Roberts, 2012; Wegner, 2011). The gill filaments are the functional and anatomical units of the organ (Figure 7). The filaments have different types of cells and tissues. The long, slender, and flat gill filaments support numerous respiratory secondary lamellae (Figure 7). The gill filaments resist the flow of water minimally and the counter current flow of blood and water ensures gaseous exchange (Olson, 2011; Wegner, 2011).

The secondary lamellae of the gill filaments are plate-like structures projected at right angles from both sides of the filaments (primary lamellae). They help circulate the blood out into the periphery of the tissue (Ferguson, 2006). Each secondary lamella consists of two rows of epithelial cells held apart by a series of centrally located cells called pillar cells (Figure 7, 8). Pillar cells provide structural support to hold two squamous epithelial cell layers together. Pillar cells are unique in shape (spool-shaped cells) with a large central nucleus and broad cytoplasmic flanges that radiate out from the top and bottom of the cell. The adjacent pillar cells are joined together through pillar cell flange forming vascular lacunae called pillar channels that are enriched with red blood cells (RBCs) (Figure 7). The secondary lamellae dramatically increase the surface area of the respiratory epithelium (Evans et al., 2005; Ferguson, 2006).

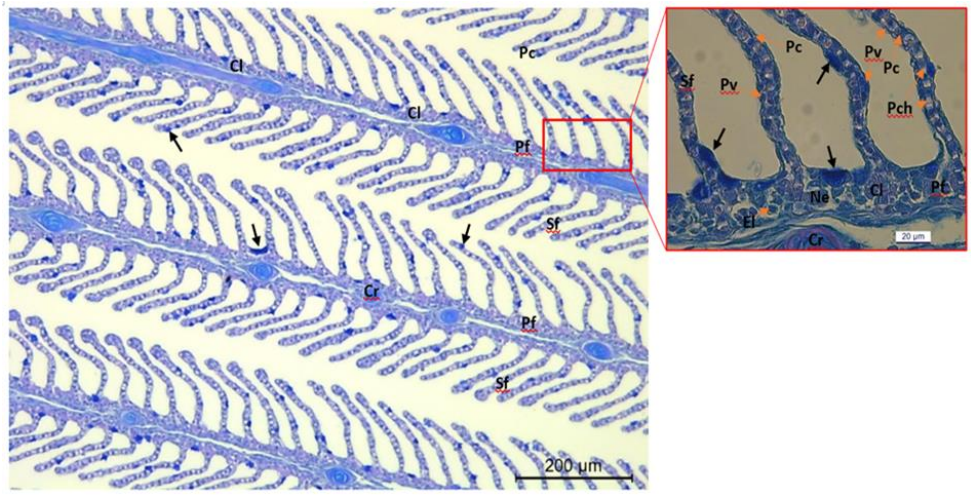

Figure 8: Photomicrograph of Atlantic salmon gills stained with AB-PAS. Arrows indicate the mucous cells. Abbreviation: Pf - Primary lamellae (gill filaments), Cr - cartilaginous rod running in the centre, Sf - Secondary lamellae extending as projections from the primary lamellae, Cl - Chloride cells, Pc- pillar cells, Ne – Neuroepithelial cell, Pv – Pavement cell, Pch – Pillar channel, and El – Epithelial cell.

Secondary lamellae have different cell populations, including pavement cells, chloride cells, mucous cells, neuroepithelial cells, and undifferentiated cells located on germinal epithelium (Figure 8) (Evans et al., 2005). Pavement cells, the most abundant epithelial cells found on the epithelium form a relatively impermeable barrier between

the water and the tissue. These relatively thin, hexagonal cells have a surface enriched with micro-ridges or microvilli that are believed to help trap a protective coat of mucus, as well as increase the surface area for gas exchange (Mallatt, 1985).

Mucous cells are scattered throughout the gills including the gill arches, filaments and more predominantly on the edge of the filament facing the water current and basal regions of the lamella (Evans et al., 2005). These cells are filled with large membrane bound mucus droplets, basal nucleus, and tightly packed rough endoplasmic reticulum, and Golgi apparatus. Mucous cells contain acidic glycoproteins, neutral glycoproteins, or a combination of the two (Fletcher et al., 1976). Gill mucus also has antibacterial properties (Dash et al., 2018; Rakers et al., 2013). Gill mucous cell number, size, and mucus secretion change during parasitic infections (Peyghan and Powell, 2006; Roberts and Powell, 2003). Histomorphometry has been employed to examine the changes in the number of mucous cells when the fish were exposed to seawater of high salinity (Olson, 1996), ion-poor water, mechanical abrasion, high water temperature, and a variety of waterborne contaminants including metal ions, therapeutic drugs, organophosphates, and aquatic pathogens. In addition to the mucous cells, another cell type that has been reported in the gills is granular cells, which are embedded slightly deeper in the epithelium and secretes mucus intermittently (Jayasuriya, 2014). Their secretory products contained glycoproteins with abundant sialic acid residues, and they appear similar to mucous cells.

Another type of cells known as ionocytes or chloride cells which are rich in mitochondria and involved in ion-transporting, are located on the body or the lamellar portion of the filament, especially along the afferent margin of the filament and interlamellar filament epithelium located between adjacent lamellae (Evans et al., 2005). Neuroepithelial cells, which serve as chemoreceptor cells and monitor the oxygen tension in both water and blood (Bailly et al., 1992; Dunel-Erb et al., 1994) are thinly scattered along the efferent margin beneath the epithelium. In addition, interstitial and undifferentiated cells are found throughout the body of the filament.

Undifferentiated cells embedded in the area of the margin of the lamella differentiate into lamellar pillar cells (Ostrander, 2000). Rodlet cells, also called X cells, are weakly stained, and they are sparsely present on the gill arches, the body of the filament, on the interlamellar filamental epithelium, and the basal areas of the lamellae. Rodlet cells appear to be secretory in nature and it is unsure if it is granular leukocyte, or it has a grandular nature or it is a cell type that is similar to eosinophilic granulocytes (Reite and Evensen, 2006). Rodlet cells may act in response to stimuli, in a similar way to mast cells acting against parasites (Reite and Evensen, 2006).

1.7.3 Intestine (structure and function)

The GIT of fish is basically a tube running from the mouth to the anus, which accommodate the needs for food processing and nutrient absorption. The GIT can be divided into stomach, foregut with a various number of blind appendages known as pyloric caeca, mid intestine, distal intestine (DI) and rectum (Figure 9). Each of these compartments is specialized for different roles in the digestive processes.

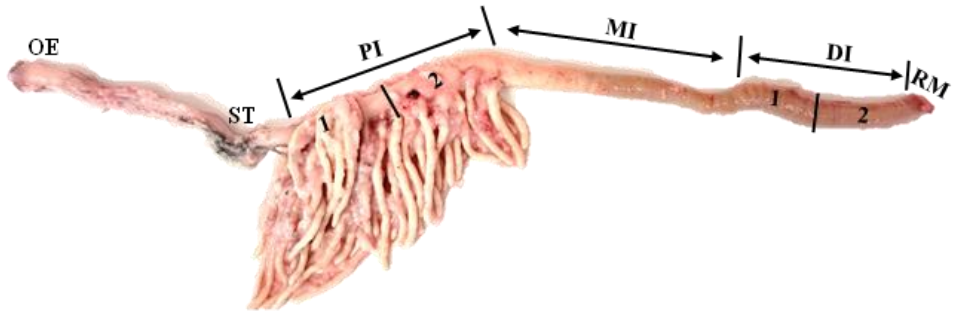

Figure 9: Gastrointestinal tract (GIT) of Atlantic salmon and its compartments: Abbreviation: OE - Oesophagus, ST - Stomach, PI, Pyloric intestine, MI - Mid intestine, DI - Distal intestine, and RM – Rectum. PI and DI can be further subdivided into two compartments as 1 and 2 denoting anterior and posterior, respectively.

Presence of sphincters between each compartment influences retention time as well as the inner environment of the compartment. Salmon has a multitude of pyloric caeca placed directly after the stomach and pyloric sphincter. The long caeca increase the digestive area and capacity (Buddington et al., 1987) and is also a prime site for

absorption of nutrients (Nordrum et al., 2000). The distal intestine is the part of the digestive tract that is thoroughly studied to understand feed ingredient-induced inflammation.

The intestinal wall can be divided into four main layers – mucosa, submucosa, tunica muscularis, and serosa (Kryvi and Poppe, 2021). The characteristics of these layers are visible at a relatively low magnification and as such can be easily evaluated using light microscopy. The mucosa is the layer that is in direct contact with the intestinal lumen, and consists of enterocytes in a single layer and the underlying lamina propria (Figure 10).

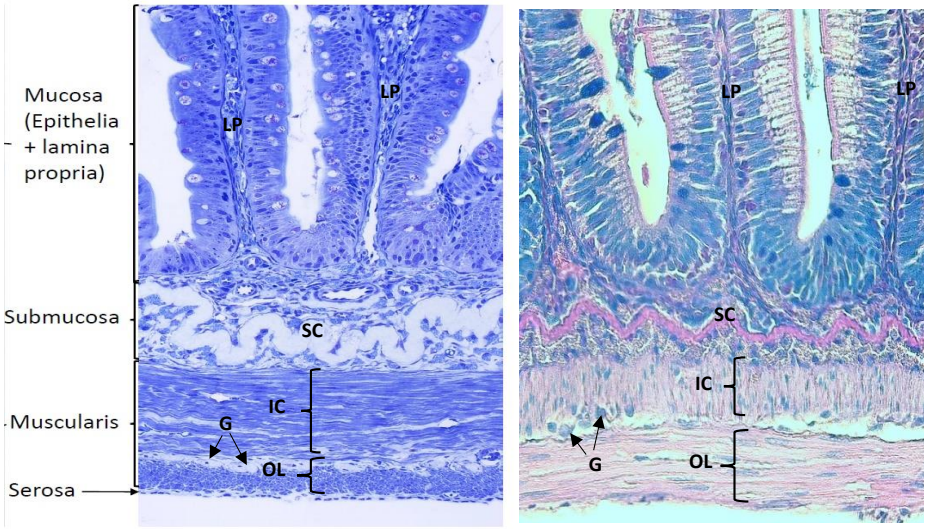

Figure 10: Histo-photomicrograph of the distal intestine of Atlantic salmon stained with toluidine blue (Left – cross section) and AB-PAS (right - longitudinal section). LP: Lamina propria. SC: Stratum compactum IC: Inner circular muscularis. OL: Outer longitudinal muscularis. G: Ganglia cells. The four main layers are marked. Left image was used with permission from Prof. Aina Cathrine, University of Bergen, Norway.

The mucosal folds extend into the lumen to increase the surface area for protection. The layer underlying the mucosa is the submucosa (Figure 10). This layer supports the mucosa, and consists mostly of loose connective tissue, but which also has a prominent layer of compact connective tissue called stratum compactum (Figure 10). On both

sides of the stratum compactum there are eosinophilic granular cells, which contain lysozymes and proteases, enzymes proposed to be active in the defence against pathogenic microorganisms (Hartviksen, 2015).

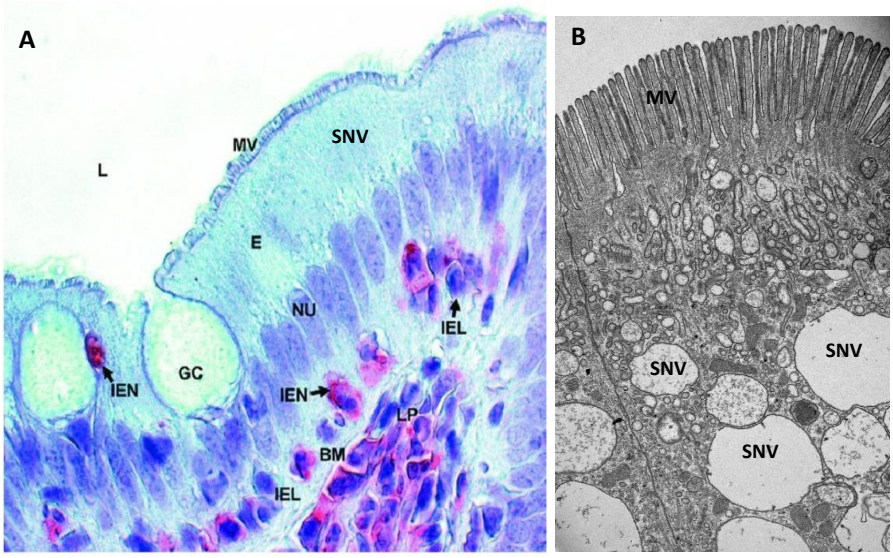

Figure 11: Mucosa of Atlantic salmon under light microscopy (A). Immunohistochemical staining for neutrophils (red) and general Giemsa staining. L, lumen; MV, microvilli; E, enterocyte; NU, nucleus; GC, goblet cell; LP, lamina propria; IEN, intra epithelial neutrophil (in red); IEL, intra epithelial lymphocyte; BM, basement membrane; SNV, supranuclear vacuoles. Source: Jutfelt, (2011). Image A is used with permission from the author. Columnar epithelium (B), distal intestine of Atlantic salmon (143g) visualised using a transmission electron microscope.

The stratum compactum is considered the border between the submucosa and the muscularis which is a layer of circular and longitudinal muscles running the length of the intestine. Between the muscular layers are ganglia cells from the enteric nervous system. The outermost layer of the intestine that has a single layer of cuboidal cells surrounded by connective tissue is known as the serosa (Figure 10). Extending from the apical membrane of the enterocytes are numerous extensions called microvilli which are collectively called the brush-border membrane (BBM) (Figure 11).

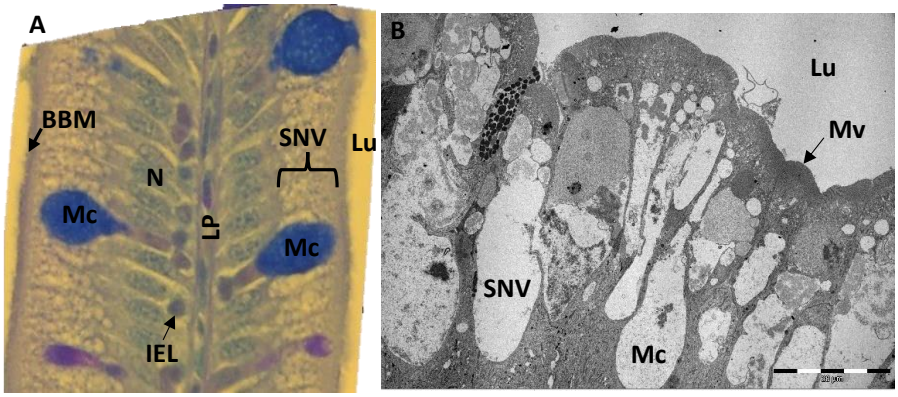

Figure 12: Enterocytes and mucous cells in the distal intestine of Atlantic salmon. A: Histological section of a simple villus stained with AB-PAS and captured under a light microscope. Lu: lumen; BBM: brush border membrane; SNV: supranuclear vacuoles; N: nucleus; LP: Lamina propria. B: Mucosa visualised using a transmission electron microscope. Mc: Mucous cell; Mv: Microvilli; Lu: lumen.

Because of the small size and tight packaging of these protrusions, visualization using a light microscope (LM) shows only fuzzy lines of the microvilli. The function of the BBM or microvilli (Figure 12) is to increase the area of digestion by membrane-bound enzymes as well as absorption of the finely digested nutrients. Goblet cells or (so called mucous cells in Atlantic salmon) are embedded in between the enterocytes. The mucous cells (Figure 12) secrete their content, mucus, from between the enterocytes, into the intestinal lumen (Hartviksen, 2015).

2. Objectives

In this study, it was hypothesized that feed additives such as probiotics and butyrate may prevent enteritis in Atlantic salmon fed plant-based diets. It was also hypothesized that microbial ingredients (probiotics and microalgae) or use of organic acid may improve the health of Atlantic salmon. The aim was to investigate the effect of feed ingredients on histomorphometric indices of the mucosal surfaces of Atlantic salmon fed marine or plant-based diets with or without feed additives. Atlantic salmon were reared in tanks under controlled environment or in sea cages (on-growth phase) under variable environmental conditions. Though health was assessed by evaluating the histomorphometric indices, other parameters such as growth performance, proximate composition, short chain fatty acids in distal intestine, and gene expression were also examined to understand the effects of feeds on health. The specific objectives were to study:

a) The effect of lactic acid bacteria on health of the fish (**Papers I-II**).
b) The effect of marine or plant derived ingredients on the health of the fish (**Papers I-IV**).
c) Efficacy of short chain fatty acid in feed to prevent enteritis (**Paper III**).
d) The effect of microalgae in plant-based diets on gut health (**Paper IV**).

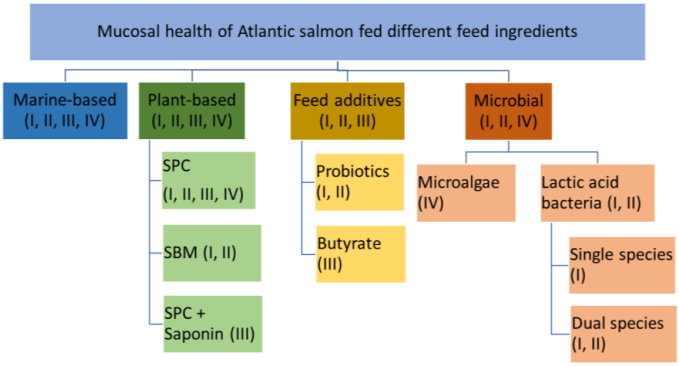

Figure 13: The objectives of the study are simplified in terms of main feed ingredients or feed additives. SBM – soybean meal, SPC – Soy protein concentrate.

3. Main findings

3.1 Paper I

Supplementation of lactic acid bacteria has positive effects on the mucosal health of Atlantic salmon (*Salmo salar*) fed soybean meal.

Published journal: Aquaculture Reports

DOI: 10.1016/j.aqrep.2022.101461

The experiment was designed to study the ability of lactic acid bacteria, either single (*Lactobacillus plantarum* or *L. fermentum*) or a blend of the two species in feed, to prevent soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*). A feed, based on marine ingredients with 20% soybean meal (CT), was designed to induce enteritis in Atlantic salmon. In total four diets were produced by coating the CT with *L. plantarum* (LP) or *L. fermentum* (LF) or a blend of the two (LP&LF). Samples were collected to study the histomorphometry of the distal intestine including mucous cell size and number in the skin and gills. The other parameters studied were weight gain, content of short chain fatty acids in digesta, mucin and AMP gene expression in the skin, gills, and intestine.

The results showed that the growth performance was not affected by the probiotic species. The individual and total short chain fatty acids were altered by the probiotics, notably acetoacetic, succinic acid; the total short chain fatty acids were significantly higher in fish fed the combination of LAB species. Mucous cell evaluation revealed that the mucous cell indices in the skin, SME (area of skin mucous cells per unit epidermis) and SNE (number of skin mucous cells per unit epidermis) area were not significantly affected by probiotics. Approximately 14% of skin epidermis area in Atlantic salmon was covered by mucous cells that correspond to an average of 1148 mucous cells in 1 mm^2 of epidermis. The mRNA level of skin antimicrobial peptide gene *defensin1* was significantly higher in the fish fed the LAB (LP) compared to the CT and the LAB blend

(LP&LF) feeds. The gill mucous cell indices, GME (area of gill mucous cells per unit epithelium area) and GNE (number of gill mucous cells per unit epithelium area) showed a significantly elevated response to the probiotic blend compared to the group without probiotics. The gill *muc5ac2* mRNA levels tended to be higher in fish fed the combination of the two probiotic species. Fish fed the control diet developed enteritis and showed the symptoms of enteritis. The mixture of the two lactic acid bacteria species tended to improve gut health; this inference is based on the increased villi height, reduced width of lamina propria, and reappearance of supranuclear vacuoles in enterocytes, compared to the fish fed the single probiotic species (diet groups LP or LF). Our study showed that the development of soybean meal-induced enteritis in Atlantic salmon, can be prevented using probiotic bacteria. Feeding a mixture of two probiotic species had a better effect compared to the single probiotic species.

3.2 Paper II

Mucosal barrier status in Atlantic salmon fed marine or plant-based diets supplemented with probiotics.

Published journal: Aquaculture

DOI: 10.1016/j.aquaculture.2021.737516

The aim of the study was to investigate the effect of ingredient composition as well as lactic acid bacteria supplementation on the mucosal health of Atlantic salmon. Three basal feeds based on either marine ingredient (BG1), plant ingredients (commercial like ingredient composition, BG5), or a marine-based diet with 20% soybean meal (BG2) were fed to fish with or without probiotics (*L. plantarum* and *L. fermentum*) coated to the feed. A 3 × 2 factorial design was adopted to study the effect of lactic acid bacteria on mucosal barrier organs (skin, gills, and intestine). Atlantic salmon were fed for 38-days and the same parameters that were evaluated in **Paper I** were also assessed in **Paper II**.

The results showed that total short chain fatty acid concentration was significantly affected by feed ingredients. The fish fed BG1 had significantly more total short chain fatty acids compared to the commercial-like diet and soybean meal-based diet. The fish fed probiotics had significantly lower concentration of total short chain fatty acids than those without probiotics. The fish fed marine-based diet had significantly higher expression of *muc5ac2* in the skin. The mucin gene in the gills, *muc5b* was significantly higher in the fish fed commercial-like diet compared to soybean meal-based diet. In the intestine, the fish fed soybean meal-based diet had significantly lower mucin (*muc2*) mRNA levels compared to the other two feed groups. Histomorphometric indices of the skin indicated that the fish fed soybean meal-based diet had significantly higher number of skin mucous cells per unit epidermis area (SNE) compared to the fish fed other diets. Notably, the probiotic groups had significantly higher SNE, area of gill mucous cells per unit epithelium area (GME), and number of gill mucous cells per unit epithelium area (GNE) compared to the groups without probiotics.

Evaluation of the distal intestinal histomorphometric indices revealed that the fish fed the marine-based feed had higher value for most of the indices except those of the lamina propria. The values for the fish fed the commercial-like diet had the same trend but ranked in between the values of the other two feed groups. Fish fed the soybean meal-based diet had the lowest values for most indices and had all the signs of enteritis. Probiotic application improved the distal intestinal histomorphology of the fish fed soybean meal-based diet, mainly considering the increased villi height, reduced lamina propria width, increased reappearance of supranuclear vacuoles, reduced number of mucous cells as well as intraepithelial lymphocytes. The results suggest that feed ingredients can alter the mucosal protective barrier of the tissue. Supplementation of probiotics alleviated the inflammatory responses and activated selected innate immune defence molecules, without impairing the growth of Atlantic salmon. The positive effect of the probiotics was similar regardless of the feed ingredients,

suggesting that these probiotics can be used to evoke favourable responses on the skin, gills, and intestine, possibly through their impact on the immune system.

3.3 Paper III

Saponin-induced inflammation of the distal intestine of Atlantic salmon (*Salmo salar*) and its prevention using butyrate as a feed additive.

Manuscript

The aim of this study was to investigate if butyrate in Atlantic salmon feed can prevent saponin-induced enteritis. Three basal diets were formulated with either no saponin (CT), low saponin (0.4%) or high saponin (0.8%). Each of these feeds were produced without or with ButiPEARL® (calcium butyrate encapsulated in palm oil), for a 3 x 2 factorial study design. The 6 feeds were fed to Atlantic salmon (250.5 g start weight) for 8 weeks. A total of 540 fish were randomly distributed into 18 tanks. At the end of the experiment, fish weights and lengths were recorded. In addition, distal intestinal tissues from 4 fish per tank were obtained for histomorphometric analysis.

The weight of the experimental fish almost doubled during the feeding period. The fish fed the control diet had better growth and intestinal health. The fish groups fed low or high levels of saponin in the feeds were diagnosed with enteritis regardless of the inclusion level. Ca-butyrate supplementation improved the growth as well as the microscopic structure of the fish irrespective of diet groups. Ca-butyrate supplementation significantly increased the mucous cells, intraepithelial cells per villus, and supranuclear vacuoles in the distal intestine of Atlantic salmon. Villi height was significantly reduced in the fish fed high saponin compared to the groups fed low saponin or the control diet. Dose-dependent effects of saponin was not evident, based on the distal intestinal histomorphometry. The conclusion from the study is that Ca-butyrate can prevent soybean meal-induced enteritis. More research is needed to investigate if a higher dose of Ca-butyrate can be used to completely cure or prevent enteritis.

3.4 Paper IV

Growth performance, feed conversion ratio, proximate composition, and distal intestinal health of Atlantic salmon (*Salmo salar*) fed different microalgae.

Manuscript

The study was designed to investigate the effect of different microalgae on the growth, feed conversion ratio, proximate composition, and distal intestinal (DI) health of Atlantic salmon reared in sea pens. Five experimental feeds were produced; one control feed (CT) based on plant ingredients and four feeds wherein 5% fishmeal was replaced with 7.5% of microalgae (*Desmodesmus* sp. - defatted, DX; *Nannochloropsis oceanica*- extruded, NW; *Phaeodactylum tricornutum* – bead-milled, PB; *P. tricornutum* – unprocessed, PW).

The results did not indicate any diet-induced differences in the growth and proximate composition of Atlantic salmon. The condition factor was significantly lower in the fish fed DX compared to the CT and NW groups. A significantly higher feed conversion ratio was observed for the algae fed groups (except for the NW group), compared to the CT group. The distal intestinal health of Atlantic salmon was affected by different microalgae species.

The fish fed NW had significantly higher values for villi height, submucosa thickness, muscle layer, villi width, and thickness of enterocytes compared to the CT and PB groups. Wider lamina propria was observed in the fish fed CT compared to those fed PB. PW significantly increased the mucous cells and DX increased the intraepithelial lymphocytes compared to the other diet groups. NW significantly increased the supranuclear vacuoles in the enterocytes compared to DX.

A principal component (PC) analysis was performed to get a better understanding of the distal intestine histomorphometric indices and the morphological changes induced by the different microalgae. The PC1 explained 38.9% of the variance, and PC2

corresponded for 16.2%. The analysis revealed the differential responses of the diets NW, PW, and DX and the similar responses of the diets CT and PB. However, none of the microalgae fed fish showed classical symptoms of enteritis. The fish fed the diet NW performed well in terms of distal intestine health compared to the fish fed the other diets.

4. Methodological considerations

4.1 Histomorphometry of the intestine, skin, and gills

Histological evaluation of tissues from organs such as the skin, intestine, and gills, which are at the forefront of defense, can provide visual evidence and quantitative data to obtain a better understanding of the effects of different feed ingredients on the health status of farmed fish (Sørensen et al., 2021). The main steps of the histological (from sampling to staining) and analytical methods for arriving at the semiquantitative or quantitative morphometrics will be discussed in this section.

Sampling

Tissue samples from the gills, skin, or intestine were collected to study the effect of feed ingredients on the health status, including the inflammatory phenotypes of the intestine. For all the papers in this thesis, 1 – 3 cm of tissue from the anterior part of the distal intestine was usually taken for the histology study following the protocols described by Krogdahl et al. (2015). As for the skin and gill tissues, the 2nd gill arch from the left side and skin from the left dorsal area were collected for the analysis.

The number of samples or replicates influence the statistical power of the study. Underpowered studies may yield false-negative and unreproducible results. Generally, fish feeding experiments employ triplicate feeding tanks, with six to twelve sampling units (fish) per diet group (Booman et al., 2018; Øverland et al., 2009; Urán et al., 2008). Based on different feeding trials, the number of samples per feed group in our studies was usually 9-12 fish per treatment (Sørensen et al., 2021b). A dietary study suggests that a minimum of 9 fish is adequate to derive valid conclusions (Panteli et al., 2020). Using individual fish as the statistical unit for histological evaluation can raise concerns about pseudo replication (Bansemer et al., 2015; Cerezuela et al., 2013; Urán et al., 2008). It should be noted that individual fish within a tank is not an independent sample from the population and is termed a pseudo replicate (Hurlbert, 1984). In the

studies described here fish from replicate tanks (2 tanks for **Papers I** and **II**, and 3 tanks or pens for **Papers III** and **IV**) of a particular treatment were employed for the analysis. For the first three experiments described in this thesis, the sample size was at least 12 fish per diet group (15 fish per diet, i.e., 5 fish/tank of a particular treatment for **Paper IV**). A power of > 80 - 85% can be obtained for an effect size of f > 0.4 - 0.5, as analysed by a priori power analysis using a software, G* power (version 3.1.9.2) (Faul et al., 2009). The effect size is in an acceptable range for the histology studies described in **Papers I**-**IV**. Nonetheless, maximizing the experimental units (tanks) and sampling units (fish) per treatment would enhance the statistical power. Moreover, a mixed model analysis would also enhance the power as well as reduce Type II error (i.e. accepting a false null hypothesis) (Ling and Cotter, 2003; Searcy-Bernal, 1994; Thorarensen et al., 2015).

Fixation

Fixatives halt enzymatic reactions and metabolism to prevent autolysis and putrefaction, and thereby help in preserving tissues of interest. Fixatives can also kill microbes in the tissues. There are several fixatives like glutaraldehyde solution (i.e., Karnovsky's Fixative), McDowells fixative, Carnoy's solution, and alcohol-based fixative (EMA - mixture of ethanol, methanol, and acetic acid) (Caballero et al., 2003; EMS, 2023; Rahman et al., 2022; Ringø et al., 2007; Urán et al., 2008). However, the widely used fixative is 10% neutrally buffered formalin (NBF). In all our experiments, the standard steps of histology were adopted: used the same 10% NBF (4% formaldehyde, pH 7.0) for 24 hours, ethanol gradient (70%, 80%, 90% 96% and 100 %) for up to 17 h, paraffin for embedding, and a day to harden the tissue blocks at -18 °C. This strategy helped to minimize organelle-loss, nuclear-shrinkage and clumping that may arise if we use different fixatives, chemicals or different duration for the aforementioned steps in preservation (Chatterjee, 2014; Webb, 2020). In addition, the skin tissues were decalcified with 10% formic acid for 5 h to avoid fish scales from interfering with the sectioning using microtome. To avoid introducing the body site as a factor in analysis

(e.g. location based effect on mucous cells), all the skin tissues were always obtained from the dorsal area (Pittman et al., 2013), and the gill tissues from the second arch.

Processing and cutting

The tissues can be embedded in paraffin, along one of the following planes; cross (transverse), tangential, sagittal, frontal or longitudinal planes according to the parameters of interest and the detailed morphological assessment (Dang et al., 2020; Fiedler et al., 2020; Øverland et al., 2009; Penn et al., 2011). For the different studies described in this thesis longitudinal sections of the DI tissues were prepared to get more features, i.e., enough number of villi per fish compared to cross section. A transverse orientation was adopted for the skin tissue to have enough tissue section for semi-automated mucous cell quantification. Sagittal orientation was adopted for the gill sections because such a strategy will help visualize secondary lamellae (Fiedler et al., 2020). The trimming and feeding thickness can interfere with the quality of tissue on the slides (Shields and Heinbockel, 2019). However, having the same thickness (15 µm for trimming and 4 µm for feeding) for all the experiments would ensure less deviations during the cutting process.

Staining

A set of slides stained with H&E was employed for general evaluation. It should be noted that the results of the analysis of mucous cells will depend on the type of staining (Jensen, 2015). Salmon skin tissues stained with different stains are shown in Figure 14 (Jensen, 2015). Among them, AB-PAS staining (e), which stains the mucopolysaccharides, is the best for mucous cell evaluation. The neutral mucous cells stain magenta with PAS staining, while acidic mucous cells turn blue with AB staining. We have evaluated total (neutral and acidic) mucous cells in this thesis. The AB-PAS stains are detected by employing the colour threshold in ImageJ software. Hence, AB-PAS staining was used in the different experiments described in this thesis.

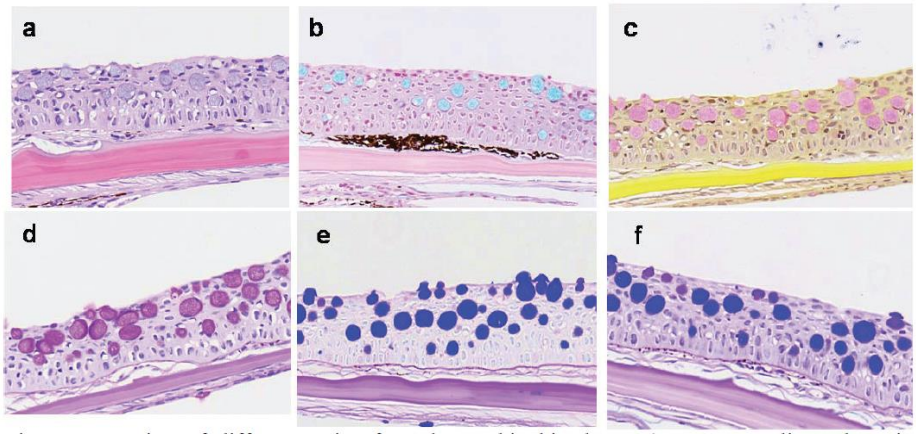

Figure 14: Skin mucous cells of Atlantic salmon visualized using different stains. a) Haemotoxylin and Eosin (H&E), b) Alcian Blue (AB) c) Mucicarmine d) Periodic acid-Schiff reaction (PAS) with diastase e) AB-PAS and f) AB-PAS & H. Source: (Jensen, 2015). Permission was granted by author.

Image processing

Light microscopy (LM) is an important tool to study tissues or cell structure. Electron microscopes (transmission or scanning) can reveal the ultrastructure or organelles of interest. However, in the latter case, the tissues should be fixed in a specific way which might be more time consuming and requires resources compared to standard paraffin histology. Different software (i.e., Image Pro Plus, AnalySiS Extended Pro, and Video Pro) are available for analyzing digital images. Among these, ImageJ software is commonly used for histological analysis because it is an open source and user-friendly software (Bakke-McKellep et al., 2007; Bansemer et al., 2015; Urán et al., 2008).

Histomorphometric indices

Pre-defined morphological parameters can be evaluated qualitatively, quantitatively, or semi-quantitatively. Many studies on Atlantic salmon have reported the severity of SBMIE based on qualitative, quantitative, and semi-quantitative assessments (Agboola et al., 2022; Sørensen et al., 2011b; van den Ingh et al., 1991; Øverland et al., 2009). The reliability of a qualitative assessment depends on the skill of a researcher and the results cannot be statistically validated. On the other hand, semi-quantitative scoring

and quantitative assessment could be validated with statistical analysis if the artifacts are minimized (Baeverfjord and Krogdahl, 1996; Knudsen et al., 2008; Urán et al., 2008).

Nutritional studies focus on mucosa, submucosa, muscularis and serosa layers of the intestine to understand the effect of different feed ingredients (Øverland et al., 2009; Urán et al., 2008). The papers discussed in this thesis also focused on villi height, villi width, enterocytes height, lamina propria width, submucosa thickness (SM), muscle layer thickness (ML), serosa thickness (SA) of DI. Such values were obtained through quantitative assessment of the parameters. It should be noted that salmon has complex and simple villi depending on the life stage and regions of intestinal tract (i.e., mid intestine has simple villi) (Bjørgen et al., 2020; Løkka et al., 2013).

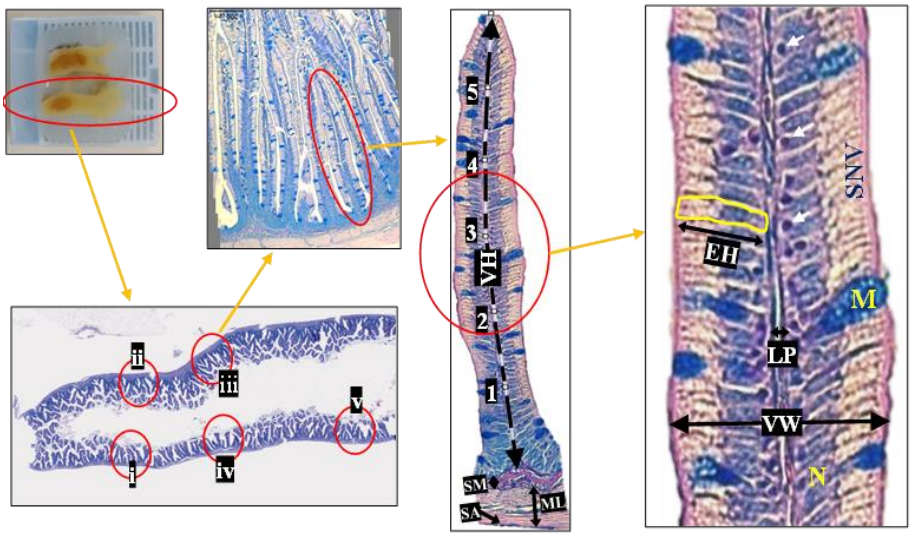

Figure 15: Distal intestinal histomorphometric indices that were studied for papers I-IV. Villi height (VH), villi width (VW), enterocytes height (EH), lamina propria width (LP), submucosa thickness (SM), muscle layer thickness (ML), serosa thickness (SA), mucous cell (M), and supranuclear vacuoles (SNV). Small white arrows indicate intraepithelial lymphocytes (IEL). "N" denotes nucleus of enterocytes. The single enterocyte is highlighted with a yellow box line. Lower case roman numerals i-v indicate different locations at which the measurements were taken to obtain the indices. The number 1-5 indicate the partition of villi along the lamina propria.

At least 5 or 10 simple, intact villi (whole length from tip to base of villus was visible) were selected from 3 - 5 different locations of DI per fish (Figure 15). One can argue that cutting angle can affect the measured villi height. The protocols were standardized for all the treatment groups to obtain reliable results. The height was measured along the lamina propria from bottom to tip of the villus. When the samples are from fish that has developed intestine inflammation, the width of villi varies along its length. Therefore, the villus was partitioned into 6 equal portions and obtained average values for villi width, enterocyte height, and lamina propria width measured at the 5 locations shown in Figure 15 (**Papers I** and **II**).

For the semi-quantitative assessment, a scoring strategy was developed after examining all the DI slides (Knudsen et al., 2007; Krogdahl et al., 2015; Urán et al., 2008). In this way qualitative tissue data were transformed into numerical values. We assumed that staining distribution and density might interfere with the quantification of the number of mucous cells (NMC), intraepithelial lymphocytes (IELs), and supranuclear vacuoles (SNVs). Therefore, a scale of 1 to 5 was employed for NMC and IEL; 1 for many NMC or IEL and 5 for fewer counts. As for the scores of SNV, 1 was chosen for very few or absence of SNV and 5 for fully present (**Papers I**-**III**). For **Paper IV**, a scale of 1 to 4 was employed to obtain the values for SNV and IEL. Each villus received a score only after completing the evaluation of the associated slide and the average value was reported as median plus first and third interquartile. Semi-quantitative score data can be obtained relatively quickly and the method is inexpensive compared to quantitative methods or rigorous compared to descriptive texts (Silva et al., 2015). A transition from comprehensive masking or blinding to group masking or post examination masking was practiced by the author of this thesis to avoid bias (**Papers I**-**IV**) (Price, 2008).

Moreover, a semi-quantitative assessment was adopted to obtain more information from complex villi. Simple villi were evaluated quantitatively. Only experienced researchers could identify IELs because they are small in size and occasionally, they

appear as doublets. As regards NMC, quantification of squeezed mucous cells or those cells with missing parts will be difficult. Regarding SNVs, the staining intensity, as well as lipid droplets might mislead the quantitative assessment. Therefore, semi-quantitative assessment of NMC, IEL, and SNV of DI tissues is considered easier and better than quantitative assessment of these three variables.

Ordinal scoring system is employed to grade the severity of a disease; hierarchical or progressive grades can reflect the severity. Such a system enhances the reproducibility of data. Studies suggest that the levels of the score range can be about 4 to 5 (Knudsen et al., 2008; Silva et al., 2015). Fewer levels decrease the sensitivity to detect treatment effects while more levels reduce the repeatability. Studies also suggest that having multiple indices for assessing lesions is better than a wholistic score for the enteritis (Agboola et al., 2022). Moreover, ordinal scores are discontinuous data which are not normally distributed. Therefore, statistical analyses of the score data are performed using non-parametric tests (Meyerholz and Beck, 2018).

Enteritis model-based studies mainly focus on the intestine but not on the skin or gills (Urán et al., 2009). Studies seldom focus on the mucosal health of the intestine, skin and gills which are all key immune defense organs of fish (Sørensen et al., 2021). Therefore, in this thesis (**Papers I** and **II**) we focused on mucosal health of these three organs. To assess the mucous cell size or number in the skin and gills, we developed a quantitative methodology (Figure 16). Rather than counting the cells manually, this semi-automated image analysis helped us to get information about: a) the number of mucous cells present in the epithelium in relation to unit area of mucosa (epidermis or lamellae), and b) mucosa area covered by mucous cell area. For the histological assessment of the skin tissue, “total area of skin epithelium” (SE) was determined from a defined area of epidermis from 9 different locations per fish (Figure 16).

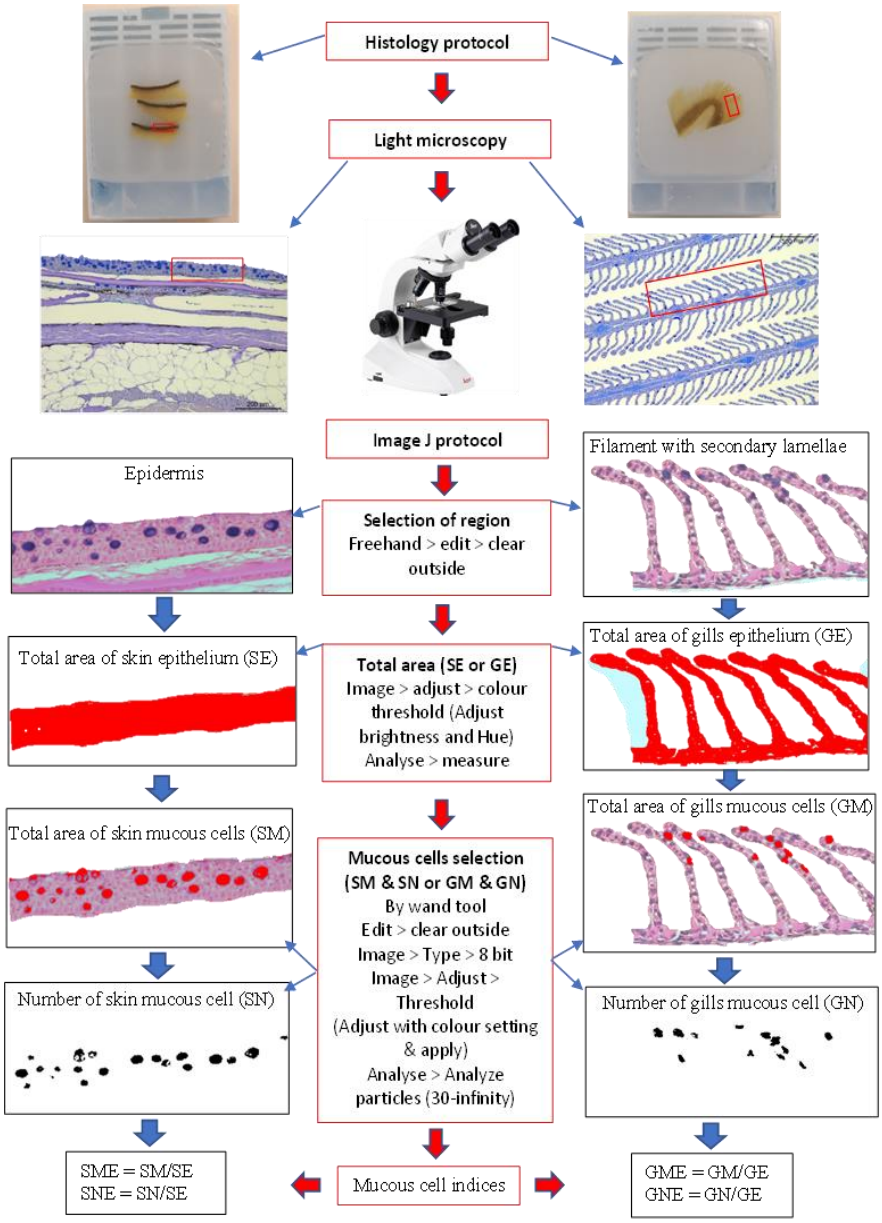

Figure 16: Histomorphometric procedures adapted to evaluate the size and number related mucous cell indices in the skin and gills for Papers I and II.

The color threshold in ImageJ was employed to define “total area of skin mucous cells” (SM). The “analyze particle” command in ImageJ was used to derive “the number of mucous cells” (SN). The index SME (ratio between total area of skin mucous cells and total area of skin epithelium) was calculated by dividing SM by SE. Likewise index SNE (ratio between number of skin mucous cells and total area of skin epithelium) was calculated by dividing SN by SE. For the gills, 10 secondary lamellae (including one side of filament) from the mid region of gills (avoiding tips and near arch region) were selected from 5 different filaments. Similar approach, as stated for the skin, was adopted to obtain the two indices GME and GNE (Figure 16), based on the gill mucous cell counts (both on filaments and lamellae).

There are other novel stereology-based methods to quantify the skin mucous cells, for example the one suggested by Pittman et al. 2011. Such stereology-based methods use tangential sections where more surface area can be obtained compared to the traditional method. This mucosal mapping gives volumetric density of the mucous cells in the mucosa. Moreover, mucosal mapping estimates mucous cell size at its equator (Dang et al., 2020). Although time consuming and resource demanding, this method is more efficient than the traditional mucous cell counting methods (Pittman et al., 2011). The present study reports the number and size of the mucous cells in the dorsal skin. However, it should be noted that the mucous cell density varies depending on the body site, the rearing condition, the life stage, the species as well as individual. For instance, caudal fin has less mucous cell density. Moreover, the largest diameter of mucous cell is obtained when the cell is cut along its diameter assuming that they are perfectly spherical (Pittman et al., 2011). This effect is less relevant as we report the number and size of mucous cells per unit area of epidermis.

In addition to the standard paraffin embedded histological techniques, researchers also use immunohistochemistry using suitable antibodies to detect molecules that are implicated in inflammation and to understand cell proliferation. However, lack of

antibodies is a limiting factor for advancing immunohistochemistry in inflammation studies of Atlantic salmon.

4.2 qPCR for gene expression

Mucins are high molecular weight glycoproteins comprising a protein core with branching oligosaccharide chains attached by glycoside bonds (Lievin-Le Moal and Servin, 2006). On the other hand, AMPs are small peptides mostly cationic with low molecular weight and direct microbicidal activities. Among different AMPs, cathelicidins and defensins belong to the cationic peptides with amphipathic properties (Kościuczuk et al., 2012). AMPs have also been reported in other tissues including spleen, eyes, gonads, liver, and kidney (Beck and Peatman, 2015). Therefore, mucin and AMP genes are often used as bio markers to assess the mucosal health of fish (Marcos-López et al., 2018).

The mucin and AMP gene expression was analysed in **Papers I** and **II**, but not for **Papers III** and **IV** (limited resources and considering the time frame of the study). Mucin related genes; *muc5ac1*, *muc5ac2*, *muc5b*, and *muc2* and AMP genes; *defensin1* (*def1*), *defensin3* (*def3*), *defensin4* (*def4*) and *cathelicidin1* (*cathl1*) were studied. The following mucin genes were studied in the skin, gills, and distal intestine: *muc2*, *muc5ac1*, *muc5ac2*, and *muc5b* and the following AMP genes were studied in the skin and distal intestine: *def1*, *def2*, *def3*, *def4*, and *cathl1*.

Extracting high quality RNA is vital for the gene expression analysis. E-Z 96 Total RNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) was used to extract the total RNA from the tissues. Separation of RNA from other cellular molecules, avoiding genomic DNA contamination, and getting high standard purified mRNA are dependent on many factors including the techniques used, good laboratory practices, and the researcher's skills. The purity can be tested using Nanodrop spectrophotometer. The quality and quantity of RNA can be assessed by microfluidic capillary electrophoresis systems.

In the studies undertaken for this thesis, cDNA synthesis was done with QuantiTect™ Reverse Transcription Kit (Quiagen GmbH, Hilden, Germany) (Vasanth et al., 2015). qPCR was performed on a LightCycler® 96 (Roche Life Science) using Fast SYBR® Green Real-Time PCR Master Mix (Applied Biosystems, Carlsbad, USA). The chosen reference genes were *elongation factor 1AB* (*ef1ab*), *ribosomal protein L13* (*rpl13*), *ribosomal protein S29* (*rps29*), and *ubiquitin* (*ubi*) (Sørensen et al., 2021). Although gene expression can indicate a condition for example inflammation, other techniques such as histology should be employed to confirm the "phenomenon". Interestingly, gene expression analysis provides information about changes in expression of known marker genes. On the other hand, histology provides information about the changes in the whole tissue/cells. Therefore, histology is a strong tool to assess the quality of feed ingredients.

4.3 A capillary isotachophoresis for quantifying short chain fatty acids

Exact and reliable quantification of SCFAs is important for understanding the type of SCFA that prevents enteritis in fish. Many methods are available (Bihan et al., 2022) to detect SCFAs. A derivatization-free method, the gas chromatography mass spectrometry (GC-MS) can quantify SCFAs not only in faeces but also in plasma, cecum, liver, and adipose tissue (Rohde et al., 2022). The present study used a capillary isotachophoresis (cITP) method (Electrophoretic analyzer EA 202M, VILLA LABECO spol. s.r.o., Spisska Nova Ves, Slovakia) as described by Gancarcikova et al., (2020). It should be noted that this methodology only quantifies the SCFAs found in digesta. The total amount of SCFAs produced by the intestine microbes or the amount that is absorbed in the intestine is not quantified by this method. When the SCFA production is high in the lumen the pH will be low, and the microbial composition will be shaped accordingly.

4.4 Assessment of fish performance

Three of the studies in this thesis were performed as short-term feeding studies; 38 feeding days for **Papers I** and **II,** and 51 feeding days for **Paper III**. As a general rule, for proper evaluation of fish growth performance, the final weight of the fish should be equal to at least double the initial weight (Table 7). In addition, feed intake should be calculated using the actual feed consumption values. The leftover feed was not quantified for the studies reported in **Papers I**-**III**. Only **Paper IV** was properly done as a long-term (32 weeks) study where the final fish weight was double that of the initial weight and the actual feed intake was determined. The other studies in this thesis were mainly performed to examine the health aspects linked to feed additives and not growth performance. The growth parameters are used only to support the results from the other analyses. Therefore, the growth data from **Papers I**-**III** should only be used to understand the appetite and health status of the experimental fish.

The growth performance in terms of weight gain, thermal growth coefficient, specific growth rate, condition factor was calculated mainly to ensure that the experimental fish had a good growth, and they did not have any abnormal behaviour (**Papers I**-**IV**). It should be noted that **Papers I** and **II** were part of a larger project that intended to assess the health of fish fed plant or marine-based diets and reported by Solveig Sørensen as part of her Ph.D. thesis (Sørensen et al., 2021). In my experiments, tank was used as the experimental unit for growth performance calculations (Kiron et al., 2016). Feed utilization was also determined in the growth study reported in **Paper IV**. Hence, weight gain, specific growth rate and feed utilization are included along with proximate chemical composition.

4.5 The experimental diets and composition

The effects of feed ingredients derived from marine, terrestrial, or low trophic (microbial) sources on the growth and health (based on histomorphometric indices and

gene expression) of Atlantic salmon were studied for this thesis (Table 2). The diets were formulated and produced by a feed producer in Portugal.

The first two studies were conducted to understand the compromised gut health caused by SBM in marine-based diets (**Papers I** & **II**), or soyasaponin in plant-based diets (Paper III). The last study (**Paper IV**) was designed to describe the effect of microalgae in plant-based diets. Soybean meal, containing saponin, is extensively used to study enteritis in fish studies (Sørensen et al., 2011b) though other ANFs found in plants might also induce inflammation (Bureau et al., 1998; Knudsen et al., 2007; Penn et al., 2011; Van Den Ingh et al., 1996). For **Paper III**, saponin extracted from soybean was used to induce the SSIE instead of soybean meal, at an inclusion level that is known to cause enteritis (Kiron et al., 2020; Krogdahl et al., 2015). There are other sources of saponin such as Quillaja bark (Bureau et al., 1998) and their effects are dependent on the structure of the saponin product. Feed additives such as probiotics and organic acids are used in functional aquafeeds for different purposes. Functional feeds are defined as non-nutritive products incorporated to the basic feed mix to improve feed utilization, enhance growth and health, ensure welfare of the animal, and thus to enrich the quality of the product (Lee et al., 2015). The butyrate employed in the experimental feeds of the studies in **Paper III** was intended to reduce inflammation.

In **Paper IV**, inclusion level of three different microalgae was kept constant and a particular form of the alga product (defatted *Desmodesmus* sp., a pre-extruded *Nannochloropsis oceanica*, bead-milled *Phaeodactylum tricornutum*, unprocessed *P. tricornutum*) was added to each diet. Since earlier studies have reported that microalgae inclusion can reduce the nutrient digestibility (Kiron et al., 2016, 2012; Sørensen et al., 2017, 2016), 7.5% microalgae was added to a plant-based feed, keeping fishmeal level at 10%.

This PhD project investigated the effect of feed additives such as probiotic lactic acid bacteria, or the organic acid butyrate, on the health of Atlantic salmon. For **Papers I**

and **II**, two different *Lactobacillus* species (*L. plantarum* and *L. fermentum*) isolated from healthy rainbow trout, cultured *in vitro*, were added to the feed by using a vacuum coater.

Table 2: Overview of experimental feeds and ingredients used in different experiments.

Papers/	Paper I				Paper II			Paper III			Paper IV				
Diet name/ Ingredients	CT	LP	LF	LP&LF	BG1 (÷ / +)	BG5 (÷ / +)	BG2 (÷ / +)	C (O / B)	L (O / B)	H (O / B)	CT	DX	PB	PW	NW
Marine-based:															
FM %	30	30	30	30	50	10	30	15	15	15	15	10	10	10	10
FO %	26.4	26.4	26.4	26.4	25	7.7	26.4	7.5	7.5	7.5	7	7	7	7	7
Plant-based:															
SPC %	0	0	0	0	0	20	0	22	22	22	15	15	15	15	15
PPC %	0	0	0	0	0	9	0	0	0	0	10	10	10	10	10
RO %	0	0	0	0	0	19.8	0	14.7	14.7	14.7	16	16	15	15	15
SBM %	20	20	20	20	0	0	20	-	-	-	-	-	-	-	-
Saponin %	-	-	-	-	-	-	-	0	0.4	0.8	-	-	-	-	-
Probiotics: (CFU / g feed)					BG1+	BG5+	BG2+								
L. plantarum	0	10^8	0	10^4	10^4	10^4	10^4	-	-	-	-	-	-	-	-
L. fermentum	0	0	10^8	10^4	10^4	10^4	10^4	-	-	-	-	-	-	-	-
Organic acid:								CB	LB	HB					
Ca-Butyrate %	-	-	-	-	-	-	-	0.15	0.15	0.15	-	-	-	-	-
Microalgae %	-	-	-	-	-	-	-	-	-	-	0	7.5	7.5	7.5	7.5

CT in Paper I, control diet without probiotics; LP, CT diet with *Lactobacillus plantarum*; LF, CT diet with *L. fermentum*; and LP&LF, CT diet with both *L. plantarum* and *L. fermentum*. In Paper II, BG1, marine-based feed; BG5, plant-based commercial feed; BG2, soybean meal-based feed; ÷, without probiotics; +, with probiotics. In **Paper III**, C, plant-based control diet; L, C diet with low level of saponin; H, C diet with high level of saponin; O, without butyrate; B, with butyrate. CT in **Paper IV**, plant-based control diet; DX, CT diet with defatted *Desmodesmus* sp.; NW, CT diet with double extruded *Nannochloropsis oceanica*; PB, CT diet with bead-milled *Phaeodactylum tricornutum*; and PW, CT diet with unprocessed *P. tricornutum*. FM, fishmeal; FO, fish oil; SPC, soy protein concentrate; PPC, pea protein concentrate; RO, rapeseed oil; SBM, soybean meal. **Papers I** and **IV** studied the effect of one factor (used a one-way ANOVA). The design in **Papers II** and **III** was a 3*2 factorial design (used a two-way ANOVA).

The binomial nomenclature of the LAB strains *L. fermentum* and *L. plantarum* has been renamed to *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum*, respectively (Schoch et al., 2020; Zheng et al., 2020). The new nomenclature was not used when **Papers I** and **II** were published. To avoid confusion with the published paper, *L. fermentum* and *L. plantarum* will be used in the discussion.

The LABs were chosen based on their characteristics such as high tolerance to different pH values, bile, temperature, best growth properties and viability (Fečkaninová et al., 2019). A previous study has also documented that these probiotic bacteria can colonize the gastrointestinal tract (GIT) of post-smolt salmon (Gupta et al., 2019). The latter study was not designed to investigate potential health benefits of the probiotics. The studies presented in **Papers I** and **II** was therefore undertaken to investigate if the LABs can impart health benefits to the host.

For **Paper III**, a product ButiPEARL® (calcium butyrate encapsulated in hydrogenated palm oil) was added at 0.15% as feed additive. In the gut of Atlantic salmon where the pH is 8, the product is expected to dissociate/dissolve and release calcium and butyrate. However, other forms of butyrate such as sodium butyrate which is fat soluble might not reach the distal intestine and absorbed by enterocytes (Liu et al., 2014; Tran-Ngoc et al., 2019).

More than 80% of salmon feed is used during on-growth from 1 kg to slaughter size (4-6 kg) (Mowi, 2012; Thoresen, 2022). However, most laboratory scale studies on microalgae as protein source for salmonids have used parr and smolt-size fish (Thoresen, 2022). A grow-out study at sea was therefore undertaken, to study the effects of microalgae under fluctuating environmental conditions at sea and results are reported in **Paper IV**.

5. Discussion of main findings

5.1 Mucosal health of Atlantic salmon

5.1.1 Effects of main feed ingredients (marine- or plant-based)

Three basal diets were tested in **Paper II**; a marine-, or a plant-based (commercial-like), and SBM-included in a marine-based diet. The histomorphometric indices in **Paper II** revealed that the mucosal barrier health was superior in fish fed marine-based diet compared to SBM-based diet. The histomorphometric indices of the fish fed the commercial-like diet (dominated by plant-based ingredients) were in between those of fish fed the other two diets. Antinutritional factors present in plant ingredients, mainly saponins, can cause enteritis. Signs of SBMIE were observed in the fish fed SBM-based diet (**Papers I** and **II**), while most of the characteristics of enteritis were observed when diets contained saponin (**Paper III**, LO-low, 0.4% or HO-high, 0.8%). In both cases, enteritis was characterized by shortened intestinal villi and enterocytes, many intraepithelial lymphocytes and mucous cells, fewer supranuclear vacuoles, wider lamina propria and submucosa, both regions with many inflammatory cells (Table 3).

The enteritis observed in the studies described in **Papers I**, **II**, and **III** are similar to those reported by previous studies that investigated the effect of soybean meal in marine-based diets on Atlantic salmon (Baeverfjord and Krogdahl, 1996; Sørensen et al., 2021). The plant-based control diet, dominated by soy protein concentrate (SPC) did not induce inflammation (**Paper III**), while the control diet used in **Paper IV**, with a combination of SPC and pea protein concentrate (PPC), induced mild inflammation characterized by wider lamina propria and thicker serosa (Table 4).

The effects of SBM on histological indices are dependent on the dose of the SBM (Krogdahl et al., 2003). At inclusion levels below 10%, the effects vary from being absent to very distinct changes depending on the fish species and rearing conditions (Baeverfjord and Krogdahl, 1996; Urán et al., 2008, 2008). Adverse effects were

reported when the inclusion level of SBM was above 20% (Sørensen et al., 2021b). Therefore, soybean meal at 20% inclusion was used to induce enteritis in **Papers I** and **II**, while a saponin with 98% purity was used in **Paper III**.

Saponins can cause intestine inflammation and there are various modes of action. They can form non-soluble complex with the cell membrane cholesterol and make holes in the epithelial cells of mammalian intestine (Johnson et al., 1986; Lorent et al., 2013; Verstraeten et al., 2020; Zheng and Gallot, 2020), thereby increasing the epithelial permeability (Knudsen et al., 2008; Nordrum et al., 2000). Consequently, toxins or pathogens present in the lumen may breach the epithelium, thereby causing various pathological conditions (Gu et al., 2018). Enteritis is also marked by the upregulation of proinflammatory cytokines such as IL-1 β, IL-8, and TNF- α, which can mediate the recruitment and activation of leukocytes (Gu et al., 2018).

Table 3: Histomorphometric indices of the distal intestine examined for Papers I-IV.

	Paper I				Paper II						Paper III						Paper IV				
	CT	**LP**	**LF**	**LP&LF**	**BG1÷**	**BG1+**	**BG5÷**	**BG5+**	**BG2÷**	**BG2+**	**CO**	**CB**	**LO**	**LB**	**HO**	**HB**	**CT**	**DX**	**PB**	**PW**	**NW**
VH	897.65	976.84	979.53	1021.44	1146.68	1142.51	1004.95	993.43	788.31	966.11	976.55	1078.91	737.01	1139.23	731.18	954.27	1460.94	1914.12	1243.66	1318.02	2178.51
VW	126.73	113.19	112.13	116.61	114.56	117.49	102.33	104.71	106.99	103.08	107.87	115.08	103.71	117.03	105.58	109.82	84.40	112.78	82.30	112.58	115.83
EH	48.26	43.35	43.23	46.95	55.52	59.18	47.99	48.80	44.16	43.58	48.66	51.21	44.15	50.88	45.05	45.68	32.88	42.72	35.09	47.05	56.92
WLP	27.62	22.07	24.61	18.39	9.67	6.53	6.60	6.69	20.87	15.76	8.15	6.75	14.08	9.45	11.77	10.17	11.23	9.35	6.89	9.83	9.51
NOM	3	3	4	3	3.5	2	4	3	1.5	2	4	3	4	2	4	2.5	2	3	3	3	3
IEL	4	3	3	3	5	3.5	4	3.5	1	3	4	1	2	1	2	1	3	2	3	3	3
SNV	1	2	2	2	5	5	5	5	1	2	5	5	2	4	3	3	3	2	4	3	4

CT in **Paper I**, control diet without probiotics; LP, CT diet with *Lactobacillus plantarum*; LF, CT diet with *L. fermentum*; and LP&LF, CT diet with both *L. plantarum* and *L. fermentum*. In **Paper II**, BG1, marine-based feed; BG5, plant-based commercial feed; BG2, soybean meal-based feed; ÷, without probiotics; +, with probiotics. In **Paper III**, CO, plant-based control diet without saponin & butyrate; CB, CO diet with butyrate; LO, CO diet with low level of saponin; LB, LO diet with butyrate; HO, CO diet with high level of saponin; HB, HO diet with butyrate. CT in **Paper IV**, plant-based control diet; DX, CT diet with defatted *Desmodesmus* sp.; NW, CT diet with double extruded *Nannochloropsis oceanica*; PB, CT diet with bead-milled *Phaeodactylum tricornutum*; and PW, CT diet with unprocessed *P. tricornutum*. VH, villi height; VW, villi width; EH, height of enterocyte; WLP, width of lamina propria; and median scores for: number of mucous cells (NOM), number of intraepithelial lymphocytes (IEL), and presence of supranuclear vacuoles (SNV).

The histomorphometric indices of DI mainly villus height, supranuclear vacuoles, and lamina propria were negatively altered by plant-based feed ingredients. The PCA analysis revealed that the gut health was improved by the feed additives (probiotics and butyrate) and microalgae (**Papers I**-**IV**) as we observed positive effects on the aforementioned histomorphometric indices. This is also further discussed in sections 5.1.2, 5.1.3, and 5.1.4.

Indeed, dietary saponin increased IELs in the intestine of Atlantic salmon (**Paper III**). The leucocytes present in the blood infiltrate the submucosa, and through lamina propria certain leucocytes reach the epithelium (Gu et al., 2018; Luissint et al., 2016). The epithelial cells have junctions, namely tight junctions (TJ), anchoring junctions (adherens junction, desmosomes, and hemidesmosomes) and communicating junction (gap junction) (Dong et al., 2020; Landy et al., 2016). Saponins seem also to destruct TJ proteins (Gu et al., 2018) and increase the reactivity of caspase-3, causing DNA fragmentation followed by apoptosis of cells (Bakke-McKellep et al., 2007). The dry matter content in feces of fish fed SBM or other plant protein concentrates, decreases, an indication of diarrhea (Hu et al., 2016; Refstie et al., 2005; Sørensen et al., 2021b), which maybe an outcome of mucosal damage caused by, among others, dysfunctional epithelial barrier. Overall, ANFs present in plant ingredients can compromise the mucosal barrier health. The results in **Paper III** did not indicate any difference in the severity of enteritis of the two levels of saponins used in the feed, suggesting that the lowest level (0.4%) was sufficient and should also be used in future studies. However, previous studies have reported a dose-dependent severity of enteritis caused by saponin (Chen et al., 2011; Krogdahl et al., 2015). Nevertheless, it should be noted that different fractions of soyasaponin may elicit varying effects in the host intestine.

Results presented in **Papers I** and **II** revealed that feed ingredients significantly influenced the SCFA composition (Table 4). Fish fed the marine ingredients had more total SCFAs as well as the individual fatty acids, formic, lactic, succinic, acetic, propionic, and butyric acids. The results were unexpected because plant ingredients contain more

carbohydrates and potential prebiotic sugars, serving as substrate to the microbiota to generate more SCFAs (Adorian et al., 2020; Hoseinifar et al., 2017; Rowland et al., 2018; Wu et al., 2021). The lower faecal DM might be the reason for the lower SCFA concentration in the fish fed the plant-based feeds (Sørensen et al., 2021). Changes in the microbiota composition was not examined in this experiment, but another study has reported the lactic acid bacteria-caused changes in the composition of bacteria in the mucus of Atlantic salmon (Gupta et al., 2019).

Table 4: Short chain fatty acids altered by lactic acid bacteria (Papers I and II) or feed ingredient composition (Paper II).

SCFAs	Paper I	Paper II
	LAB	Main factor feed or LAB
Formic	CT = LF	BG1 > BG5 > BG2
Acetoacetic	LP&LF > LP > LF CT = LF	BG5 > BG2 = BG1 without LAB (÷) > with LAB (+)
Lactic	LP > LF	BG1 > BG5 > BG2
Succinic	LP&LF > LF LP > LF	BG1 > BG5 = BG2
Acetic	CT = LF = LP = LP&LF	BG1 > BG5 = BG2
Propionic	LF > LP	BG1 > BG5 = BG2
Butyric	CT = LF = LP = LP&LF	BG1 > BG5 = BG2
Total acids	LP&LF > LF	BG1 > BG5 > BG2 without LAB (÷) > with LAB (+)

CT – control diet without LAB, LP – CT diet with the *Lactobacillus plantarum*, LF – CT diet with the *L. fermentum*, and LP&LF – CT diet with both *L. plantarum* and *L. fermentum*. BG1, marine-based feed; BG5, plant-based commercial feed; BG2, soybean meal-based feed; LAB – lactic acid bacteria; ÷, without LAB; +, with LAB. The diet groups which had significantly higher, lower, or equal concentration of a particular SCFA/total SCFAs are indicated using appropriate symbols (>, <, or =). For example, CT = LF indicates that the concentration of an SCFA is not significantly different.

Feed ingredients altered the mucous cell indices. The mucous cell number related scores for DI are presented in Table 3 (**Papers I**, **II**, **III**, and **IV**). The highest score denotes fewer mucous cells per villus. A general trend was that plant-based diets received lower score compared to marine-based diet. Similar trends were also observed in the skin and gill mucosal tissues (Table 5). The higher number of mucous cells in mucosa of fish fed plant-based diets may be a non-specific response to inflammation (Huang et al., 2012) caused by antinutritional factors (Sørensen et al., 2021). The increased number of mucous cells subsequently lead to more mucus and other immune molecules to enhance the protection against pathogens (Bosi et al., 2005; Pittman et al., 2013).

Table 5: Histomorphometric indices of the mucous cells of the skin and gills of Atlantic salmon (Papers I and II).

	Paper I				Paper II					
	CT	**LP**	**LF**	**LP&LF**	**BG1÷**	**BG1+**	**BG5÷**	**BG5+**	**BG2÷**	**BG2+**
SME	0.1563	0.1248	0.1317	0.1382	0.1505	0.1639	0.1761	0.1786	0.1712	0.1712
SNE	0.0011	0.0012	0.0011	0.0012	0.0009	0.0012	0.0011	0.0012	0.0012	0.0013
GME	0.0399	0.0514	0.0493	0.0657	0.0241	0.0414	0.0398	0.0565	0.0487	0.0736
GNE	0.0006	0.0007	0.0007	0.0008	0.0003	0.0007	0.0006	0.0009	0.0008	0.0012

CT in Paper I, control diet without probiotics; LP, CT diet with *Lactobacillus plantarum*; LF, CT diet with *L. fermentum*; and LP&LF, CT diet with both *L. plantarum* and *L. fermentum*. In Paper II, BG1÷, marine-based feed without probiotics; BG1+, marine-based feed with probiotics; BG5÷, plant-based feed without probiotics; BG5+, plant-based feed with probiotics; BG2÷, soybean meal-based feed without probiotics; BG2+, soybean meal-based feed with probiotics; SME, ratio between skin mucous cells area and epithelium area; SNE, ratio between number of skin mucous cells and area of epithelium; GME, ratio between gills mucous cells and area of epithelium; GNE, ratio between number of gill mucous cells and epithelium area.

The mucin gene expression was tissue specific. Skin expressed *muc5ac1*, *muc5ac2*, and *muc5b*. Gills expressed *muc5ac2* and *muc5b*. Distal intestine expressed *muc2*. Differences in the expression of genes in a particular diet group (significant $P<0.05$; tendency $0.05<P< 0.10$) are highlighted using appropriate symbols (Table 6).

Table 6: Mucin and AMPs genes altered by feed ingredients or probiotics.

Tissue	Paper I	Paper II
Skin	*def1* (LP > CT = LP&LF)	*cathl1* (BG5 > BG1 = BG2)
	muc5ac1 (tendency for LP)	*muc5ac1* (with LAB (+) > without LAB (÷))
		muc5ac2 (BG1 > BG5 = BG2)
		muc5b (with LAB (+) > without LAB (÷))
Gills	*muc5ac2* (tendency for LP&LF)	*muc5b* (BG1 = BG5 > BG2)
Intestine		*def3* (BG1 = BG5 > BG2)
		def4 (BG1 = BG5 > BG2)
		cathl1 (BG2 = BG5 > BG1) (with LAB (+) > without LAB (÷))
	muc2 (tendency for LP)	*muc2* (BG1 = BG5 > BG2))

Antimicrobial peptide (AMP) genes; *def1*, defensin1; *def3,* defensin3; *def4*, defensin4; *cathl1*, cathelicidin1. Mucin genes; *muc5ac1*, *muc5ac2*, *muc5b*. Considering a 3*2 factorial design two-way ANOVA was performed to understand the interaction and the effect of the factors. CT in Paper I, control diet without probiotics; LP, CT diet with *Lactobacillus plantarum*; LF, CT diet with *L. fermentum*; and LP&LF, CT diet with both *L. plantarum* and *L. fermentum*. In Paper II, BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed; ÷, without probiotics; +, with probiotics.

Generally, mucin gene expression can be positively attributed to mucin production (Sørensen, 2022). In **Papers I** and **II**, a trend towards negative correlation was observed between mucin gene expression and mucous cell number. Fish fed marine-based diet had significantly higher *muc5ac2* mRNA levels and fewer mucous cells in skin than those that had plant-based ingredients or soybean meal in the diet. Likewise, more mucous cells were observed in the gills and intestine of fish fed the SBM-based (BG2) diet, but the mRNA levels of mucin genes, *muc5b* in the gills, and *muc2* in the intestine were lower compared to those fed plant-based (BG5) or marine-based feed (BG1). This observation was reflected in the negative relationship between the number of mucous cells and mucin gene expression, and these parameters were altered by feed ingredients (Sørensen et al., 2021). Therefore, the relationship between mucous cells

and mucin gene expression seems to be dependent on the type of tissue or the type of feed ingredient.

The health of fish mucosal surfaces including the intestine can also be assessed by evaluating the AMP-related genes (Bridle et al., 2011; Broekman et al., 2013; Chang et al., 2006; Marcos-López et al., 2018), but not many studies have evaluated the effect of feed ingredients on the expression of these genes. The expression of AMP genes appears to be tissue specific. Skin expressed *defensin1* (*def1*) and *cathelicidin1* (*cathl1*) while distal intestine expressed *defensin3* (*def3*), *defensin4* (*def4*), and *cathelicidin1* (*cathl1*). The AMP genes *cathl1* in the skin, *def3*, and *def4* in DI were altered by feed ingredient composition (Table 6). Fish fed a plant-based diet (BG5) had significantly higher *cathl1* mRNA levels in the skin than those fed marine or SBM-based diets (BG1 and BG2; **Paper II**). Fish fed plant-based ingredients (diet BG2 and BG5) had significantly higher *cathl1* mRNA levels in DI than those fed BG1. There seems to be a positive correlation between the *cathelicidin* and number of mucous cells in DI. On the contrary, the mRNA levels of *def3* and *def4* in DI were lower in fish fed SBM-based diet (BG2) compared to those fed plant-based (BG5) or marine-based (BG1) diets. Therefore, the *defensins* in DI appear to have a negative correlation with the number of mucous cells. However, not many studies have evaluated the effect of diet on AMP gene expression. In humans, modified defensin expression is suggested to be an integral element in the pathogenesis of inflammatory bowel disease. In Crohn's disease, reduced alpha-defensin levels are reported in patients with ileal disease and beta-defensin level was reduced in those with colonic involvement (Ramasundara et al., 2009). In the present study, the down regulation of defensins could be due to mucosal surface destruction because of inflammatory changes in fish fed soybean meal, indicating that reduced defensin expression is a symptom of the enteritis and not its cause. Thus, the negative correlation between mucous cells number and defensin/mucin can be an indication of enteritis.

5.1.2 Effects of probiotics

Supplementation of lactic acid bacteria (*L. plantarum* and *L. fermentum*) imparted positive effects on the intestine of Atlantic salmon (**Paper I**). Fish fed a combination of two probiotic species had more supranuclear vacuoles, long villi, and thinner lamina propria (**Paper I**). Positive effects of using multispecies probiotics over single species were also documented by other authors (Hossain et al., 2022; Salinas et al., 2008a). Substrate-dependent mutualism between species could be the reason for this "cocktail" effect on the mucosal health. Intermediate fermentation products such as lactate produced by one group of bacteria can be converted to butyrate by another group of bacteria (Belenguer et al., 2006).

The results of **Paper I** revealed that dual probiotic species were more effective in improving the mucosal health compared to the single probiotic species. Therefore, for the experiment described in **Paper II**, the two LAB species were added to three different basal diets, to examine whether both probiotics would improve the gut health of fish. Fish fed marine-based diet and commercial-like plant-based diet supplemented with probiotics did not show a significant difference in villi height, villi width, and enterocyte height. However, numerically higher values of the parameters (reduced lamina propria width along with increased mucous cell indices and number of IELs) associated with the aforementioned diets indicated that probiotics could improve (not significantly) the mucosal health. On the other hand, significant changes were noted for the fish fed the soybean meal-based diet supplemented with probiotics (**Paper II**). Improved histomorphometric indices were also reported by other authors when different fish species were fed lactic acid bacteria. For example, increased height of villi or microvilli were reported for Nile tilapia fed *Lactobacillus rhamnosus* (Pirarat et al., 2011), rainbow trout fed *Bacillus* spp. (Merrifield et al., 2010) or European lobster fed *Bacillus* spp. (Daniels et al., 2010).

The improved distal intestine indices are likely due to the modulation of the gut microbiota toward specific SCFA producing communities, stimulated by the LABs

supplemented to feeds (**Papers I** and **II**). Fish fed *L. fermentum* had more propionic acid, while *L. plantarum* had more lactic acid (**Paper I**). Acetoacetic, succinic, and total SCFAs were significantly higher in the fish fed the mixture of two probiotic species compared to those fed *L. fermentum*. Other studies have also reported dietary probiotics-caused alteration of SCFAs in the digesta of fishes (Allameh et al., 2017; Asaduzzaman et al., 2018; Burr et al., 2005).

The SCFAs are produced by the commensal microbiota in the distal intestine, by fermenting nondigestible carbohydrates (prebiotics). The shift in SCFA suggests that microbiota was modulated in the fish fed single or dual supplementation of LABs. Shift in the microbiota composition in fish fed *the L. plantarum* and *L. fermentum* was confirmed earlier by Gupta et al. (2019). Bacterial fermentation products (peptides, SCFAs, and vitamins) help to maintain the integrity of the gut (Allameh et al., 2017; Asaduzzaman et al., 2018; Burr et al., 2005). The increased villi height and supranuclear vacuoles in this study (**Papers I** and **II**) is likely indicating SCFA-stimulated epithelial integrity (Ichikawa et al., 1999). Similar observations were also reported in other species fed single probiotics (Cerezuela et al., 2012; Pirarat et al., 2011).

In **Paper I**, the AMP gene, *def1* in the skin was significantly upregulated in fish fed *L. plantarum* compared to those fed CT and *L. plantarum* and *L. fermentum* (LP&LF). Expression of the mucin genes *muc5ac1* in the skin and *muc2* in the intestine tended to increase in fish fed *L. plantarum*, while the probiotic blend (*L. plantarum* and *L. fermentum*) triggered an increasing trend for *muc5ac2* in the gills. In **Paper II**, mucosa-related genes *def3*, *def4*, and *cathl1* were upregulated when probiotics were incorporated in the feeds. These findings are in line with the results of earlier studies about probiotics-mediated stimulation of the immune system through the production of mucins after the recognition by different receptors on goblet cells (Grondin et al., 2020). Probiotics release the proteolytic enzyme, meprin β, which is anchored to the apical membrane of enterocytes. Meprin β cleaves and releases *mucin* from the goblet cells (Schütte et al., 2014). Moreover, structural elements such as lipopolysaccharides

(LPS), flagellin A, and lipoteichoic acids (LTA), or several metabolites of the probiotics, can regulate mucin gene expression (Dharmani et al., 2009).

Probiotics also altered the number of intestinal mucous cells and intraepithelial lymphocytes in the intestine, as well as increased the number of mucous cells in the skin and gills (**Papers I** and **II**). These increased mucous cell count observations are in line with earlier findings (Das et al., 2013; Hernandez et al., 2010; Nimalan et al., 2022; Paone and Cani, 2020; Sewaka et al., 2019). The probiotics may have direct effect on the mucosal barrier function through stimulation of the beneficial commensal microflora colonizing in the distal intestine (Ohland and MacNaughton, 2010), modulating the activity and/or composition of the intestinal immune cells and microbial community (Reid et al., 2011). A more indirect mode of action can be that the probiotics may change the composition of viscoelastic mucus in the skin and gill mucosal barrier by influencing mucin expression or proliferation of mucous cells (Caballero-Franco et al., 2007; Mazziotta et al., 2023). Probiotics that adhere to the epithelial cells could, among others, modulate the production of cytokines, chemokines and mucins by epithelial cells and the produced cytokines activate the Tregulatory (Treg) cells that could eventually establish intestinal homeostasis (Mazziotta et al., 2023; Yousefi et al., 2019). Probiotics can impact the interaction between cells through pattern recognition receptors expressed on macrophages and dendritic cells to support the differentiation of T-cells to Treg cells (Cristofori et al., 2021). These abilities of the dietary probiotics likely have maintained homeostasis (i.e., reduction of infiltrating lymphocytes and lamina propria width, and altered the SCFAs profile) in the intestine of Atlantic salmon fed probiotic coated feeds.

Strengthening the mucosal surfaces of fish may have advantageous effects such as protection from pathogens. Pathogens can easily infect the host when they breach the mucosal barrier. Ectoparasitic infections such as those from sea lice, incur the largest disease-related production cost during the farming of Atlantic salmon (Bjørndal and Tusvik, 2020). Increased knowledge about Atlantic salmon mucosal health and the

interaction with microbiota is essential to manage pathogens and disease outbreaks in farms. Such information may help prevent diseases by enhancing our competence to develop efficacious supplements (for example evidence regarding a positive correlation between healthy characteristics in the intestine and the administered probiotic through the establishment of beneficial gut bacteria can support the use of a probiotics or mucosal vaccines (molecules that are associated with improved immunity can be exploited in vaccine development) that can be easily delivered via dietary probiotics (Adams, 2019; Bledsoe et al., 2022; Ringø et al., 2014).

5.1.3 Effects of Ca-butyrate

The encapsulated Ca-butyrate (ButiPEARL®) supplementation significantly improved the histomorphometric indices of the distal intestine of the fish fed saponins. The villus height, villus width, enterocyte height, supranuclear vacuoles, mucous cells and intraepithelial lymphocytes were increased, while lamina propria width was reduced in fish fed Ca-butyrate (**Paper III**). Butyrate seemed to have more protective effect in the fish fed diet with low saponin content compared to those fed high saponin diet, based on the histomorphometric indices. The results in **Paper III** are in line with other studies about butyrate-caused improvement in the intestinal morphology. Low FM (20%) and FO (10%) diet supplemented with 0.8% Na-butyrate (BP-70 ®Norel) increased mucosal folds, granulocytes in submucosa, and lymphocyte infiltration into the base of epithelium of gilthead seabream compared to the fish fed control diet without butyrate (Estensoro et al., 2016). A 0.2% Na-butyrate supplementation in a 20% SBM diet prevented SBMIE in turbot and increased the absorptive surface of the fish. Moreover, gene expression analysis in their study showed that certain tight junction (TJ) protein genes were upregulated while inflammatory genes (TNF-α and NF-κB) were downregulated significantly in the Na-butyrate group compared to the SBM control group (Liu et al., 2019).

Biological effects of organic acids may vary with product type (salt, ester, or encapsulated) and inclusion level, and rearing conditions (Castillo et al., 2014; Tran-

Ngoc et al., 2019). Nile tilapia fed high SBM (500g/kg) supplemented with Ca-butyrate at 2g/kg under hypoxic conditions had reduced lamina propria and submucosa thickness compared to the control group fed SBM only (Tran-Ngoc et al., 2019). Common carp fed oxidised soybean oil supplemented with Na-butyrate increased microvilli density and prevented intestinal mucosa damage (Liu et al., 2014). Nevertheless, Na-butyrate is a fat-soluble salt and be released quickly in the gut whereas encapsulated form of Ca-butyrate is believed to be released slowly along the gastrointestinal tract (GIT) of host. Thus, effects might be sustained over time (Tran-Ngoc et al., 2019). Only one inclusion level of Ca-butyrate was tested in **Paper III**. Maybe a higher dose would have given more protective effect at the high saponin inclusion level.

Butyrate can also be produced in the intestine via pyruvate-acetyl-CoA-butyryl-CoA pathway by primarily bacteria belonging to Firmicutes through the fermentation of mainly carbohydrates (Rowland et al., 2018). Furthermore, peptides and amino acids such as glutamate, histidine, cysteine, serine, methionine, and branched chain amino acids (lysine, leucine and isoleucine) can also be metabolized by certain bacteria, resulting in end products such as ammonia and biogenic amines (Averina et al., 2020; Diether and Willing, 2019). Fermentation of branched chain amino acids may yield branched chain fatty acids which are related to certain health conditions (Salazar et al., 2022). The bacterial metabolites, SCFAs for example butyrate, the key energy source for epithelial cells has anti-inflammatory properties and hence is also involved in intestinal homeostasis (Corrêa-Oliveira et al., 2016; Donohoe et al., 2011; Palma et al., 2022). This was most likely the mode of action in the fish fed butyrate (**Paper III**). The low concentration of butyrate in the fish fed plant-based ingredients compared to the marine-based diet in **Papers I** and **II** suggest that the prevailing substrates were not optimal in the host gut. However, probiotic (Papers **I** and **II**) or butyrate (**Paper III**) supplementation can help in establishing a healthier microenvironment in the intestine.

Supplementation of butyrate seemed to be an efficient tool to improve the gut health in Atlantic salmon fed plant-based ingredients. Gene expression analysis were not performed to support the histology data. Other studies (in mammals) have shown that SCFAs have direct or indirect effects on cell proliferation, differentiation, and gene expression (Park et al., 2016; Villodre Tudela et al., 2015). Butyrate enhances the epithelial barrier function by modulating genes involved in the TJ components. Butyrate has been shown to induce AMP (β-defensins) genes in *in vitro*, and *in vivo* models (Parada Venegas et al., 2019; Zhao et al., 2018). The anti-inflammatory property of butyrate is through the activation of G-protein coupled receptors, monocarboxylate transporter membrane proteins and Interleukin 10 receptor-mediated suppression of claudin-2 expression (Zheng et al., 2017). In addition, butyrate can enhance the content of the barrier function promoting Interleukin-18 (IL-18) in intestinal epithelial cells and it is also known that IL-18R1 signalling is key to preventing intestinal inflammation with the support of $Foxp3^+$ Treg cells (Kalina et al., 2002). Butyrate also inhibits histone deacetylases (HDACs), and suppresses NF-κB activation, essential processes in preventing inflammation (Macia et al., 2015; Parada Venegas et al., 2019; Thangaraju et al., 2009). Previous studies with butyric acids in aquafeeds of various aquatic animals have tested doses in the range 0.3 – 20 g/kg of butyrate salts (da Silva et al., 2016; Liu et al., 2014; Robles et al., 2013). However, some studies recommended a maximum inclusion level of 50 g/kg for certain organic acids; citric and succinic acids (Fauconneau, 1988; Ng and Koh, 2017; Sugiura et al., 1998). Lipid accretion was increased in Nile tilapia fed 2% or more sodium butyrate (El-Sayed Ali et al., 2018). Therefore, the concentration of organic acids can have negative effects on feed utilization or histomorphometry of intestine. However, these outcomes are likely species specific.

5.1.4 Effects of microalgae meal

Microalgae diets seemed to have improved the gut health of Atlantic salmon (**Paper IV**). The PCA analysis revealed the differential effects of the microalgae on the DI

indices. The values obtained for the fish fed *N. oceanica* can be considered best compared to those fed the plant-based control diet. Positive effects of the plant-based feed supplemented with *N. oceanica* on the intestinal indices of Atlantic salmon were reported earlier by our group (Liu et al., 2022). These observations are in line with other studies where salmonids were fed 20% *Chlorella vulgaris* to SBM-based diet (Grammes et al., 2013). The current PhD project did not evaluate microalgae in connection to an SBMIE model. Another study reported that higher levels (30%) of microalgae (in fishmeal-based feed) gave increased width of villi in the mid intestine and lower enterocyte vacuolation in the distal intestine and was interpreted as signs of immune stimulation (Sørensen et al., 2021).

The results in **Paper IV** indicated that inclusion of 7.5% microalgae to Atlantic salmon feed had no negative effects on the intestinal health, in line with other studies. No negative effects of microalgae on the intestinal morphology were reported when Atlantic salmon were fed *Schizochytrium* sp. (Kousoulaki et al., 2015), *Desmodesmus* sp. (Kiron et al., 2016), or *N. oceanica* (Sørensen et al., 2017), or when *N. oceanica* was fed to European seabass (Batista et al., 2020a), or *Scenedesmus* sp. fed to juvenile rainbow trout (Skalli et al., 2020). It should be noted that different microalgae species and their production techniques might have different effects. Hence, general conclusions should not be drawn for other species than those tested.

The mucous cells in DI were affected by the microalgae, fish fed unprocessed *P. tricornutum* had significantly lower number of mucous cells compared to those fed the control diet or diet containing *Desmodesmus* sp. or *N. oceanica* (**Paper IV**). Studies with other fish species have also reported altered intestinal goblet cell number; for example, *Scenedesmus* sp. (5%) feeding increased the goblet cell density in the anterior intestine of juvenile rainbow trout (Skalli et al., 2020), *N. oceanica* (8%) feeding increased goblet cells in the posterior intestine of European seabass (Batista et al., 2020a), *Schizochytrium* feeding (6 or 15%) increased the intestinal goblet cells in intestine (just after the pyloric caeca) of Atlantic salmon (Kousoulaki et al., 2015).

5.2 Growth performance of Atlantic salmon

5.2.1 Effects of saponin

Weight gain in fish fed saponins (**Paper III**) was lower compared to those fed a control diet without saponins. However, the weight gain in the fish fed low and high levels of saponin were similar (Table 7), suggesting that the lowest inclusion level (0.4%) is enough to simulate the effects induced by 20% SBM, which is more commonly used in enteritis models (Agboola et al., 2022). Saponin alters the expression of tight junction proteins such as claudin, thereby affecting the intercellular transport of macromolecules or permeability (Gu et al., 2018; Piechota-Polanczyk and Fichna, 2014; Rao, 2008; Utech et al., 2010). In addition, the antinutritional factor reduces the digestibility of lipids and energy and uptake of nutrients (Krogdahl et al., 2015; Sørensen et al., 2011b, 2021b; Urán et al., 2008). The negative effect of saponin on weight gain that was observed in the present study is in line with another study by Krogdahl et al. (2015). Such a negative effect of saponin is probably a reflection of gut health and lipid metabolism. The SBMIE condition also reduce brush border membrane enzyme activities (Bakke-McKellep et al., 2000; Krogdahl et al., 2003), reduce bile salt levels (Romarheim et al., 2006; Sørensen et al., 2011b), downregulate genes associated with hepatic biosynthesis (Gu et al., 2014) and cause hypocholesterolemia (Gu et al., 2014, Sørensen et al., 2021). Overall, ANFs present in plant ingredients disturb the mucosal barrier health, thereby negatively affected the growth performance.

Table 7: Growth performance of Atlantic salmon, reported in Papers I-IV.

	Paper I				Paper II						Paper III						Paper IV				
	CT	**LP**	**LF**	**LP&LF**	**BG1÷**	**BG1+**	**BG5÷**	**BG5+**	**BG2÷**	**BG2+**	**CO**	**CB**	**LO**	**LB**	**HO**	**HB**	**CT**	**DX**	**PB**	**PW**	**NW**
IW	135.0	124.7	129.6	127.4	127.0	126.8	123.4	125.7	115.1	117.6	250.6	250.6	250.5	250.5	250.5	250.5	1832.3	1831.0	1823.2	1847.3	1828.3
FW	214.0	200.7	197.7	194.5	194.3	199.5	205.6	194.2	187.4	185.3	541.2	551.3	495.6	515.3	507.2	520.8	4228.4	4100.3	3970.25	4083.1	4069.9
WG%	58.52	60.89	52.51	52.72	52.90	57.33	66.58	54.40	62.97	57.53	290.6	300.7	245.1	264.8	256.8	270.2	130.8	123.9	117.8	121.0	122.6
FL	25.7	25.1	25.1	25.0	24.58	24.76	24.85	24.43	24.80	24.76	35.95	35.84	35.13	35.63	35.33	35.51					
CF	1.27	1.27	1.25	1.25	1.31	1.32	1.34	1.33	1.23	1.22	1.16	1.20	1.14	1.14	1.15	1.16	1.32	1.26	1.31	1.29	1.30
SGR	1.21	1.25	1.11	1.11	1.12	1.19	1.34	1.14	1.29	1.20	1.51	1.55	1.34	1.41	1.38	1.43	0.42	0.41	0.39	0.40	0.40
TGC	2.95	2.97	2.65	2.63	2.64	2.84	3.20	2.69	2.98	2.77	4.64	4.76	4.05	4.31	4.20	4.38	3.75	3.59	3.45	3.54	3.56

CT in **Paper I**, control diet without probiotics; LP, CT diet with *Lactobacillus plantarum*; LF, CT diet with *L. fermentum*; and LP&LF, CT diet with both *L. plantarum* and *L. fermentum*. In **Paper II**, BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed; ÷, without probiotics; +, with probiotics. In **Paper III**, CO, plant-based control diet without saponin & butyrate; CB, diet without saponin with butyrate; LO, diet with low level of saponin without butyrate; LB, diet with low level of saponin with butyrate; HO, diet with high level of saponin without butyrate; HB, diet with high level of saponin with butyrate. CT in **Paper IV**, plant-based control diet; DX, diet with defatted *Desmodesmus* sp.; NW, diet with double extruded *Nannochloropsis oceanica*; PB, diet with bead-milled *Phaeodactylum tricornutum*; and PW, diet with unprocessed *P. tricornutum*. IW, initial weight; FW, final weight; WG%, weight gain in percentage; FL, final length; CF, condition factor; SGR, specific growth rate; TGC, thermal growth coefficient.

5.2.2 Effects of lactic acid bacteria

Weight gain of the fish fed experimental feeds were similar to those fed the control feeds in **Papers I**, **II**, and **IV**. The short experimental period in **Papers I** and **II** is most likely the reason for the insignificant differences in weight gain among the groups fed without or with lactic acid bacteria. It has been reported that LAB improved growth performance due to upregulation of growth-related genes and increased villi features (Abdelfatah and Mahboub, 2018; Dawood et al., 2019; Feng et al., 2019; Jami et al., 2019; Van Nguyen et al., 2019). Other studies have indicated the enhancement of positive effects through dual species supplementation (Aly et al., 2008; Beck et al., 2015; Hai, 2015; Hai et al., 2009). The inclusion level of lactic acid bacteria should, therefore, be optimised in future studies. Our related studies (Cingeľová Maruščáková et al., 2021; Gupta et al., 2019) employed only one dose (10^8 CFU/g feed), mainly based on the results in previous literature with other strains on salmonids (Mohammadian et al., 2022; Soltani et al., 2019; Sumon et al., 2022; Wang et al., 2019).

5.2.3 Effects of Ca-butyrate

The butyrate supplementation improved the growth performance, as described in **Paper III** (Table 7). Positive effect of butyrate on the growth of *Litopenaeus vannamei*, and broilers was reported previously (da Silva et al., 2016; Polycarpo et al., 2017). Butyrate is known to provide energy to the epithelial cells and enrich the mucosa layer. Thus, better nutrient absorption, improved mucosal barrier, and enhanced metabolism together with enteritis alleviation collectively improve the growth performance of the fish (Abdel-Latif et al., 2020; Mallioris et al., 2022; Palma et al., 2022; Tran et al., 2020). Though butyrate improved the growth of Atlantic salmon and alleviated the negative effects of saponins, dose-response studies should be performed in the future to optimise the inclusion level of butyrate.

5.2.4 Effects of microalgae on performance, feed utilization, and proximate composition

Negative effects of microalgae were not noted in Atlantic salmon fed the microalgae incorporated diets. This observation is in line with a farm study performed with slaughter size fish fed *N. oceanica* (Liu et al., 2022) and previous lab scale studies with small (parr and smolt-size) fish fed low levels of microalgae (Kiron et al., 2016; Sørensen et al., 2017). The results of the microalgae study (**Paper IV**) described in this thesis revealed the high feed consumption and feed conversion ratio (FCR) of the fish fed certain microalgae (in case of PB, PW, and DX) compared to the fish fed the control diet. Other studies have shown that lower nutrient availability due to reduced energy digestibility might be the reason for poor FCR (Kiron et al., 2016; Ribeiro et al., 2017; Sørensen et al., 2017). However, proximate composition was similar for all feed groups discussed in **Paper IV** (Table 8). This result corroborates with the findings of previous studies on small salmon fed 5% or 10% *Nanofrustulum* or *Tetraselmis*, respectively (Kiron et al., 2012) as well as 6% *P. tricornutum* (Sørensen et al., 2016).

Table 8: Proximate composition of Atlantic salmon fed different microalgae (Paper IV).

	CT	**DX**	**PB**	**PW**	**NW**
Moisture (%)	65.64	65.62	65.77	65.58	65.56
In dry matter					
Protein (%)	50.16	49.89	49.52	50.15	50.77
Lipid (%)	47.98	49.69	49.27	48.99	46.59
Ash (%)	5.55	5.68	5.80	5.54	5.94
Energy (MJ/kg)	26.84	26.71	27.06	26.89	26.71

CT, plant-based control diet; DX, CT diet with defatted *Desmodesmus* sp.; NW, CT diet with double extruded *Nannochloropsis oceanica*; PB, CT diet with bead-milled *Phaeodactylum tricornutum*; and PW, CT diet with unprocessed *P. tricornutum*.

6. Conclusion

Mucosal surfaces of the skin, gills, and intestine, with their inherent protective barriers supported by an arsenal of immune molecules, are vital for the health of fishes. The present PhD study was designed to investigate the effect of probiotics, Ca-butyrate, or microalgae on fish health. Feed ingredient composition significantly affected the morphology of mucosal surfaces of the skin, gills, and distal intestine as well as mucus related gene expression, and short chain fatty acid composition in the digesta. The specific conclusions from the different experiments are:

1. Single species of *Lactobacillus plantarum* and *L. fermentum* had positive effects on gut health, but combination of two dietary probiotics was more efficient in preventing enteritis in Atlantic salmon. Probiotics altered the short chain fatty acid composition, mucin, and AMP gene expression. Probiotics improved the intestinal indices such as height of villi, height of enterocyte, and supranuclear vacuoles.

2. Mucosal health of salmon was affected by different feed ingredients. Fish fed marine-based diets had better mucosal health than those fed commercial plant-based diet or SBM-based feed. Atlantic salmon fed 20% SBM in the feed developed enteritis. Supplementation of *L. plantarum* and *L. fermentum* improved the mucosal health of fish.

3. Both low (0.4%) and high (0.8%) levels of soy-saponin included in plant-based diet dominated by soy protein concentrate, induced enteritis in Atlantic salmon. The saponin dosage effects on the distal intestinal health were not distinguishable. Dietary encapsulated Ca-butyrate can prevent enteritis in Atlantic salmon, by preserving the features of the intestine.

4. Microalgae can be used to replace fishmeal in plant-based salmon feed without affecting the growth and proximate composition. Lower inclusion (7.5%) of microalgae meal improved the distal intestinal health. *Nannochloropsis oceanica* has the best potential to improve gut health.

7. Future perspective

As the world's leading producer of Atlantic salmon, Norway must find sustainable feed ingredients and solutions to propel the growth ambitions of the aquaculture industry. Results from the present thesis revealed that plant-based aquafeeds may have negative effect on the growth and feed utilization of Atlantic salmon. Hence, supplementation of the lactic acid bacteria *L. plantarum* or *L. fermentum* preferably in combination can be used to improve the mucosal health of the fish. Additives such as Ca-butyrate can also improve the intestinal health of the fish. Since low tropic feed ingredients such as microalgae (at 7.5% inclusion level) can have positive effects on gut health they can be ideal replacers of fishmeal and other ingredients in salmon feeds. The result in the present study showed that feed additives such as probiotics and butyrate can be used to strengthen the mucosal surfaces. The laboratory scale studies should be verified by conducting grow-out phase studies in sea pens to clarify if these additives can reduce mortality and support health of the fish under more stressful conditions.

The studies described in this thesis point to the increase in lymphocytes during enteritis as well as by additives. Future studies should try to understand if the lymphocyte subpopulations altered by feed additives are different from those altered during intestinal inflammation. That will give us a better understanding of the mode of action of probiotics or organic acids. Immunohistochemistry or transmission electron microscopy can also help us to get a deeper understanding of ultra-morphometric indices which might be altered by feed ingredients or additives.

Future studies should also include other inflammatory genes to understand the pro- or anti-inflammatory effects of feed additives, as well as genes related to tight junctions to get a deeper understanding of the changes at the mucosal barrier.

The present study did not investigate the nutrient digestibility or the microbiota profile when fish were supplemented with probiotics, Ca-butyrate, or microalgae. This information should be collected in future studies. The thesis explored the effects on a rather limited number of organs (intestine, skin, and gills). Other tissues such as pyloric ceaca, pancreas, spleen, liver, kidney, heart, and brain can also be of interest. These tissues may also react to inflammation model or show positive effects with the additives.

Pulse feeding of feed additives for a longer period until the fish reach the slaughter size should also be performed to delineate the effect of such a feeding strategy. Future studies can also investigate the product quality and socio-economic performance when the feed additives are used during the entire production cycle.

8. References

Aalamifar, H., Soltanian, S., Vazirzadeh, A., Akhlaghi, M., Morshedi, V., Gholamhosseini, A., Torfi Mozanzadeh, M., 2020. Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*). Aquac. Nutr. 26, 156–164.

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

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

Abdel-Latif, H.M.R., Abdel-Tawwab, M., Dawood, M.A.O., Menanteau-Ledouble, S., El-Matbouli, M., 2020. Benefits of dietary butyric acid, sodium butyrate, and their protected forms in aquafeeds: A review. Rev. Fish. Sci. Aquac. 28, 421–448. https://doi.org/10.1080/23308249.2020.1758899

Abdel-Mohsen, H.H., Wassef, E.A., El-Bermawy, N.M., Abdel-Meguid, N.E., Saleh, N.E., Barakat, K.M., Shaltout, O.E., 2018. Advantageous effects of dietary butyrate on growth, immunity response, intestinal microbiota and histomorphology of European Seabass (*Dicentrarchus labrax*) fry. Egypt. J. Aquat. Biol. Fish. 22, 93–110.

Acién, F.G., Molina, E., Reis, A., Torzillo, G., Zittelli, G.C., Sepúlveda, C., Masojídek, J., 2017. Photobioreactors for the production of microalgae, in: Microalgae-Based Biofuels and Bioproducts. Elsevier, pp. 1–44. https://doi.org/10.1016/B978-0-08-101023-5.00001-7

Adams, A., 2019. Progress, challenges and opportunities in fish vaccine development. Fish Shellfish Immunol. 90, 210–214. https://doi.org/10.1016/j.fsi.2019.04.066

Adarme-Vega, T.C., Lim, D.K.Y., Timmins, M., Vernen, F., Li, Y., Schenk, P.M., 2012. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb. Cell Fact. 11, 96. https://doi.org/10.1186/1475-2859-11-96

Adeshina, I., 2018. The effect of *Lactobacillus acidophilus* as a dietary supplement on nonspecific immune response and disease resistance in juvenile common carp, *Cyprinos carpio*. Int. Food Res. J. 25.

Adorian, T.J., Mombach, P.I., Fagundes, M.B., Wagner, R., Pianesso, D., Telles, Y.B., Dalcin, M.O., da Silva, L.P., 2020. Linseed fibers modulate the production of short-chain fatty acids and improve performance and plasma and skin mucus parameters of silver catfish (*Rhamdia quelen*). Fish Physiol. Biochem. https://doi.org/10.1007/s10695-020-00885-7

Agboola, J.O., Chikwati, E.M., Hansen, J.Ø., Kortner, T.M., Mydland, L.T., Krogdahl, Å., Djordjevic, B., Schrama, J.W., Øverland, M., 2022. A meta-analysis to determine factors associated with the severity of enteritis in Atlantic salmon (*Salmo salar*) fed soybean meal-based diets. Aquaculture 555, 738214. https://doi.org/10.1016/j.aquaculture.2022.738214

Ahmad, A., W. Hassan, S., Banat, F., 2022. An overview of microalgae biomass as a sustainable aquaculture feed ingredient: food security and circular economy. Bioengineered 13, 9521–9547. https://doi.org/10.1080/21655979.2022.2061148

Ahmed, H.A., Sadek, K.M., 2015. Impact of dietary supplementation of sodium butyrate and/or protexin on the growth performance, some blood parameters, and immune response of *Oreochromis niloticus*. Int. J. Agric. Innov. Res. 3, 2147–2319.

Akter, M.N., Hashim, R., Sutriana, A., Siti Azizah, M.N., Asaduzzaman, M., 2019. Effect of *Lactobacillus acidophilus* supplementation on growth performances, digestive enzyme activities and gut histomorphology of striped catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) juveniles. Aquac. Res. 50, 786–797.

Albrektsen, S., Kortet, R., Skov, P.V., Ytteborg, E., Gitlesen, S., Kleinegris, D., Mydland, L., Hansen, J.Ø., Lock, E., Mørkøre, T., James, P., Wang, X., Whitaker, R.D., Vang, B., Hatlen, B., Daneshvar, E., Bhatnagar, A., Jensen, L.B., Øverland, M., 2022. Future feed resources in sustainable salmonid production: A review. Rev. Aquac. https://doi.org/10.1111/raq.12673

Alexander, J.B., Ingram, G.A., 1992. Noncellular nonspecific defence mechanisms of fish. Annu. Rev. Fish Dis. 2, 249–279. https://doi.org/10.1016/0959-8030(92)90066-7

Alishahi, M., Tulaby Dezfuly, Z., Mohammadian, T., Mesbah, M., 2018. Effects of two probiotics, *Lactobacillus plantarum* and *Lactobacillus bulgaricus* on growth performance and intestinal lactic acid bacteria of *Cyprinus carpio*. Iran. J. Vet. Med. 12, 207–218. https://doi.org/10.22059/ijvm.2018.235444.1004816

Allameh, S.K., Ringø, E., Yusoff, F.M., Daud, H.M., Ideris, A., 2017. Dietary supplement of *Enterococcus faecalis* on digestive enzyme activities, short-chain fatty acid production, immune system response and disease resistance of Javanese carp (*Puntius gonionotus*, Bleeker 1850). Aquac. Nutr. 23, 331–338. https://doi.org/10.1111/anu.12397

Alvarez-Pellitero, P., 2008. Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. Vet. Immunol. Immunopathol. 126, 171–198. https://doi.org/10.1016/j.vetimm.2008.07.013

Ameixa, O.M.C.C., Duarte, P.M., Rodrigues, D.P., 2020. Insects, food security and sustainable aquaculture. pp. 1–11. https://doi.org/10.1007/978-3-319-69626-3_111-1

Angahar, L.T., 2016. Applications of probiotics in aquaculture. Am. J. Curr. Microbiol. 4, 66–79.

Asaduzzaman, M., Iehata, S., Akter, S., Kader, M.A., Ghosh, S.K., Khan, M.N.A., Abol-Munafi, A.B., 2018. Effects of host gut-derived probiotic bacteria on gut morphology, microbiota composition and volatile short chain fatty acids production of Malaysian Mahseer *Tor tambroides*. Aquac. Reports 9, 53–61. https://doi.org/10.1016/j.aqrep.2017.12.003

Asche, F., Oglend, A., 2016. The relationship between input-factor and output prices in commodity industries: The case of Norwegian salmon aquaculture. J. Commod. Mark. 1, 35–47. https://doi.org/10.1016/j.jcomm.2015.11.001

Averina, O. V., Zorkina, Y.A., Yunes, R.A., Kovtun, A.S., Ushakova, V.M., Morozova, A.Y., Kostyuk, G.P., Danilenko, V.N., Chekhonin, V.P., 2020. Bacterial metabolites of human gut microbiota correlating with depression. Int. J. Mol. Sci. 21, 1–40. https://doi.org/10.3390/ijms21239234

Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: A comparison with the intestines of fasted fish. J. Fish Dis. 19, 375–387. https://doi.org/10.1111/j.1365-2761.1996.tb00376.x

Bailly, Y., Dunel-Erb, S., Laurent, P., 1992. The neuroepithelial cells of the fish gill filament: Indolamine-immunocytochemistry and innervation. Anat. Rec. 233, 143–161. https://doi.org/10.1002/ar.1092330118

Baindara, P., Mandal, S.M., 2019. Antimicrobial peptides and vaccine development to control multi-drug resistant bacteria. Protein Pept. Lett. 26, 324–331. https://doi.org/10.2174/0929866526666190228162751

Bakke-McKellep, A M, Frøystad, M.K., Lilleeng, E., Dapra, F., Refstie, S., Krogdahl, Å., Landsverk, T., 2007. Response to soy: T-cell-like reactivity in the intestine of Atlantic salmon, *Salmo salar* L. J. Fish Dis. 30. https://doi.org/10.1111/j.1365-2761.2007.00769.x

Bakke-McKellep, Anne Marie, Penn, M.H., Salas, P.M., Refstie, S., Sperstad, S., Landsverk, T., Ringø, E., Krogdahl, Å., 2007. Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). Br. J. Nutr. 97, 699–713. https://doi.org/10.1017/S0007114507381397

Bakke-McKellep, A.M., Press, C.M., Baeverfjord, G., Krogdahl, A., Landsverk, T., 2000. Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. J. Fish Dis. 23, 115–127. https://doi.org/10.1046/j.1365-2761.2000.00218.x

Bansemer, M.S., Forder, R.E.A., Howarth, G.S., Suitor, G.M., Bowyer, J., Stone, D.A.J., 2015. The effect of dietary soybean meal and soy protein concentrate on the intestinal mucus layer and development of subacute enteritis in Yellowtail Kingfish (*Seriola lalandi*) at suboptimal water temperature. Aquac. Nutr. 21, 300–310. https://doi.org/10.1111/anu.12160

Bartley, D.M., 2022. World aquaculture 2020 – A brief overview. FAO. https://doi.org/10.4060/cb7669en

Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Messina, M., Silva, J.L., Abreu, H., Valente, L.M.P., 2020a. Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. J. Appl. Phycol. 32, 2041–2059. https://doi.org/10.1007/s10811-020-02118-z

Batista, S., Pintado, M., Marques, A., Abreu, H., Silva, J.L., Jessen, F., Tulli, F., Valente, L.M.P., 2020b. Use of technological processing of seaweed and microalgae as strategy to improve their apparent digestibility coefficients in European seabass (*Dicentrarchus labrax*) juveniles. J. Appl. Phycol. 32, 3429–3446. https://doi.org/10.1007/s10811-020-02185-2

Beck, B.H., Peatman, E., 2015. Mucosal health in aquaculture. Academic Press.

Belenguer, A., Duncan, S.H., Calder, A.G., Holtrop, G., Louis, P., Lobley, G.E., Flint, H.J., 2006. Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. Appl. Environ. Microbiol. 72, 3593–3599. https://doi.org/10.1128/AEM.72.5.3593-3599.2006

Belghit, I., Liland, N.S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å., Lock, E.-J., 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). Aquaculture 503, 609–619. https://doi.org/10.1016/j.aquaculture.2018.12.032

Bendiksen, E.Å., Johnsen, C.A., Olsen, H.J., Jobling, M., 2011. Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 314, 132–139. https://doi.org/10.1016/j.aquaculture.2011.01.040

Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., Gil, A., 2012. Probiotic mechanisms of action. Ann. Nutr. Metab. 61, 160–174. https://doi.org/10.1159/000342079

Bernaerts, T.M.M., Gheysen, L., Kyomugasho, C., Jamsazzadeh Kermani, Z., Vandionant, S., Foubert, I., Hendrickx, M.E., Van Loey, A.M., 2018. Comparison of microalgal biomasses as functional food ingredients: Focus on the composition of cell wall related polysaccharides. Algal Res. 32, 150–161. https://doi.org/10.1016/j.algal.2018.03.017

Betancor, M.B., Li, K., Bucerzan, V.S., Sprague, M., Sayanova, O., Usher, S., Han, L., Norambuena, F., Torrissen, O., Napier, J.A., Tocher, D.R., Olsen, R.E., 2018. Oil from transgenic *Camelina sativa* containing over 25 % n -3 long-chain PUFA as the major lipid source in feed for Atlantic salmon (*Salmo salar*). Br. J. Nutr. 119, 1378–1392. https://doi.org/10.1017/S0007114518001125

Bihan, D.G., Rydzak, T., Wyss, M., Pittman, K., McCoy, K.D., Lewis, I.A., 2022. Method for absolute quantification of short chain fatty acids via reverse phase chromatography mass spectrometry. PLoS One 17, e0267093. https://doi.org/10.1371/journal.pone.0267093

Bjørgen, H., Li, Y., Kortner, T.M., Krogdahl, Å., Koppang, E.O., 2020. Anatomy, immunology, digestive physiology and microbiota of the salmonid intestine: Knowns and unknowns under the impact of an expanding industrialized production. Fish Shellfish Immunol. 107, 172–186. https://doi.org/10.1016/j.fsi.2020.09.032

Bjørndal, T., Tusvik, A., 2020. Economic analysis of on-growing of salmon post-smolts. Aquac. Econ. Manag. 24, 355–386. https://doi.org/10.1080/13657305.2020.1737272

Bledsoe, J.W., Pietrak, M.R., Burr, G.S., Peterson, B.C., Small, B.C., 2022. Functional feeds marginally alter immune expression and microbiota of Atlantic salmon (*Salmo salar*) gut, gill, and skin mucosa though evidence of tissue-specific signatures and host–microbe coadaptation remain. Anim. Microbiome 4. https://doi.org/10.1186/s42523-022-00173-0

Booman, M., Forster, I., Vederas, J.C., Groman, D.B., Jones, S.R.M., 2018. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*). Aquaculture 483, 238–243. https://doi.org/10.1016/j.aquaculture.2017.10.025

Bosi, G., Arrighi, S., Di Giancamillo, A., Domeneghini, C., 2005. Histochemistry of glycoconjugates in mucous cells of *Salmo trutta* uninfected and naturally parasitized with intestinal helminths. Dis. Aquat. Organ. https://doi.org/10.3354/dao064045

Bou, M., Berge, G.M., Baeverfjord, G., Sigholt, T., Østbye, T.K., Ruyter, B., 2017. Low levels of very-long-chain n-3 PUFA in Atlantic salmon (*Salmo salar*) diet reduce fish robustness under challenging conditions in sea cages. J. Nutr. Sci. https://doi.org/10.1017/jns.2017.28

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

Bridle, A., Nosworthy, E., Polinski, M., Nowak, B., 2011. Evidence of an antimicrobial-immunomodulatory role of Atlantic salmon cathelicidins during infection with *Yersinia ruckeri*. PLoS One 6, e23417. https://doi.org/10.1371/journal.pone.0023417

Brinchmann, M., Patel, D., Pinto, N., Iversen, M., 2018. Functional aspects of fish mucosal lectins—Interaction with non-self. Molecules 23, 1119. https://doi.org/10.3390/molecules23051119

Broekman, D.C., Guðmundsson, G.H., Maier, V.H., 2013. Differential regulation of cathelicidin in salmon and cod. Fish Shellfish Immunol. 35, 532–538. https://doi.org/10.1016/j.fsi.2013.05.005

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture, in: Aquaculture. https://doi.org/10.1016/S0044-8486(96)01501-3

Bruni, L., Randazzo, B., Cardinaletti, G., Zarantoniello, M., Mina, F., Secci, G., Tulli, F., Olivotto, I., Parisi, G., 2020. Dietary inclusion of full-fat *Hermetia illucens* prepupae meal in practical diets for rainbow trout (*Oncorhynchus mykiss*): Lipid metabolism and fillet quality investigations. Aquaculture 529, 735678. https://doi.org/10.1016/j.aquaculture.2020.735678

Buddington, R.K., Chen, J.W., Diamond, J., 1987. Genetic and phenotypic adaptation of intestinal nutrient transport to diet in fish. J. Physiol. 393, 261–281. https://doi.org/10.1113/jphysiol.1987.sp016823

Bullock, A.M., Marks, R., Roberts, R.J., 1978. The cell kinetics of teleost fish epidermis: Epidermal mitotic activity in relation to wound healing at varying temperatures in plaice (*Pleuronectes platessa*). J. Zool. 185, 197–204. https://doi.org/10.1111/j.1469-7998.1978.tb03321.x

Bureau, D.P., Harris, A.M., Young Cho, C., 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161, 27–43. https://doi.org/10.1016/S0044-8486(97)00254-8

Burr, G., Gatlin, D., Ricke, S., 2005. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in Finnish aquaculture. J. World Aquac. Soc. 36, 425–436. https://doi.org/10.1111/j.1749-7345.2005.tb00390.x

Butprom, S., Phumkhachorn, P., Rattanachaikunsopon, P., 2013. Effect of *Lactobacillus plantarum* C014 on innate immune response and disease resistance against *Aeromonas hydrophila* in hybrid catfish. Sci. World J. 2013, 1–6. https://doi.org/10.1155/2013/392523

Caballero-Franco, C., Keller, K., De Simone, C., Chadee, K., 2007. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am. J. Physiol. Liver Physiol. 292, G315–G322. https://doi.org/10.1152/ajpgi.00265.2006

Caballero, M.., Izquierdo, M.., Kjørsvik, E., Montero, D., Socorro, J., Fernández, A.., Rosenlund, G., 2003. Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. Aquaculture 225, 325–340. https://doi.org/10.1016/S0044-8486(03)00299-0

Cai, J., Lovatelli, A., Aguilar-Manjarrez, J. Cornish, L., Dabbadie, L., Desrochers, A., Diffey, S., Garrido Gamarro, E., Geehan, J., Hurtado, A., Lucente, D., Mair, G., Miao, W., Potin, P., Przybyla, C., Reantaso, M., Roubach, R., Tauati, M., Yuan, X., 2021. Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. FAO, Rome. https://doi.org/10.4060/cb5670en

Castillo, S., Rosales, M., Pohlenz, C., Gatlin, D.M., 2014. Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*. Aquaculture 433, 6–12. https://doi.org/10.1016/j.aquaculture.2014.05.038

Castro, C., Couto, A., Diógenes, A.F., Corraze, G., Panserat, S., Serra, C.R., Oliva-Teles, A., 2019. Vegetable oil and carbohydrate-rich diets marginally affected intestine histomorphology, digestive enzymes activities, and gut microbiota of gilthead sea bream juveniles. Fish Physiol. Biochem. 45, 681–695. https://doi.org/10.1007/s10695-018-0579-9

Cerezuela, R., Guardiola, F.A., Meseguer, J., Esteban, M.Á., 2012. Enrichment of gilthead seabream (*Sparus aurata* L.) diet with microalgae: Effects on the immune system. Fish Physiol. Biochem. 38, 1729–1739. https://doi.org/10.1007/s10695-012-9670-9

Chang, C.-I., Zhang, Y.-A., Zou, J., Nie, P., Secombes, C.J., 2006. Two cathelicidin genes are present in both rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Antimicrob. Agents Chemother. 50, 185–195. https://doi.org/10.1128/AAC.50.1.185-195.2006

Chatterjee, S., 2014. Artefacts in histopathology. J. Oral Maxillofac. Pathol. 18, 111. https://doi.org/10.4103/0973-029X.141346

Chen, W., Ai, Q., Mai, K., Xu, W., Liufu, Z., Zhang, W., Cai, Y., 2011. Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). Aquaculture 318, 95–100. https://doi.org/10.1016/j.aquaculture.2011.04.050

Chu, E.W., Karr, J.R., 2017. Environmental impact: Concept, consequences, measurement ☆, in: Reference Module in Life Sciences. Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.02380-3

Cingeľová Maruščáková, I., Schusterová, P., Popelka, P., Gancarčíková, S., Csank, T., Fečkaninová, A., Ratvaj, M., Mudroňová, D., 2021. Effect of autochthonous lactobacilli on immunologically important molecules of rainbow trout after bacterial infection studied on intestinal primoculture. Fish Shellfish Immunol. 119, 379–383. https://doi.org/10.1016/j.fsi.2021.10.021

Corrêa-Oliveira, R., Fachi, J.L., Vieira, A., Sato, F.T., Vinolo, M.A.R., 2016. Regulation of immune cell function by short-chain fatty acids. Clin. Transl. Immunol. 5, e73. https://doi.org/10.1038/cti.2016.17

Cottrell, R.S., Blanchard, J.L., Halpern, B.S., Metian, M., Froehlich, H.E., 2020. Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. Nat. Food 1, 301–308. https://doi.org/10.1038/s43016-020-0078-x

Cristofori, F., Dargenio, V.N., Dargenio, C., Miniello, V.L., Barone, M., Francavilla, R., 2021. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: A door to the body. Front. Immunol. 12, 1–21. https://doi.org/10.3389/fimmu.2021.578386

Current World Population [WWW Document], 2022. URL https://www.worldometers.info/world-population/#:~:text=Population in the world is,it was at around 2%25.

D.C. Shields, V., Heinbockel, T., 2019. Introductory chapter: Histological microtechniques, in: Histology. IntechOpen. https://doi.org/10.5772/intechopen.82017

da Silva, B.C., Vieira, F. do N., Mouriño, J.L.P., Bolivar, N., Seiffert, W.Q., 2016. Butyrate and propionate improve the growth performance of *Litopenaeus vannamei*. Aquac. Res. 47, 612–623. https://doi.org/10.1111/are.12520

Dang, M., Pittman, K., Sonne, C., Hansson, S., Bach, L., Søndergaard, J., Stride, M., Nowak, B., 2020. Histological mucous cell quantification and mucosal mapping reveal different aspects of mucous cell responses in gills and skin of shorthorn sculpins (*Myoxocephalus scorpius*). Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2020.03.020

Das, A., Nakhro, K., Chowdhury, S., Kamilya, D., 2013. Effects of potential probiotic *Bacillus amyloliquifaciens* FPTB16 on systemic and cutaneous mucosal immune responses and disease resistance of catla (*Catla catla*). Fish Shellfish Immunol. 35, 1547–1553. https://doi.org/10.1016/j.fsi.2013.08.022

Dash, G., Raman, R.P., Prasad, K.P., Makesh, M., Pradeep, M.A., Sen, S., 2014. Evaluation of *Lactobacillus plantarum* as feed supplement on host associated microflora, growth, feed efficiency, carcass biochemical composition and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Aquaculture 432, 225–236.

Dash, S., Das, S.K., Samal, J., Thatoi, H.N., 2018. Epidermal mucus, a major determinant in fish health: a review. Iran. J. Vet. Res. 19, 72–81.

Dawood, M.A.O., Koshio, S., Esteban, M.Á., 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquac. 10, 950–974. https://doi.org/10.1111/raq.12209

De Santis, C., Tocher, D.R., Ruohonen, K., El-Mowafi, A., Martin, S.A.M., Dehler, C.E., Secombes, C.J., Crampton, V., 2016. Air-classified faba bean protein concentrate is efficiently utilized as a dietary protein source by post-smolt Atlantic salmon (*Salmo salar*). Aquaculture 452, 169–177. https://doi.org/10.1016/j.aquaculture.2015.10.035

Diether, N.E., Willing, B.P., 2019. Microbial fermentation of dietary protein: An important factor in diet–microbe–host interaction. Microorganisms 7. https://doi.org/10.3390/microorganisms7010019

Dong, D., Xie, W., Liu, M., 2020. Alteration of cell junctions during viral infection. Thorac. Cancer 11, 519–525. https://doi.org/10.1111/1759-7714.13344

Donohoe, D.R., Garge, N., Zhang, X., Sun, W., O'Connell, T.M., Bunger, M.K., Bultman, S.J., 2011. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab. 13, 517–526. https://doi.org/10.1016/j.cmet.2011.02.018

Dunel-Erb, S., Chevalier, C., Laurent, P., 1994. Distribution of neuroepithelial cells and neurons in the trout gill filament: comparison in spring and winter. Can. J. Zool. 72, 1794–1799. https://doi.org/10.1139/z94-243

Easy, R.H., Ross, N.W., 2010. Changes in Atlantic salmon *Salmo salar* mucus components following short- and long-term handling stress. J. Fish Biol. 77, 1616–1631. https://doi.org/10.1111/j.1095-8649.2010.02796.x

Easy, R.H., Ross, N.W., 2009. Changes in Atlantic salmon (*Salmo salar*) epidermal mucus protein composition profiles following infection with sea lice (*Lepeophtheirus salmonis*). Comp. Biochem. Physiol. Part D Genomics Proteomics 4, 159–167. https://doi.org/10.1016/j.cbd.2009.02.001

Einarsson, M.I., Jokumsen, A., Bæk, A.M., Jacobsen, C., Pedersen, S.A., Samuelsen, T.A., Pálsson, J., Eliasen, O., Flesland, O., 2019. Nordic centre of excellence network in fishmeal and fish oil.

El-Sayed Ali, T., El-Sayed, A.M., Eissa, M.A.-R., Hanafi, H.M., 2018. Effects of dietary Biogen and sodium butyrate on hematological parameters, immune response, and histological characteristics of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquac. Int. 26, 139–150. https://doi.org/10.1007/s10499-017-0205-3

Elliott, D.G., 2011. The skin | Functional morphology of the integumentary system in fishes, in: Encyclopedia of Fish Physiology. Elsevier, pp. 476–488. https://doi.org/10.1016/B978-0-12-374553-8.00108-8

Elliott, D.G., 2000. Integumentary system, in: The Laboratory Fish. Elsevier, pp. 95–108. https://doi.org/10.1016/B978-012529650-2/50008-1

EMS, 2023. Paraformaldehyde - glutaraldehyde solution (Karnovsky's fixative) [WWW Document]. URL https://www.emsdiasum.com/docs/technical/datasheet/15720
Encarnação, P., 2016. Functional feed additives in aquaculture feeds, in: Nates, S.F. (Ed.), Aquafeed Formulation. Elsevier Science & Technology, pp. 217–238.
Enferadi, M.H.N., Mohammadizadeh, F., Soltani, M., Bahri, A.H., Sheikhzadeh, N., 2018. Effects of *Lactobacillus plantarum* on growth performance, proteolytic enzymes activity and intestine morphology in rainbow trout (*Oncorhynchus mykiss*). Turkish J. Fish. Aquat. Sci. 18, 351–356.
Esteban, M.Á., 2012. An overview of the immunological defenses in fish skin. ISRN Immunol. 2012, 1–29. https://doi.org/10.5402/2012/853470
Estensoro, I., Ballester-Lozano, G., Benedito-Palos, L., Grammes, F., Martos-Sitcha, J.A., Mydland, L.-T., Calduch-Giner, J.A., Fuentes, J., Karalazos, V., Ortiz, Á., Øverland, M., Sitjà-Bobadilla, A., Pérez-Sánchez, J., 2016. Dietary butyrate helps to restore the intestinal status of a marine teleost (*Sparus aurata*) fed extreme diets low in fish meal and fish oil. PLoS One 11, e0166564. https://doi.org/10.1371/journal.pone.0166564
European Union, 2017. Commission Regulation (EU) 2017/ 893 - of 24 May 2017 - amending Annexes I and IV to Regulation (EC) No 999 / 2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142 / 2011 as regards the provision. Off. J. Eur. Union 60, 92–116.
Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177. https://doi.org/10.1152/physrev.00050.2003
Fagan, M.S., O'Byrne-Ring, N., Ryan, R., Cotter, D., Whelan, K., Mac Evilly, U., 2003. A biochemical study of mucus lysozyme, proteins and plasma thyroxine of Atlantic salmon (*Salmo salar*) during smoltification. Aquaculture 222, 287–300. https://doi.org/10.1016/S0044-8486(03)00128-5
FAO, 2022. The state of world fisheries and aquaculture 2022. Towards blue transformation. FAO, Rome. https://doi.org/10.4060/cc0461en
FAO, 2020. The state of world fisheries and aquaculture 2020. FAO. https://doi.org/10.4060/ca9229en
FAO, 2010. Sustainable diets and biodiversity. Directions and solutions for policy, research and action [WWW Document]. URL http://www.fao.org/3/i3004e/i3004e.pdf
FAO, 2002. Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food.
Farmery, A.K., Alexander, K., Anderson, K., Blanchard, J.L., Carter, C.G., Evans, K., Fischer, M., Fleming, A., Frusher, S., Fulton, E.A., Haas, B., MacLeod, C.K., Murray, L., Nash, K.L., Pecl, G.T., Rousseau, Y., Trebilco, R., van Putten, I.E., Mauli, S., Dutra, L., Greeno, D., Kaltavara, J., Watson, R., Nowak, B., 2022. Food for all: designing sustainable and secure future seafood systems. Rev. Fish Biol. Fish. 32, 101–121. https://doi.org/10.1007/s11160-021-09663-x
Fast, M.., Sims, D.., Burka, J.., Mustafa, A., Ross, N.., 2002. Skin morphology and humoral non-specific defence parameters of mucus and plasma in rainbow trout, coho and Atlantic salmon. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 132, 645–657. https://doi.org/10.1016/S1095-6433(02)00109-5
Fast, M., Ross, N., Mustafa, A., Sims, D., Johnson, S., Conboy, G., Speare, D., Johnson, G., Burka, J., 2002. Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. Dis. Aquat. Organ. 52, 57–68. https://doi.org/10.3354/dao052057
Fauconneau, B., 1988. Partial substitution of protein by a single amino acid or an organic acid in rainbow trout diets. Aquaculture 70, 97–106. https://doi.org/10.1016/0044-8486(88)90009-9
Faul, F., Erdfelder, E., Buchner, A., Lang, A.-G., 2009. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav. Res. Methods 41, 1149–1160. https://doi.org/10.3758/BRM.41.4.1149

Faure, M., Moënnoz, D., Montigon, F., Mettraux, C., Breuillé, D., Ballèvre, O., 2005. Dietary threonine restriction specifically reduces intestinal mucin synthesis in rats. J. Nutr. 135, 486–491. https://doi.org/10.1093/jn/135.3.486

Fečkaninová, A., Koščová, J., Mudroňová, D., Schusterová, P., Cingeľová Maruščáková, I., Popelka, P., 2019. Characterization of two novel lactic acid bacteria isolated from the intestine of rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Slovakia. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.03.026

Ferguson, H., 2006. Systemic pathology of fish: a text and atlas of normal tissue responses in teleosts, and their responses in disease, 2nd ed. Scotian Press, London.

Ferguson, H.W., 1989. Skin, in: Ferguson, H.W. (Ed.), Systemic Pathology of Fish. Iowa State University Press, Ames, Iowa, pp. 43–63.

Fiedler, S., Wünnemann, H., Hofmann, I., Theobalt, N., Feuchtinger, A., Walch, A., Schwaiger, J., Wanke, R., Blutke, A., 2020. A practical guide to unbiased quantitative morphological analyses of the gills of rainbow trout (*Oncorhynchus mykiss*) in ecotoxicological studies. PLoS One 15, e0243462. https://doi.org/10.1371/journal.pone.0243462

Fiskeridirektoratet, 2022. Statistics for aquaculture [WWW Document]. URL https://www.fiskeridir.no/English/Aquaculture/Statistics

Fletcher, T.C., Jones, R., Reid, L., 1976. Identification of glycoproteins in goblet cells of epidermis and gill of plaice (*Pleuronectes platessa* L.), flounder (*Platichthys flesus* (L.)) and rainbow trout (*Salmo gairdneri* Richardson). Histochem. J. 8, 597–608. https://doi.org/10.1007/BF01003961

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. https://doi.org/10.1016/S0044-8486(01)00526-9

Gancarcikova, S., Lauko, S., Hrckova, G., Andrejcakova, Z., Hajduckova, V., Madar, M., Kolesar Fecskeova, L., Mudronova, D., Mravcova, K., Strkolcova, G., Nemcova, R., Kacirova, J., Staskova, A., Vilcek, S., Bomba, A., 2020. Innovative animal model of DSS-induced ulcerative colitis in pseudo germ-free mice. Cells. https://doi.org/10.3390/cells9122571

Gao, Q., Xiao, C., Min, M., Zhang, C., Peng, S., Shi, Z., 2016. Effects of probiotics dietary supplementation on growth performance, innate immunity and digestive enzymes of silver pomfret, Pampus argenteus. Indian J. Anim. Res. 50, 936–941.

Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, E., Mola, P., Chatzifotis, S., 2016. Tenebrio molitor meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220, 34–45. https://doi.org/10.1016/j.anifeedsci.2016.07.003

Gatesoupe, F.-J., 2008. Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. Microb. Physiol. 14, 107–114.

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

Gong, Y., Bandara, T., Huntley, M., Johnson, Z.I., Dias, J., Dahle, D., Sørensen, M., Kiron, V., 2019. Microalgae *Scenedesmus* sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (*Salmo salar* L.). Aquaculture. https://doi.org/10.1016/j.aquaculture.2018.11.049

Gong, Y., Guterres, H.A.D.S., Huntley, M., Sørensen, M., Kiron, V., 2018. Digestibility of the defatted microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon, *Salmo salar*. Aquac. Nutr. 24, 56–64. https://doi.org/10.1111/anu.12533

Grammes, F., Reveco, F.E., Romarheim, O.H., Landsverk, T., Mydland, L.T., Øverland, M., 2013. *Candida utilis* and *Chlorella vulgaris* counteract intestinal inflammation in Atlantic salmon (*Salmo salar* L.). PLoS One 8. https://doi.org/10.1371/journal.pone.0083213

Grondin, J.A., Kwon, Y.H., Far, P.M., Haq, S., Khan, W.I., 2020. Mucins in intestinal mucosal defense and inflammation: Learning from clinical and experimental studies. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.02054

Gu, M., Bai, N., Zhang, Y., Krogdahl, Å., 2016. Soybean meal induces enteritis in turbot *Scophthalmus maximus* at high supplementation levels. Aquaculture 464, 286–295. https://doi.org/10.1016/j.aquaculture.2016.06.035

Gu, M., Jia, Q., Zhang, Z., Bai, N., Xu, X., Xu, B., 2018. Soya-saponins induce intestinal inflammation and barrier dysfunction in juvenile turbot (*Scophthalmus maximus*). Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2018.04.004

Gu, M., Kortner, T.M., Penn, M., Hansen, A.K., Krogdahl, Å., 2014. Effects of dietary plant meal and soya-saponin supplementation on intestinal and hepatic lipid droplet accumulation and lipoprotein and sterol metabolism in Atlantic salmon (*Salmo salar* L.). Br. J. Nutr. 111, 432–444. https://doi.org/10.1017/S0007114513002717

Guardiola, F.A., Cuesta, A., Abellán, E., Meseguer, J., Esteban, M.A., 2014. Comparative analysis of the humoral immunity of skin mucus from several marine teleost fish. Fish Shellfish Immunol. 40, 24–31. https://doi.org/10.1016/j.fsi.2014.06.018

Gupta, S., Fečkaninová, A., Lokesh, J., Koščová, J., Sørensen, M., Fernandes, J., Kiron, V., 2019. *Lactobacillus* dominate in the intestine of Atlantic salmon fed dietary probiotics. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.03247

Hai, N. V., 2015. The use of probiotics in aquaculture. J. Appl. Microbiol. 119, 917–935. https://doi.org/10.1111/jam.12886

Hansen, A.K.G., Kortner, T.M., Krasnov, A., Björkhem, I., Penn, M., Krogdahl, Å., 2020. Choline supplementation prevents diet induced gut mucosa lipid accumulation in post-smolt Atlantic salmon (*Salmo salar* L.). BMC Vet. Res. 16, 32. https://doi.org/10.1186/s12917-020-2252-7

Hansen, L., 2019. The weak sustainability of the salmon feed transition in Norway – A bioeconomic case study. Front. Mar. Sci. 6. https://doi.org/10.3389/fmars.2019.00764

Harris, J.E., Hunt, S., 1975. The fine structure of the epidermis of two species of salmonid fish, the Atlantic salmon (*Salmo salar* L.) and the brown trout (*Salmo trutta* L.). Cell Tissue Res. 163. https://doi.org/10.1007/BF00218498

Hartviksen, M.B., 2015. Replacement of fishmeal with alternative proteins in diets for Atlantic salmon (*Salmo salar* L.): A study on the microbiota, morphology and function of the intestine.

Haugarvoll, E., Bjerkås, I., Nowak, B.F., Hordvik, I., Koppang, E.O., 2008. Identification and characterization of a novel intraepithelial lymphoid tissue in the gills of Atlantic salmon. J. Anat. 213, 202–209. https://doi.org/10.1111/j.1469-7580.2008.00943.x

Hawkes, J.W., 1974. The structure of fish skin. Cell Tissue Res. 149, 147–158. https://doi.org/10.1007/bf00222270

Hedrera, M.I., Galdames, J.A., Jimenez-Reyes, M.F., Reyes, A.E., Avendaño-Herrera, R., Romero, J., Feijóo, C.G., 2013. Soybean meal induces intestinal inflammation in Zebrafish larvae. PLoS One 8, e69983. https://doi.org/10.1371/journal.pone.0069983

Hekmatpour, F., Torfi Mozanzadeh, M., 2021. Legumes, sustainable alternative protein sources for aquafeeds, in: Legumes - Volume 2 [Working Title]. IntechOpen. https://doi.org/10.5772/intechopen.99778

Helfman, G., Collette, B.B., Facey, D.E., Bowen, B.W., 2009. The diversity of fishes: Biology, evolution, and ecology, 2nd ed. John Wiley & Sons.

Hemaiswarya, S., Raja, R., Ravikumar, R., Carvalho, I.S., 2013. Mechanism of action of probiotics. Brazilian Arch. Biol. Technol. 56, 113–119. https://doi.org/10.1590/S1516-89132013000100015

Hernandez, L.H.H., Barrera, T.C., Mejia, J.C., Mejia, G.C., Del Carmen, M., Dosta, M., de Lara Andrade, R., Sotres, J.A.M., 2010. Effects of the commercial probiotic *Lactobacillus casei* on the growth, protein content of skin mucus and stress resistance of juveniles of the Porthole livebearer *Poecilopsis gracilis* (Poecilidae). Aquac. Nutr. 16, 407–411. https://doi.org/10.1111/j.1365-2095.2009.00679.x

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., Sanders, M.E., 2014. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11, 506–514. https://doi.org/10.1038/nrgastro.2014.66

Hoseinifar, S.H., Roosta, Z., Hajimoradloo, A., Vakili, F., 2015. The effects of *Lactobacillus acidophilus* as feed supplement on skin mucosal immune parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (*Xiphophorus helleri*). Fish Shellfish Immunol. 42, 533–538. https://doi.org/10.1016/j.fsi.2014.12.003

Hoseinifar, S.H., Sun, Y.Z., Caipang, C.M., 2017. Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquac. Res. https://doi.org/10.1111/are.13239

Hoseinifar, S.H., Sun, Y.Z., Wang, A., Zhou, Z., 2018. Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Front. Microbiol. 9, 1–18. https://doi.org/10.3389/fmicb.2018.02429

Hossain, M.K., Hossain, M.M., Mim, Z.T., Khatun, H., Hossain, M.T., Shahjahan, M., 2022. Multi-species probiotics improve growth, intestinal microbiota and morphology of Indian major carp mrigal *Cirrhinus cirrhosus*. Saudi J. Biol. Sci. 29, 103399. https://doi.org/10.1016/j.sjbs.2022.103399

Hu, H., Kortner, T.M., Gajardo, K., Chikwati, E., Tinsley, J., Krogdahl, Å., 2016. Intestinal fluid permeability in Atlantic salmon (*Salmo salar* L.) is affected by dietary protein source. PLoS One 11, e0167515. https://doi.org/10.1371/journal.pone.0167515

Hua, K., Cobcroft, J.M., Cole, A., Condon, K., Jerry, D.R., Mangott, A., Praeger, C., Vucko, M.J., Zeng, C., Zenger, K., Strugnell, J.M., 2019. The future of aquatic protein: Implications for protein sources in aquaculture diets. One Earth 1, 316–329. https://doi.org/10.1016/j.oneear.2019.10.018

Huang, B.L., Chandra, S., Shih, D.Q., 2012. Skin manifestations of inflammatory bowel disease. Front. Physiol. 3 FEB, 1–13. https://doi.org/10.3389/fphys.2012.00013

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54, 187–211. https://doi.org/10.2307/1942661

Ichikawa, H., Kuroiwa, T., Inagaki, A., Shineha, R., Nishihira, T., Satomi, S., Sakata, T., 1999. Probiotic bacteria stimulate gut epithelial cell proliferation in rat. Dig. Dis. Sci. 44, 2119–2123. https://doi.org/10.1023/a:1026647024077

Iger, Y., Balm, P.H.M., Jenner, H.A., Bonga, S.E.., 1995. Cortisol induces stress-related changes in the skin of rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. IVE Endocrinol. 97, 188–198.

Jami, M.J., Abedian Kenari, A., Paknejad, H., Mohseni, M., 2019. Effects of dietary b-glucan, mannan oligosaccharide, *Lactobacillus plantarum* and their combinations on growth performance, immunity and immune related gene expression of Caspian trout, *Salmo trutta caspius* (Kessler, 1877). Fish Shellfish Immunol. 91, 202–208. https://doi.org/10.1016/j.fsi.2019.05.024

Jayasuriya, N.S., 2014. Gill image analysis: a tool for assessing pathophysiological and morphometric changes in the gill of Atlantic salmon (*Salmo salar* L.).

Jensen, L.B., 2015. Nutritional and environmental impacts on skin and mucus condition in Atlantic salmon (*Salmo salar*). PhD Thesis.

Jesus, G.F.A., Pereira, S.A., Owatari, M.S., Syracuse, N., Silva, B.C., Silva, A., Pierri, B.S., Lehmann, N.B., Figueiredo, H.C.P., Fracalossi, D.M., 2019. Protected forms of sodium butyrate improve the growth and health of Nile tilapia fingerlings during sexual reversion. Aquaculture 499, 119–127.

Johnson, I.T., Gee, J.M., Price, K., Curl, C., Fenwick, G.R., 1986. Influence of saponins on gut permeability and active nutrient transport in vitro. J. Nutr. 116, 2270–2277. https://doi.org/10.1093/jn/116.11.2270

Jones, S.R.., 2001. The occurrence and mechanisms of innate immunity against parasites in fish. Dev. Comp. Immunol. 25, 841–852. https://doi.org/10.1016/S0145-305X(01)00039-8

Kalina, U., Koyama, N., Hosoda, T., Nuernberger, H., Sato, K., Hoelzer, D., Herweck, F., Manigold, T., Singer, M. V., Rossol, S., Böcker, U., 2002. Enhanced production of IL-18 in butyrate-treated intestinal epithelium by stimulation of the proximal promoter region. Eur. J. Immunol. 32, 2635–2643. https://doi.org/10.1002/1521-4141(200209)32:9<2635::AID-IMMU2635>3.0.CO;2-N

Karlsen, C., Ytteborg, E., Timmerhaus, G., Høst, V., Handeland, S., Jørgensen, S.M., Krasnov, A., 2018. Atlantic salmon skin barrier functions gradually enhance after seawater transfer. Sci. Rep. 8, 9510. https://doi.org/10.1038/s41598-018-27818-y

Katerina, K., Berge, G.M., Turid, M., Aleksei, K., Grete, B., Trine, Y., Mats, C., John, S., Bente, R., 2020. Microalgal *Schizochytrium limacinum* biomass improves growth and filet quality when used long-term as a replacement for fish oil, in modern salmon diets. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.00057

Kazuń, B., Małaczewska, J., Kazuń, K., Żylińska-Urban, J., Siwicki, A.K., 2018. Immune-enhancing activity of potential probiotic strains of *Lactobacillus plantarum* in the common carp (*Cyprinus carpio*) fingerling. J. Vet. Res. 62, 485.

Kiron, V., Park, Y., Siriyappagouder, P., Dahle, D., Vasanth, G.K., Dias, J., Fernandes, J.M.O., Sørensen, M., Trichet, V.V., 2020. Intestinal transcriptome analysis reveals soy derivative-linked changes in Atlantic salmon. Front. Immunol. 11, 1–14. https://doi.org/10.3389/fimmu.2020.596514

Kiron, V., Phromkunthong, W., Huntley, M., Archibald, I., De Scheemaker, G., 2012. Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. Aquac. Nutr. https://doi.org/10.1111/j.1365-2095.2011.00923.x

Kiron, V., Sørensen, M., Huntley, M., Vasanth, G.K., Gong, Y., Dahle, D., Palihawadana, A.M., 2016. Defatted biomass of the microalga, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. Front. Mar. Sci. 3. https://doi.org/10.3389/fmars.2016.00067

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

Knudsen, D., Urán, P., Arnous, A., Koppe, W., Frøkiær, H., 2007. Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. J. Agric. Food Chem. 55, 2261–2267. https://doi.org/10.1021/jf0626967

Knutsen, H.R., Ottesen, O.H., Palihawadana, A.M., Sandaa, W., Sørensen, M., Hagen, Ø., 2019. Muscle growth and changes in chemical composition of spotted wolffish juveniles (*Anarhichas minor*) fed diets with and without microalgae (*Scenedesmus obliquus*). Aquac. Reports 13, 100175. https://doi.org/10.1016/j.aqrep.2018.11.001

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., Bäckhed, F., 2016. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. Cell. https://doi.org/10.1016/j.cell.2016.05.041

Kongnum, K., Hongpattarakere, T., 2012. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. Fish Shellfish Immunol. 32, 170–177.

Kościuczuk, E.M., Lisowski, P., Jarczak, J., Strzałkowska, N., Jóźwik, A., Horbańczuk, J., Krzyżewski, J., Zwierzchowski, L., Bagnicka, E., 2012. Cathelicidins: family of antimicrobial peptides. A review. Mol. Biol. Rep. 39, 10957–10970. https://doi.org/10.1007/s11033-012-1997-x

Kotzamanis, Y., Kumar, V., Tsironi, T., Grigorakis, K., Ilia, V., Vatsos, I., Brezas, A., van Eys, J., Gisbert, E., 2020. Taurine supplementation in high-soy diets affects fillet quality of European sea bass (*Dicentrarchus labrax*). Aquaculture 520, 734655. https://doi.org/10.1016/j.aquaculture.2019.734655

Kousoulaki, K., Østbye, T.-K.K., Krasnov, A., Torgersen, J.S., Mørkøre, T., Sweetman, J., 2015. Metabolism, health and fillet nutritional quality in Atlantic salmon (*Salmo salar*) fed diets containing n-3-rich microalgae. J. Nutr. Sci. 4, e24. https://doi.org/10.1017/jns.2015.14

Koyande, A.K., Chew, K.W., Rambabu, K., Tao, Y., Chu, D.-T., Show, P.-L., 2019. Microalgae: A potential alternative to health supplementation for humans. Food Sci. Hum. Wellness 8, 16–24. https://doi.org/10.1016/j.fshw.2019.03.001

Kroeckel, S., Harjes, A.-G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A., Schulz, C., 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364–365, 345–352. https://doi.org/10.1016/j.aquaculture.2012.08.041

Krogdahl, Å., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 9, 361–371. https://doi.org/10.1046/j.1365-2095.2003.00264.x

Krogdahl, Å., Gajardo, K., Kortner, T.M., Penn, M., Gu, M., Berge, G.M., Bakke, A.M., 2015. Soya saponins induce enteritis in Atlantic salmon (*Salmo salar* L.). J. Agric. Food Chem. 63, 3887–3902. https://doi.org/10.1021/jf506242t

Krogdahl, Å., Kortner, T.M., Jaramillo-Torres, A., Gamil, A.A.A., Chikwati, E., Li, Y., Schmidt, M., Herman, E., Hymowitz, T., Teimouri, S., Storebakken, T., 2020. Removal of three proteinaceous antinutrients from soybean does not mitigate soybean-induced enteritis in Atlantic salmon (Salmo salar, L). Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.734495

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: An update on recent findings regarding responses in salmonids. Aquac. Res. https://doi.org/10.1111/j.1365-2109.2009.02426.x

Kryvi, H., Poppe, T., 2021. Fiskeanatomi (2. utgave.).

Kryvi, H., Totland, G., 1997. Fiskeanatomi. Norwegian Academic Press, Krisitansand.

Landy, J., Ronde, E., English, N., Clark, S.K., Hart, A.L., Knight, S.C., Ciclitira, P.J., Al-Hassi, H.O., 2016. Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. World J. Gastroenterol. 22, 3117. https://doi.org/10.3748/wjg.v22.i11.3117

Lara-Flores, M., Olvera-Novoa, M.A., Guzmán-Méndez, B.E., López-Madrid, W., 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216, 193–201.

Lazado, C.C., Caipang, C.M.A., 2014. Mucosal immunity and probiotics in fish. Fish Shellfish Immunol. 39, 78–89. https://doi.org/10.1016/j.fsi.2014.04.015

Lee, C.-S., Lim, C., Gatlin, D.M., Webster, C.D. (Eds.), 2015. Dietary nutrients, additives, and fish health. John Wiley & Sons, Inc, Hoboken, NJ, USA. https://doi.org/10.1002/9781119005568

Ling, E.N., Cotter, D., 2003. Statistical power in comparative aquaculture studies. Aquaculture 224, 159–168. https://doi.org/10.1016/S0044-8486(03)00225-4

Liu, C., Palihawadana, A.M., Nadanasabesan, N., Vasanth, G.K., Vatsos, I.N., Dias, J., Valente, L.M.P., Micallef, G., Sørensen, M., Kiron, V., 2022. Utilization of *Nannochloropsis oceanica* in plant-based feeds by Atlantic salmon (*Salmo salar*). Aquaculture 561, 738651. https://doi.org/10.1016/j.aquaculture.2022.738651

Liu, M., Guo, W., Wu, F., Qu, Q., Tan, Q., Gong, W., 2017. Dietary supplementation of sodium butyrate may benefit growth performance and intestinal function in juvenile grass carp (*Ctenopharyngodon idellus*). Aquac. Res. 48, 4102–4111.

Liu, W., Yang, Y., Zhang, J., Gatlin, D.M., Ringø, E., Zhou, Z., 2014. Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil. Br. J. Nutr. 112, 15–29.

Liu, Y., Chen, Z., Dai, J., Yang, P., Xu, W., Ai, Q., Zhang, W., Zhang, Yongan, Zhang, Yanjiao, Mai, K., 2019. Sodium butyrate supplementation in high-soybean meal diets for turbot (*Scophthalmus maximus* L.): effects on inflammatory status, mucosal barriers and microbiota in the intestine. Fish Shellfish Immunol. 88, 65–75.

Løkka, G., Austbø, L., Falk, K., Bjerkås, I., Koppang, E.O., 2013. Intestinal morphology of the wild Atlantic salmon (*Salmo salar*). J. Morphol. 274, 859–876. https://doi.org/10.1002/jmor.20142

Lorent, J., Le Duff, C.S., Quetin-Leclercq, J., Mingeot-Leclercq, M.P., 2013. Induction of highly curved structures in relation to membrane permeabilization and budding by the triterpenoid saponins,, α- And δ-hederin. J. Biol. Chem. 288, 14000–14017. https://doi.org/10.1074/jbc.M112.407635

Lourenço, F., Calado, R., Medina, I., Ameixa, O.M.C.C., 2022. The Potential impacts by the invasion of insects reared to feed livestock and pet animals in Europe and other regions: A critical review. Sustainability 14, 6361. https://doi.org/10.3390/su14106361

Lü, A., Hu, X., Xue, J., Zhu, J., Wang, Y., Zhou, G., 2012. Gene expression profiling in the skin of zebrafish infected with *Citrobacter freundii*. Fish Shellfish Immunol. 32, 273–283. https://doi.org/10.1016/j.fsi.2011.11.016

Luissint, A.-C., Parkos, C.A., Nusrat, A., 2016. Inflammation and the intestinal barrier: Leukocyte–epithelial cell interactions, cell junction remodeling, and mucosal repair. Gastroenterology 151, 616–632. https://doi.org/10.1053/j.gastro.2016.07.008

Macia, L., Tan, J., Vieira, A.T., Leach, K., Stanley, D., Luong, S., Maruya, M., Ian McKenzie, C., Hijikata, A., Wong, C., Binge, L., Thorburn, A.N., Chevalier, N., Ang, C., Marino, E., Robert, R., Offermanns, S., Teixeira, M.M., Moore, R.J., Flavell, R.A., Fagarasan, S., Mackay, C.R., 2015. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nat. Commun. 6, 6734. https://doi.org/10.1038/ncomms7734

Makkar, H.P.S., 1993. Antinutritional factors in foods for livestock. BSAP Occas. Publ. 16, 69–85. https://doi.org/10.1017/S0263967X00031086

Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Can. J. Fish. Aquat. Sci. 42, 630–648.

Mallioris, P., Kotzamanis, Y., Vardali, S., Roussos, E., Ilia, V., Paschali, E., Kampantais, D., Bouziotis, D., Khadem, A., Vatsos, I.N., 2022. Modulation of intestinal health and hepatic vacuolation in gilthead sea bream (*Sparus aurata*) juveniles by a mixture of dietary esterified butyrins, emulsifiers from plants and yeast extracts at low and high fish meal inclusion. Anim. Feed Sci. Technol. 284, 115194. https://doi.org/10.1016/j.anifeedsci.2021.115194

Marcos-López, M., Calduch-Giner, J.A., Mirimin, L., MacCarthy, E., Rodger, H.D., O'Connor, I., Sitjà-Bobadilla, A., Pérez-Sánchez, J., Piazzon, M.C., 2018. Gene expression analysis of Atlantic salmon gills reveals mucin 5 and interleukin 4/13 as key molecules during amoebic gill disease. Sci. Rep. 8, 1–15. https://doi.org/10.1038/s41598-018-32019-8

Martínez Cruz, P., Ibáñez, A.L., Monroy Hermosillo, O.A., Ramírez Saad, H.C., 2012. Use of probiotics in aquaculture. ISRN Microbiol 2012: 916845.

Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., Rotondo, J.C., 2023. Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells 12, 184. https://doi.org/10.3390/cells12010184

Mbow, C., Rosenzweig, C., Barioni, L.G., Benton, T.G., Herrero, M., Krishnapillai, M., Liwenga, E., Pradhan, P., Rivera-Ferre, M.G., Sapkota, T., Tubiello, F.N., Xu, Y., 2019. Fodd security. In: Climate change and land: An IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems, in: Shukla, P.R., Skea, J., Buendia, E.C., Masson-Delmotte, V., Pörtner, H.-O., Roberts, D.C., Zhai, P., Slade, R., Connors, S., Diemen, R. van, Ferrat, M., Haughey, E., Luz, S., Neogi, S., Pathak, M., Petzold, J., Pereira, J.P., Vyas, P., Huntley, E., Kissick, K., M., J.M. (Eds.), . In Press, pp. 437–550.

Meyerholz, D.K., Beck, A.P., 2018. Fundamental concepts for semiquantitative tissue scoring in translational research. ILAR J. 59, 13–17. https://doi.org/10.1093/ilar/ily025

Mohammadian, T., Ghanei-Motlagh, R., Jalali, M., Nasirpour, M., Mohtashamipour, H., Osroush, E., Nejad, A.J., 2022. Protective effects of non-encapsulated and microencapsulated *Lactobacillus delbrueckii* Subsp. *bulgaricus* in rainbow trout (*Oncorhynchus mykiss*) exposed to lead (Pb) via diet. Ann. Anim. Sci. 22, 325–348. https://doi.org/10.2478/aoas-2021-0026

Moldal, T., Løkka, G., Wiik-Nielsen, J., Austbø, L., Torstensen, B.E., Rosenlund, G., Dale, O.B., Kaldhusdal, M., Koppang, E.O., 2014. Substitution of dietary fish oil with plant oils is associated with shortened mid intestinal folds in Atlantic salmon (*Salmo salar*). BMC Vet. Res. 10, 60. https://doi.org/10.1186/1746-6148-10-60

Morrison, D.J., Preston, T., 2016. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes. https://doi.org/10.1080/19490976.2015.1134082

Mowi, 2012. Salmon farming industry handbook 2012.

Nayak, S.K., 2010. Probiotics and immunity: A fish perspective. Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2010.02.017

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

Newton, R.W., Little, D.C., 2018. Mapping the impacts of farmed Scottish salmon from a life cycle perspective. Int. J. Life Cycle Assess. 23, 1018–1029. https://doi.org/10.1007/s11367-017-1386-8

Ng, W.-K., Koh, C.-B., 2017. The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. Rev. Aquac. 9, 342–368. https://doi.org/10.1111/raq.12141

Nimalan, N., Sørensen, S.L., Fečkaninová, A., Koščová, J., Mudroňová, D., Gancarčíková, S., Vatsos, I.N., Bisa, S., Kiron, V., Sørensen, M., 2022. Mucosal barrier status in Atlantic salmon fed marine or plant-based diets supplemented with probiotics. Aquaculture 547, 737516. https://doi.org/10.1016/j.aquaculture.2021.737516

Nogal, A., Valdes, A.M., Menni, C., 2021. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. Gut Microbes 13. https://doi.org/10.1080/19490976.2021.1897212

Nordrum, S., Bakke-McKellep, A.., Krogdahl, Å., Buddington, R.., 2000. Effects of soybean meal and salinity on intestinal transport of nutrients in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 125, 317–335. https://doi.org/10.1016/S0305-0491(99)00190-X

NRC, 2011. Nutrient requirements of fish and shrimp. National Academies Press, Washington, D.C. https://doi.org/10.17226/13039

Ohland, C.L., MacNaughton, W.K., 2010. Probiotic bacteria and intestinal epithelial barrier function. Am. J. Physiol. Liver Physiol. 298, G807–G819. https://doi.org/10.1152/ajpgi.00243.2009

Olson, K.R., 2011. Hydrogen sulfide is an oxygen sensor in the carotid body. Respir. Physiol. Neurobiol. 179, 103–110. https://doi.org/10.1016/j.resp.2011.09.010

Olson, K.R., 1996. Scanning electron microscopy of the fish gill, in: Fish Morphology. Science Publishers, Lebanon, pp. 31–45.

Ostrander, G.K., 2000. The laboratory fish. Elsevier.
Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., Skrede, A., 2009. Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)—Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture 288, 305–311. https://doi.org/10.1016/j.aquaculture.2008.12.012
Palma, M., Magnoni, L.J., Morais, S., Viegas, I., 2022. Tributyrin supplementation in fish and crustacean nutrition: A review. Rev. Aquac. https://doi.org/10.1111/raq.12759
Pandey, S., Stockwell, C.A., Snider, M.R., Wisenden, B.D., 2021. Epidermal club cells in fishes: A case for ecoimmunological analysis. Int. J. Mol. Sci. 22, 1440. https://doi.org/10.3390/ijms22031440
Panigrahi, A., Kiron, V., Kobayashi, T., Puangkaew, J., Satoh, S., Sugita, H., 2004. Immune responses in rainbow trout *Oncorhynchus mykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM 1136. Vet. Immunol. Immunopathol. 102, 379–388. https://doi.org/10.1016/j.vetimm.2004.08.006
Paone, P., Cani, P.D., 2020. Mucus barrier, mucins and gut microbiota: The expected slimy partners? Gut 69, 2232–2243. https://doi.org/10.1136/gutjnl-2020-322260
Parada Venegas, D., De la Fuente, M.K., Landskron, G., González, M.J., Quera, R., Dijkstra, G., Harmsen, H.J.M., Faber, K.N., Hermoso, M.A., 2019. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for Inflammatory Bowel Diseases. Front. Immunol. 10. https://doi.org/10.3389/fimmu.2019.00277
Park, J., Kotani, T., Konno, T., Setiawan, J., Kitamura, Y., Imada, S., Usui, Y., Hatano, N., Shinohara, M., Saito, Y., Murata, Y., Matozaki, T., 2016. Promotion of intestinal epithelial cell turnover by commensal bacteria: Role of short-chain fatty acids. PLoS One 11, e0156334. https://doi.org/10.1371/journal.pone.0156334
Pelletier, N., Klinger, D.H., Sims, N.A., Yoshioka, J.-R., Kittinger, J.N., 2018. Nutritional attributes, substitutability, scalability, and environmental intensity of an illustrative subset of current and future protein sources for aquaculture feeds: Joint consideration of potential synergies and trade-offs. Environ. Sci. Technol. 52, 5532–5544. https://doi.org/10.1021/acs.est.7b05468
Penn, M.H., Bendiksen, E.A., Campbell, P., Krogdahl, A.S., 2011. High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (*Salmo salar* L.). Aquaculture. https://doi.org/10.1016/j.aquaculture.2010.10.040
Pérez-Sánchez, J., Terova, G., Simó-Mirabet, P., Rimoldi, S., Folkedal, O., Calduch-Giner, J.A., Olsen, R.E., Sitjà-Bobadilla, A., 2017. Skin mucus of gilthead sea bream (*Sparus aurata* L.). Protein mapping and regulation in chronically stressed fish. Front. Physiol. 8. https://doi.org/10.3389/fphys.2017.00034
Peyghan, R., Powell, M.D., 2006. Histopathological study of gills in experimentally amoebic gill disease (AGD) infected Atlantic salmon, *Salmo salar*, L. Iran. J. Vet. Res. 7, 8–13. https://doi.org/10.22099/ijvr.2006.2628
Piechota-Polanczyk, A., Fichna, J., 2014. Review article: the role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. Naunyn. Schmiedebergs. Arch. Pharmacol. 387, 605–620. https://doi.org/10.1007/s00210-014-0985-1
Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., Maita, M., 2011. Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Res. Vet. Sci. 91, e92–e97. https://doi.org/10.1016/j.rvsc.2011.02.014
Pittman, K., Pittman, A., Karlson, S., Cieplinska, T., Sourd, P., Redmond, K., Ravnøy, B., Sweetman, E., 2013. Body site matters: An evaluation and application of a novel histological methodology on the quantification of mucous cells in the skin of Atlantic salmon, *Salmo salar* L. J. Fish Dis. 36, 115–127. https://doi.org/10.1111/jfd.12002

Pittman, K., Sourd, P., Ravnøy, B., Espeland, Ø., Fiksdal, I.U., Oen, T., Pittman, A., Redmond, K., Sweetman, J., 2011. Novel method for quantifying salmonid mucous cells. J. Fish Dis. 34, 931–936. https://doi.org/10.1111/j.1365-2761.2011.01308.x

Plaza-Diaz, J., Ruiz-Ojeda, F.J., Gil-Campos, M., Gil, A., 2019. Mechanisms of action of probiotics. Adv. Nutr. 10, S49–S66. https://doi.org/10.1093/advances/nmy063

Polycarpo, G.V., Andretta, I., Kipper, M., Cruz-Polycarpo, V.C., Dadalt, J.C., Rodrigues, P.H.M., Albuquerque, R., 2017. Meta-analytic study of organic acids as an alternative performance-enhancing feed additive to antibiotics for broiler chickens. Poult. Sci. 96, 3645–3653. https://doi.org/10.3382/ps/pex178

Price, S.J. and T., 2008. Principles for histopathologic scoring. Vet. Pathol. 23, 1–7. https://doi.org/10.1177/0300985813485099.Principles

Rahman, M.A., Sultana, N., Ayman, U., Bhakta, S., Afrose, M., Afrin, M., Haque, Z., 2022. Alcoholic fixation over formalin fixation: A new, safer option for morphologic and molecular analysis of tissues. Saudi J. Biol. Sci. 29, 175–182. https://doi.org/10.1016/j.sjbs.2021.08.075

Rakers, S., Niklasson, L., Steinhagen, D., Kruse, C., Schauber, J., Sundell, K., Paus, R., 2013. Antimicrobial peptides (AMPs) from fish epidermis: Perspectives for investigative dermatology. J. Invest. Dermatol. 133, 1140–1149. https://doi.org/10.1038/jid.2012.503

Ramasundara, M., Leach, S.T., Lemberg, D.A., Day, A.S., 2009. Defensins and inflammation: The role of defensins in inflammatory bowel disease. J. Gastroenterol. Hepatol. 24, 202–208. https://doi.org/10.1111/j.1440-1746.2008.05772.x

Ranjan, A., Arora, J., Chauhan, A., Basniwal, R.K., Kumari, A., Rajput, V.D., Prazdnova, E. V., Ghosh, A., Mukerjee, N., Mandzhieva, S.S., Sushkova, S., Minkina, T., Jindal, T., 2022. Advances in characterization of probiotics and challenges in industrial application. Biotechnol. Genet. Eng. Rev. 1–44. https://doi.org/10.1080/02648725.2022.2122287

Rao, R., 2008. Oxidative stress-induced disruption of epithelial and endothelial tight junctions. Front. Biosci. Volume, 7210. https://doi.org/10.2741/3223

Refstie, S., Sahlström, S., Bråthen, E., Baeverfjord, G., Krogedal, P., 2005. Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). Aquaculture 246, 331–345. https://doi.org/10.1016/j.aquaculture.2005.01.001

Reid, G., Younes, J.A., Van der Mei, H.C., Gloor, G.B., Knight, R., Busscher, H.J., 2011. Microbiota restoration: natural and supplemented recovery of human microbial communities. Nat. Rev. Microbiol. 9, 27–38. https://doi.org/10.1038/nrmicro2473

Reite, O.B., Evensen, Ø., 2006. Inflammatory cells of teleostean fish: A review focusing on mast cells/eosinophilic granule cells and rodlet cells. Fish Shellfish Immunol. 20, 192–208. https://doi.org/10.1016/j.fsi.2005.01.012

Reyes-Becerril, M., Guardiola, F., Rojas, M., Ascencio-Valle, F., Esteban, M.Á., 2013. Dietary administration of microalgae *Navicula* sp. affects immune status and gene expression of gilthead seabream (*Sparus aurata*). Fish Shellfish Immunol. 35, 883–889. https://doi.org/10.1016/j.fsi.2013.06.026

Reyes, M., Rodríguez, M., Montes, J., Barroso, F.G., Fabrikov, D., Morote, E., Sánchez-Muros, M.J., 2020. Nutritional and growth effect of insect meal inclusion on seabass (*Dicentrarchuss labrax*) feeds. Fishes 5, 16. https://doi.org/10.3390/fishes5020016

Riaz Rajoka, M.S., Wu, Y., Mehwish, H.M., Bansal, M., Zhao, L., 2020. *Lactobacillus* exopolysaccharides: New perspectives on engineering strategies, physiochemical functions, and immunomodulatory effects on host health. Trends Food Sci. Technol. 103, 36–48. https://doi.org/10.1016/j.tifs.2020.06.003

Ribeiro, A.R., Gonçalves, A., Barbeiro, M., Bandarra, N., Nunes, M.L., Carvalho, M.L., Silva, J., Navalho, J., Dinis, M.T., Silva, T., Dias, J., 2017. *Phaeodactylum tricornutum* in finishing diets for gilthead seabream: effects on skin pigmentation, sensory properties and nutritional value. J. Appl. Phycol. 29, 1945–1956. https://doi.org/10.1007/s10811-017-1125-3

Richards, J.L., Yap, Y.A., McLeod, K.H., MacKay, C.R., Marinõ, E., 2016. Dietary metabolites and the gut microbiota: An alternative approach to control inflammatory and autoimmune diseases. Clin. Transl. Immunol. https://doi.org/10.1038/cti.2016.29

Ringø, E., Hoseinifar, S.H., Ghosh, K., Doan, H. Van, Beck, B.R., Song, S.K., 2018. Lactic acid bacteria in finfish-An update. Front. Microbiol. 9, 1–37. https://doi.org/10.3389/fmicb.2018.01818

Ringø, E., Olsen, R.E., Jensen, I., Romero, J., Lauzon, H.L., 2014. Application of vaccines and dietary supplements in aquaculture: possibilities and challenges. Rev. Fish Biol. Fish. 24, 1005–1032. https://doi.org/10.1007/s11160-014-9361-y

Ringø, E., Salinas, I., Olsen, R.E., Nyhaug, A., Myklebust, R., Mayhew, T.M., 2007. Histological changes in intestine of Atlantic salmon (*Salmo salar* L.) following in vitro exposure to pathogenic and probiotic bacterial strains. Cell Tissue Res. 328, 109–116. https://doi.org/10.1007/s00441-006-0323-0

Roberts, R., 2012. Fish pathology, 4th ed. Blackwell Publishing Ltd.

Roberts, S.D., Powell, M.D., 2005. The viscosity and glycoprotein biochemistry of salmonid mucus varies with species, salinity and the presence of amoebic gill disease. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. https://doi.org/10.1007/s00360-004-0453-1

Roberts, S.D., Powell, M.D., 2003. Comparative ionic flux and gill mucous cell histochemistry: effects of salinity and disease status in Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 134, 525–537. https://doi.org/10.1016/S1095-6433(02)00327-6

Robles, R., Lozano, A.B., Sevilla, A., Márquez, L., Nuez-Ortín, W., Moyano, F.J., 2013. Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (*Sparus aurata*). Fish Physiol. Biochem. 39, 1567–1580.

Rocha, C.P., Cabral, H.N., Marques, J.C., Gonçalves, A.M.M., 2022. A global overview of aquaculture food production with a focus on the activity's development in transitional systems—The sase study of a south European country (Portugal). J. Mar. Sci. Eng. 10, 417. https://doi.org/10.3390/jmse10030417

Rohde, J.K., Fuh, M.M., Evangelakos, I., Pauly, M.J., Schaltenberg, N., Siracusa, F., Gagliani, N., Tödter, K., Heeren, J., Worthmann, A., 2022. A gas chromatography mass spectrometry-based method for the quantification of short chain fatty acids. Metabolites 12, 170. https://doi.org/10.3390/metabo12020170

Romarheim, O.H., Landsverk, T., Mydland, L.T., Skrede, A., Øverland, M., 2013. Cell wall fractions from *Methylococcus capsulatus* prevent soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*). Aquaculture 402–403, 13–18. https://doi.org/10.1016/j.aquaculture.2013.03.011

Romarheim, O.H., Øverland, M., Mydland, L.T., Skrede, A., Landsverk, T., 2011. Bacteria grown on natural gas prevent soybean meal-induced enteritis in Atlantic Salmon. J. Nutr. https://doi.org/10.3945/jn.110.128900

Romarheim, O.H., Skrede, A., Gao, Y., Krogdahl, Å., Denstadli, V., Lilleeng, E., Storebakken, T., 2006. Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 256, 354–364. https://doi.org/10.1016/j.aquaculture.2006.02.006

Rose, M.C., Voynow, J.A., 2006. Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol. Rev. 86, 245–278. https://doi.org/10.1152/physrev.00010.2005

Rossi, M., Amaretti, A., Raimondi, S., 2011. Folate production by probiotic bacteria. Nutrients 3, 118–134. https://doi.org/10.3390/nu3010118

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., Tuohy, K., 2018. Gut microbiota functions: metabolism of nutrients and other food components. Eur. J. Nutr. 57, 1–24. https://doi.org/10.1007/s00394-017-1445-8

Ruiz, J., Wijffels, R.H., Dominguez, M., Barbosa, M.J., 2022. Heterotrophic vs autotrophic production of microalgae: Bringing some light into the everlasting cost controversy. Algal Res. 64, 102698. https://doi.org/10.1016/j.algal.2022.102698

Rumsey, G.L., Siwicki, A.K., Anderson, D.P., Bowser, P.R., 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Vet. Immunol. Immunopathol. 41, 323–339. https://doi.org/10.1016/0165-2427(94)90105-8

Sahlmann, C., Sutherland, B.J.G., Kortner, T.M., Koop, B.F., Krogdahl, Å., Bakke, A.M., 2013. Early response of gene expression in the distal intestine of Atlantic salmon (*Salmo salar* L.) during the development of soybean meal induced enteritis. Fish Shellfish Immunol. 34, 599–609. https://doi.org/10.1016/j.fsi.2012.11.031

Salazar, N., González, S., de los Reyes Gavilan, C.G., Rios-Covian, D., 2022. Branched short-chain fatty acids as biological indicators of microbiota health and links with anthropometry. pp. 1–17. https://doi.org/10.1007/978-3-030-81304-8_4-1

Salinas, I., Abelli, L., Bertoni, F., Picchietti, S., Roque, A., Furones, D., Cuesta, A., Meseguer, J., Esteban, M.Á., 2008a. Monospecies and multispecies probiotic formulations produce different systemic and local immunostimulatory effects in the gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol. 25, 114–123. https://doi.org/10.1016/j.fsi.2008.03.011

Salinas, I., Fernández-Montero, Á., Ding, Y., Sunyer, J.O., 2021. Mucosal immunoglobulins of teleost fish: A decade of advances. Dev. Comp. Immunol. 121, 104079. https://doi.org/10.1016/j.dci.2021.104079

Salinas, I., Myklebust, R., Esteban, M.A., Olsen, R.E., Meseguer, J., Ringø, E., 2008b. In vitro studies of *Lactobacillus delbrueckii* subsp. *lactis* in Atlantic salmon (*Salmo salar* L.) foregut: Tissue responses and evidence of protection against *Aeromonas salmonicida* subsp. *salmonicida* epithelial damage. Vet. Microbiol. 128, 167–177. https://doi.org/10.1016/j.vetmic.2007.10.011

Schoch, C.L., Ciufo, S., Domrachev, M., Hotton, C.L., Kannan, S., Khovanskaya, R., Leipe, D., McVeigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J.P., Sun, L., Turner, S., Karsch-Mizrachi, I., 2020. NCBI Taxonomy: A comprehensive update on curation, resources and tools. Database 2020, 1–21. https://doi.org/10.1093/database/baaa062

Schrock, R.M., Smith, S.D., Maule, A.G., Doulos, S.K., Rockowski, J.J., 2001. Mucous lysozyme levels in hatchery coho salmon (*Oncorhynchus kisutch*) and spring chinook salmon (*O. tshawytscha*) early in the parr–smolt transformation. Aquaculture 198, 169–177. https://doi.org/10.1016/S0044-8486(00)00585-8

Schulze, P.S.C., Hulatt, C.J., Morales-Sánchez, D., Wijffels, R.H., Kiron, V., 2019. Fatty acids and proteins from marine cold adapted microalgae for biotechnology. Algal Res. 42, 101604. https://doi.org/10.1016/j.algal.2019.101604

Searcy-Bernal, R., 1994. Statistical power and aquacultural research. Aquaculture 127, 371–388. https://doi.org/10.1016/0044-8486(94)90239-9

Serra, C.R., Almeida, E.M., Guerreiro, I., Santos, R., Merrifield, D.L., Tavares, F., Oliva-Teles, A., Enes, P., 2019. Selection of carbohydrate-active probiotics from the gut of carnivorous fish fed plant-based diets. Sci. Rep. 9, 6384. https://doi.org/10.1038/s41598-019-42716-7

Sevgili, H., Sezen, S., Yılayaz, A., Aktaş, Ö., Pak, F., Aasen, I.M., Reitan, K.I., Sandmann, M., Rohn, S., Turan, G., Kanyılmaz, M., 2019. Apparent nutrient and fatty acid digestibilities of microbial raw materials for rainbow trout (*Oncorhynchus mykiss*) with comparison to conventional ingredients. Algal Res. 42. https://doi.org/10.1016/j.algal.2019.101592

Sewaka, M., Trullas, C., Chotiko, A., Rodkhum, C., Chansue, N., Boonanuntanasarn, S., Pirarat, N., 2019. Efficacy of synbiotic Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile re. Fish Shellfish Immunol. 86, 260–268. https://doi.org/10.1016/j.fsi.2018.11.026

Shah, A.A., Totakul, P., Matra, M., Cherdthong, A., Harnboonsong, Y., Wanapat, M., 2022. Nutritional composition of various insects and potential uses as alternative protein sources in animal diets. Anim. Biosci. 35, 317–331. https://doi.org/10.5713/ab.21.0447

Shephard, K.L., 1994. Functions for fish mucus. Rev. Fish Biol. Fish. 4, 401–429. https://doi.org/10.1007/BF00042888

Silva, P.F., McGurk, C., Knudsen, D.L., Adams, A., Thompson, K.D., Bron, J.E., 2015. Histological evaluation of soya bean-induced enteritis in Atlantic salmon (*Salmo salar* L.): Quantitative image analysis vs. semi-quantitative visual scoring. Aquaculture 445, 42–56. https://doi.org/10.1016/j.aquaculture.2015.04.002

Skalli, A., Firmino, J.P., Andree, K.B., Salomón, R., Estévez, A., Puig, P., Sabater-Martínez, M., Hechavarria, T., Gisbert, E., 2020. The Inclusion of the microalga *Scenedesmus* sp. in diets for rainbow trout, *Onchorhynchus mykiss*, juveniles. Animals 10, 1656. https://doi.org/10.3390/ani10091656

Skrede, A., Mydland, L., Ahlstrøm, Ø., Reitan, K., Gislerød, H., Øverland, M., 2011. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. J. Anim. Feed Sci. https://doi.org/10.22358/jafs/66164/2011

Soltani, M., Abdy, E., Alishahi, M., Mirghaed, A.T., Hosseini-Shekarabi, P., 2017. Growth performance, immune-physiological variables and disease resistance of common carp (*Cyprinus carpio*) orally subjected to different concentrations of *Lactobacillus plantarum*. Aquac. Int. 25, 1913–1933.

Soltani, M., Pakzad, K., Taheri-Mirghaed, A., Mirzargar, S., Shekarabi, S.P.H., Yosefi, P., Soleymani, N., 2019. Dietary application of the probiotic *Lactobacillus plantarum* 426951 enhances immune status and growth of rainbow trout (*Oncorhynchus mykiss*) vaccinated against *Yersinia ruckeri*. Probiotics Antimicrob. Proteins 11, 207–219. https://doi.org/10.1007/s12602-017-9376-5

Sørensen, M., Berge, G.M., Reitan, K.I., Ruyter, B., 2016. Microalga *Phaeodactylum tricornutum* in feed for Atlantic salmon (*Salmo salar*) —Effect on nutrient digestibility, growth and utilization of feed. Aquaculture 460, 116–123. https://doi.org/10.1016/j.aquaculture.2016.04.010

Sørensen, M., Berge, G.M., Thomassen, M., Ruyter, B., Hatlen, B., Ytrestøyl, T., Aas, T.S., Åsgård, T., 2011a. Today's and tomorrow's feed ingredients in Norwegian aquaculture.

Sørensen, M., Gong, Y., Bjarnason, F., Vasanth, G.K., Dahle, D., Huntley, M., Kiron, V., 2017. *Nannochloropsis oceania*-derived defatted meal as an alternative to fishmeal in Atlantic salmon feeds. PLoS One 12, e0179907. https://doi.org/10.1371/journal.pone.0179907

Sørensen, M., Penn, M., El-Mowafi, A., Storebakken, T., Chunfang, C., Øverland, M., Krogdahl, Å., 2011b. Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L). Aquaculture 314, 145–152. https://doi.org/10.1016/j.aquaculture.2011.02.013

Sørensen, S.L., 2022. Influence of feed ingredients and additives on mucosal health with focus on the intestine of Atlantic salmon (*Salmo salar*).

Sørensen, S.L., Ghirmay, A., Gong, Y., Dahle, D., Vasanth, G., Sørensen, M., Kiron, V., 2021a. Growth, chemical composition, histology and antioxidant genes of Atlantic salmon (*Salmo salar*) fed whole or pre-processed *Nannochloropsis oceanica* and *Tetraselmis* sp. Fishes 6, 23. https://doi.org/10.3390/fishes6030023

Sørensen, S.L., Park, Y., Gong, Y., Vasanth, G.K., Dahle, D., Korsnes, K., Phuong, T.H., Kiron, V., Øyen, S., Pittman, K., Sørensen, M., 2021b. Nutrient digestibility, growth, mucosal barrier status, and activity of leucocytes from head kidney of Atlantic salmon fed marine- or plant-derived protein and lipid sources. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.623726

Storebakken, T., Zhang, Y., Ma, J., Øverland, M., Mydland, L.T., Kraugerud, O.F., Apper, E., Feneuil, A., 2015. Feed technological and nutritional properties of hydrolyzed wheat gluten when used as a main source of protein in extruded diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 448, 214–218. https://doi.org/10.1016/j.aquaculture.2015.05.029

Sugiura, S.H., Dong, F.M., Hardy, R.W., 1998. Effects of dietary supplements on the availability of minerals in fish meal; preliminary observations. Aquaculture 160, 283–303. https://doi.org/10.1016/S0044-8486(97)00302-5

Sukenik, A., 1991. Ecophysiological considerations in the optimization of eicosapentaenoic acid production by *Nannochloropsis* sp. (Eustigmatophyceae). Bioresour. Technol. 35, 263–269. https://doi.org/10.1016/0960-8524(91)90123-2

Sumon, M.A.A., Sumon, T.A., Hussain, M.A., Lee, S.J., Jang, W.J., Sharifuzzaman, S.M., Brown, C.L., Lee, E.W., Hasan, M.T., 2022. Single and multi-strain probiotics supplementation in commercially prominent finfish aquaculture: Review of the current knowledge. J. Microbiol. Biotechnol. 32, 681–698. https://doi.org/10.4014/jmb.2202.02032

Sveen, L.R., Grammes, F.T., Ytteborg, E., Takle, H., Jørgensen, S.M., 2017. Genome-wide analysis of Atlantic salmon (*Salmo salar*) mucin genes and their role as biomarkers. PLoS One. https://doi.org/10.1371/journal.pone.0189103

Teran, M. del M., de Moreno de LeBlanc, A., Savoy de Giori, G., LeBlanc, J.G., 2021. Thiamine-producing lactic acid bacteria and their potential use in the prevention of neurodegenerative diseases. Appl. Microbiol. Biotechnol. 105, 2097–2107. https://doi.org/10.1007/s00253-021-11148-7

Teuling, E., Schrama, J.W., Gruppen, H., Wierenga, P.A., 2017. Effect of cell wall characteristics on algae nutrient digestibility in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarus gariepinus*). Aquaculture 479, 490–500. https://doi.org/10.1016/j.aquaculture.2017.06.025

Tevatia, R., Allen, J., Rudrappa, D., White, D., Clemente, T.E., Cerutti, H., Demirel, Y., Blum, P., 2015. The taurine biosynthetic pathway of microalgae. Algal Res. 9, 21–26. https://doi.org/10.1016/j.algal.2015.02.012

Thangaraju, M., Cresci, G.A., Liu, K., Ananth, S., Gnanaprakasam, J.P., Browning, D.D., Mellinger, J.D., Smith, S.B., Digby, G.J., Lambert, N.A., Prasad, P.D., Ganapathy, V., 2009. GPR109A Is a G-protein–coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. Cancer Res. 69, 2826–2832. https://doi.org/10.1158/0008-5472.CAN-08-4466

Thorarensen, H., Kubiriza, G.K., Imsland, A.K., 2015. Experimental design and statistical analyses of fish growth studies. Aquaculture 448, 483–490. https://doi.org/10.1016/j.aquaculture.2015.05.018

Thoresen, L., 2022. Digestibility and performance of microalgae in salmonid feed.

Tibbetts, S.M., Milley, J.E., Lall, S.P., 2015. Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. J. Appl. Phycol. 27, 1109–1119. https://doi.org/10.1007/s10811-014-0428-x

Tocher, D.R., Betancor, M.B., Sprague, M., Olsen, R.E., Napier, J.A., 2019. Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: Bridging the gap between supply and demand. Nutrients 11, 1–20. https://doi.org/10.3390/nu11010089

Tran-Ngoc, K.T., Huynh, S.T., Sendão, J., Nguyen, T.H., Roem, A.J., Verreth, J.A.J., Schrama, J.W., 2019. Environmental conditions alter the effect of organic acid salts on digestibility and intestinal morphology in Nile tilapia (*Oreochromis niloticus*). Aquac. Nutr. 25, 134–144. https://doi.org/10.1111/anu.12837

Tran, N.T., Li, Z., Wang, S., Zheng, H., Aweya, J.J., Wen, X., Li, S., 2020. Progress and perspectives of short-chain fatty acids in aquaculture. Rev. Aquac. 12, 283–298. https://doi.org/10.1111/raq.12317

Turchini, G.M., Torstensen, B.E., Ng, W.-K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. 1, 10–57. https://doi.org/10.1111/j.1753-5131.2008.01001.x
Urán, P. A., Aydin, R., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M., 2008. Soybean meal-induced uptake block in Atlantic salmon *Salmo salar* distal enterocytes. J. Fish Biol. https://doi.org/10.1111/j.1095-8649.2008.02091.x
Urán, P.A., Gonçalves, A.A., Taverne-Thiele, J.J., Schrama, J.W., Verreth, J.A.., Rombout, J.H.W.., 2008. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). Fish Shellfish Immunol. 25, 751–760. https://doi.org/10.1016/j.fsi.2008.02.013
Urán, P.A., Schrama, J.W., Rombout, J.H.W.M., Obach, A., Jensen, L., Koppe, W., Verreth, J.A.J., 2008. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar* L.) at different temperatures. Aquac. Nutr. 14, 324–330. https://doi.org/10.1111/j.1365-2095.2007.00534.x
Urán, P.A., Schrama, J.W., Rombout, J.H.W.M., Taverne-Thiele, J.J., Obach, A., Koppe, W., Verreth, J.A.J., 2009. Time-related changes of the intestinal morphology of Atlantic salmon, *Salmo salar* L., at two different soybean meal inclusion levels. J. Fish Dis. 32, 733–744. https://doi.org/10.1111/j.1365-2761.2009.01049.x
Utech, M., Mennigen, R., Bruewer, M., 2010. Endocytosis and recycling of tight junction proteins in inflammation. J. Biomed. Biotechnol. 2010, 1–6. https://doi.org/10.1155/2010/484987
Vågsholm, I., Arzoomand, N.S., Boqvist, S., 2020. Food security, safety, and sustainability—Getting the trade-offs right. Front. Sustain. Food Syst. 4. https://doi.org/10.3389/fsufs.2020.00016
Valente, L.M.P., Custódio, M., Batista, S., Fernandes, H., Kiron, V., 2019. Defatted microalgae (*Nannochloropsis* sp.) from biorefinery as a potential feed protein source to replace fishmeal in European sea bass diets. Fish Physiol. Biochem. 45, 1067–1081. https://doi.org/10.1007/s10695-019-00621-w
Valipour, A., Nedaei, S., Noori, A., Khanipour, A.A., Hoseinifar, S.H., 2019. Dietary *Lactobacillus plantarum* affected on some immune parameters, air-exposure stress response, intestinal microbiota, digestive enzyme activity and performance of narrow clawed crayfish (*Astacus leptodactylus*, Eschscholtz). Aquaculture 504, 121–130.
van den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J.J., Hendriks, H.G.C.J.M., Koninkx, J.G.J.F., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. Aquaculture 94, 297–305. https://doi.org/10.1016/0044-8486(91)90174-6
Van Den Ingh, T.S.G.A.M., Olli, J.J., Krogdahl, Å., 1996. Alcohol-soluble components in soybeans cause morphological changes in the distal intestine of Atlantic salmon, *Salmo salar* L. J. Fish Dis. https://doi.org/10.1111/j.1365-2761.1996.tb00119.x
van der Marel, M., Adamek, M., Gonzalez, S.F., Frost, P., Rombout, J.H.W.M., Wiegertjes, G.F., Savelkoul, H.F.J., Steinhagen, D., 2012. Molecular cloning and expression of two β-defensin and two mucin genes in common carp (*Cyprinus carpio* L.) and their up-regulation after β-glucan feeding. Fish Shellfish Immunol. 32, 494–501. https://doi.org/10.1016/j.fsi.2011.12.008
van Raamsdonk, L.W.D., van der Fels-Klerx, H.J., de Jong, J., 2017. New feed ingredients: the insect opportunity. Food Addit. Contam. Part A 34, 1384–1397. https://doi.org/10.1080/19440049.2017.1306883
Verstraeten, S.L., Lorent, J.H., Mingeot-Leclercq, M.P., 2020. Lipid membranes as key targets for the pharmacological actions of ginsenosides. Front. Pharmacol. 11, 1–13. https://doi.org/10.3389/fphar.2020.576887
Vieira, F.N., Buglione, C.C., Mourino, J.P.L., Jatobá, A., Martins, M.L., Schleder, D.D., Andreatta, E.R., Barraco, M.A., Vinatea, L.A., 2010. Effect of probiotic supplemented diet on marine shrimp survival after challenge with *Vibrio harveyi*. Arq. Bras. Med. Veterinária e Zootec. 62, 631–638.
Villamil, L., Reyes, C., Martínez-Silva, M.A., 2014. In vivo and in vitro assessment of *Lactobacillus acidophilus* as probiotic for tilapia (*Oreochromis niloticus*, Perciformes: Cichlidae) culture improvement. Aquac. Res. 45, 1116–1125.

Villodre Tudela, C., Boudry, C., Stumpff, F., Aschenbach, J.R., Vahjen, W., Zentek, J., Pieper, R., 2015. Down-regulation of monocarboxylate transporter 1 (MCT1) gene expression in the colon of piglets is linked to bacterial protein fermentation and pro-inflammatory cytokine-mediated signalling. Br. J. Nutr. 113, 610–617. https://doi.org/10.1017/S0007114514004231

Wang, C., Liu, Y., Sun, G., Li, X., Liu, Z., 2019. Growth, immune response, antioxidant capability, and disease resistance of juvenile Atlantic salmon (*Salmo salar* L.) fed *Bacillus velezensis* V4 and *Rhodotorula mucilaginosa* compound. Aquaculture 500, 65–74. https://doi.org/10.1016/j.aquaculture.2018.09.052

Wang, Y., Gu, Q., 2010. Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. Mar. Biol. Res. 6, 327–332. https://doi.org/10.1080/17451000903300893

Webb, S., 2020. Fixative artefacts in histology: Mitigation and interpretation.

Wegner, N., 2011. Ventilation and animal respiration | Gill respiratory morphometrics, in: Encyclopedia of Fish Physiology. Elsevier, pp. 803–811. https://doi.org/10.1016/B978-0-12-374553-8.00166-0

Whitear, M., 1986. Epidermis, in: Biology of the Integument. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 8–38. https://doi.org/10.1007/978-3-662-00989-5_2

Wilson, J.M., Laurent, P., 2002. Fish gill morphology: Inside out. J. Exp. Zool. 293, 192–213. https://doi.org/10.1002/jez.10124

Wu, H.-X., Li, W.-J., Shan, C.-J., Zhang, Z.-Y., Lv, H.-B., Qiao, F., Du, Z.-Y., Zhang, M.-L., 2021. Oligosaccharides improve the flesh quality and nutrition value of Nile tilapia fed with high carbohydrate diet. Food Chem. Mol. Sci. 3, 100040. https://doi.org/10.1016/j.fochms.2021.100040

Wuertz, S., Schroeder, A., Wanka, K.M., 2021. Probiotics in fish nutrition—Long-standing household remedy or native nutraceuticals? Water 13, 1348. https://doi.org/10.3390/w13101348

Xu, X., Peng, Q., Zhang, Y., Tian, D., Zhang, P., Huang, Y., Ma, L., Qiao, Y., Shi, B., 2020. A novel exopolysaccharide produced by *Lactobacillus coryniformis* NA-3 exhibits antioxidant and biofilm-inhibiting properties in vitro. Food Nutr. Res. 64. https://doi.org/10.29219/fnr.v64.3744

Yongmanitchai, W., Ward, O.P., 1991. Growth of and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. Appl. Environ. Microbiol. 57, 419–425. https://doi.org/10.1128/aem.57.2.419-425.1991

Yousefi, B., Eslami, M., Ghasemian, A., Kokhaei, P., Salek Farrokhi, A., Darabi, N., 2019. Probiotics importance and their immunomodulatory properties. J. Cell. Physiol. 234, 8008–8018. https://doi.org/10.1002/jcp.27559

Zhai, Q., Yu, L., Li, T., Zhu, J., Zhang, C., Zhao, J., Zhang, H., Chen, W., 2017. Effect of dietary probiotic supplementation on intestinal microbiota and physiological conditions of Nile tilapia (*Oreochromis niloticus*) under waterborne cadmium exposure. Antonie Van Leeuwenhoek 110, 501–513.

Zhao, H., Wang, G., Wang, H., Mo, W., Huang, Y., Cao, J., Li, P., 2021. Effects of dietary sodium butyrate on growth, digestive enzymes, body composition and nutrient retention-related gene expression of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Anim. Nutr. 7, 539–547. https://doi.org/10.1016/j.aninu.2020.12.007

Zhao, Y., Chen, F., Wu, W., Sun, M., Bilotta, A.J., Yao, S., Xiao, Y., Huang, X., Eaves-Pyles, T.D., Golovko, G., Fofanov, Y., D'Souza, W., Zhao, Q., Liu, Z., Cong, Y., 2018. GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. Mucosal Immunol. 11, 752–762. https://doi.org/10.1038/mi.2017.118

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., O'toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M.G., Lebeer, S., 2020. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int. J. Syst. Evol. Microbiol. 70, 2782–2858. https://doi.org/10.1099/ijsem.0.004107

Zheng, L., Kelly, C.J., Battista, K.D., Schaefer, R., Lanis, J.M., Alexeev, E.E., Wang, R.X., Onyiah, J.C., Kominsky, D.J., Colgan, S.P., 2017. Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor–dependent repression of claudin-2. J. Immunol. 199, 2976–2984. https://doi.org/10.4049/jimmunol.1700105

Zheng, X., Gallot, G., 2020. Dynamics of cell membrane permeabilization by saponins using terahertz attenuated total reflection. Biophys. J. 119, 749–755. https://doi.org/10.1016/j.bpj.2020.05.040

Zhou, C., Lin, H., Huang, Z., Wang, J., Wang, Y., Yu, W., 2019. Effect of dietary sodium butyrate on growth performance, enzyme activities and intestinal proliferation-related gene expression of juvenile golden pompano *Trachinotus ovatus*. Aquac. Nutr. 25, 1261–1271.

Zorriehzahra, M.J., Delshad, S.T., Adel, M., Tiwari, R., Karthik, K., Dhama, K., Lazado, C.C., 2016. Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. Vet. Q. https://doi.org/10.1080/01652176.2016.1172132

Paper I

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Supplementation of lactic acid bacteria has positive effects on the mucosal health of Atlantic salmon (*Salmo salar*) fed soybean meal

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ABSTRACT

We investigated the ability of lactic acid bacteria, when added individually or in combination in feeds, to prevent soybean meal-induced enteritis in Atlantic salmon. A control diet, designed to induce enteritis, was formulated with marine ingredients and 20% soybean meal. Three more diets were produced by coating the control diet with two bacteria, either singly (*Lactobacillus plantarum*; *L. fermentum*) or in combination. The fish were fed the abovementioned diets for 38 days. We performed histological assessments and evaluated the expression of selected mucin and antimicrobial peptide genes in the dorsal skin, gills, and distal intestine. Digesta were also collected to study the short chain fatty acids. Feeding bacteria, individually or in combination, altered the short chain fatty acids−acetoacetic acid, lactic acid, succinic acid, propionic acid−and the total fatty acids in the digesta significantly. Of all the determined short chain fatty acids, the concentration of acetoacetic acid was the highest, and the fish fed the combination of the two bacteria had the significantly highest value. Succinic acid was also significantly higher in fish fed the combination compared to the control group and the *L. fermentum* group. Total fatty acids were significantly higher in fish fed the combination than those fed *L. fermentum*. Compared to the control and probiotic combination-fed fish, those fed *L. plantarum* had higher *defensin1* expression in the skin. We also observed significantly higher number of gill mucous cells in the fish fed the blend compared to the control group. Lamina propria width was significantly reduced in fish fed the blend. Supra nuclear vacuoles were higher in fish fed the single species or the blend, compared to the control group. Thus, adding the probiotics to a soybean meal diet can elevate the digesta short chain fatty acids and intestine supranuclear vacuoles, and reduce the lamina propria width, which probably indicate prevention of enteritis.

Abbreviations[1,2]: AB, Alcian blue; AMPs, Antimicrobial peptides; ANOVA, Analysis of variance; CF, Condition factor; CFU, Colony forming unit; CT, Control diet; DNA, Deoxyribonucleic acid; FDU, Forsøksdyrutvalget; FL, Final fork length; FW, Final body weight; GE, Total area of gill epithelium; GM, Total area of gill mucous cells; GM2, Gills *muc5ac2*; GM5, Gill *muc5b*,; GME, Total area of gill mucous cells (GM) / Total area of gill epithelium (GE); GN, Number of gill mucous cells; GNE, Number of gill mucous cells (GN) / Total area of gill epithelium (GE); HOE, Height of enterocytes; HOV, Height of villi; HSB, Hue, saturation and brightness; HSD, Honestly significant difference; IC1, Intestine *cathelicidin1*,; ID3, Intestine *defensin3*; ID4, Intestine *defensin4*; IEL, Number of intraepithelial lymphocytes; IL, Initial fork length; IM2, Intestine *muc2*; IW, Initial body weight; LAB, Lactic acid bacteria; LAS, Leica Application Suite; LF, Diet with probiotic bacteria *Lactobacillus fermentum*; LP, Diet with probiotic bacteria *Lactobacillus plantarum*; LP&LF, Diet with both probiotic bacteria *L. fermentum* & *L. plantarum*; MRS, Man, Rogosa and Sharpe; NBF, Neutral buffered formalin; NOM, Number of mucous cells (in distal intestine); PAS, Periodic acid–Schiff; PC, Principal component; PCA, Principal component analysis; PCR, Polymerase chain reaction; RNA, Ribonucleic acid; RVC, Rotating vacuum coater; SBM, Soybean meal; SBMIE, Soybean meal induced enteritis; SC1, Skin *cathelicidin1*; SCFAs, Short chain fatty acids; SD1, Skin *defensin1*; SE, Total area of skin epithelium; SEM, Standard error of mean; SGR, Specific growth rate; SM, Total area of skin mucous cells; SM1, Skin *muc5ac1*; SM2, Skin *muc5ac2*; SM5, Skin *muca5b*; SME, Total area of skin mucous cells (SM) / Total area of skin epithelium (SE); SN, Number of skin mucous cells; SNE, Number of skin mucous cells (SN) / Total area of skin epithelium (SE); SNV, Supra nuclear vacuoles; TGC, Thermal growth coefficient; TJ, Tight junction; WG, Weight gain; WLP, Width of lamina propria; WOV, Width of villi.

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1. Introduction

Feeds are formulated with various ingredients to deliver appropriate nutrients to farmed fish. Feed ingredients may affect the barrier status and health of mucosal surfaces (Nimalan et al., 2022; Sørensen et al., 2021). Barrier functions are mediated by microbiota, which is greatly affected by ingredients and additives such as prebiotics and probiotics in feeds (Ringø et al., 2016; Gupta et al., 2019; Wang et al., 2020). Administration of feeds with probiotics can be considered an environment-friendly disease management tool that targets the host innate immune system (Andani et al., 2012; Beck et al., 2015; Jahangiri and Esteban, 2018; Ringø et al., 2020, 2018; Zorriehzahra et al., 2016). Oligosaccharides (prebiotics) in feeds are fermented by the administered probiotics and certain resident microbes. The fermentation products including short chain fatty acids (SCFAs) such as acetate, propionate and butyrate (Asaduzzaman et al., 2018; Hills et al., 2019; Markowiak-Kopeć and Śliżewska, 2020; Rimoldi et al., 2018) display pleiotropic functions to maintain microbial homeostasis and host health (Louis et al., 2014; Rivière et al., 2016). In a human colon carcinoma (Caco-2) epithelial cell model, butyrate promoted epithelial barrier function by suppressing the expression of certain barrier proteins that increase gut permeability, facilitating assembly of tight junction (TJ) complexes, and modulating the epigenetic landscape in host cells (Chang et al., 2014; Kelly et al., 2015; Peng et al., 2009; Zheng et al., 2017). Notably, in salmonids too, dietary butyrate has been shown to upregulate the expression of TJ molecules and innate immune parameters in vivo (Hoseinifar et al., 2017; Mirghaed et al., 2019). Such studies are beginning to unravel the complex relationship between fish and microbiota and how microbial metabolites can promote intestinal integrity, though more research is warranted to identify strains that produce butyrate in situ in the salmonid gut. Evidence from in vivo studies substantiates the concept that the intestinal barrier is a key determinant of host health. Therefore, if probiotic strains for salmonids can increase nutrient uptake, improve barrier function, and reduce overall mortality, such products have great potential for application in aquaculture.

The mode of action of probiotics includes antimicrobial activity (by decreasing luminal pH, competitive exclusion of pathogens, production of antimicrobial substances), barrier function enhancement, and immune system modulation (Angahar, 2016; Cordero et al., 2015; Jahangiri and Esteban, 2018; Moriarty, 1997; Nayak, 2010; Ng et al., 2009; Ringø, 1999; Ringø et al., 2020). Among the innate immune responses, pro- or anti-inflammatory genes have key roles in regulating intestinal homeostasis (Bäuerl et al., 2013; Krishnaveni et al., 2021). An in vitro study with rainbow trout (*Oncorhynchus mykiss*) intestinal cells in primoculture reported the anti-inflammatory and pro-inflammatory responses evoked by the probiotic strain, *Lactobacillus plantarum* R2, depending on the type of bacterial infection (Cingeľová Maruščáková et al., 2021). The authors of the aforementioned study have demonstrated the ability of probiotics to appropriately regulate inflammation based on the immune status and demand of an organism. However, the effectiveness of probiotics depends on several factors such as mode of administration (through diet or directly into the water or as a vaccine), type and number of species (single or combination of species), source (terrestrial or aquatic host), duration (feeding days) and viability in the gastrointestinal tract of the host (Asaduzzaman et al., 2018; Beck et al., 2015; Ringø et al., 2020).

Some of the commercially available non-fish-derived probiotics may have limited viability in fish gut (Lazado et al., 2015). Therefore, viable candidates with proven ability to adhere to the intestine mucus and eventually exert positive effects on hosts are vital for applications in aquaculture. Several bacterial candidates including lactic acid bacteria (LAB), pseudomonads, and yeast have been recognized as viable and efficient probiotics in aquaculture (Fečkaninová et al., 2019; Gildberg et al., 1995; Lobo et al., 2014; Suzer et al., 2008; Tapia-Paniagua et al., 2010). Among the LAB species, *L. plantarum* was known to have an anti-inflammatory property (Duary et al., 2012; Sherif et al., 2021) and *L. fermentum* was found to have antibacterial activity (Song et al., 2021). Zheng et al. (2020) reported that whiteleg shrimp (*Penaeus vannamei*) intestine harboured specific beneficial bacteria (the genera *Demequina*, *Rubritalea*, *Tenacibaculum*, *Marinicella* and *Phaeobacter*) when the shrimp received *L. plantarum* supplemented (cell-free extract) diet. Their study also indicated the highest abundance of *Acidobacteria*, with a 70-fold increase compared to those fed the control diet. Krishnaveni et al. (2021) reported that dietary supplementation *of L. fermentum* URLP18 increased the LAB population and improved the growth performance and feed utilization in *Cyprinus carpio*.

There are also studies on the application of two or more probiotic bacterial species, including those belonging to the genus *Lactobacillus*; the combined effect was found to enhance the growth and immune performance of the host aquatic animals (Alishahi et al., 2018; Foysal et al., 2020; Wang and Gu, 2010). Moreover, a mixture of LAB (*L. plantarum* and *Lactococcus lactis*), both isolated from the hindgut of olive flounder (*Paralichthys olivaceus*) are effective against *Streptococcus iniae* (Beck et al., 2015). Probiotics for aquaculture should have an excellent ability to strengthen mucosal health not only to control pathogen-caused diseases but also to endure inflammatory reactions associated with new/novel plant ingredients in aquafeeds.

Soybean meal (SBM) (defatted and dehulled product of soybeans), or saponins isolated from soy are commonly used to create enteritis models in Atlantic salmon (*Salmo salar*) (Baeverfjord and Krogdahl, 1996; Kiron et al., 2022; Sørensen et al., 2011, 2021; Urán et al., 2008a). SBM contains a range of antinutritional factors, and a 20% inclusion can adversely affect the growth and gut health of salmonids, mainly due to saponins (Booman et al., 2018; Knudsen et al., 2008, 2007; Krogdahl et al., 2015, 2010). SBM-induced enteritis (SBMIE) model is an ideal tool to study the protective effect of probiotics on fish gut. Previous studies have documented the properties of the two strains *Lactobacillus plantarum* BiocenolTM (CCM 8674) and *Lactobacillus fermentum* BiocenolTM (CCM 8675) as potential probiotic strains (Fečkaninová et al., 2019). The ability of these two strains to adhere to the enterocytes was documented by Gupta et al. (2019) and the capacity to modulate intestinal health was reported by Nimalan et al. (2022). In our two previous studies, a mix of the two strains at a concentration of $\sim 10^8$ cells per g feed were used. In the study of Nimalan et al. (2022), we reported the effects of supplementing three different diets (marine-, plant- and soybean meal-based) with a mixture of *L. plantarum* R2 Biocenol™ (CCM 8674) and *L. fermentum* R3 Biocenol™ (CCM 8675); mucosal health was assessed by studying the histomorphology and expression of selected mucin and AMP genes in the dorsal skin, gills and distal intestine as well as changes in short chain fatty acids (Nimalan et al., 2022). The two LABs improved the mucosal health of Atlantic salmon but did not alleviate SBMIE signs (Nimalan et al., 2022).

There is only limited information regarding the ability of LABs to prevent SBMIE in Atlantic salmon. Also, there is a need to clarify if a single LAB is equally efficient as a mix of the two species. A feeding experiment was therefore designed to understand whether the individual effects of the two probiotic species (*L. plantarum* R2 Biocenol™ (CCM 8674) and *L. fermentum* R3 Biocenol™ (CCM 8675)) are as effective as their combined influence on the mucosal barriers to prevent SBMIE in Atlantic salmon. It should be noted that the probiotics were added in the feed, at the same concentration that we employed in previous studies. This study investigated the mucosal health of the skin, gills, and distal intestine by evaluating histomorphometric parameters and mucin and antimicrobial peptide (AMP) gene expression in post-smolt Atlantic salmon fed SBM-based feed. In addition, the concentration of SCFAs in the faeces was also evaluated to study the effect of probiotics on the

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metabolites.

2. Materials and methods

This study was approved by the National Animal Research Authority (FDU: Forsøksdyrutvalget ID-5887) in Norway. The experiment was the second phase of a study that assessed the effects of plant and marine feed ingredients on the performance of Atlantic salmon (Sørensen et al., 2021).

2.1. Basal feed formulation, probiotic culture and coating, and experimental diets

A feed based on fish meal, fish oil, soybean meal (SBM), wheat meal, wheat gluten and micronutrients was produced at the Feed Technology Center, Nofima, Bergen, Norway (Table 1). The nutrient and amino acid composition of the feed is given in Table 2. Details about the extrusion process and experimental feed production can be found in Nimalan et al. (2022).

Two lactic acid bacteria (LAB) species; *L. plantarum* R2 Biocenol™ (CCM 8674); and *L. fermentum* R3 Biocenol™ (CCM 8675) were obtained from the intestinal content of rainbow trout obtained from a fish farm, Rybárstvo – Požehy s.r.o. Dubové in the Slovak Republic (Fečkaninová et al., 2019). The strains are part of the culture collection of the Czech Republic (Czech Collection of Microorganisms, Brno). Pure cultures of LAB were grown anaerobically (Oxoid Gas Pack Anaerobic system) on De Man, Rogosa and Sharpe (MRS) agar plates (HiMedia Laboratories, Mumbai, India) at 37 °C for 48 h. Next, the culture was inoculated into 1000 ml of MRS broth and incubated at 37 °C for 18 h on a shaker, before they were centrifuged at 4500 rpm for 20 min at 4 °C in a cooling centrifuge (Universal 320 R, Hettich, Germany). The resulting cell pellets were washed twice and resuspended in 30 ml of 0.9% (w/v) sterile saline. The experimental diets (batches of 1800 g) were thoroughly coated with the LAB suspensions (single species and mixture of both species) using a vacuum coater (Rotating Vacuum Coater F-6-RVC, Forberg International AS, Oslo, Norway) at 70 kPa at the feed laboratory of Nord University, Bodø, Norway.

Table 1
Ingredient composition (%) of the four experimental diets.

	CT	LP	LF	LP&LF
Fishmeal[a]	30	30	30	30
Wheat meal[b]	6.55	6.55	6.55	6.55
Wheat gluten[c]	10	10	10	10
Soybean meal[d]	20	20	20	20
Fish oil[e]	26.4	26.4	26.4	26.4
Mineral premix[f]	0.59	0.59	0.59	0.59
Vitamin premix[f]	2	2	2	2
Monosodium Phosphate[g]	2.5	2.5	2.5	2.5
Choline chloride[g]	0.5	0.5	0.5	0.5
Methionine[g]	0.6	0.6	0.6	0.6
Lysine[g]	0.5	0.5	0.5	0.5
Threonine[g]	0.1	0.1	0.1	0.1
Histidine[g]	0.2	0.2	0.2	0.2
Sterile saline, %	0.9	0	0	0
Carop. Pink (10% Astax)[h]	0.05	0.05	0.05	0.05
Lactobacillus plantarum (cells/g)[i]	0	$\sim 10^8$	0	$\sim 10^4$
Lactobacillus fermentum (cells/g)[i]	0	0	$\sim 10^8$	$\sim 10^4$

CT – control diet without probiotics, LP – control diet with the probiotic species *Lactobacillus plantarum*, LF - control diet with the probiotic species *Lactobacillus fermentum*, and LP&LF – control diet with both *L. plantarum* and *L. fermentum*.

[a] Fishmeal - LT fiskemel – Pelagia Protein (Ryttervik, Egersund, Norway)

[b] Wheat – Norgesmøllene AS (Bergen, Norway)

[c] Wheat gluten – Tereos Syral (Nicaise, France)

[d] Soybean meal – Fiskå mølle (Etne, Norway)

[e] Fishoil – Vedde Sildoljefabrikk (Langevåg, Norway)

[f] Nofima mineral premix, 0.59% inclusion give per kg diet: Fe: 60 mg, Mn: 30 mg, Zn:130 mg, Cu: 6 mg, Mg: 750 mg, K: 800 mg, Se: 0.3 mg; Nofima vitamin premix, 2% inclusion give per kg diet: Vitamin A: 2 000IE, vitamin D3: 2 500IE, vitamin E: 200 mg, vitamin K3: 20 mg, vitamin B1: 20 mg, vitamin B2: 30 mg, vitamin B6: 25 mg, vitamin B12: 0.05 mg, niacin: 200 mg, Ca-D-pantonat: 60 mg, biotin: 1.0 mg, folic acid: 10 mg, vitamin C: 200 mg

[g] Monosodium Phosphate, Choline chloride, Methionine, Lysine, Histidin, Threonin – Vilomiks (Hønefoss, Norway)

[h] Carophyll Pink – DSM Nutritional Products (Village-Neuf, France)

[i] Autochtohonous *Lactobacillus plantarum* BiocenolTM (CCM 8674) and *Lactobacillus fermentum* BiocenolTM (CCM 8675) from the intestinal content of healthy rainbow trout.

Table 2
Analyzed proximate composition (% as is) and amino acid composition (% as is) of the basal feed.

Composition	Values
Moisture	4.9
Protein	42.2
Lipid	28.6
Ash	9.45
Energy (KJ/100 g)	2029
Amino acids	
Alanine	2.03
Arginine	2.33
Aspartic acid	3.43
Glutamic acid	8.03
Glycine	2.18
Histidine	1.02
Hydroxyproline	0.22
Isoleucine	1.64
Leucine	2.93
Lysine	2.85
Phenylalanine	1.79
Proline	2.47
Serine	1.91
Threonine	1.64
Tyrosine	1.35
Valine	1.86
Tryptophan	0.44
Cysteine	0.50
Methionine	1.67

The LAB were coated on to the pellets of the probiotic feeds. Experimental feed pellets were first transferred to the vacuum coater, then vacuum pump was started to create a vacuum inside the coater. Paddles were started before spraying the oil on to the pellets to ensure constant mixing for even distribution of the LAB suspension. In total, four experimental feeds were used in this study; a control diet without probiotics, CT; a diet with *L. plantarum*, LP; a diet with *L. fermentum*, LF and one diet with a combination of both LAB (*L. plantarum* and *L. fermentum*), LP&LF. After coating, the bacterial counts on each diet were $\sim 10^8$ CFU/g as determined by spread plating on MRS agar plates and incubating at 37 °C for 48 h. In the probiotic combination diet, the bacterial counts ($\sim 10^4$ CFU/g) of each species were kept similar to maintain identical counts in all the three diets (LP, LF, and LP&LF). The control diet (CT) was coated with 0.9% of sterile saline. The coated diets were stored at 4 °C until they were fed to the experimental fish.

The SBM-based feed (without LAB) was intended to induce enteritis and served as the negative control. A reader can clarify that the CT diet-caused enteritis, by considering a marine-based feed without LAB (BG1) from our previous publications (Nimalan et al., 2022; Sørensen et al., 2021) as the positive control. The BG1-based histomorphometric results are used in the discussion section to emphasize the ability of the negative control (CT in this study) to induce inflammation compared to the positive control (BG1).

2.2. Animal, rearing condition and feeding

Atlantic salmon post-smolts were purchased from Cermaq, Hopen, Bodø, Norway (Aquagen strain, Aquagen AS, Trondheim, Norway). These fish were reared in 8 circular fiberglass tanks (1100 L) connected to a flow-through system and each tank contained 40–43 fish, with an

average initial weight of 129.2 ± 2.2 g (mean ± standard error of mean, SEM). The water was pumped at 1000 L per h from Saltenfjorden, from a depth of 250 m. The average temperature and salinity of the water were 7.6 °C and 35‰, respectively. Oxygen saturation was always above 85%, measured at the water outlet. A 24 h photoperiod was maintained throughout the 38-day feeding trial. The fish were fed ad libitum using automatic feeders (Arvo Tec, Huutokoski, Finland) for 12 h per day between 08:00 and 20:00 (7 feedings: 08:00–10:00, 10:00–12:00, 12:00–14:00, 14:00–16:00, 16:00–18:00, 18:00–19:00 and 19:00–20:00). The experimental fish used in the current study were first offered feeds without the LAB for a period of 65 days before the start of LAB feeding (Sørensen et al., 2021).

2.3. Sampling strategy for assessing the growth, histology, gene expression and SCFAs

Prior to handling, fish were anesthetized using tricainemethanesulfonate (MS 222, 140 mg/L). The weight and fork length of fish were individually recorded, at the beginning and end of the experiment. The dorsal skin (left), gills (second arch) and intestinal (approximately 2 cm of the anterior part of the distal intestine) (Sanden and Olsvik, 2009; Sundell and Sundh, 2012) tissues were obtained from 12 fish per tank, of which tissues from 6 fish were immediately placed in 10% neutral buffered formalin (NBF) for 24 h at room temperature, for the histological evaluation. Tissues from the remaining 6 fish were transferred to tubes filled with RNA later® (Ambion Inc., Austin, Texas, United States), and stored at − 20 °C for gene expression analysis. For SCFA analysis, 5 fish per tank were stripped to collect the digesta and the samples were stored at − 20 °C.

2.4. Growth performance

Fish growth performance indicators were calculated as follows: Weight gain (WG%) = ((FW - IW) / IW) × 100. Specific Growth Rate (SGR) = ((Ln (FW) - Ln (IW)) / D) × 100. Thermal growth coefficient (TGC) = (((FW)^(1/3) - (IW)^(1/3)) / ((T × D))) × 1000. Condition factor (CF) = (FW / FL^3) × 100. Where, FW = final body weight of fish (g), IW = initial body weight of fish (g), T is the water temperature in °C, D is feeding duration in days. IL and FL are the initial and final fork length (cm) of fish, respectively.

2.5. Short chain fatty acid composition analysis

The digesta (approximately 1 g per fish) were first thoroughly mixed with deionized water (50 ml). Then membrane filter paper with 0.45 μm pore size (Supor®−450, PALL Life Sciences, Emiliano Zapata, Mexico) was used to filter the solution. Until further analysis, the filtrates (5 ml per fish) were kept in cryotubes and stored at − 20 °C. The produced SCFAs (acetic, acetoacetic, butyric, formic, lactic, propionic, valeric and succinic acids) were quantified by capillary isotachophoresis (Electrophoretic analyzer EA 202 M, VILLA LABECO spol. s.r.o., Spisska Nova Ves, Slovakia) as described by Gancarcikova et al. (2020).

2.6. Mucin and AMP gene expression analysis

Primers were purchased from Eurofins Genomics (Luxembourg, Luxembourg) and the sequences and details of the primers of all target and reference genes are described in Sørensen et al. (2021). For this experiment, relative mRNA levels of mucin genes (*muc2*, *muc5ac1*, *muc5ac2* and *muc5b*) in the skin, gills and distal intestine, and AMP genes (*defensin1*, *defensin2*, *defensin3*, *defensin4*, and *cathelicidin1*) in the skin and distal intestine were studied. The RNA extraction, cDNA synthesis and qPCR were performed as described elsewhere (Sørensen et al., 2021).

2.7. Histomorphometric evaluations

Tissues were processed and embedded in paraffin following standard histological procedures (Øverland et al., 2009; Sørensen et al., 2011) at the histology laboratory of Nord University, Bodø, Norway. The skin tissues (approximately 2 cm) were sliced transversely into 3 equal parts and decalcified with 10% formic acid (25 blocks per L) for 5 h, prior to processing. As for the gill samples, the arches were trimmed before processing. Regarding the intestine samples, the contents were first rinsed off with 10% NBF prior to fixation, and the tissues were embedded longitudinally. The Leica microtome was used to cut tissue sections of 4 μm and the prepared slides (one per fish) were stained with Alcian blue - periodic acid–Schiff (AB-PAS) at pH 2.5. A camera (Leica MC170HD, Heersbrugg, Switzerland) fitted on a light microscope (Leica DM1000, Wetzlar, Germany) was used to generate microphotographs at 40 × magnification by using a software, Leica Application Suite (LAS V4.12. INK, Heerbrugg, Switzerland). ImageJ 1.52a (Schneider et al., 2012) software was used to assess all the images.

2.7.1. Dorsal skin mucous cell image analyses

Approximately 600–900 μm (length) of skin microphotographs (9 per fish) were generated to evaluate the mucous cells. 'Freehand selections' tool of ImageJ was selected to demarcate the total area of skin epithelium (SE) and then 'Brightness and Hue' under 'Colour threshold' of the 'Image' menu was adjusted, while keeping 'Thresholding method' as 'Default', 'Threshold colour' set to red and 'Colour space' to HSB (hue, saturation and brightness). The next step was to select the 'Analyze' menu to measure the SE (Gong et al., 2020). The 'Wand tool' was used to select individual mucous cells. The background was cleared using 'Edit' and then the image was converted to 8 bits to retain only the mucous cells. The total area of the skin mucous cells (SM) and the number of skin mucous cells (SN) were determined by selecting 'Threshold' under 'Image' menu, and by setting 'Analyze particles' to '30 to infinity' under the 'Analyze' menu in ImageJ (Nimalan et al., 2022). SE, SM and SN were used to calculate 2 indices: SME (SM ÷ SE) and SNE (SN ÷ SE).

2.7.2. Gill mucous cell image analyses

To evaluate the area and number of mucous cells in the gills, 10 secondary lamellae from 5 different filaments per fish were chosen. Thus, in this study 50 secondary lamellae per fish were examined. The same image analysis procedure that is described for the skin, was employed for the gills also to examine the total area of gill epithelium (GE), the total area of gill mucous cells (GM), and the number of gill mucous cells (GN). The obtained values were used to calculate 2 indices: GME (GM ÷ GE) and GNE (GN ÷ GE) (Nimalan et al., 2022).

2.7.3. Distal intestine image analyses

For the morphometric analysis, 10 simple, long, well-oriented, and intact villi per fish were selected from 3 to 5 different locations. Approximately, 10 microphotographs per fish were generated. Height of villi (HOV), the width of villi (WOV), the height of enterocytes (HOE), and the width of the associated lamina propria (WLP) were quantitatively measured to understand the diet-induced aberrations in intestinal structure. The width of the villus varies along its height, and hence, to measure WOV, each villus was partitioned into 6 equal parts from the base to the tip (Nimalan et al., 2022). From these 5 points, WOV, HOE and WLP were gauged by employing the analysing tools ('straight' and 'segmented lines') of the ImageJ, and the average of the 5 values were registered. Semi-quantitative assessment was adopted to study the morphological changes in the following indices: number of mucous cells (NOM), number of intraepithelial lymphocytes (IEL), and supra nuclear vacuoles (SNV) of intestinal villi. An ordinal scoring strategy (from 1 to 5) for each index was developed (Supplementary Figure 1–3) based on Baeverfjord and Krogdahl (1996); Bakke-McKellep et al. (2007); Knudsen et al. (2008); Silva et al. (2015); Urán et al. (2008a, 2008b, 2009).

2.8. Statistics

All statistical analyses were executed in R studio (version 1.2.5042) for windows. In this experiment, the probiotic treatment effect was analysed by one-way analysis of variance (one-way ANOVA). The Shapiro–Wilk test was used to check the normality of data. Levene's test was used to assess the homogeneity of variance. One-way ANOVA was performed on SCFAs, most of the histology and gene expression data. Significant differences were revealed by carrying out Tukey's honestly significant difference (HSD) post-hoc test. When necessary, data were log-transformed (GNE, WOV, HOE, *defensin3,* and *cathelicidin1* in the intestine, *muc5b* in the gills, *muc5ac2* and *muc5b* in the skin and all the SCFAs data except formic and acetoacetic acids). Welch's ANOVA was performed for data that showed heteroscedasticity (growth performance, skin *muc5b*, and *muc2*). Kruskal-Wallis was performed for the gill data (GME) and semi-quantitively assessed ordinal data (NOM, IEL and SNV). Significant differences were revealed by performing Dunn's multiple comparison test. The function from the package "corrplot" in R was used to run Spearman correlations for all the combinations of histologically evaluated mucous cell indices and the selected mucin and AMP genes. To assess the ability of CT to induce enteritis, the histomorphometric indices, HOV, HOE, WOV, WLP, NOM, IEL, and SNV were compared with those of the BG1 diet reported in our previous publication (Nimalan et al., 2022); employing either parametric unpaired two-samples t-test or non-parametric Wilcoxon test (Supplementary file 1). The statistical significance is reported when $p < 0.05$. The gene expression data (missing values were replaced with group average) and distal intestinal histomorphometric indices were subjected to principal component analysis (PCA). In this study, tank was used as the experimental unit for growth performance calculations (Kiron et al., 2016). However, individual fish was considered as the experimental unit for histological evaluation (Bansemer et al., 2015; Cerezuela et al., 2013; Urán et al., 2008a), gene expression and SCFAs analyses (Bansemer et al., 2015). Means ± SEM of parameters are presented in all tables and figures.

3. Results

3.1. Growth performance indicators

During the course of the experiment, the mean weight of the fish increased from 129.2 g to 198.8 g. There were no significant differences in FW, FL, CF, SGR, TGC and WG% among diet groups. The growth parameters are presented in Table 3.

3.2. Effects of probiotics on the short chain fatty acids in the digesta

The concentration of faecal SCFAs is presented in Table 4. Formic, acetoacetic, lactic, succinic, acetic, propionic and butyric acids were detected in all the samples. The concentration of acetoacetic was high and butyric acid was low in all the groups, regardless of the probiotic treatment. Among all SCFAs, formic, acetic and butyric acids were not significantly affected by the probiotics, when applied singly or in combination. However, other SCFAs such as acetoacetic, lactic, succinic and propionic acids as well as total SCFAs were altered by probiotics. Fish fed a combination of the two probiotics (LP&LF) had a significantly higher concentration of acetoacetic (compared to CT, LP and LF), succinic (compared to CT and LF) and total SCFAs (compared to LF). In addition, fish fed LF had more propionic acid, compared to LP, which had a higher content of acetoacetic acid compared to fish fed LF and CT. Fish fed LP had more lactic acid, compared to LF (Table 4).

3.3. Effects of probiotics on mucin and AMP gene expression

The uncorrelated variables (principal components) of the expressed AMPs and mucin-related genes and their loadings are shown in Fig. 1, with principal component (PC) 1 and PC 2 explaining 23.3% and 14.6% of the variance in the data, respectively. The cumulative proportion of variance explained by 5 PCs was 70% (Supplementary Fig. 4). The variation in skin *muc5ac1* (SM1), skin *muc5ac2* (SM2), skin *muca5b* (SM5), and intestine *muc2* (IM2), were mainly explained by PC1, while the skin *defensin1* (SD1), skin *cathelicidin1* (SC1), intestine *defensin3* (ID3), and intestine *defensin4* (ID4) were explained by PC2. All the genes, except SC1, had positive loading on PC1, while only SM2, SM5, GM2, and IC1 had positive loading on PC2 (Supplementary Fig. 5). Mucin genes had the highest loadings on PC1 (e.g.: SM1, SM2, SM5 and

Table 3
Growth performance of Atlantic salmon fed soybean meal-based diet and the three diets coated with a single probiotic species or a combination of two probiotic species.

Growth parameters	Diet groups				*p*-value
	CT	LP	LF	LP&LF	
IW (g/fish)	135.0 ± 2.57	124.7 ± 7.14	129.6 ± 3.97	127.4 ± 2.35	0.497
IL (cm)	21.9 ± 0.25	21.5 ± 0.23	21.6 ± 0.28	21.6 ± 0.07	0.802
FW (g/fish)	214.0 ± 2.54	200.7 ± 11.53	197.7 ± 9.38	194.5 ± 5.65	0.311
FL (cm)	25.7 ± 0.15	25.1 ± 0.47	25.1 ± 0.48	25.0 ± 0.25	0.469
CF (g/cm^3)	1.27 ± 0.01	1.27 ± 0.00	1.25 ± 0.01	1.25 ± 0.00	0.149
SGR	1.21 ± 0.02	1.25 ± 0.00	1.11 ± 0.04	1.11 ± 0.12	0.300
TGC	2.95 ± 0.03	2.97 ± 0.06	2.65 ± 0.14	2.63 ± 0.30	0.505
WG (%)	58.52 ± 1.14	60.89 ± 0.03	52.51 ± 2.56	52.72 ± 7.24	0.293

CT – control diet without probiotics, LP – control diet with the probiotic species *Lactobacillus plantarum*, LF - control diet with the probiotic species *Lactobacillus fermentum*, and LP&LF – control diet with both *L. plantarum* and *L. fermentum*. IW, initial weight; IL, initial length; FW, final weight; FL, final length; CF, condition factor; SGR, specific growth rate; TGC, thermal growth coefficient; WG%, weight gain in percentage. Values are presented as means ± SEM of two replicates. Significant difference ($p < 0.05$) among diet groups on each row was revealed by a one-way Welch test or Kruskal-Wallis test.

Table 4
Short-chain fatty acid concentration (mmol/L) in the digesta of the study groups.

SCFAs	Diet groups				*p*-value
	CT	LP	LF	LP&LF	
Formic acid	2.60 ± 0.30	2.20 ± 0.25	1.92 ± 0.24	2.30 ± 0.17	0.287
Acetoacetic acid	9.18 ± 0.55^{a}	12.84 ± 0.36^{b}	9.85 ± 0.26^{a}	14.64 ± 0.42^{c}	< 0.001
Lactic acid	5.03 ± 0.42^{b}	5.13 ± 0.25^{b}	3.98 ± 0.18^{a}	4.93 ± 0.17^{ab}	0.008
Succinic acid	5.63 ± 0.15^{ab}	6.46 ± 0.39^{bc}	5.41 ± 0.14^{a}	6.89 ± 0.19^{c}	< 0.001
Acetic acid	12.72 ± 1.49	9.52 ± 0.49	9.79 ± 0.48	11.73 ± 1.25	0.866
Propionic acid	2.76 ± 0.37^{ab}	2.27 ± 0.32^{a}	3.59 ± 0.32^{b}	2.23 ± 0.16^{a}	0.005
Butyric acid	1.25 ± 0.35	1.85 ± 0.21	1.93 ± 0.29	1.42 ± 0.22	0.235
Total acids	37.47 ± 2.08^{ab}	39.16 ± 1.03^{ab}	36.09 ± 0.87^{a}	43.43 ± 1.99^{b}	0.007

CT – control diet without probiotics, LP – control diet with the probiotic species *Lactobacillus plantarum*, LF - control diet with the probiotic species *Lactobacillus fermentum*, and LP&LF – control diet both *L. plantarum* and *L. fermentum*. SCFAs; short chain fatty acids. Values are presented as means ± SEM, n = 10 per diet group. Significant difference ($p < 0.05$) among diet groups, indicated on each row, was revealed by a one-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test.

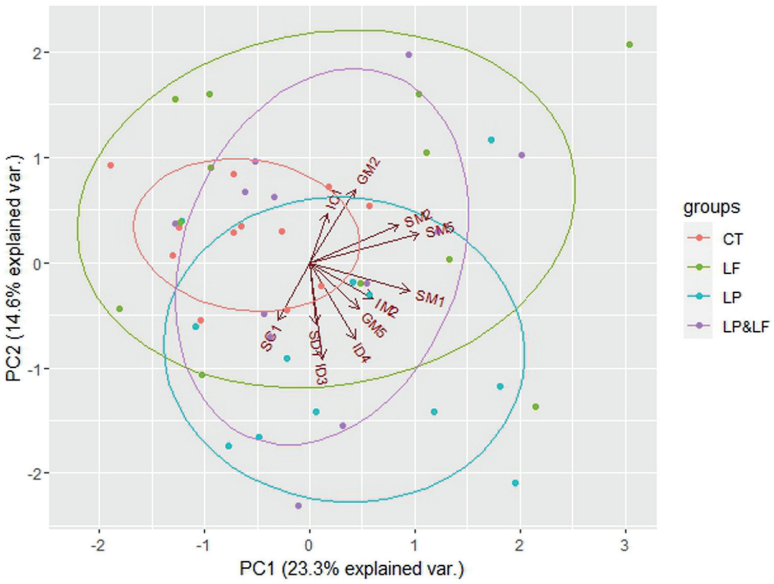

Fig. 1. Principal component analysis (PCA) biplot showing the skin, gill and intestine samples and loading vectors. Expression of AMP and mucin genes were used for this dimensionality reduction. CT, control diet without probiotics; LF, control diet with the probiotics *Lactobacillus fermentum*; LP, control diet with the probiotics *Lactobacillus plantarum*; and LP&LF, control diet with a mixture of *L. plantarum* and *L. fermentum*. SM1, skin *muc5ac1*; SM2, skin *muc5ac2*; SM5, skin *muca5b*; SD1, skin *defensin1*; SC1, skin *cathelicidin1*, GM2, gills *muc5ac2*; GM5, gill *muc5b*; IM2, intestine *muc2*; IC1, intestine *cathelicidin1*; ID3; intestine *defensin3*; ID4, intestine *defensin4*.

IM2), and are therefore the "mucin gene component" while the AMP component was PC2, with the highest loadings of AMP genes (e.g.: SD1 and SC1). SM1, SM2 and SM5 are positively related to each other, likewise the following pairs have shown positive correlation: IC1 and GM2; SD1 and ID3.

3.3.1. Mucin and AMP genes in the dorsal skin

Among the studied mucin and AMP genes, *muc5ac1*, *muc5ac2*, *muc5b, defensin1* and *cathelicidin1* were expressed in the skin. Almost all the mucin and AMP genes were not affected by the individual or combined administration of probiotics. The gene *defensin1* was an exception; fish fed LP had significantly higher *defensin1* mRNA level compared to CT and LP&LF (Table 5). A trend towards statistical significance was noted in the case of *muc5ac1*, with the highest value for the LP fed fish and the lowest for the CT group.

3.3.2. Mucin genes in the gills

The gills expressed *muc5ac2* and *muc5b*, among the studied mucin genes. The *muc5ac2* mRNA levels tended to be higher in fish fed LP&LF (Table 5), while no other mucin genes were significantly altered by the LAB supplementation in the diets.

3.3.3. Mucin and AMP genes in the distal intestine

The distal intestine expressed the *muc2*, *defensin3*, *defensin4* and *cathelicidin1*, among the assessed genes. The single or combined administration of probiotics did not affect any of the mucin or AMP genes. However, a trend towards significance was observed for *muc2* mRNA levels in fish fed LP and LP&LF compared to CT (Table 5).

3.4. Effects of probiotics on the architecture of the skin, gills and distal intestine

3.4.1. Mucous cell indices in the dorsal skin

The results revealed that approximately 100 μm^2 of skin epidermis in Atlantic salmon was covered by mucous cells of a total average area 14 μm^2, corresponding to an average of 1148 mucous cells in 1 mm^2 of

Table 5
Gene expression in the skin, gills and intestine of Atlantic salmon.

Tissues	Type of genes	Name of genes	Diet groups				*p*-value
			CT	LP	LF	LP&LF	
Skin	**AMP**	*defensin1*	0.58 ± 0.06[a]	0.93 ± 0.08[b]	0.71 ± 0.07[ab]	0.67 ± 0.06[a]	0.005
		cathelicidin1	0.47 ± 0.07	0.60 ± 0.07	0.48 ± 0.05	0.43 ± 0.07	0.318
	Mucin	*muc5ac1*	0.31 ± 0.04	0.65 ± 0.12	0.42 ± 0.07	0.35 ± 0.06	0.076
		muc5ac2	0.25 ± 0.05	0.41 ± 0.10	0.69 ± 0.19	0.52 ± 0.10	0.164
		muc5b	0.28 ± 0.03	0.34 ± 0.03	0.36 ± 0.07	0.30 ± 0.04	0.527
Gills	**Mucin**	*muc5ac2*	0.95 ± 0.06	0.90 ± 0.04	1.04 ± 0.10	1.18 ± 0.10	0.080
		muc5b	0.13 ± 0.02	0.18 ± 0.03	0.17 ± 0.04	0.21 ± 0.04	0.701
Intestine	**AMP**	*defensin3*	0.38 ± 0.10	0.70 ± 0.14	0.48 ± 0.18	0.64 ± 0.18	0.512
		defensin4	0.72 ± 0.06	0.74 ± 0.08	0.56 ± 0.07	0.72 ± 0.08	0.310
		cathelicidin1	0.11 ± 0.03	0.13 ± 0.04	0.15 ± 0.05	0.07 ± 0.01	0.901
	Mucin	*muc2*	0.75 ± 0.06	0.93 ± 0.05	0.78 ± 0.04	0.89 ± 0.10	0.096

CT – control diet without probiotics, LP – control diet with the probiotic species *Lactobacillus plantarum*, LF - control diet with the probiotic species *Lactobacillus fermentum*, and LP&LF – control diet with both *L. plantarum* and *L. fermentum*. AMP; Antimicrobial peptide. Values are presented as means ± SEM, n = 12 per diet group. Significant difference ($p < 0.05$) among diet groups, indicated on each row, was revealed by a one-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test or Welch test.

epidermis. The mucous cell indices, SME and SNE were not significantly affected by the LAB supplementation. However, SME values revealed a decreasing trend in LP fed fish compared to the other diet groups (Table 6). Representative histological images of skin epidermis and dermis regions for each diet group are presented in Fig. 2.

3.4.2. Mucous cell indices in the gills

Histological evaluation of gill mucous cells revealed the probiotic supplementation-induced changes in both GME and GNE. Fish fed LP&LF had significantly more GME (compared to CT) and GNE (compared to CT, LP and LF) (Table 6). Representative histological micro-photographs of primary and secondary gill filaments for each diet group are presented in Fig. 3.

3.4.3. Distal intestine histomorphometry

Effects of supplementing a single or a mixture of the two probiotics on the distal intestine indices of Atlantic salmon were examined. For all diet groups, representative histological images of the distal intestine are presented in Fig. 4. The PCA biplot shows the intestinal health indicators and the different experimental groups (Fig. 5), with PC1 explaining 38.7% of the variance, and PC2 corresponding for 27.8%. Approximately 80% of the cumulative proportion of variance could be explained when a 3rd. PC was included (Supplementary Figure 6). The indices HOE, NOM and WOV are explained by PC1, while the HOV, WLP, IEL and SNV are explained by PC2. All the indices, except NOM, SNV and IEL had positive loading on PC1, while HOV and SNV had negative loading on PC2 (Supplementary Figure 7). The indices WOV and HOE are positively related to each other. Likewise, the following pairs are positively correlated: HOV and SNV; NOM and IEL. The indices WOV and NOM are negatively correlated. The other pairs that are negatively correlated are: HOV and IEL, WLP and SNV. We observed differential clustering of the groups LP&LF and CT. However, the clusters of LP and LF cannot be differentiated.

The examined histomorphometric indices such as HOV, WOV, WLP and SNV were significantly but differently altered by the single or combined administration of probiotics. Fish fed the combination of probiotics (LP&LF) had significantly higher villi compared to CT which is SBM-based feed without probiotics (CT). While the WOV value of LP fed fish showed a decreasing tendency, LF fed fish had significantly reduced WOV compared to CT fed fish. Fish fed the CT had significantly wider lamina propria compared to fish fed the LP or LP&LF. Fish fed LP&LF had significantly narrower lamina propria than LF. The fish fed LP, LF and LP&LF had significantly higher SNV scores than those fed CT. Other indices (HOE, NOM and IEL) were not significantly affected by probiotics. The HOE varied between 43 and 48 μm. A tendency towards significance was noted for the NOM values, with the highest value for LF and the lowest for the LP&LF (Table 6).

Table 6
Histomorphometric indices in the skin, gills and intestine of Atlantic salmon.

Indices	Diet groups				*p*-value
	CT	LP	LF	LP&LF	
SME (ratio)	0.1563 ± 0.009	0.1248 ± 0.008	0.1317 ± 0.007	0.1382 ± 0.010	0.059
SNE (number / μm^2)	1.1099 ± 0.051	1.1710 ± 0.045	1.1336 ± 0.042	1.1768 ± 0.060	0.751
GME (ratio)	0.0399 ± 0.005^a	0.0514 ± 0.004^{ab}	0.0493 ± 0.003^{ab}	0.0657 ± 0.005^b	< 0.001
GNE (number / μm^2)	0.0006 ± 8e-05^a	0.0007 ± 3e-05^a	0.0007 ± 5e-05^a	0.0008 ± 4e-05^b	0.008
NOM (score)	3.00 ± 0.30	3.42 ± 0.23	3.67 ± 0.22	2.75 ± 0.25	0.073
HOV (μm)	897.65 ± 30.23^a	976.84 ± 26.08^{ab}	979.53 ± 29.40^{ab}	1021.44 ± 30.12^b	0.033
WOV (μm)	126.73 ± 5.28^b	113.19 ± 2.40^{ab}	112.13 ± 3.51^a	116.61 ± 2.73^{ab}	0.044
HOE (μm)	48.26 ± 2.13	43.35 ± 1.28	43.23 ± 1.34	46.95 ± 1.14	0.063
WLP (μm)	27.62 ± 1.62^c	22.07 ± 0.98^{ab}	24.61 ± 1.71^{bc}	18.39 ± 1.08^a	< 0.001
IEL (score)	3.67 ± 0.19	3.00 ± 0.33	3.25 ± 0.18	2.92 ± 0.23	0.127
SNV (score)	1.00 ± 0.00^a	2.08 ± 0.08^b	1.75 ± 0.18^b	2.17 ± 0.11^b	< 0.001

CT, control diet without probiotics; LP, control diet with the probiotic species *Lactobacillus plantarum*; LF, control diet with the probiotic species *Lactobacillus fermentum*; and LP&LF, control diet with both *L. plantarum* and *L. fermentum*. SME, total area of skin mucous cells per total area of skin epithelium; SNE, number of skin mucous cells per total area of skin epithelium; GME, total area of gill mucous cells per total area of gill epithelium; GNE, number of gill mucous cells per total area of gill epithelium; NOM, number of intestinal mucous cells; HOV, height of villi; WOV, width of villi; HOE, height of enterocytes; WLP, width of lamina propria; IEL, number of intraepithelial lymphocytes; SNV, supra nuclear vacuoles. Values are presented as means ± SEM, n = 12 per diet group. If present, significant differences ($p < 0.05$) among diet groups are indicated by different superscripts (a, b, c, or d) on each row after conducting a one-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test or Kruskal-Wallis (for GNE, NOM, IEL and SNV) followed by Dunn's post hoc test.

3.5. Correlation between mucous cell-based histological indices and mucin and AMP gene expression data

Spearman correlation-based analysis revealed a significant correlation between histologically analysed mucous cell indices and mucin and AMP gene expression data (Fig. 6). Significant positive correlations, with correlation coefficient > 0.50) between mucous cell indices were observed for the following pairs: SME and SNE ($r = 0.60$, $p < 0.001$), GME and GNE ($r = 0.74$, $p < 0.001$). The histological index SNE tended to be positively correlated to the skin AMP gene SD1 ($r 0.28$, $p = 0.058$). A significant positive correlation between gene expression data was also observed for the following pair: SC1 and SD1 ($r = 0.35$, $p = 0.016$). The SM1 positively correlated with SM2 ($r = 0.49$, $p < 0.001$), SM5 ($r = 0.84$, $p < 0.001$), and ID4 ($r = 0.29$, $p = 0.049$). Likewise, SM5 positively correlated with SM2 ($r = 0.51$, $p < 0.001$), GM2 ($r = 0.31$, $p = 0.033$), and IM2 ($r = 0.30$, $p = 0.039$). In addition, ID4 and GM5 ($r = 0.22$, $p = 0.037$) were also positively correlated.

4. Discussion

In the present study, *L. plantarum* R2 Biocenol™ (CCM 8674) and *L. fermentum* R3 Biocenol™ (CCM 8675) were fed to Atlantic salmon to understand if the probiotic bacteria, when applied singly or in combination, could prevent soybean meal-induced enteritis. The LAB species were isolated from the intestinal content of rainbow trout, and they were selected based on features including tolerance to different pH values, bile acids and temperature, antagonistic activity against the two salmonid pathogens *A. salmonicida* subsp. *salmonicida* CCM 1307 and *Y. ruckeri* CCM 6093 and growth properties in vitro (Fečkaninová et al., 2019). These probiotic species have the capacity to prevent diseases in aquaculture. The results from the present study indicate that the single and combined administration of the probiotic bacteria are effective to prevent soybean meal-induced enteritis, the latter approach was found to have a better effect.

An earlier study with Atlantic salmon showed a shift in gut microbiota composition when Atlantic salmon was fed diets supplemented with *L. plantarum* and *L. fermentum* (Gupta et al., 2019), and is most likely the reason for the changes in SCFAs observed in the present experiment. The enterocytes in the villi absorb metabolites and utilize the SCFAs as energy sources. A legume-based diet (mix of soybean meal and wheat gluten – SBMWG) presented a high relative abundance of LAB (Gajardo et al., 2017) and increased the plasma osmolality and water content of the distal intestine chyme (Hu et al., 2016). A shift in microbiota composition can sometimes favour SCFA production. In the

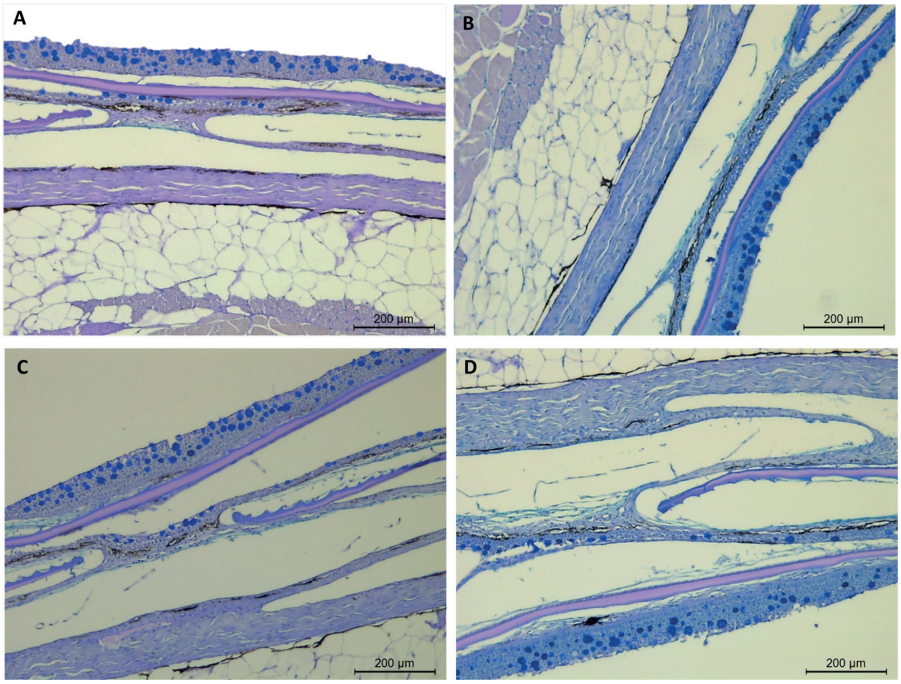

Fig. 2. Histological micro-photographs of the skin from post-smolt Atlantic salmon fed with soybean meal-based control diet (A), control diet coated with *Lactobacillus plantarum* (B), control diet coated with *Lactobacillus fermentum* (C) and control diet coated with both *L. plantarum* and *L. fermentum* (D). Images were acquired with Leica camera fitted on DM 3000 light microscope at 10X magnification.

present study, supplementation of feeds with LAB, singly or in combination, did not alter the concentration of formic, acetic or butyric acids. However, acetoacetic, lactic, succinic and propionic acids including total SCFAs were altered by probiotics. Fish fed the LP diet had more lactic acid compared to those fed LF, while those fed the LF diet had more propionic acid than the LP group as well as those fed the LP&LF diet. The end product of *L. fermentum* might have influenced the propionate-producing bacteria; either by shifting the microbiota composition to favour the pyruvate-lactate-propionate pathway or succinate-propionate pathway under anaerobic conditions (Hati et al., 2019; Kusumo et al., 2019; Meenakshi Malhotra, 2015). Propionate is absorbed through enterocytes and transported via vena porta to the liver where it plays a role in lipid synthesis in hepatocytes. An overview of the potential effects of propionate on cholesterol level and lipid synthesis in humans is given by Hosseini et al. (2011). In brief, this review cited different in vivo and in vitro studies that reported propionate intake-caused decrease in hepatic and blood cholesterol levels and liver lipogenesis. Newer literature also points to the role of propionic acid in autoimmune and neurodegenerative diseases in humans (Duscha et al., 2020). Long-term consumption of propionic acid had positive effects on the health of these patients; their relapse rate was less, and brain atrophy was reduced. The LP&LF fed fish (mixture of two LAB species) had more acetoacetic acids compared to LP or LF fed groups (one of the species of LAB), as well as a higher concentration of succinic and total SCFAs. These observations suggest that each LAB species has its metabolic pathways and metabolic fingerprint. Our findings are in line with other studies on the influence of probiotic supplementation on the SCFAs in the fish gastrointestinal tract (Allameh et al., 2017; Asaduzzaman et al., 2018; Burr et al., 2005). In fish, acetic acid is transported from the intestinal lumen into the portal blood and used either as an energy source for skeletal muscle or lipid synthesis (Asaduzzaman et al., 2018; Titus and Ahearn, 1991). The SCFA metabolites of bacterial fermentation are also known to stimulate gut epithelial cell proliferation resulting in increased villi height (Ichikawa et al., 1999). Our study also demonstrated the LP&LF-caused beneficial effects such as increased SCFA production in the digesta, increased villi height, and reappearance of supranuclear vacuoles in Atlantic salmon fed SBM-based diet. It seems that different species of bacteria depend on the carbon sources produced by the other cohabitants (Hosseini et al., 2011; Smid and Lacroix, 2013). Thus, the two approaches had differential effects on the SCFA content and the subsequent alteration of the micromorphology of the intestine.

Earlier studies on fishes have reported the influence of probiotics on immune-related gene expression (Cingeľová Maruščáková et al., 2021; Hasan et al., 2018; Van Doan et al., 2018). The present study evaluated the expression of four mucin genes in the skin, gills and intestine and four AMP genes in the skin and intestine. The expression pattern was tissue-specific, and probiotics influenced the studied mucin and AMP genes differently. Fish fed the LP diet had a significantly higher level of skin *defensin1* mRNA, a tendency towards a significantly higher expression of skin *muc5ac1* and intestine *muc2* compared to those fed the control diet (CT). These observations in the skin are in line with a study that reported an upregulation of *beta defensin* in juvenile Atlantic cod, when probiotics were added to the rearing water (Ruangsri et al., 2014). In our study, fish fed a mixture of the LAB species (diet LP&LF) also had a strong tendency towards elevating the expression of *muc5ac2* in the gills compared to the control group (CT). These observations may suggest that the fish fed LP&LF had improved gill barrier status compared to fish fed the CT. Our observations in the skin and gills of Atlantic salmon are in line with several other studies that reported positive effects of LAB on the host innate immune responses. For example, common carp

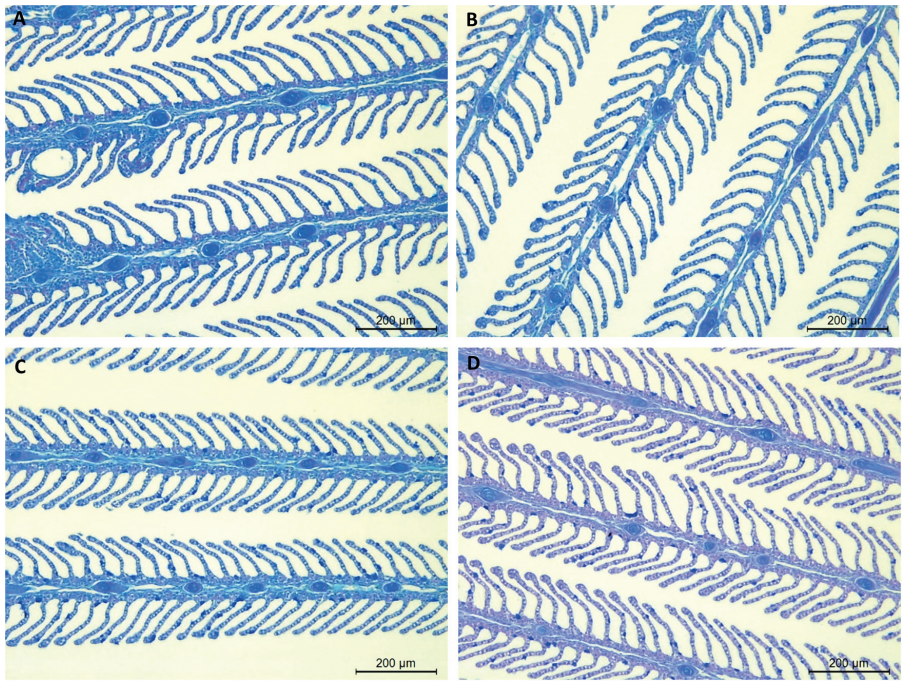

Fig. 3. Histological micro-photographs of the gills from post-smolt Atlantic salmon fed a soybean meal-based control diet (A), control diet coated with *Lactobacillus plantarum* (B), control diet coated with *Lactobacillus fermentum* (C) and control diet coated with both *L. plantarum* and *L. fermentum* (D). Images were acquired with Leica camera fitted on DM 3000 light microscope at 10X magnification.

(*Cyprinus carpio*) fed *P. acidilactici* (6×10^8 CFU per g) for 60 days had increased mucus protease activity and skin lysozyme gene expression (Hoseinifar et al., 2019), Nile tilapia (*Oreochromis niloticus*) fed *L. plantarum* (10^8 CFU per g) and *Bifidobacterium velezensis* (10^7 CFU per g) for 15 or 30 days had significantly higher innate immune markers (Van Doan et al., 2018); Olive flounder fed *L. sakei* and *L. plantarum* (10^{11} CFU /g) for 42 days had increased expression of immune genes in the gills and head kidney (Feng et al., 2018). It seems that the effects of single and combined application of the bacteria on the immune genes can vary and the target area can also be different.

Moreover, the present study found a significant positive correlation between mucus-related genes. For example, skin *muc5b* (SM5) is positively correlated with skin *muc5ac1* (SM1), skin *muc5ac2* (SM2), gills *muc5ac2* (GM2), and intestine *muc2* (IM2). These results indicate the interactions between the mucosal surfaces and the tendency to follow a pattern in gene expression in multiple mucosal sites. Moreover, the mucin and AMP gene expression positively correlated with the histologically evaluated mucous cell indices (in some cases) supporting the hypothesis that probiotics have the ability to influence gene expression and alter the histomorphometry of mucosal tissues. The gut microbiome and its metabolites are known to influence the skin microbiome and the associated immune defence (Salem et al., 2018). Gut health status in fishes like yellowtail kingfish was found to influence the skin and gill bacterial assemblages, which have a bearing on the barrier systems of these mucosal surfaces during the early onset of enteritis (Legrand et al., 2018). Hence, the possible connection, which we observed in our study, between diet, the intestine and other organs (skin and gills) could be that the metabolites absorbed by the gut epithelium are transported to the skin, gills and brain through the circulatory system where metabolites aid in modulating the mucosal tissues (Ghosh et al., 2021; Guo et al., 2022; Sharon et al., 2014; Silva et al., 2020; Thursby and Juge, 2017; Wiatrak et al., 2022). Moreover, the mechanism or the pathway could be that the dietary probiotics influence the production of mucins via stimulation of different receptors on lymphocytes resulting in a modulation of the immune system (Grondin et al., 2020).

Based on the present study, around 14% of the skin epidermis area is covered by skin mucous cells, irrespective of LAB supplementation. Mucous cell area-related indices (SME and GME) indicate the fraction of epithelium area that is covered by mucous cell area. These indices depend on mucous cell size and count, both of which reflect the overall mucus production. It should be noted that if the size or count of mucous cells increases, the SME or GME will increase, but if only one of these cell attributes increases and the other decreases, SME or GME will not change. Dietary probiotics can stimulate mucous cell formation, which is counted as one of the innate immune responses in fish (Sewaka et al., 2019). The present study revealed a non-significant, but numerical increase in skin mucous cell count when LAB was administrated via feeds compared to the feed without LAB. This is in line with our previous experiment that reported an increase in skin mucous cell count in salmon fed a combination of the two LAB *L. plantarum* and *L. fermentum* (Nimalan et al., 2022). Other studies have also reported responses in the skin when fish were fed probiotics; Porthole livebearer (*Poecilopsis gracilis*) fed *Lactobacillus* enriched *Artemia* (Hernandez et al., 2010) and catla (*Catla catla*) fed the LAB, *Bacillus* (Das et al., 2013) had a high content of protein in skin mucus. Unfavourable environmental factors may also increase the mucus cell counts of the skin (Vatsos et al., 2010). Genes encoding for secretory processes-linked proteins in mucus were altered with an increase in mucus-producing cells and hypertrophic mucous cell modelling in the gills of Atlantic salmon was a response to an oxidizing agent (Karlsen et al., 2018; Haddeland et al., 2020). The

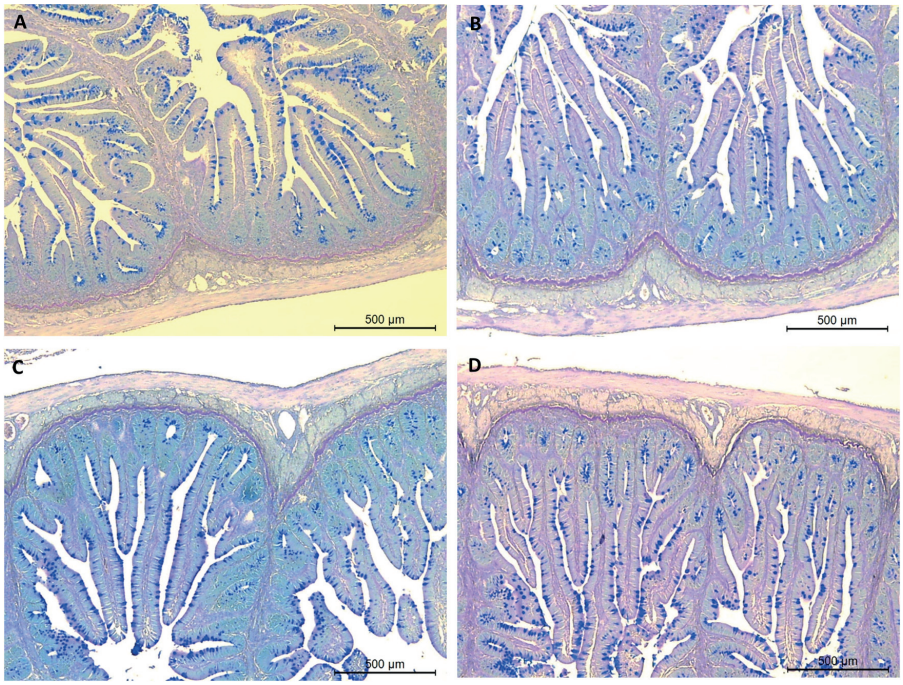

Fig. 4. Histological micro-photographs of the distal intestine from post-smolt Atlantic salmon fed a soybean meal-based control diet (A), control diet coated with *Lactobacillus plantarum* (B), control diet coated with *Lactobacillus fermentum* (C) and control diet coated with both *L. plantarum* and *L. fermentum* (D). Images were acquired with Leica camera fitted on DM 3000 light microscope at 5X magnification.

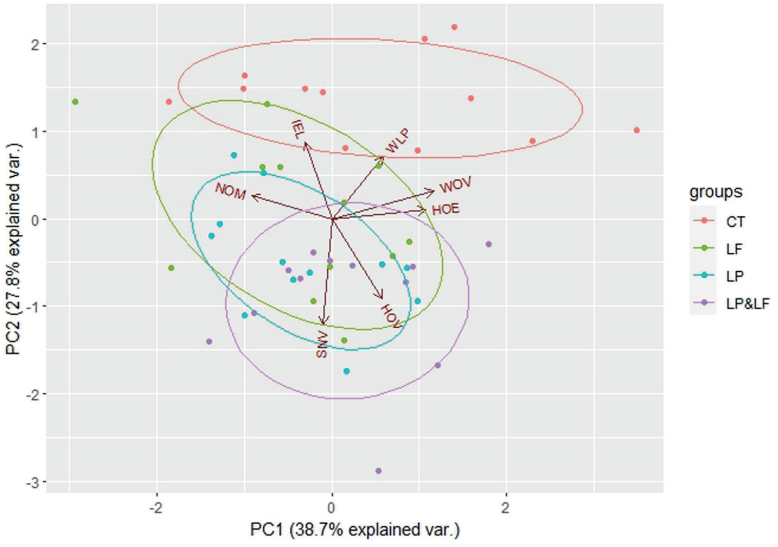

Fig. 5. Principal component analysis (PCA) biplot showing the intestine samples and loading vectors. Expression of AMP and mucin genes were used for the intestinal histology data-based dimensionality reduction. CT, control diet without probiotics; LF, control diet with probiotic species *Lactobacillus fermentum*; LP, control diet with probiotic species *Lactobacillus plantarum*; and LP&LF, control diet with a combination of *L. plantarum* and *L. fermentum*. NOM, score for number of intestinal mucous cells; HOV, height of villi; WOV, width of villi; HOE, height of enterocytes; WLP, width of lamina propria; IEL, score for number of intraepithelial lymphocytes; SNV, score for supra nuclear vacuoles.

present study showed that the gills of fish fed LP&LF had almost two times higher GME and GNE than the CT group, and mucous cell count in the distal intestine tended to be higher than in the fish of the CT group.

Our observations are in line with a study that reported an increase in mucin-secreting goblet cells in red tilapia (*Oreochromis* spp.) fed dried *L. rhamnosus* (10^8 CFU/g) for 30 days (Sewaka et al., 2019). Not only the

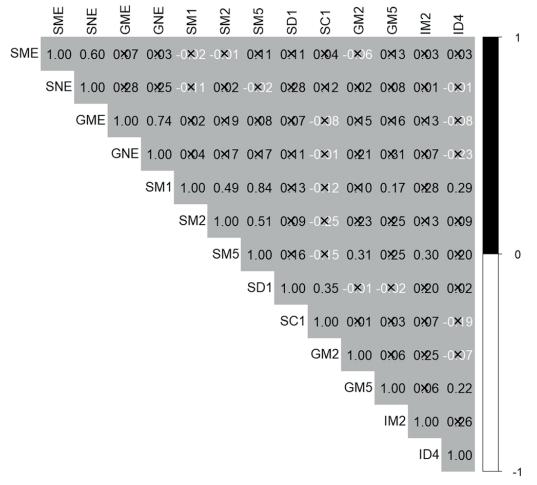

Fig. 6. Correlation plot for histologically evaluated mucous cell indices and selected mucus-related gene expression. Intestinal mucous cell number (NOM), intestine *defensin3*, intestine *cathelicidin1* are not shown because they were not significantly correlated with any of the other parameters. A cross indicates non-significant correlations ($p > 0.05$; Spearman rank correlation test). Correlation coefficients are color coded; black font indicates positive correlations and white font indicates negative correlations. We did not detect any significant negative correlations. SME, total area of mucous cells per total area of epithelium in the dorsal skin. SNE, number of mucous cells per total area of epithelium in the dorsal skin. GME, total area of mucous cells per total area of epithelium in the gills. GNE, number of mucous cells per total area of epithelium in the gills. SM1, skin *muc5ac1*. SM2, skin *muc5ac2*. SM5, skin *muc5b*. SC1, skin *cathelicidin1*. GM2, gill *muc5ac2*. GM5, gills *muc5b*. IM2, intestine *muc2*. ID4, intestine *defensin4*.

mucin secretion and the number of mucus cells, but also the mucus composition, viscosity and thickness can be changed in response to host factors and external factors such as probiotics in feeds (Paone and Cani, 2020). Interestingly, our study showed that singly or in combination, LAB can be effective to target different mucosal surfaces.

In the present study, we found that feeding a mix of *L. plantarum* and *L. fermentum* can even increase the villi height. These observations are similar to those of several other studies that reported histomorphometric effects of probiotics on the intestine of fishes (Daniels et al., 2010; Merrifield et al., 2010; Pirarat et al., 2011). A probiotic species *L. rhamnosus* significantly increased villi height in Nile tilapia fed at a rate of 10^{10} CFU/g in feed for 30 days (Pirarat et al., 2011). Yet another probiotic *L. pediococcus* enhanced the enterocyte microvilli in the anterior intestine in rainbow trout (Merrifield et al., 2010). Similarly, *Bacillus* spp. increased microvilli length and density in larvae and post-larvae of European lobster (*Homarus gammarus*) (Daniels et al., 2010). It should be noted that the aforementioned studies have assessed the effect of a single probiotic species and, most of them did not report the impact on the absorptive surface or SNVs. However, the present study results revealed the significant reappearance of SNVs in the enterocytes, likely associated with the observed increase in SCFAs and improvement in other intestine features such as villi height of fish fed the probiotics, *L. plantarum* and *L. fermentum*. These observations point to improved mucosal health of post-smolt Atlantic salmon. Therefore, both *L. plantarum* and *L. fermentum* have the potential to prevent SBMIE in Atlantic salmon. In this study, the combination of two LAB species showed a better response in mucosal tissues as they might have promoted cross feeding through positive interactions that benefit the host.

Enteritis is defined as inflammation of the intestine, and the condition is characterised by shortened intestinal villi, changes in mucus production, epithelial abnormalities, widened lamina propria as well as submucosa mainly due to the infiltration of different immune cells including neutrophils, macrophages and lymphocytes (Agboola et al., 2022; Baeverfjord and Krogdahl, 1996; Nimalan et al., 2022). In our previous studies also, we observed intestinal inflammation in Atlantic salmon fed 20% SBM in the diet (Nimalan et al., 2022; Sørensen et al., 2021). In those studies, we tested, amongst others, a marine-based diet (BG1) mainly with fish meal and fish oil, and one diet with 20% SBM diluting the marine ingredients (BG2) to study the gut health of Atlantic salmon. Though we did not have a positive control in the present study, BG1 can be considered as the positive control. The BG2 diet used in our previous studies is the CT diet in the present study. The gut barrier biomarker mucin 2, *muc2*, was significantly reduced in fish fed BG2 compared to BG1 (Nimalan et al., 2022; Sørensen et al., 2021). A comparison of the positive control in Nimalan et al. (2022) with the CT diet in the present study showed that HOV, HOE, WLP, IEL and SNV were significantly reduced and WOV tended ($P = 0.070$) to be lower in fish fed the CT (Supplementary Table 1). The number of intraepithelial lymphocytes (IELs) in CT (less score = more cells in this study, 3.67) was significantly higher compared to BG1÷ (score 4.75) in Nimalan et al. (2022). Moreover, *muc2* in the intestine was significantly reduced in CT (0.75) compared to BG1 (2.79). Therefore, reduction in supranuclear vacuoles, villi height and enterocyte height and increase in width of lamina propria and IELs indicate that fish fed the CT diet could develop enteritis in the present study.

Generally, fish fed plant ingredients tend to have more small-sized mucous cells in the intestine (Sørensen et al., 2021). This is a general response feature of inflammation. Though studies have reported probiotics-induced increase in the mucous cells, under inflammatory condition also we observed more mucous cells (Nimalan et al., 2022). In the present study, an increasing trend was observed for the distal intestinal mucous cell indices of the fish fed lactic acid bacteria, when applied singly or in combination. Since the response was not significant compared to the control group (without probiotics) NOM score may not indicate an alleviation of enteritis. Nevertheless, for more responsive organs like the gills, the mucous cell indices can be considered as a good indicator.

Assessment of various forms of enteritis like ulcerative colitis and Crohn's disease in humans is based on established histological scores (Erben et al., 2014; Ma et al., 2021). It was reported that the percentage of tissue occupied by $CD4^+$ T cells was significantly lower in inflamed tissue while that covered by macrophages were significantly higher (Naser et al., 2011). Furthermore, infiltrating intestinal T cells in active cases of inflammatory bowel disease had increased percentages of $CD4^+$ T cells, T_{reg}, and lower percentages of $CD8^+$ T cells and $CD103^+$ T cells (Smids et al., 2018). Although we were not able to immunophenotype the subpopulation of the IELs, this subset was lower in the soybean meal fed fish ($p = 0.127$). The T cell population in the IEL compartment of humans is populated mostly by induced TCRαβ CD8αβ and barely by TCRαβ CD4 (Mayassi and Jabri, 2018). Flow cytometry studies are necessary to ascertain the type of T cells that were decreased in the soybean fed fish. As for the higher number of IELs in the probiotic fed groups, another study has also reported similar results. *Lactobacillus rhamnosus* feeding increased the number of IELs in the intestine of tilapia (Pirarat et al., 2015).

Probiotic feeding can have benefits beyond immune system stimulation. *Lactobacillus* feeding can improve the growth of the probiotic-consumed fish (Abdelfatah and Mahboub, 2018; Dawood et al., 2019; Feng et al., 2019; Jami et al., 2019; Van Nguyen et al., 2019). Supplementation of a mixture of probiotic species may have stronger growth-promoting effects than a single probiotic species (Aly et al., 2008; Beck et al., 2015; Hai, 2015; Hai et al., 2009). Nonetheless, we did not observe growth-promoting effects of the two LAB in the present study, when Atlantic salmon post-smolts were fed SBM-based feed with either *L. plantarum* or *L. fermentum* or a mix of the two, at 10^8 CFU/g of

feed for 38 days. On the other hand, supplementation of a multi-strain commercial product containing probiotic strains of *Saccharomyces cerevisiae*, *Enterococcus faecium*, *L. acidophilus*, *L. casei*, *L. plantarum*, and *L. brevis*, in a diet with 20% SBM, prevented SBM-caused growth retardation in rainbow trout. Furthermore, the fish fed this product and the SBM starter diets exhibited higher digestibility and growth during the grow-out phases (Sealey et al., 2009). These findings suggest that certain microbes can promote intestinal health and prevent inflammation induced by antinutrients. Notably, these short-term effects will be erased following the cessation of supplementation, presumably because the strains do not colonize the intestinal tract to impart a sustained health-promoting effect (Sealey et al., 2009).

5. Conclusion

The present study has shown that the fish fed SBM alone had enteritis symptoms. Single or combined application of *L. plantarum* and *L. fermentum* can stimulate the formation of goblet cells at different mucosal surfaces such as the skin, gills and intestine. Though probiotics did not completely prevent the SBMIE, they had positive effects—such as increased villi height, reduced lamina propria width, and reappearance of supra nuclear vacuoles—on intestinal micro-morphometric structures. This study has demonstrated that the probiotics can prevent enteritis, possibly by altering the SCFA composition, mucous cell count, mucin and AMP genes expression, and improved endocytosis.

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CRediT authorship contribution statement

Mette Sørensen conceptualized the study, developed methodology, acquired resources, and was involved in investigation, writing, reviewing and editing the manuscript, besides supervising and coordinating the study and leading the project. Dagmar Mudroňová, Jana Koščová, Soňa Gancarčíková and Adriána Fečkaninová developed methodology, acquired resources for probiotics and short chain fatty acid study, and were involved in investigation, writing, reviewing and editing the manuscript. Solveig Lysfjord Sørensen developed the protocol and conducted the gene expression study. Nadanasabesan Nimalan carried out histological studies, analysed data and wrote the first version of the manuscript. Saraswathy Bisa, Ioannis N. Vatsos and Viswanath Kiron were involved in developing, editing and reviewing the manuscript. All co-authors have read and edited the manuscript and have approved the submission to Aquaculture Reports.

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

We have included all the data in the manuscript and the supplementary files.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2022.101461.

References

Abdelfatah, E.N., Mahboub, H.H.H., 2018. Studies on the effect of *Lactococcus garvieae* of dairy origin on both cheese and Nile tilapia (*O. niloticus*). Int. J. Vet. Sci. Med. 6, 201–207. https://doi.org/10.1016/j.ijvsm.2018.11.002.

Agboola, J.O., Chikwati, E.M., Hansen, J.Ø., Kortner, T.M., Mydland, L.T., Krogdahl, Å., Djordjevic, B., Schrama, J.W., Øverland, M., 2022. A meta-analysis to determine factors associated with the severity of enteritis in Atlantic salmon (*Salmo salar*) fed soybean meal-based diets. Aquaculture 555, 738214. https://doi.org/10.1016/j.aquaculture.2022.738214.

Alishahi, M., Tulaby Dezfuly, Z., Mohammadian, T., Mesbah, M., 2018. Effects of two probiotics, *Lactobacillus plantarum* and *Lactobacillus bulgaricus* on growth performance and intestinal lactic acid bacteria of *Cyprinus carpio*. Iran. J. Vet. Med. 12, 207–218. https://doi.org/10.22059/ijvm.2018.235444.1004816.

Allameh, S.K., Ringø, E., Yusoff, F.M., Daud, H.M., Ideris, A., 2017. Dietary supplement of *Enterococcus faecalis* on digestive enzyme activities, short-chain fatty acid production, immune system response and disease resistance of Javanese carp (*Puntius gonionotus*, Bleeker 1850). Aquac. Nutr. 23, 331–338. https://doi.org/10.1111/anu.12397.

Aly, S.M., Abdel-Galil Ahmed, Y., Abdel-Aziz Ghareeb, A., Mohamed, M.F., 2008. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish. Shellfish Immunol. 25, 128–136. https://doi.org/10.1016/j.fsi.2008.03.013.

Andani, H.R.R., Tukmechi, A., Meshkini, S., Sheikhzadeh, N., 2012. Antagonistic activity of two potential probiotic bacteria from fish intestines and investigation of their effects on growth performance and immune response in rainbow trout (*Oncorhynchus mykiss*). J. Appl. Ichthyol. 28, 728–734. https://doi.org/10.1111/j.1439-0426.2012.01974.x.

Angahar, L.T., 2016. Applications of probiotics in aquaculture. Am. J. Curr. Microbiol. 4, 66–79.

Asaduzzaman, M., Iehata, S., Akter, S., Kader, M.A., Ghosh, S.K., Khan, M.N.A., et al., 2018. Effects of host gut-derived probiotic bacteria on gut morphology, microbiota composition and volatile short chain fatty acids production of Malaysian Mahseer *Tor tambroides*. Aquac. Rep. 9, 53–61. https://doi.org/10.1016/j.aqrep.2017.12.003.

Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: A comparison with the intestines of fasted fish. J. Fish. Dis. 19, 375–387. https://doi.org/10.1111/j.1365-2761.1996.tb00376.x.

Bakke-McKellep, A.M., Penn, M.H., Salas, P.M., Refstie, S., Sperstad, S., Landsverk, T., et al., 2007.). Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). Br. J. Nutr. 97, 699–713. https://doi.org/10.1017/S0007114507381397.

Bansemer, M.S., Forder, R.E.A., Howarth, G.S., Suitor, G.M., Bowyer, J., Stone, D.A.J., 2015. The effect of dietary soybean meal and soy protein concentrate on the intestinal mucus layer and development of subacute enteritis in Yellowtail Kingfish (*Seriola lalandi*) at suboptimal water temperature. Aquac. Nutr. 21, 300–310. https://doi.org/10.1111/anu.12160.

Bäuerl, C., Llopis, M., Antolín, M., Monedero, V., Mata, M., Zúñiga, M., et al., 2013. *Lactobacillus paracasei* and *Lactobacillus plantarum* strains downregulate proinflammatory genes in an ex vivo system of cultured human colonic mucosa. Genes Nutr. 8, 165–180. https://doi.org/10.1007/s12263-012-0301-y.

Beck, B.R., Kim, D., Jeon, J., Lee, S.M., Kim, H.K., Kim, O.J., et al., 2015. The effects of combined dietary probiotics *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 on innate immunity and disease resistance in olive flounder (*Paralichthys olivaceus*). Fish. Shellfish Immunol. 42, 177–183. https://doi.org/10.1016/j.fsi.2014.10.035.

Booman, M., Forster, I., Vederas, J.C., Groman, D.B., Jones, S.R.M., 2018. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*). Aquaculture 483, 238–243. https://doi.org/10.1016/j.aquaculture.2017.10.025.

Burr, G., Gatlin, D., Ricke, S., 2005. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in Finnish aquaculture. J. World Aquac. Soc. 36, 425–436. https://doi.org/10.1111/j.1749-7345.2005.tb00390.x.

Cerezuela, R., Fumanal, M., Tapia-Paniagua, S.T., Meseguer, J., Moriñigo, M. ángel, Esteban, M. ángeles, 2013. Changes in intestinal morphology and microbiota caused by dietary administration of inulin and *Bacillus subtilis* in gilthead sea bream (*Sparus aurata* L.) specimens. Fish. Shellfish Immunol. 34, 1063–1070. https://doi.org/10.1016/j.fsi.2013.01.015.

Chang, P.V., Hao, L., Offermanns, S., Medzhitov, R., 2014. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc. Natl. Acad. Sci. U. S. A. 111, 2247–2252. https://doi.org/10.1073/pnas.1322269111.

Cingeľová Maruščáková, I., Schusterová, P., Popelka, P., Gancarčíková, S., Csank, T., Fečkaninová, A., et al., 2021. Effect of autochthonous lactobacilli on immunologically important molecules of rainbow trout after bacterial infection

studied on intestinal primoculture. Fish. Shellfish Immunol. 119, 379–383. https://doi.org/10.1016/j.fsi.2021.10.021.
Cordero, H., Guardiola, F.A., Tapia-Paniagua, S.T., Cuesta, A., Meseguer, J., Balebona, M. C., et al., 2015.). Modulation of immunity and gut microbiota after dietary administration of alginate encapsulated *Shewanella putrefaciens* Pdp11 to gilthead seabream (*Sparus aurata* L.). Fish. Shellfish Immunol. https://doi.org/10.1016/j.fsi.2015.05.010.
Daniels, C.L., Merrifield, D.L., Boothroyd, D.P., Davies, S.J., Factor, J.R., Arnold, K.E., 2010. Effect of dietary *Bacillus* spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. Aquaculture 304, 49–57. https://doi.org/10.1016/j.aquaculture.2010.03.018.
Das, A., Nakhro, K., Chowdhury, S., Kamilya, D., 2013. Effects of potential probiotic *Bacillus amyloliquifaciens* FPTB16 on systemic and cutaneous mucosal immune responses and disease resistance of catla (*Catla catla*). Fish. Shellfish Immunol. 35, 1547–1553. https://doi.org/10.1016/j.fsi.2013.08.022.
Dawood, M.A.O., Magouz, F.I., Salem, M.F.I., Abdel-Daim, H.A., 2019. Modulation of digestive enzyme activity, blood health, oxidative responses and growth-related gene expression in GIFT by heat-killed *Lactobacillus plantarum* (L-137). Aquaculture 505, 127–136. https://doi.org/10.1016/j.aquaculture.2019.02.053.
Duary, R.K., Bhausaheb, M.A., Batish, V.K., Grover, S., 2012. Anti-inflammatory and immunomodulatory efficacy of indig enous probiotic *Lactobacillus plantarum* Lp91 in colitis mouse model. Mol. Biol. Rep. 39, 4765–4775. https://doi.org/10.1007/s11033-011-1269-1.
Erben, U., Christoph Loddenkemper, K.D., Simone Spieckermann, Haller, D., Heimesaat, M.M., Zeitz, M., Siegmund, B., Kühl, A.A., 2014. A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int. J. Clin. Exp. Pathol. 7, 4557–4576.
Fečkaninová, A., Koščová, J., Mudroňová, D., Schusterová, P., Cingeľová Maruščáková, I., Popelka, P., 2019. Characterization of two novel lactic acid bacteria isolated from the intestine of rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Slovakia. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.03.026.
Feng, J., Li, D., Liu, L., Tang, Y., Du, R., 2018. Characterization and comparison of the adherence and immune modulation of two gut *Lactobacillus* strains isolated from *Paralichthys olivaceus*. Aquaculture 499, 381–388. https://doi.org/10.1016/j.aquaculture.2018.08.026.
Feng, J., Chang, X., Zhang, Y., Yan, X., Zhang, J., Nie, G., 2019. Effects of *Lactococcus lactis* from *Cyprinus carpio* L. as probiotics on growth performance, innate immune response and disease resistance against *Aeromonas hydrophila*. Fish. Shellfish Immunol. 93, 73–81. https://doi.org/10.1016/j.fsi.2019.07.028.
Foysal, M.J., Fotedar, R., Siddik, M.A.B., Tay, A., 2020. *Lactobacillus acidophilus* and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (*Cherax cainii*). Sci. Rep. 10. https://doi.org/10.1038/s41598-020-62655-y.
Gajardo, K., Jaramillo-Torres, A., Kortner, T.M., Merrifield, D.L., Tinsley, J., Bakke, A.M., et al., 2017. Alternative protein sources in the diet modulate microbiota and functionality in the distal intestine of Atlantic salmon (*Salmo salar*). Appl. Environ. Microbiol. 83, 1–16. https://doi.org/10.1128/AEM.02615-16.
Gancarcikova, S., Lauko, S., Hrckova, G., Andrejcakova, Z., Hajduckova, V., Madar, M., et al., 2020. Innovative animal model of DSS-induced ulcerative colitis in pseudo germ-free mice. Cells. https://doi.org/10.3390/cells9122571.
Ghosh, S., Whitley, C.S., Haribabu, B., Jala, V.R., 2021. Regulation of intestinal barrier function by microbial metabolites. Cell. Mol. Gastroenterol. Hepatol. 11, 1463–1482. https://doi.org/10.1016/j.jcmgh.2021.02.007.
Gildberg, A., Johansen, A., Bøgwald, J., 1995. Growth and survival of Atlantic salmon (*Salmo salar*) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida*. Aquaculture 138, 23–34. https://doi.org/10.1016/0044-8486(95)01144-7.
Gong, Y., Sørensen, S.L., Dahle, D., Nadanasabesan, N., Dias, J., Valente, L.M.P., et al., 2020. Approaches to improve utilization of *Nannochloropsis oceanica* in plant-based feeds for Atlantic salmon. Aquaculture 522, 735122. https://doi.org/10.1016/j.aquaculture.2020.735122.
Grondin, J.A., Kwon, Y.H., Far, P.M., Haq, S., Khan, W.I., 2020. Mucins in intestinal mucosal defense and inflammation: Learning from clinical and experimental studies. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.02054.
Guo, C., Huo, Y.-J., Li, Y., Han, Y., Zhou, D., 2022. Gut-brain axis: Focus on gut metabolites short-chain fatty acids. World J. Clin. Cases 10, 1754–1763. https://doi.org/10.12998/wjcc.v10.i6.1754.
Gupta, S., Fečkaninová, A., Lokesh, J., Koščová, J., Sørensen, M., Fernandes, J., et al., 2019. *Lactobacillus* dominate in the intestine of Atlantic salmon fed dietary probiotics. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.03247.
Haddeland, S., Lazado, C.C., Merkin, G.V., Myre, O.J., Okubamichael, M., Pedersen, L.-F., et al., 2020. Dynamic morphometrics of mucous cells reveal the minimal impact of therapeutic doses of peracetic acid on Atlantic salmon gill health. Aquaculture 534, 736315. https://doi.org/10.1016/j.aquaculture.2020.736315.
Hai, N.V., 2015. The use of probiotics in aquaculture. J. Appl. Microbiol. 119, 917–935. https://doi.org/10.1111/jam.12886.
Hai, N.Van, Buller, N., Fotedar, R., 2009. Effects of probiotics (*Pseudomonas synxantha* and *Pseudomonas aeruginosa*) on the growth, survival and immune parameters of juvenile western king prawns (*Penaeus latisulcatus* Kishinouye, 1896). Aquac. Res. 40, 590–602. https://doi.org/10.1111/j.1365-2109.2008.02135.x.
Hasan, T., Jang, W.J., Tak, J.Y., Lee, B.-J., Kim, K.W., Hur, S.W., et al., 2018. Effects of *Lactococcus lactis* subsp. *lactis* I2 with β-glucooligosaccharides on growth, innate immunity and Streptococcosis resistance in Olive flounder (*Paralichthys olivaceus*). J. Microbiol. Biotechnol. 28, 1433–1442. https://doi.org/10.4014/jmb.1805.05011.
Hati, S., Patel, M., Mishra, B.K., Das, S., 2019. Short-chain fatty acid and vitamin production potentials of *Lactobacillus* isolated from fermented foods of Khasi Tribes, Meghalaya, India. Ann. Microbiol. 69, 1191–1199. https://doi.org/10.1007/s13213-019-01500-8.
Hernandez, L.H.H., Barrera, T.C., Mejia, J.C., Mejia, G.C., Del Carmen, M., Dosta, M., et al., 2010. Effects of the commercial probiotic *Lactobacillus casei* on the growth, protein content of skin mucus and stress resistance of juveniles of the Porthole livebearer *Poecilopsis gracilis* (Poecilidae). Aquac. Nutr. 16, 407–411. https://doi.org/10.1111/j.1365-2095.2009.00679.x.
Hills, R.D., Pontefract, B.A., Mishcon, H.R., Black, C.A., Sutton, S.C., Theberge, C.R., 2019. Gut microbiome: Profound implications for diet and disease. Nutrients 11, 1–40. https://doi.org/10.3390/nu11071613.
Hoseinifar, S.H., Sun, Y.Z., Caipang, C.M., 2017. Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquac. Res. https://doi.org/10.1111/are.13239.
Hoseinifar, S.H., Hosseini, M., Paknejad, H., Safari, R., Jafar, A., Yousefi, M., et al., 2019. Enhanced mucosal immune responses, immune related genes and growth performance in common carp (*Cyprinus carpio*) juveniles fed dietary *Pediococcus acidilactici* MA18/5M and raffinose. Dev. Comp. Immunol. 94, 59–65. https://doi.org/10.1016/j.dci.2019.01.009.
Hosseini, E., Grootaert, C., Verstraete, W., Van de Wiele, T., 2011. Propionate as a health-promoting microbial metabolite in the human gut. Nutr. Rev. 69, 245–258. https://doi.org/10.1111/j.1753-4887.2011.00388.x.
Hu, H., Kortner, T.M., Gajardo, K., Chikwati, E., Tinsley, J., Krogdahl, Å., 2016. Intestinal fluid permeability in Atlantic salmon (*Salmo salar* L.) Is Affected by Dietary Protein Source. PLoS One 11, e0167515. https://doi.org/10.1371/journal.pone.0167515.
Ichikawa, H., Kuroiwa, T., Inagaki, A., Shineha, R., Nishihira, T., Satomi, S., et al., 1999. Probiotic bacteria stimulate gut epithelial cell proliferation in rat. Dig. Dis. Sci. 44, 2119–2123. https://doi.org/10.1023/a:1026647024077.
Jahangiri, L., Esteban, M.Á., 2018. Administration of probiotics in the water in finfish aquaculture systems: A review. Fishes. https://doi.org/10.3390/fishes3030033.
Jami, M.J., Abedian Kenari, A., Paknejad, H., Mohseni, M., 2019. Effects of dietary b-glucan, mannan oligosaccharide, *Lactobacillus plantarum* and their combinations on growth performance, immunity and immune related gene expression of Caspian trout, *Salmo trutta caspius* (Kessler, 1877). Fish. Shellfish Immunol. 91, 202–208. https://doi.org/10.1016/j.fsi.2019.05.024.
Karlsen, C., Ytteborg, E., Timmerhaus, G., Høst, V., Handeland, S., Jørgensen, S.M., et al., 2018. Atlantic salmon skin barrier functions gradually enhance after seawater transfer. Sci. Rep. 8, 9510. https://doi.org/10.1038/s41598-018-27818-y.
Kelly, C.J., Zheng, L., Campbell, E.L., Saeedi, B., Scholz, C.C., Bayless, A.J., et al., 2015. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe 17, 662–671. https://doi.org/10.1016/j.chom.2015.03.005.
Kiron, V., Sørensen, M., Huntley, M., Vasanth, G.K., Gong, Y., Dahle, D., et al., 2016. Defatted biomass of the microalga, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. Front. Mar. Sci. 3. https://doi.org/10.3389/fmars.2016.00067.
Kiron, V., Kathiresan, P., Fernandes, J.M.O., Sørensen, M., Vasanth, G.K., Lin, Q., et al., 2022. Clues from the intestinal mucus proteome of Atlantic salmon to counter inflammation. J. Proteom. 255, 104487 https://doi.org/10.1016/j.jprot.2022.104487.
Knudsen, D., Urán, P., Arnous, A., Koppe, W., Frøkiær, H., 2007. Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. J. Agric. Food Chem. https://doi.org/10.1021/jf0626967.
Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., Frøkiær, H., 2008. Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (*Salmo salar* L.). Br. J. Nutr. https://doi.org/10.1017/S0007114507886338.
Krishnaveni, G., Vignesh, S., Vidhyalakshmi, N., Vijay, V., Ramesh, U., 2021. Effects of dietary supplementation of *Lactobacillus fermentum* URLP18 on growth, innate immunity and survival against *Aeromonas hydrophila* ATCC 7966 challenge in freshwater fish *Cyprinus carpio* (common carp). Aquac. Res. 52, 1160–1176. https://doi.org/10.1111/are.14974.
Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: An update on recent findings regarding responses in salmonids. Aquac. Res. https://doi.org/10.1111/j.1365-2109.2009.02426.x.
Krogdahl, Å., Gajardo, K., Kortner, T.M., Penn, M., Gu, M., Berge, G.M., et al., 2015. Soya saponins induce enteritis in Atlantic salmon (*Salmo salar* L.). J. Agric. Food Chem. 63, 3887–3902. https://doi.org/10.1021/jf506242t.
Kusumo, P.D., Maulahela, H., Utari, A.P., Surono, I.S., Soebandrio, A., Abdullah, M., 2019. Probiotic *Lactobacillus plantarum* is 10506 supplementation increase SCFA of women with functional constipation. Iran. J. Microbiol. 11, 389–396. https://doi.org/10.18502/ijm.v11i5.1957.
Lazado, C.C., Caipang, C.M.A., Estante, E.G., 2015. Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions. Fish. Shellfish Immunol. 45, 2–12. https://doi.org/10.1016/j.fsi.2015.02.023.
Legrand, T.P.R.A., Catalano, S.R., Wos-Oxley, M.L., Stephens, F., Landos, M., Bansemer, M.S., et al., 2018. The inner workings of the outer surface: Skin and gill microbiota as Indicators of changing gut health in yellowtail kingfish. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02664.
Lobo, C., Moreno-Ventas, X., Tapia-Paniagua, S., Rodríguez, C., Moriñigo, M.A., de La Banda, I.G., 2014. Dietary probiotic supplementation (*Shewanella putrefaciens* Pdp11) modulates gut microbiota and promotes growth and condition in Senegalese

sole larviculture. Fish. Physiol. Biochem. 40, 295–309. https://doi.org/10.1007/s10695-013-9844-0.
Louis, P., Hold, G.L., Flint, H.J., 2014. The gut microbiota, bacterial metabolites and colorectal cancer. Nat. Rev. Microbiol. https://doi.org/10.1038/nrmicro3344.
Ma, H., Qiu, Y., Yang, H., 2021. Intestinal intraepithelial lymphocytes: Maintainers of intestinal immune tolerance and regulators of intestinal immunity. J. Leukoc. Biol. 109, 339–347. https://doi.org/10.1002/JLB.3RU0220-111.
Markowiak-Kopeć, P., Śliżewska, K., 2020. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients 12. https://doi.org/10.3390/nu12041107.
Mayassi, T., Jabri, B., 2018. Human intraepithelial lymphocytes. Mucosal Immunol. 11, 1281–1289. https://doi.org/10.1038/s41385-018-0016-5.
Meenakshi Malhotra, I.K., 2015. Identification of *Lactobacillus fermentum* strains with potential against colorectal cancer by characterizing short chain fatty acids production, anti-proliferative activity and survival in an intestinal fluid: *In vitro* analysis. J. Bioanal. Biomed. 07. https://doi.org/10.4172/1948-593X.1000132.
Merrifield, D.L., Dimitroglou, A., Bradley, G., Baker, R.T.M., Davies, S.J., 2010. Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. Aquac. Nutr. 16, 504–510. https://doi.org/10.1111/j.1365-2095.2009.00689.x.
Mirghaed, A.T., Yarahmadi, P., Soltani, M., Paknejad, H., Hoseini, S.M., 2019. Dietary sodium butyrate (Butirex® C4) supplementation modulates intestinal transcriptomic responses and augments disease resistance of rainbow trout (*Oncorhynchus mykiss*). Fish. Shellfish Immunol. 92, 621–628. https://doi.org/10.1016/j.fsi.2019.06.046.
Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. *Aquaculture*. https://doi.org/10.1016/S0044-8486(96)01487-1.
Naser, S.A., Romero, C., Urbina, P., Naser, N., Valentine, J., 2011. Cellular infiltration and cytokine expression correlate with fistulizing state in Crohn's disease. Clin. Vaccin. Immunol. 18, 1416–1419. https://doi.org/10.1128/CVI.05095-11.
Nayak, S.K., 2010. Probiotics and immunity: A fish perspective. Fish. Shellfish Immunol. https://doi.org/10.1016/j.fsi.2010.02.017.
Ng, S.C., Hart, A.L., Kamm, M.A., Stagg, A.J., Knight, S.C., 2009. Mechanisms of action of probiotics: Recent advances. Inflamm. Bowel Dis. 15, 300–310. https://doi.org/10.1002/ibd.20602.
Nimalan, N., Sørensen, S.L., Fečkaninová, A., Koščová, J., Mudroňová, D., Gancarčíková, S., et al., 2022. Mucosal barrier status in Atlantic salmon fed marine or plant-based diets supplemented with probiotics. Aquaculture 547, 737516. https://doi.org/10.1016/j.aquaculture.2021.737516.
Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., Skrede, A., 2009. Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)—Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture 288, 305–311. https://doi.org/10.1016/j.aquaculture.2008.12.012.
Paone, P., Cani, P.D., 2020. Mucus barrier, mucins and gut microbiota: The expected slimy partners. Gut 69, 2232–2243. https://doi.org/10.1136/gutjnl-2020-322260.
Peng, L., Li, Z.R., Green, R.S., Holzman, I.R., Lin, J., 2009. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J. Nutr. 139, 1619–1625. https://doi.org/10.3945/jn.109.104638.
Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., et al., 2011. Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Res. Vet. Sci. 91, e92–e97. https://doi.org/10.1016/j.rvsc.2011.02.014.
Pirarat, N., Pinpimai, K., Rodkhum, C., Chansue, N., Ooi, E.L., Katagiri, T., Maita, M., 2015. Viability and morphological evaluation of alginate-encapsulated *Lactobacillus rhamnosus* GG under simulated tilapia gastrointestinal conditions and its effect on growth performance, intestinal morphology and protection against *Streptococcus agalactiae*. Anim. Feed Sci. Technol. 207, 93–103. https://doi.org/10.1016/j.anifeedsci.2015.03.002.
Rimoldi, S., Gliozheni, E., Ascione, C., Gini, E., Terova, G., 2018. Effect of a specific composition of short- and medium-chain fatty acid 1-monoglycerides on growth performances and gut microbiota of gilthead sea bream (*Sparus aurata*). PeerJ 2018, 1–27. https://doi.org/10.7717/peerj.5355.
Ringø, E., 1999. Does *Carnobacterium divergens* isolated from Atlantic salmon, *Salmo salar* L., colonize the gut of early developing turbot, *Scophthalmus maximus* L., larvae? Aquac. Res. https://doi.org/10.1046/j.1365-2109.1999.00269.x.
Ringø, E., Zhou, Z., Vecino, J.L.G., Wadsworth, S., Romero, J., Krogdahl, et al., 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? Aquac. Nutr. 22, 219–282. https://doi.org/10.1111/anu.12346.
Ringø, E., Hoseinifar, S.H., Ghosh, K., Doan, H., Van, Beck, B.R., Song, S.K., 2018. Lactic acid bacteria in finfish-An update. Front. Microbiol. 9, 1–37. https://doi.org/10.3389/fmicb.2018.01818.
Ringø, E., Van Doan, H., Lee, S.H., Soltani, M., Hoseinifar, S.H., Harikrishnan, R., et al., 2020. Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. J. Appl. Microbiol. 129, 116–136. https://doi.org/10.1111/jam.14628.
Rivière, A., Selak, M., Lantin, D., Leroy, F., De Vuyst, L., 2016. Bifidobacteria and butyrate-producing colon bacteria: Importance and strategies for their stimulation in the human gut. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00979.
Ruangsri, J., Lokesh, J., Fernandes, J.M.O., Kiron, V., 2014. Transcriptional regulation of antimicrobial peptides in mucosal tissues of Atlantic cod *Gadus morhua* L. in response to different stimuli. Aquac. Res. https://doi.org/10.1111/are.12136.
Salem, I., Ramser, A., Isham, N., Ghannoum, M.A., 2018. The gut microbiome as a major regulator of the gut-skin axis. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.01459.
Sanden, M., Olsvik, P.A., 2009. Intestinal cellular localization of PCNA protein and CYP1A mRNA in Atlantic salmon *Salmo salar* L. exposed to a model toxicant. BMC Physiol. 9, 1–11. https://doi.org/10.1186/1472-6793-9-3.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods. https://doi.org/10.1038/nmeth.2089.
Sealey, W.M., Barrows, F.T., Smith, C.E., Overturf, K., LaPatra, S.E., 2009. Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout *Oncorhynchus mykiss* to utilize high levels of soybean meal during grow-out. Aquaculture 293, 195–203. https://doi.org/10.1016/j.aquaculture.2009.04.013.
Sewaka, M., Trullas, C., Chotiko, A., Rodkhum, C., Chansue, N., Boonanuntanasarn, S., et al., 2019. Efficacy of synbiotic Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile re. Fish. Shellfish Immunol. 86, 260–268. https://doi.org/10.1016/j.fsi.2018.11.026.
Sharon, G., Garg, N., Debelius, J., Knight, R., Dorrestein, P.C., Mazmanian, S.K., 2014. Specialized metabolites from the microbiome in health and disease. Cell Metab. 20, 719–730. https://doi.org/10.1016/j.cmet.2014.10.016.
Sherif, A.H., Gouda, M.Y., Al-Sokary, E.T., Elseify, M.M., 2021. *Lactobacillus plantarum* enhances immunity of Nile tilapia *Oreochromis niloticus* challenged with *Edwardsiella tarda*. Aquac. Res. 52, 1001–1012. https://doi.org/10.1111/are.14955.
Silva, P.F., McGurk, C., Knudsen, D.L., Adams, A., Thompson, K.D., Bron, J.E., 2015. Histological evaluation of soya bean-induced enteritis in Atlantic salmon (*Salmo salar* L.): Quantitative image analysis vs. semi-quantitative visual scoring. Aquaculture 445, 42–56. https://doi.org/10.1016/j.aquaculture.2015.04.002.
Silva, Y.P., Bernardi, A., Frozza, R.L., 2020. The Role of short-chain fatty acids from gut microbiota in gut-brain communication. Front. Endocrinol. (Lausanne) 11. https://doi.org/10.3389/fendo.2020.00025.
Smid, E.J., Lacroix, C., 2013. Microbe–microbe interactions in mixed culture food fermentations. Curr. Opin. Biotechnol. 24, 148–154. https://doi.org/10.1016/j.copbio.2012.11.007.
Smids, C., Horjus Talabur Horje, C.S., Drylewicz, J., Roosenboom, B., Groenen, M.J.M., van Koolwijk, E., van Lochem, E.G., Wahab, P.J., 2018. Intestinal T cell profiling in inflammatory bowel disease: Linking T cell subsets to disease activity and disease course. J. Crohn's Colitis 12, 465–475. https://doi.org/10.1093/ecco-jcc/jjx160.
Song, J., Peng, S., Yang, J., Zhou, F., Suo, H., 2021. Isolation and identification of novel antibacterial peptides produced by *Lactobacillus fermentum* SHY10 in Chinese pickles. Food Chem. 348, 129097 https://doi.org/10.1016/j.foodchem.2021.129097.
Sørensen, M., Penn, M., El-Mowafi, A., Storebakken, T., Chunfang, C., Øverland, M., et al., 2011. Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L). Aquaculture 314, 145–152. https://doi.org/10.1016/j.aquaculture.2011.02.013.
Sørensen, S.L., Park, Y., Gong, Y., Vasanth, G.K., Dahle, D., Korsnes, K., et al., 2021. Nutrient digestibility, growth, mucosal barrier status, and activity of leucocytes from head kidney of Atlantic salmon fed marine- or plant-derived protein and lipid sources. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.623726.
Sundell, K.S., Sundh, H., 2012. Intestinal fluid absorption in anadromous salmonids: Importance of tight junctions and aquaporins. Front. Physiol. (3 SEP), 1–13. https://doi.org/10.3389/fphys.2012.00388.
Suzer, C., Çoban, D., Kamaci, H.O., Saka, Ş., Firat, K., Otgucuoğlu, Ö., et al., 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: Effects on growth performance and digestive enzyme activities. Aquaculture 280, 140–145. https://doi.org/10.1016/j.aquaculture.2008.04.020.
Tapia-Paniagua, S.T., Chabrillón, M., Díaz-Rosales, P., de la Banda, I.G., Lobo, C., Balebona, M.C., et al., 2010. Intestinal microbiota diversity of the flat fish *Solea senegalensis* (Kaup, 1858) following probiotic administration. Microb. Ecol. 60, 310–319. https://doi.org/10.1007/s00248-010-9680-z.
Thursby, E., Juge, N., 2017. Introduction to the human gut microbiota. Biochem. J. 474, 1823–1836. https://doi.org/10.1042/BCJ20160510.
Titus, E., Ahearn, G.A., 1991. Transintestinal acetate transport in a herbivorous teleost: Anion exchange at the basolateral membrane. J. Exp. Biol. 156, 41–61.
Urán, P.A., Aydin, R., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M., 2008a. Soybean meal-induced uptake block in Atlantic salmon *Salmo salar* distal enterocytes. J. Fish. Biol. https://doi.org/10.1111/j.1095-8649.2008.02091.x.
Urán, P.A., Schrama, J.W., Rombout, J.H.W.M., Obach, A., Jensen, L., Koppe, W., et al., 2008b. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar* L.) at different temperatures. Aquac. Nutr. 14, 324–330. https://doi.org/10.1111/j.1365-2095.2007.00534.x.
Urán, P.A., Schrama, J.W., Rombout, J.H.W.M., Taverne-Thiele, J.J., Obach, A., Koppe, W., et al., 2009. Time-related changes of the intestinal morphology of Atlantic salmon, *Salmo salar* L., at two different soybean meal inclusion levels. J. Fish. Dis. 32, 733–744. https://doi.org/10.1111/j.1365-2761.2009.01049.x.
Van Doan, H., Hoseinifar, S.H., Khanongnuch, C., Kanpiengjai, A., Unban, K., Van Kim, V., et al., 2018. Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 491, 94–100. https://doi.org/10.1016/j.aquaculture.2018.03.019.
Van Nguyen, N., Onoda, S., Van Khanh, T., Hai, P.D., Trung, N.T., Hoang, L., et al., 2019. Evaluation of dietary heat-killed *Lactobacillus plantarum* strain L-137 supplementation on growth performance, immunity and stress resistance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 498, 371–379. https://doi.org/10.1016/j.aquaculture.2018.08.081.
Vatsos, I.N., Kotzamanis, Y., Henry, M., Angelidis, P., Alexis, M., 2010. Monitoring stress in fish by applying image analysis to their skin mucous cells. Eur. J. Histochem. 54, 22. https://doi.org/10.4081/ejh.2010.e22.

Wang, J., Kortner, T.M., Chikwati, E.M., Li, Y., Jaramillo-Torres, A., Jakobsen, J.V., et al., 2020. Gut immune functions and health in Atlantic salmon (*Salmo salar*) from late freshwater stage until one year in seawater and effects of functional ingredients: A case study from a commercial sized research site in the Arctic region. Fish. Shellfish Immunol. 106, 1106–1119. https://doi.org/10.1016/j.fsi.2020.09.019.

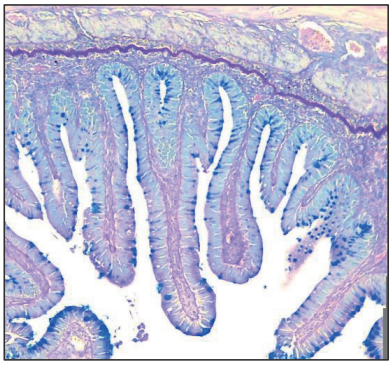
Wang, Y., Gu, Q., 2010. Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. Mar. Biol. Res. 6, 327–332. https://doi.org/10.1080/17451000903300893.

Wiatrak, B., Balon, K., Jawień, P., Bednarz, D., Jęśkowiak, I., Szeląg, A., 2022. The role of the microbiota-gut-brain axis in the development of Alzheimer's disease. Int. J. Mol. Sci. 23, 4862. https://doi.org/10.3390/ijms23094862.

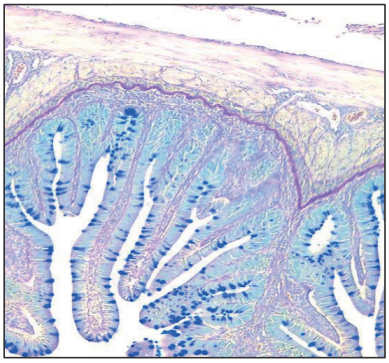
Zheng, L., Kelly, C.J., Battista, K.D., Schaefer, R., Lanis, J.M., Alexeev, E.E., et al., 2017. Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor–dependent repression of Claudin-2. J. Immunol. 199, 2976–2984. https://doi.org/10.4049/jimmunol.1700105.

Zheng, X., Duan, Y., Dong, H., Zhang, J., 2020. The effect of *Lactobacillus plantarum* administration on the intestinal microbiota of whiteleg shrimp *Penaeus vannamei*. Aquaculture 526, 735331. https://doi.org/10.1016/j.aquaculture.2020.735331.

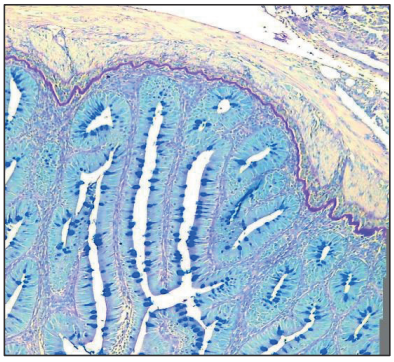
Zorriehzahra, M.J., Delshad, S.T., Adel, M., Tiwari, R., Karthik, K., Dhama, K., et al., 2016. Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. Vet. Q. https://doi.org/10.1080/01652176.2016.1172132.



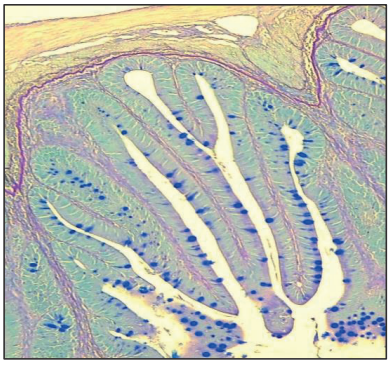
Score 1: More than 38 mucous cells per simple villi, densely distributed small mucous cells throughout the complex villi



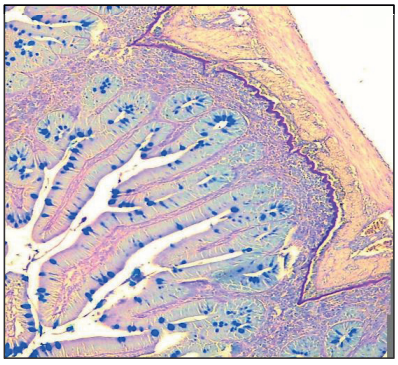
Score 2: In between 33 to 38 mucous cells per simple villi, small and large mucous cells throughout the complex villi



Score 3: Around 27 to 32 mucous cells per simple villi, small and few large mucous cells throughout the complex villi

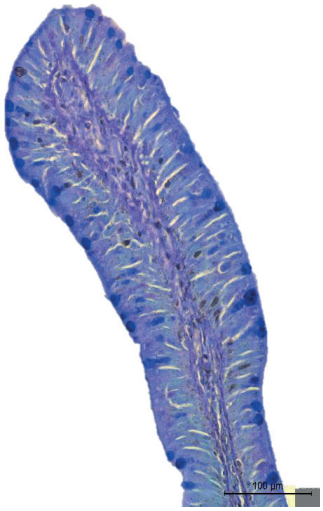


Score 4: Around 21 to 26 mucous cells per simple villi, many large and few small mucous cells throughout the complex villi

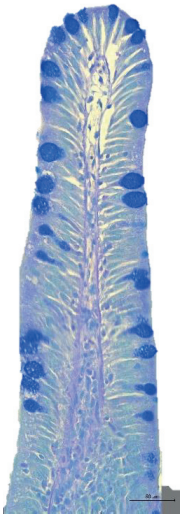


Score 5: Below 21 mucous cells per simple villi, large mucous cells evenly distributed throughout the complex villi

Supplementary Figure 1. Semi-quantitative scoring system adopted to study the number of mucous cells (NOM) and representative histological images of the distal intestine from Atlantic salmon. Images were acquired with a magnification of 10X. The scale of the images is 1.06 µm per pixels.

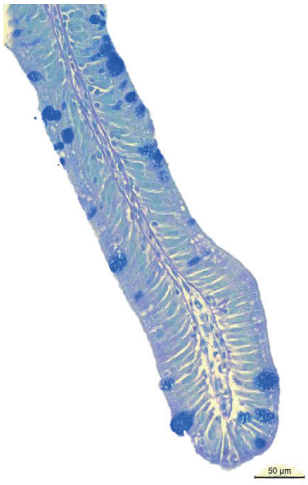


Score 1: More than 17 IEL per simple villi, densely distributed in the complex villi



Score 2: In between 15 to 17 IEL per simple villi

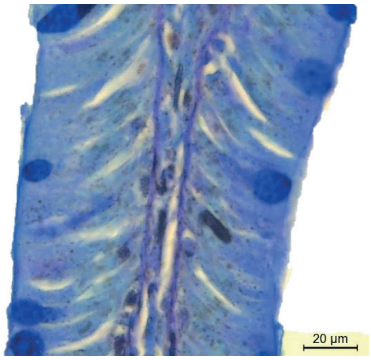
Score 3: Around 12 to 14 IEL per simple villi



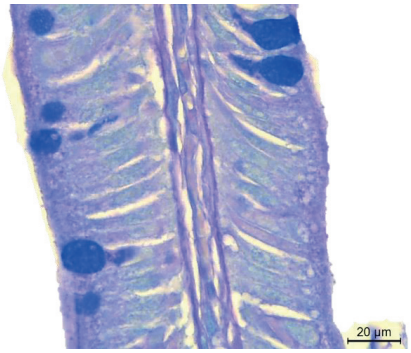
Score 4: Around 9 to 11 IEL per simple villi

Score 5: Below 9 IEL per simple villi, evenly distributed throughout the complex villi

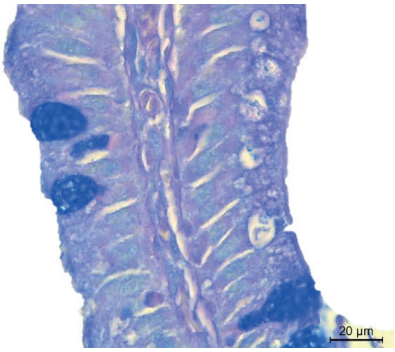
Supplementary Figure 2. Semi-quantitative scoring system adopted to study the number of intraepithelial lymphocytes (IEL) and representative histological images of the distal intestine from Atlantic salmon. Images were acquired with a magnification of 63X. The scale of the images is 1.68 µm per pixels.



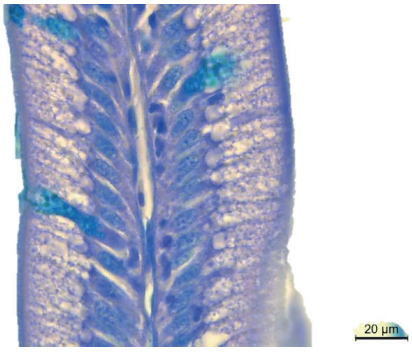
Score 1: Vacuoles completely absent or not present



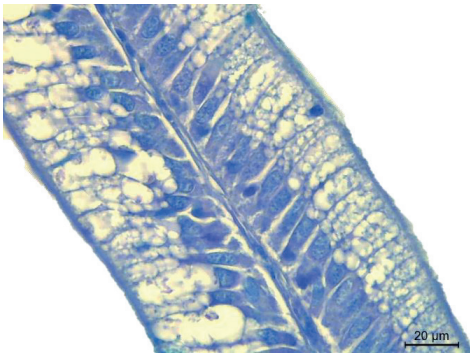
Score 2: Few tiny vacuoles appear scattered in certain regions of enterocytes.



Score 3: Sizes of few small vacuoles still present in many enterocytes were obviously reduced.

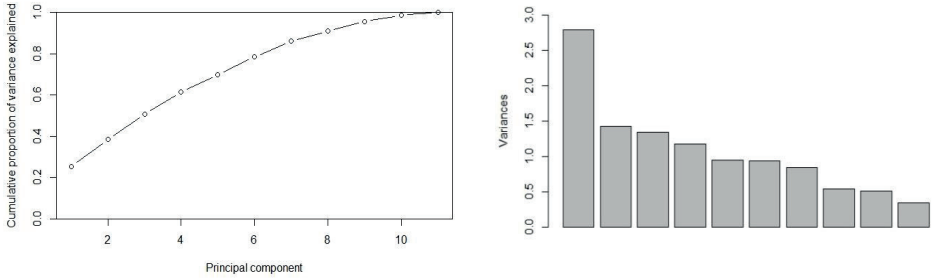


Score 4: Sizes of many medium- sized vacuoles that were present in almost 50% of the enterocyte area were mildly reduced.



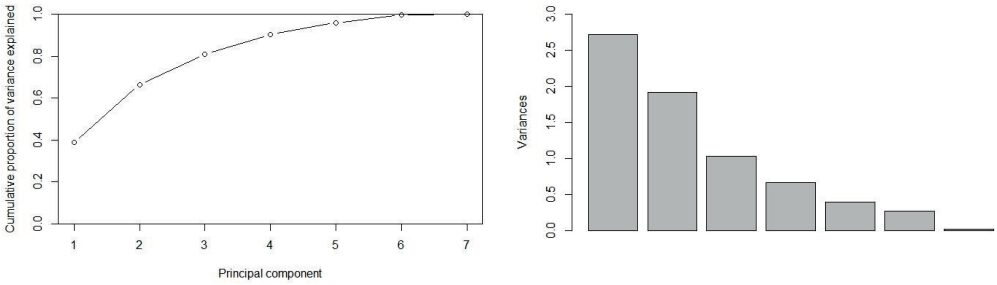
Score 5: Highly vacuolated enterocyte; large vacuoles were present along the apical part of enterocytes

Supplementary Figure 3. Semi-quantitative scoring system adopted in the study to assess the presence of supranuclear vacuoles in enterocytes (SNV) and representative histological images of the distal intestine from Atlantic salmon. Images were acquired with a magnification of 63X. The scale of the images is 1.68 µm per pixels.

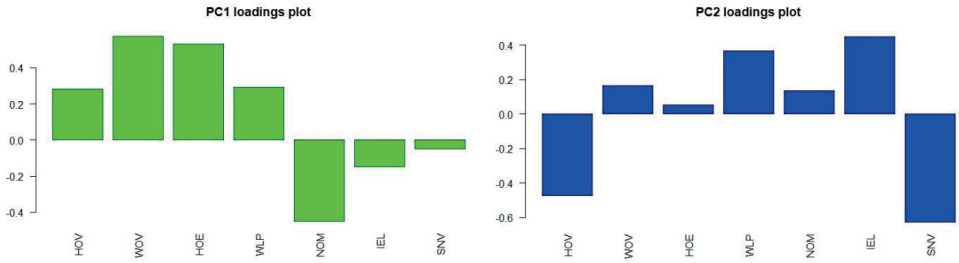


Supplementary Figure 4. Proportion of variance explained in the principal component analysis (PCA) plots of selected mucus-related genes. Left figure shows the cumulative proportion of variance. Right figure shows scree plot as a bar chart.

Supplementary Figure 5. Loadings of mucus-related genes employed in the principal component analysis plot shown in figure 1. Variable loading on PC1 is shown on the left (green). Variable loading on PC2 is shown on the right (blue). SM1, skin *muc5ac1*; SM2, skin *muc5ac2*; SM5, skin *muca5b*; SD1, skin *defensin1*; SC1, skin *cathelicidin1*, GM2, gills *muc5ac2*; GM5, gill *muc5b*; IM2, intestine *muc2*; ID3; intestine *defensin3*; ID4, intestine *defensin4*; IC1, intestine *cathelicidin1*.



Supplementary Figure 6. Proportion of variance explained in the principal component analysis (PCA) plots of distal intestinal histomorphometric indices. Left figure shows the cumulative proportion of variance. Right figure shows scree plot as a bar chart.



Supplementary figure 7. Loadings of the distal intestinal histomorphometric indices employed in the principal component analysis plot shown in figure 5. Variable loading on PC1 is shown on the left (green). Variable loading on PC2 is shown on the right (blue). HOV, height of villi; WOV, width of villi; HOE, height of enterocytes; WLP, width of lamina propria; NOM, score for number of intestinal mucous cells; IEL, score for number of intraepithelial lymphocytes; SNV, score for supra nuclear vacuoles.

Supplementary Table 1

Development of enteritis assessed through intestinal histomorphometric indices of Atlantic salmon fed the soybean meal-based control (CT) in this study in comparison to the marine-based positive control feed (BG1÷) from Nimalan et al., (2022).

Indices	Diet groups		*p*-values
	BG1	**CT**	
HOV (µm)	1146.68 ± 42.86^{b}	897.65 ± 30.23^{a}	<0.001
WOV (µm)	114.56 ± 3.60	126.73 ± 5.28	0.070
HOE (µm)	55.52 ± 1.69^{b}	48.26 ± 2.13^{a}	0.014
WLP (µm)	9.67 ± 0.65^{a}	27.62 ± 1.62^{b}	<0.001
NOM (score)	3.50 ± 0.34	3.00 ± 0.30	0.339
IEL (score)	4.75 ± 0.13^{b}	3.67 ± 0.19^{a}	<0.001
SNV (score)	5.00 ± 0.00^{b}	1.00 ± 0.00^{a}	<0.001

CT, SBM-based control diet without probiotics; BG1÷, marine-based diet from our previous publication. HOV, height of villi; WOV, width of villi; HOE, height of enterocytes; WLP, width of lamina propria; NOM, score for number of intestinal mucous cells; IEL, score for number of intraepithelial lymphocytes; SNV, score for supranuclear vacuoles. Values are presented as means ± SEM, n = 12 per diet group. If present, significant differences ($p < 0.05$) among diet groups are indicated by different superscripts (a or b) on each row after conducting unpaired two-samples t-test or non-parametric Wilcoxon test for score data. Less scores for NOM and IEL indicate more cells per villi, while less score for SNV implies less vacuoles and vice versa.

Paper II

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Mucosal barrier status in Atlantic salmon fed marine or plant-based diets supplemented with probiotics

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ABSTRACT

Feed ingredients and additives significantly affect the mucosal health of fish. A 3 × 2 factorial experiment was conducted to investigate the mucosal health of Atlantic salmon (*Salmo salar*) fed three basal feeds (namely, BG1, BG5 and BG2; marine-, plant-, and soybean meal-based feeds, respectively) or the basal feeds with (+) or without (÷) probiotics, *Lactobacillus fermentum* and *Lactobacillus plantarum*. Six diets were fed to fish distributed into 12 tanks (approximately 43 fish/tank). The average start weight of the experimental fish was about 122.6 g. After 38-days of feeding, the dorsal skin, gills and distal intestine were obtained for analysing histomorphometry and mucus-related genes. Digesta were also collected to study short chain fatty acids (SCFAs).

Fish fed BG2 had significantly higher number of mucous cells/μm^2 skin epithelium (SNE) than those fed BG1 and BG5. Addition of probiotics significantly increased SNE in BG5+ and BG2+ group compared to BG1÷ group. Similarly, the area and number of mucous cells/μm^2 gill epithelium (GME and GNE) were significantly higher in BG2 group, followed by BG5 and BG1 fish groups. Probiotics significantly increased GME and GNE in all feed types. Concerning intestine, villi height (VH) and enterocyte height (EH) were significantly higher for BG1 group, followed by BG5 and BG2 groups. Compared to fish offered BG2, fish fed BG1 had significantly wider villi (VW) and narrower lamina propria (LPW). The number of mucous cells (NM) and intraepithelial lymphocytes (IEL) in the intestine were significantly higher in BG2 fed fish than those offered BG5 and BG1. The indices VH, VW, EH, and IEL were not affected by probiotics. Although higher NM and IEL were observed in BG2÷ fish compared to those fed the other two diets, probiotics reduced NM and IEL. Fish fed BG2÷ showed symptoms of inflammation, including disappearance of supranuclear vacuoles (SNV). Probiotics improved VH, significantly reduced LPW and aided in the reappearance of SNV in BG2+ fed fish. Some of the gene expression data supported histological findings; notably, levels of *muc5ac1* in the skin and *defensin3* and *cathelcidin1* in the intestine were correlated with histology data. Moreover, the total SCFA concentration was significantly affected by feed ingredients. Only acetoacetic acid was affected by both factors.

Our findings suggest that feed ingredients can significantly alter the mucosal protective barrier of the organs. Supplementation of probiotics alleviated the inflammatory responses and activated selected innate immune defence molecules, without affecting growth. The positive effect of the probiotics was similar regardless of the feed ingredients, suggesting that these probiotics can be utilized as immune regulators to evoke favourable responses on the skin, gills and intestine.

1. Introduction

Fish feed accounts for a significant part of the variable costs in the farming of Atlantic salmon (*Salmo salar*). Efficient utilization of feeds by the fish is the key to keep the production cost low and the economic turnover high (Iversen et al., 2020). Since 1990, salmon feed industry

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has gradually shifted its dependence from marine- to plant-derived ingredients (Aas et al., 2019); now soy protein concentrate is a key salmon feed ingredient, and pea protein concentrate, wheat gluten, corn gluten, fava beans, sunflower meal and sunflower protein are incorporated at lower levels (Aas et al., 2019; Øverland et al., 2009; Ytrestøyl et al., 2015). Regarding the fish oil, it has been replaced to a large extent by rapeseed oil in European salmon feeds (Aas et al., 2019; Sprague et al., 2016; Ytrestøyl et al., 2015).

Use of more plant-based ingredients has taken its toll on the health of fish (Sørensen et al., 2021). Less refined feed ingredients, in particular soybean meal (SBM) that contains various antinutritional factors such as saponins, cause enteritis in fish (Baeverfjord and Krogdahl, 1996; Booman et al., 2018; Knudsen et al., 2007; Krogdahl et al., 2015; Sanden et al., 2005; Sørensen et al., 2011; Vasanth et al., 2015). SBM derivatives-induced intestinal inflammation was characterized by abnormal intestinal villi and lamina propria, enterocytes lacking supranuclear vacuoles, immune cell-infiltrated lamina propria and submucosa, and intestinal ion and water transport disturbances (Baeverfjord and Krogdahl, 1996; Buttle et al., 2001; Kiron et al., 2020; Kortner et al., 2012; Krogdahl et al., 2000; Refstie et al., 2000; Urán et al., 2008a, 2008c, 2009; Van Den Ingh et al., 1996, 1991). These unfavourable conditions are known to affect the growth of the fish, shift the microbiota and weaken the local immune defences, thereby making the fish prone to diseases (Egerton et al., 2020; Gajardo et al., 2017; Krogdahl et al., 2000; Torrecillas et al., 2017).

Disease prevention and control coupled with reduced mortality during the grow-out phase of fishes is vital for sustainable development of aquaculture as well as to keep the production costs in check (Bang-Jensen et al., 2019; Iversen et al., 2020; Minniti et al., 2019). Fish body is exposed to various adverse environmental conditions, including many opportunistic pathogens that thrive in the rearing water. However, a rather complex immune system that encompasses innate and adaptive branches, exists to fight these threats and to maintain the health (Brunner et al., 2020; Magnadóttir, 2006). The first lines of defence include the mucosal barriers in the skin, gills and intestine (Cain and Swan, 2010; Kiron, 2012; Wang et al., 2011). The epithelial cells in these organs are covered by a mucus layer, which is mainly secreted by mucous cells. The mucus consists of several innate immune molecules such as mucins, protease, lysozyme, esterase, complement proteins, antibodies and antimicrobial peptides (AMPs) which could chemically inactivate the pathogens or arrest the formation of their colonies (Aranishi and Mano, 2000; Concha et al., 2003; Firth et al., 2000; Hatten et al., 2001; Johansson et al., 2008; Núñez-Acuña et al., 2018, 2016). In addition, the intestine mucus creates an optimum environment for the action of the digestive enzymes and aids in lubricating the digesta to ensure the integrity of intestinal mucosa during digestion (Kim and Ho, 2010). Therefore, a healthy mucosal surface with adequate number of mucous cells is essential to maintain the barrier functions and deploy appropriate molecules such as mucins and AMPs during defence (Pittman et al., 2011).

Commensal microbiota at the mucosal surfaces are also vital to maintain the mucosal barrier functions and to prevent colonization by potential pathogens (Lowrey et al., 2015). It is now known that microbiota can be modulated by feed ingredients (Hoseinifar et al., 2015; Nayak, 2010; Pérez-Sánchez et al., 2014) and additives such as probiotics (Gupta et al., 2019a) and pre-biotics (Gupta et al., 2019b). Lactic acid bacteria (LAB) such as *Lactobacillus* spp., *Lactococcus* spp., *Pediococcus* spp., *Carnobacterium* spp. and those belonging to the genus *Leuconostoc* can be considered as probiotics for aquaculture applications; for enhancement of gut health, to reduce the use of chemotherapeutics and to maintain fish welfare (Alonso et al., 2019; Andani et al., 2012; Hai, 2015; Merrifield et al., 2010; Ringø et al., 2018).

LABs have anti-inflammatory and antibacterial properties, and they are classified as GRAS (generally recognized as safe) (van Baarlen et al., 2013). Bacteria belonging to the genus, *Lactobacillus* are acid-tolerant facultative anaerobes, and they are either homo- or heterofermentative (Ringø et al., 2018). Kraus (1961) was the first to reveal the presence of lactobacilli in the gastrointestinal tract (GI) of a fish, herring (*Clupea harengus* L.). Since then, the existence of bacteria belonging to the genus *Lactobacillus* in the GI tract of several finfish species, including Atlantic salmon, has been reported by many authors (Gatesoupe, 2007; Hovda et al., 2007; Lauzon and Ringø, 2011; Merrifield et al., 2014; Ringø, 2004; Ringø et al., 2005; Ringø and Gatesoupe, 1998). Our group has reported the establishment of lactobacilli delivered through feeds in the intestinal mucus of Atlantic salmon (Gupta et al., 2019a).

It is now known that certain members of the microbiota can produce short chain fatty acids (SCFAs) by fermenting nondigestible carbohydrates in feeds (Adorian et al., 2020; Hoseinifar et al., 2017). The dominant SCFAs such as acetate, propionate and butyrate (Den Besten et al., 2013) act as energy providers, signalling molecules, gene expression regulators, inflammation suppressors and immune cell development regulators. Thus, they play a critical role in maintaining intestinal integrity and health (Koh et al., 2016; Louis et al., 2014; Morrison and Preston, 2016; Richards et al., 2016).

Important information on the fish mucosal health status can also be collected by studying the associated mucin and AMP related genes (Bridle et al., 2011; Broekman et al., 2013; Chang et al., 2006; Marcos-López et al., 2018). In Atlantic salmon, seven mucin secreting genes were reported previously; two *muc2* genes were mainly found in the intestine while five *muc5* were observed in other tissues such as pyloric caeca, gill or skin (Sveen et al., 2017). AMPs are a diverse group of defence molecules, and among them *cathelicidins* and *defensins* are powerful antimicrobials (Chang et al., 2006; Reyes-Becerril et al., 2013). Our previous study results indicated the ability of *muc2* to denote intestinal barrier status and the feed ingredient-induced alteration of AMP genes in the skin, gills and intestine (Sørensen et al., 2021). Furthermore, in mammals, SCFAs are suggested as biomarkers to assess the host health status (Farup et al., 2016). Hence, it is essential to gather more evidence on feed component-induced modulation of mucins and AMPs that are important gatekeepers of the mucosal barriers and SCFAs that support the health of the intestine.

The aim of the present short-term feeding study was to investigate the growth performance, the architecture of the mucosa of the first-line defence organs, expression of selected mucins and AMP genes in these organs and SCFAs in the digesta of Atlantic salmon post smolts fed plant-based or marine-based diets, with or without two lactic acid bacteria strains, *Lactobacillus fermentum* and *Lactobacillus plantarum* (1:1).

2. Materials and methods

The National Animal Research Authority (FDU: Forsøksdyrutvalget ID-5887) in Norway has approved the experiment, and the handling of the animals were in accordance with the approved protocols.

2.1. Experimental feed preparation

2.1.1. Feed preparation

For this trial, three basal feeds were prepared at the Feed Technology Center, Nofima, Bergen, Norway (Table 1). Extruded feeds were formulated based on the following ingredients: fish meal and fish oil (BG1), a mixture of plant and marine ingredients at a ratio of 70:30 (BG5) and SBM with marine ingredients (BG2). The ingredients of the experimental feeds were first homogenized (30 min) using a horizontal ribbon mixer and then they were subjected to a preconditioning step. During this step, water and steam were added into an atmospheric double differential preconditioner (DDC). The preconditioning step was followed by extrusion through a TX-52 co-rotating, fully intermeshing twin-screw extruder (Wenger Manufacturing Inc., Sabetha, KS, USA). While the temperature of the feed mash that was fed into the extruder was 86–88 °C, temperatures of the extruded feeds were different; 120, 128, and 137 °C for BG1, BG2 and BG5, respectively. Two of the feeds,

Table 1
Ingredient composition (%) of the three basal feeds employed in the study.

Ingredients	BG1	BG5	BG2
Fishmeal	50	10	30
Wheat meal	13.85	6.05	6.55
Wheat gluten	5	10	10
Soy protein concentrate	0	20	0
Soybean meal	0	0	20
Corn gluten	0	9	0
Pea protein concentrate	0	9	0
Fish oil	25	7.7	26.4
Rapeseed oil	0	19.8	0
Mineral premix	0.59	0.59	0.59
Vitamin premix	2	2	2
Monosodium phosphate	2.5	2.5	2.5
Carop. Pink (10% Astax)	0.05	0.05	0.05
Yttrium oxide	0.01	0.01	0.01
Choline	0.5	0.5	0.5
Methionine	0.3	0.9	0.6
Lysine	0	1.2	0.5
Threonine	0	0.4	0.1
Histidine	0.2	0.3	0.2

BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed. Three more experimental diets were prepared by coating two probiotic organisms to the three basal feeds.

BG2 and BG5 had lower wheat content; consequently, more moisture in the form of steam was added into the DDC to ensure good expansion of the feed pellets. The wet extrudates, expelled out of the 24 circular 2.5 mm dies at the extruder outlet, were cut with a rotating knife of the extruder. The extruded pellets were dried in a hot air dual layer carousel dryer (Paul Klockner, Nistertal, Germany) at constant air temperature (77 °C) to obtain pellets with approximately 7–8% moisture. Next, the feeds were coated with oil using a vacuum coater (Pegasus PG-10VC LAB, Dinnissen B.V., the Netherlands). Immediately after the oil coating, feeds were packed in sealed plastic buckets and shipped to the Research Station, Nord University, Bodø, Norway.

2.1.2. Probiotics coating on feed pellets

Two species of probiotics, *L. plantarum* R2 Biocenol™ (CCM 8674) and *L. fermentum* R3 Biocenol™ (CCM 8675) were isolated from the intestinal content of rainbow trout (*Oncorhynchus mykiss*) obtained from a fish farm, Rybárstvo – Požehy s.r.o. Dubové in the Slovak Republic (Fečkaninová et al., 2019). Pure cultures of probiotics were grown on de Man, Rogosa and Sharpe (MRS) agar plates (HiMedia Laboratories, Mumbai, India) under anaerobic condition (Oxoid Gas Pack Anaerobic system) at 37 °C for 48 h before they were inoculated into 1000 mL of MRS broth and incubated for 18 h at 37 °C on a shaker. The culture was centrifuged at 4500 rpm for 20 min at 4 °C in a cooling centrifuge (Universal 320 R, Hettich, Germany). The resulting cell pellets were washed twice and resuspended in 30 mL of 0.9% (w/v) sterile saline. The feeds (batches of 1800 g) were thoroughly coated with the bacterial suspensions using a vacuum coater (Rotating Vacuum Coater F-6-RVC, Forberg International AS, Norway) at 70 kPa at the feed laboratory of Nord University, Bodø, Norway. Post coating, the bacterial counts on diets were &10^8 cells/g as determined by spread plating on MRS agar plates and incubating anaerobically (Oxoid Gas Pack Anaerobic system) for 48 h at 37 °C. The feeds without probiotics were coated with 0.9% of sterile saline. The coated diets were stored at 4 °C until they were fed to the experimental fish.

2.1.3. Experimental feeds

In total, six experimental feeds were prepared for this study at the feed laboratory of Nord University, Bodø, Norway. The basal feeds without probiotics were named as BG1÷ (marine- based feed without probiotics), BG5÷ (plant-based feed without probiotics) and BG2÷ (SBM-based feed without probiotics). The basal feeds with probiotics were named as BG1+ (marine-based feed with probiotics), BG5+ (plant-based feed with probiotics) and BG2+ (SBM-based feed with probiotics). The nutrient and amino acid composition of the basal feeds is given in Table 2.

2.2. Fish, experimental design and feeding

Atlantic salmon post-smolts were obtained from Cermaq, Hopen, Bodø, Norway (Aquagen strain, Aquagen AS, Trondheim, Norway). The present experiment was the second phase of a large study (Sørensen et al., 2021) performed at the Research Station, Nord University, Bodø, Norway to test the effects of different combinations of plant and marine ingredients on the performance of Atlantic salmon. There were two replicate tanks for each treatment, and each tank contained 40–43 fish. The average initial weight of the fish was 122.6 ± 2.1 g (mean ± standard error of mean, SEM).

The feeding experiment was carried out in 12 circular fiberglass tanks (1100 L) that were connected to a flow-through system. Each tank was supplied with water pumped from Saltenfjorden, from a depth of 250 m. During the experiment, water flow rate was maintained at 1000 L per h, and the average temperature and salinity of the rearing water were 7.6 °C and 35 ‰, respectively. Oxygen saturation was always above 85%, measured at the water outlet. A 24 h photoperiod was maintained throughout the 38-day feeding trial. The fish were fed *ad libitum* using automatic feeders (Arvo Tech, Finland) during a 12-h period every day between 08:00 and 20:00 (7 feedings, 08:00–10:00, 10:00–12:00, 12:00–14:00, 14:00–16:00, 16:00–18:00, 18:00–19:00 and 19:00–20:00).

2.3. Sampling and data collection

At the beginning and end of the feeding experiment, all fish were individually weighed, and their fork lengths recorded. Fish were anesthetized using tricaine methanesulfonate (MS 222, 140 mg/L) before handling. Twelve fish per tank were sacrificed for obtaining the dorsal skin (left), gills (second arch) and intestine (approximately 2 cm of the anterior part of the distal intestine) (Sanden and Olsvik, 2009; Sundell

Table 2
Analyzed proximate composition (% as is) and amino acid composition (% as is) of the three experimental feeds.

Composition	BG1	BG5	BG2
Moisture	5.3	6.3	4.9
Protein	42.5	42.8	42.2
Lipid	29.0	26.0	28.6
Ash	11.2	7.02	9.45
Energy (KJ/100 g)	2000	1994	2029
Amino acids			
Alanine	2.44	2.04	2.03
Arginine	2.35	2.35	2.33
Aspartic acid	3.50	3.51	3.43
Glutamic acid	6.92	9.04	8.03
Glycine	2.61	1.75	2.18
Histidine	1.01	1.17	1.02
Hydroxyproline	0.31	0.16	0.22
Isoleucine	1.66	1.66	1.64
Leucine	3.01	3.54	2.93
Lysine	2.89	3.05	2.85
Phenylalanine	1.67	2.10	1.79
Proline	2.19	2.88	2.47
Serine	1.81	2.04	1.91
Threonine	1.64	1.9	1.64
Tyrosine	1.25	1.50	1.35
Valine	1.96	1.88	1.86
Tryptophan	0.43	0.41	0.44
Cysteine	0.41	0.53	0.50
Methionine	1.37	1.68	1.67
[a]Σ EPA/DHA	5.90	1.7	5.8

BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed.
[a] Σ EPA/DHA was calculated based on the content in the fish oil.

and Sundh, 2012). Tissues from 6 fish were immediately placed in 10% neutral buffered formalin (NBF) for 24 h at room temperature for the histological evaluation, and tissues from remaining 6 fish were transferred to tubes filled with RNA later® (Ambion Inc., Austin, Texas, United States), and stored at −20 °C for gene expression analysis. Another 5 fish per tank were stripped for digesta and stored at −20 °C for analysing SCFA composition.

2.4. Growth performance calculations

Fish growth performance was analysed using the following equations.

$$\text{Weight gain (WG\%)} = ((\text{FW} - \text{IW})/\text{IW}) \times 100$$

$$\text{Specific growth rate (SGR)} = ((\text{Ln (FW)} - \text{Ln (IW)})/\text{D}) \times 100$$

$$\text{Thermal growth coefficient (TGC)} = \left(\left((\text{FW})^{(1/3)} - (\text{IW})^{(1/3)}\right)\Big/(\text{T} \times \text{D})\right) \times 1000$$

$$\text{Condition factor (CF)} = \left(\text{FW}/\text{FL}^3\right) \times 100$$

Where, FW = mean final body weight of fish (g), IW = mean initial body weight of fish (g), T is the water temperature in °C, D is feeding duration in days. IL and FL are the initial and final fork length (cm) of fish, respectively.

2.5. Histomorphometry

Standard histological procedures were adopted, and the analyses were performed at the histology laboratory of the Research station, Nord University, Bodø, Norway. Fixed tissues were dehydrated with increasing concentrations of ethanol, followed by immersion in xylene and paraffin (Sørensen et al., 2011). Next, tissue sections of 4 μm were prepared using microtome and mounted onto a glass slide, after which they were stained with Alcian blue - periodic acid–Schiff (pH 2.5). Stained slides (one section per fish) were covered with a coverslip after adding a drop of glue, Pertex® (Histolab Products AB, Askim, Sweden). Thereafter, microphotographs were captured at 40× magnification by a camera (Leica MC170HD, Heersbrugg, Switzerland) fitted on a light microscope (Leica DM1000, Wetzlar, Germany), and using a software, Leica Application Suite (LAS V4.12.INK, Heerbrugg, Switzerland). All the images were examined with ImageJ 1.52a (Schneider et al., 2012).

2.5.1. Collection of skin samples from the dorsal area

Tissues (approximately 2 cm) were sliced transversely into 3 equal parts after removing most of the muscles that were attached to the skin and decalcified with 10% formic acid (25 blocks per L) for 5 h. The tissues were rinsed with phosphate-buffered saline (PBS) prior to standard histological procedure. Approximately 600–900 μm (length) skin microphotographs (9 per fish) were generated to investigate the skin mucous cells.

2.5.2. Collection of gill samples

To measure the area or count the number of mucous cells in the gills, 10 secondary lamellae from 5 different filaments per fish were chosen. Thus, in this study 50 secondary lamellae per fish were examined to understand the effect of the diets.

2.5.3. Histomorphometric analysis of the dorsal skin and gills

First, 'Freehand selections' tool of ImageJ was employed to demarcate the total area of skin epithelium (SE) and then 'Brightness and Hue' under 'Colour threshold' of the 'Image' menu was adjusted, while keeping 'Thresholding method' as 'Default', 'Threshold colour' set to red and 'Colour space' to HSB (hue, saturation and brightness). Next, using the measure option under the 'Analyze' menu SE was calculated (Gong et al., 2020). Thereafter, the 'Wand tool' was used to select individual mucous cells. Next, the background was cleared using 'Edit' and then the image was converted to 8 bits to retain only the mucous cells. The total area of skin mucous cells (SM) and number of skin mucous cells (SN) were determined by selecting 'Threshold' under 'Image' menu, and by setting 'Analyze particles' to '30 to infinity' under the 'Analyze' menu in ImageJ (Supplementary Fig. 1). SE, SM and SN were used to calculate 2 indices: SME (SM per SE) and SNE (SN per SE). The same image analysis procedure that is described for skin was employed for gills to examine the total area of gill epithelium (GE), the total area of gill mucous cells (GM) and number of gill mucous cells (GN). The obtained values were used to calculate 2 indices: GME (GM per GE) and GNE (GN per GE) (Supplementary Fig. 2).

2.5.4. Collection of intestine samples

The intestine contents were first rinsed off with 10% NBF prior to fixation. After trimming off the excess tissues, the intestine segment was processed and embedded longitudinally. For the histomorphometric analysis, 10 simple, long, well-oriented and intact villi per fish were selected from 3 to 5 different locations. Approximately, 10 microphotographs per fish were generated.

2.5.5. Histomorphometric analysis of distal intestine

The evaluation of the intestine histomorphology included a quantitative and a semi-quantitative assessment. For the quantitative assessment, height (VH) and width (VW) of villi, height of enterocytes (EH), and width of the associated lamina propria (LPW) were measured; these parameters helped us to evaluate the diet-induced alterations in the intestinal microscopic structure. Width of a villus varies along its height, and hence to measure VW, each villus was partitioned into 6 equal parts from the base to tip (Supplementary Fig. 3). From these 5 points, VW, EH and LPW were gauged employing the analysing tools ('straight' and 'segmented lines') of the ImageJ, and the average of the 5 values was registered. The semi-quantitative assessment included the evaluation of the number of intestinal mucous cells (NM), number of intraepithelial lymphocytes (IEL), and presence of supra nuclear vacuoles (SNV) in enterocytes of intestinal villi. A scoring system was developed (Supplementary Table 1) based on previous articles (Baeverfjord and Krogdahl, 1996; Bakke-McKellep et al., 2007; Knudsen et al., 2008; Silva et al., 2015; Urán et al., 2008a). Each index of interest received a score from 1 to 5, and these scores were used for the downstream analyses.

2.6. Gene expression analysis

For the present study, relative mRNA levels of mucin genes (*muc2*, *muc5ac1*, *muc5ac2,* and *muc5b*) in the skin, gills and distal intestine, and AMP genes (*defensin 1 - def1, defensin 2 - def2, defensin 3 - def3, defensin 4 - def4,* and *cathelicidin 1 - cathl1*) in the skin and distal intestine were studied. Primers were purchased from Eurofins Genomics (Luxembourg, Luxembourg) and the sequences and details of all target and reference genes are described in Sørensen et al. (2021). The RNA extraction, cDNA synthesis and qPCR were performed as described by Sørensen et al. (2021).

2.7. Quantification of short chain fatty acids by isotachophoresis

Approximately 1 g of digesta per fish was homogenized with deionized water (50 ml). The solution was filtered through normal filter paper. The filtrates (5 ml per fish) were kept in cryotubes at −20 °C until further analysis. The produced short chain fatty acids (formic, acetoacetic, lactic, succinic, acetic, propionic, valeric and butyric acids) were determined by capillary isotachophoresis (Electrophoretic analyzer EA 202 M, VILLA LABECO spol. s.r.o., Spisska Nova Ves, Slovakia) as described by Gancarcikova et al. (2020).

2.8. Statistical analysis

In the current study, tank was used as the experimental unit for growth performance calculations (Kiron et al., 2016). However, individual fish was considered as the experimental unit for histological evaluation (Bansemer et al., 2015; Cerezuela et al., 2013; Urán et al., 2008b), gene expression and SCFAs composition analyses. All statistical analyses were executed using R (version 3.6.3) and R studio (version 1.2.5033) for windows. Normality of the data was checked with Shapiro–Wilk test and the homogeneity of variance was assessed by Levene's test. In this experiment, feed type (based on ingredients composition) was taken as the first factor (FeedIn: BG1, BG5 and BG2) and probiotic treatment as the second factor (ProbTr: "÷" (without probiotics) and "+" (with probiotics)). The effects of the factors and their interaction (FeedIn×ProbTr) were assessed by analysing the data using two-way analysis of variance (two-way ANOVA). Hereafter, the term 'BG1' shows or represents the average value of both 'BG1÷' and 'BG1+' (marine-based feed with and without probiotics). We have adopted the same strategy for 'BG5' and 'BG2'. For the groups with (+) and without (÷) probiotics, the average values of the different FeedIn are shown in the tables. Parametric two-way ANOVA was employed for data (IW, IL, FW, FL, WG, SGR, TGC, CF, SME, SNE, GME, GNE, VH, VW, EH, LPW, *muc2*, *muc5ac1*, *muc5ac2*, *muc5b*, *def1*, *def3*, *def4*, and *cathl1*) that followed gaussian distribution and had equal variance. When necessary, data were log transformed (gene expression and SCFAs data). Significant differences among the means of the experimental groups were revealed by Tukey's honestly significant difference (HSD) test. Two-way aligned rank transform analysis of variance (ART ANOVA) from ARTool package (version 0.10.7) was used for non-parametric semi-quantitative data (NM, IEL and SNV). Here, post-hoc comparisons, based on estimated marginal means (emmeans), were performed on a linear model for the response aligned and ranked data (Feys, 2016; Wobbrock et al., 2011). Spearman correlations for all the combinations of histologically evaluated mucous cell indices and the selected mucus-related genes were evaluated using the function from the package "psych" in R software. Statistical differences are reported at a significance level of $p < 0.05$. Means ± SEM of parameters are presented in all tables and figures, except Table 4, Figs. 3 and 7.

3. Results

3.1. Growth performance

There were no mortalities during the experiment. The growth performance parameters are presented in Table 3. The fish weight increased from an average range of 116–127 g to a range of 186–200 g during the experiment. There were no significant differences in FW, FL, SGR, TGC and WG of the diet groups. On the other hand, CF was significantly affected by feed type (factor FeedIn, Table 3); fish fed BG2 had lower CF compared to BG1 and BG5. None of the growth performance parameters was affected by feeding the probiotics (factor ProbTr). Furthermore, we did not find any interaction (FeedIn×ProbTr) effect on the parameters.

3.2. Histomorphometry

The results of the two-way ANOVA for the main factors, FeedIn and ProbTr are presented in Table 4 and Figs. 1–3; employing the data on dorsal skin, gill and distal intestine morphometric indices from the histology study.

3.2.1. Mucous cells in the dorsal skin

The results did not reveal any significant differences in SME of the diet groups (Fig. 1A). However, SNE was significantly influenced by both factors, feed type (FeedIn) and probiotics (ProbTr). Fish fed BG2 had significantly more SNE compared to the fish fed BG1 and BG5 (Table 4 and Fig. 1B). Note that the probiotic groups (+) had significantly more SNE compared to groups without probiotics (÷) (Table 4; the main factor effect). Diet groups BG2÷, BG2+ and BG5+ had significantly more SNE compared to the diet group BG1÷ (Fig. 1B). We did not find any significant interaction of the two factors (FeedIn×ProbTr). However, all the probiotic fed groups showed similar increasing tendency for SNE (Table 4 and Fig. 1B).

3.2.2. Mucous cells in the gills

Histological evaluation of mucous cells in the gills revealed significant effects of feed type (factor FeedIn) and probiotics (factor ProbTr) on GME (Fig. 1C and Table 4) and GNE (Fig. 1D and Table 4). Fish fed BG2 had significantly more GME and GNE compared to BG5 followed by those fed BG1. Addition of probiotics to all the feed type significantly increased GME and GNE (Fig. 1C and D). We did not detect a significant

Table 3
Growth performance indicators of Atlantic salmon offered feeds with different combination of marine and plant ingredients, and with or without probiotic supplementation.

Parameters:		IW (g/fish)	IL (cm)	FW (g/fish)	FL (cm)	CF (g/cm^3)	SGR	TGC	WG (%)
Means of main effect:									
FeedIn	BG1	126.91 ± 1.56	21.51 ± 0.10	196.92 ± 5.45	24.67 ± 0.25	1.31 ± 0.01^B	1.15 ± 0.05	2.74 ± 0.14	55.11 ± 2.98
	BG5	124.58 ± 2.22	21.33 ± 0.09	199.90 ± 7.51	24.64 ± 0.19	1.34 ± 0.02^B	1.24 ± 0.09	2.94 ± 0.23	60.47 ± 5.29
	BG2	116.34 ± 2.59	21.16 ± 0.11	186.36 ± 3.37	24.78 ± 0.15	1.22 ± 0.00^A	1.24 ± 0.03	2.88 ± 0.06	60.25 ± 1.71
ProbTr	÷	121.83 ± 2.85	21.30 ± 0.01	195.78 ± 5.01	24.74 ± 0.15	1.29 ± 0.02	1.25 ± 0.05	2.94 ± 0.13	60.82 ± 3.13
	+	123.38 ± 2.35	21.37 ± 0.01	193.01 ± 5.16	24.65 ± 0.17	1.29 ± 0.03	1.18 ± 0.04	2.77 ± 0.11	56.41 ± 2.57
Means of interaction effect:									
BG1	÷	126.99 ± 2.03	21.44 ± 0.14	194.30 ± 11.47	24.58 ± 0.47	1.31 ± 0.01	1.12 ± 0.11	2.64 ± 0.30	52.90 ± 6.58
	+	126.82 ± 3.23	21.57 ± 0.19	199.54 ± 5.74	24.76 ± 0.36	1.32 ± 0.02	1.19 ± 0.01	2.84 ± 0.05	57.33 ± 0.51
BG5	÷	123.43 ± 4.85	21.34 ± 0.22	205.56 ± 7.12	24.85 ± 0.15	1.34 ± 0.02	1.34 ± 0.01	3.20 ± 0.01	66.58 ± 0.78
	+	125.72 ± 1.86	21.31 ± 0.08	194.24 ± 14.96	24.43 ± 0.34	1.33 ± 0.05	1.14 ± 0.16	2.69 ± 0.43	54.40 ± 9.61
BG2	÷	115.08 ± 4.36	21.10 ± 0.16	187.48 ± 5.30	24.80 ± 0.19	1.23 ± 0.01	1.29 ± 0.03	2.98 ± 0.03	62.97 ± 1.57
	+	117.61 ± 4.26	21.22 ± 0.20	185.25 ± 6.15	24.76 ± 0.33	1.22 ± 0.01	1.20 ± 0.01	2.77 ± 0.01	57.53 ± 0.48
p-values	FeedIn (F)	0.286	0.207	0.364	0.900	0.008	0.518	0.648	0.495
	ProbTr (P)	0.977	0.619	0.724	0.726	0.882	0.323	0.371	0.305
	F × P	0.948	0.849	0.681	0.665	0.913	0.298	0.338	0.295

BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed; FeedIn, factor feed ingredients; ProbTr, factor probiotics; ÷, without probiotics; +, with probiotics. F × P, Interaction between feed type and probiotics. IW, initial weight; IL, initial length; FW, final weight; FL, final length; CF, condition factor; SGR, specific growth rate; TGC, thermal growth coefficient; WG%, weight gain in percentage. Values are expressed as means ± SEM of two replicates. The uppercase letters A, B and C (based on post-hoc results) represent significant differences ($p < 0.05$) among feed groups (BG1, BG5 and BG2). Interaction effect was not detected for any of the growth parameters.

Table 4
Atlantic salmon skin, gills and intestinal histomorphometric indices.

Tissues:		Skin		Gills		Intestine						
Parameters:		SME (ratio)	SNE (number /μm)	GME (ratio)	GNE (number /μm)	VH (μm)	VW (μm)	EH (μm)	LPW (μm)	NM (score)	IEL (score)	SNV (score)
Means of main effect:												
FeedIn	BG1	0.1573 ± 0.01	0.0010 ± 0.00^{A}	0.0327 ± 0.00^{A}	0.0005 ± 0.00^{A}	1144.59 ± 46.97^{C}	116.03 ± 3.73^{B}	57.35 ± 1.93^{C}	8.10 ± 0.46^{A}	$3\ (1.8)^{B}$	$5\ (1.0)^{B}$	$5\ (0.0)^{B}$
	BG5	0.1774 ± 0.01	0.0012 ± 0.00^{AB}	0.0481 ± 0.00^{B}	0.0007 ± 0.00^{B}	999.19 ± 31.57^{B}	103.52 ± 2.61^{A}	48.40 ± 1.23^{B}	6.64 ± 0.38^{A}	$4\ (1.3)^{C}$	$4\ (1.0)^{B}$	$5\ (0.0)^{B}$
	BG2	0.1712 ± 0.01	0.0013 ± 0.00^{B}	0.0612 ± 0.00^{C}	0.0010 ± 0.00^{C}	877.21 ± 50.65^{A}	105.03 ± 4.18^{A}	43.87 ± 1.79^{A}	18.31 ± 1.22^{B}	$2\ (1.0)^{A}$	$2\ (2.0)^{A}$	$1\ (1.0)^{A}$
ProbTr	÷	0.1660 ± 0.01	0.0011 ± 0.00^{X}	0.0375 ± 0.00^{X}	0.0006 ± 0.00^{X}	979.98 ± 37.83	107.96 ± 3.32	49.22 ± 1.43	12.38 ± 0.74^{Y}	$4\ (2.3)^{Y}$	4 (3.0)	$5\ (1.8)^{X}$
	+	0.1713 ± 0.01	0.0012 ± 0.00^{Y}	0.0571 ± 0.00^{Y}	0.0009 ± 0.00^{Y}	1034.02 ± 48.30	108.43 ± 3.69	50.52 ± 1.87	9.66 ± 0.64^{X}	$2\ (1.0)^{X}$	3 (1.0)	$5\ (3.0)^{Y}$
p-values	FeedIn (F)	0.149	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	ProbTr (P)	0.534	0.008	<0.001	<0.001	0.249	0.752	0.27	<0.001	<0.001	0.888	0.002
	F × P	0.789	0.452	0.366	0.263	0.083	0.603	0.448	0.003	0.008	<0.001	<0.001

BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed; FeedIn, factor feed ingredients; ProbTr, factor probiotics; ÷, without probiotics; +, with probiotics. SME, total area of skin mucous cells per total area of skin epithelium; SNE, number of skin mucous cells per total area of skin epithelium; GME, total area of gill mucous cells per total area of gill epithelium; GNE, number of gill mucous cells per total area of gill epithelium; VH, height of villi; VW, width of villi; EH, height of enterocyte; LPW, width of lamina propria; NM, number of intestinal mucous cells; IEL, number of intraepithelial lymphocytes; SNV, supra nuclear vacuoles. Significant differences ($p < 0.05$) among feed groups (BG1, BG5 and BG2) are indicated by uppercase superscripts A, B and C (based on the post-hoc tests for the group) and those between probiotic groups (without, ÷ and with, +) are indicated by X and Y in each column (based on the probiotic main effect). For interaction effects (F × P) and post-hoc results for each feed type, please refer to corresponding figures. Values for SME, SNE, GME, GNE, VH, VW, EH and LPW are presented as means ± SEM, n = 12 per treatment group. Parametric data were analysed by two-way ANOVA followed by Tukey's HSD test. The nonparametric score data (NM, IEL and SNV) were analysed with functions from ARTool package (nonparametric two-way ANOVA) followed by post-hoc tests using functions from emmeans package. Median, interquartile range (IQR) is reported for score data (NM, IEL, SNV).

interaction effect between feed ingredients and probiotics for the indices, GME or GNE.

3.2.3. Distal intestine histomorphometry

The morphological indices of the distal intestine, VH, VW, EH, LPW, NM, IEL and SNV, were differently affected by feed type (factor FeedIn) and probiotic treatment (factor ProbTr). In addition, the interaction (FeedIn×ProbTr) effects also depended on the indices. The marine-based feed group (BG1) had higher value for most of the indices except LPW (Table 4). The values for fish fed the plant-based feed (BG5) showed the same trend but ranked in between the values of the other two feed groups. Fish fed the feed with SBM (BG2) had the lowest values for most indices and had all the signs of enteritis (Table 4).

3.2.3.1. Height of villi (VH). The VHs of the feed groups were significantly different (Fig. 2A). Fish fed BG1 had longest villi, followed by fish fed BG5 and shortest villi was observed for fish fed BG2. Addition of probiotics did not alter the VH in fish fed BG1 and BG5, but VH tended to increase in the diet group, BG2+ (Fig. 2A). The interaction between feed ingredients and probiotics was not significant.

3.2.3.2. Width of villi (VW). Average VW for the fish fed BG1 was significantly higher compared to BG5 and BG2 (Table 4). Addition of probiotics did not alter the average VW in any of the feed groups (Fig. 2B). However, the probiotics tended to increase the VW in fish fed diets BG1+ and BG5+. The interaction FeedIn×ProbTr was not significant.

3.2.3.3. Height of enterocyte (EH). Feed type had a significant effect on the EH. Significantly shortest enterocytes were observed in fish fed BG2. The average EH in the fish fed BG1 was significantly 1.18- and 1.31-fold higher than BG5 and BG2, respectively (Fig. 2C). The probiotic treatment or interaction (FeedIn×ProbTr) did not have a significant effect on EH.

3.2.3.4. Width of lamina propria (LPW). The LPW was significantly affected by feed type and supplementation of probiotics. In addition, the two factors were found to interact with each other. Significantly wider lamina propria was observed in fish fed BG2 compared to BG1 and BG5. The LPW was significantly reduced in fish fed BG1+ and BG2+ while no changes were observed for fish fed the BG5+ (Fig. 2D).

3.2.3.5. Number of distal intestinal mucous cells (NM). The score for NM was significantly affected by feed ingredients (factor FeedIn) and probiotics (factor ProbTr) (Table 4 and Fig. 3A). Furthermore, the two factors were found to interact significantly (Table 4 and Fig. 3A). The scores for NM were significantly lower (more mucous cells, Supplementary Table 1) for fish fed BG2 compared to the other two feed groups. The NM per villus of fish fed diet groups BG1+ and BG5+ were significantly increased compared to the respective groups (BG1÷ and BG5÷) without probiotics. However, fish fed the BG2+ showed a decreased NM (higher score; less mucous cells). The percentage of the score for NM is shown in Fig. 3A.

3.2.3.6. Number of intraepithelial lymphocytes (IEL). The IELs were also significantly affected by feed ingredient composition (Table 4 and Fig. 3B). Although the factor ProbTr did not have an effect on IEL, the interaction of the factors was significantly different. The score for IEL was significantly lower (more IEL per simple villi, Supplementary table 1) for fish fed BG2 compared to those fed the other two feeds. The IEL score for fish fed diet groups BG1+ and BG5+ were significantly reduced compared to those without probiotics. However, the BG2+ group had a higher score (less IELs) compared to BG2÷ (Fig. 3B).

3.2.3.7. Supranuclear vacuoles (SNV). Fish fed BG1 and BG5 feeds had

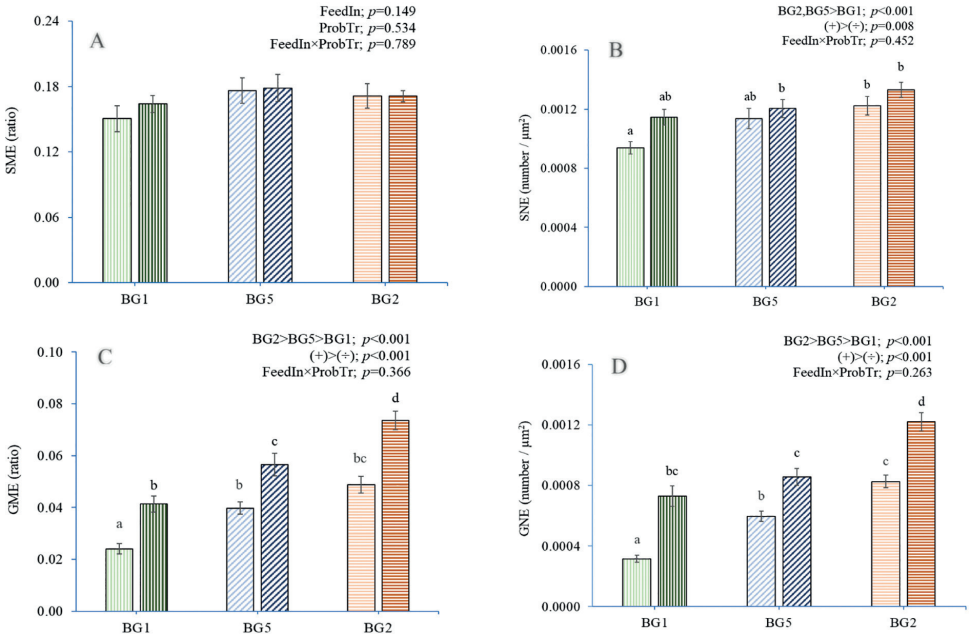

Fig. 1. Mucous cell-based indices associated with the skin and gills of Atlantic salmon offered different experimental feeds. (A) SME - the total area of mucous cells per total area of epithelium in the dorsal skin, (B) SNE - the number of mucous cells per total area of epithelium in the dorsal skin, (C) GME - the total area of mucous cells per total area of epithelium in the gills and (D) GNE - the number of mucous cells per total area of epithelium in the gills. BG1, BG5 and BG2 are marine-, plant- and soybean meal-based feeds, respectively. For each feed, light colour bar (left side) represents diet without probiotics (÷) and dark colour (right side) represents diet with probiotics (+). The effects of main factors (FeedIn and ProbTr) and their interaction (FeedIn×ProbTr) were determined by two-way ANOVA and *p* values are indicated in the upper right corner. Different lowercase letters denote significant difference ($p < 0.05$) among all diet groups; based on post-hoc (Tukey's HSD) tests. Values are presented as mean ± SEM.

larger SNVs along the entire apical part of the enterocytes in the villi. The SNV score (almost 5) of these two groups were not affected by probiotics (Table 4). On the other hand, the BG2÷ fed fish had the lowest score of 1 (almost no SNV in the enterocytes) and when the fish were fed probiotics we observed a significant increase in SNV. Scattered small SNV seemed to reappear in some enterocytes of the fish fed the diet BG2+ and it had received an average score of 1.7 out of 5 (Table 4 and Fig. 3C).

3.3. Gene expression

Relative expression of mucin genes in the skin, gills and intestine were found to be tissue specific (Table 5). The skin expressed *muc5ac1*, *muc5ac2* and *muc5b*. The gills expressed *muc5ac2* and *muc5b*. The distal intestine expressed only *muc2*. Relative expression of AMP genes in the skin and distal intestine of Atlantic salmon were also tissue specific. The skin expressed *def1* and *cathl1*. The distal intestine expressed *def3, def4* and *cathl1*. Results showed that feed ingredients (factor FeedIn), probiotics (factor ProbTr) and their interaction (FeedIn×ProbTr) affected the expression patterns of the genes in the 3 tissues differently. The results of the two-way ANOVA are presented in Table 5, and the effect of the interaction could be deciphered from Figs. 4–6.

3.3.1. Dorsal skin

The transcription of mucin and AMP genes in the dorsal skin were significantly affected either by feed ingredients or probiotics (either with or without an interaction effect), the exception was the expression of *def1* (Fig. 4D). Feed ingredient composition (main effect of the factor FeedIn) significantly altered the transcription of *muc5ac2* (Fig. 4B) and *cathl1* (Fig. 4E), but not those of the other two mucin genes. Fish fed BG1 and BG5 had significantly higher expression of *muc5ac2* and *cathl1*, respectively (Table 5, Fig. 4B and E). Probiotics significantly upregulated the expression of mucin genes in BG5 and BG2; *muc5ac1* (Fig. 4A, factor ProbTr) and *muc5b* (Fig. 4C, factor ProbTr). On the other hand, the expression of these two genes were downregulated in BG1+ fed fish. As for *cathl1*, fish fed diet BG2+ showed upregulation, while other diets tended to downregulate the expression of the AMP gene. The interaction (FeedIn×ProbTr) was significant for *muc5ac1* and *muc5b* as well as for the AMP gene, *cathl1*.

3.3.2. Gills

Expression of the mucin gene, *muc5ac2* in the gills was not significantly altered by either feed ingredient composition or probiotics (Fig. 5A). The mucin gene, *muc5b*, by contrast, was significantly affected by feed composition, and significantly higher expression was observed for the fish fed BG5 compared to BG2 (Table 5, factor FeedIn). When compared to fish fed plant-based feed (BG5), expression of *muc5b* was downregulated by 1.8-fold in fish fed SBM-based feed (BG2). Addition of probiotics to feed did not significantly affect the expression of *muc5b*. However, there was an upregulation (1.3-fold) and downregulation (1.4-fold) tendency in fish fed BG5+ and BG2+, respectively compared to BG5÷ and BG2÷ (Fig. 5B).

3.3.3. Distal intestine

Expression of the mucin gene, *muc2* was affected only by the feed ingredient composition (factor FeedIn). Fish fed BG2 had significantly lower mucin mRNA levels compared to the other two feed groups. Probiotics did not influence the mucin expression in any of the feed

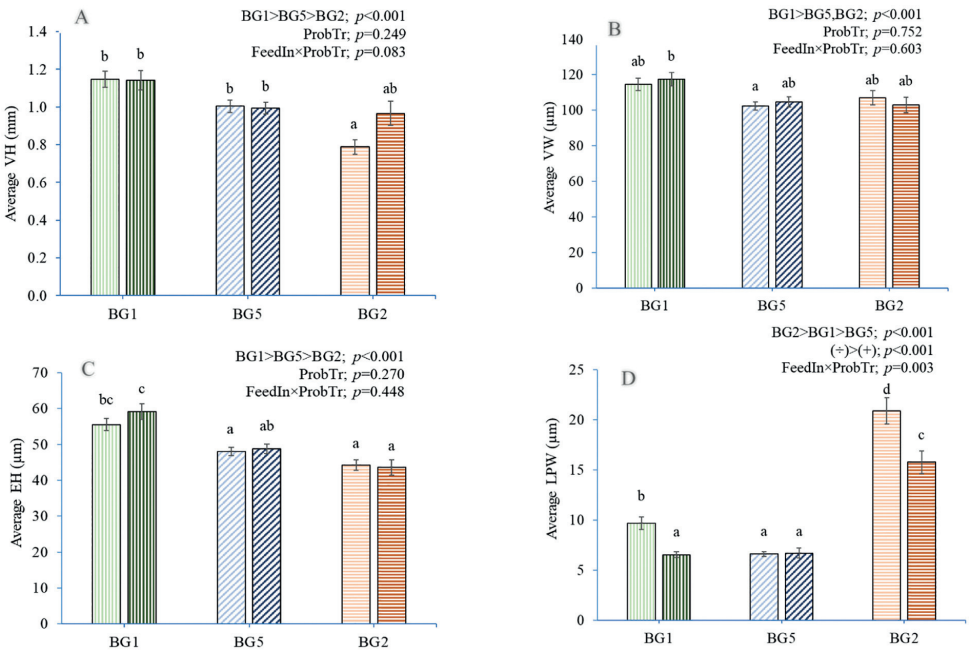

Fig. 2. Mucosa-based indices associated with the distal intestine of Atlantic salmon offered different feeds. (A) VH - height of villi, (B) VW - width of villi, (C) EH - height of enterocytes and (D) LPW - width of lamina propria. BG1, BG5 and BG2 are marine-, plant- and soybean meal-based feeds, respectively. For each feed, light colour bar (left side) represents diet without probiotics (÷) and dark colour (right side) represents diet with probiotics (+). The effects of main factors (FeedIn and ProbTr) and their interaction (FeedIn×ProbTr) were determined by two-way ANOVA and p values are indicated in the upper right corner. Different lowercase letters denote significant difference ($p < 0.05$) among all diet groups; based on post-hoc (Tukey's HSD) tests. Values are presented as mean ± SEM.

groups (Fig. 6A). Feed ingredient composition affected the expression of all AMP genes. Compared to other feed groups, fish fed BG2 had lower mRNA levels of *def3* (Fig. 6B) and *def4* (Fig. 6C, factor FeedIn). We observed an increasing trend in *def4* expression in the BG5+ fed fish (factor ProbTr; $p = 0.052$). However, fish fed BG1 had lower mRNA levels of *cathl1* (Fig. 6D). Supplementation of probiotics to the diet groups significantly influenced the AMP genes, especially *cathl1*. All probiotics-incorporated diet groups had significantly increased the expression of *cathl1* compared to their respective groups without probiotics. A significant interaction ($p = 0.056$) between feed ingredients and probiotics was observed for *def3*. The relative mRNA level of *def3* was upregulated in fish fed BG1+ and BG5+ compared to BG1÷ and BG5÷, while such a change was not observed for BG2. The mRNA level of *def4* was downregulated in fish fed BG1, while the mRNA levels in fish fed BG5 and BG2 were upregulated (not significantly; after probiotic feeding). The interaction between feed ingredients and probiotics (FeedIn×ProbTr) was not statistically significant.

3.4. Correlation between mucous cell indices and mucus-related gene expression data

Analysis of the data using Spearman correlation test revealed significant correlation between most of the histologically analysed mucous cell indices (Fig. 7). Significant positive correlations were observed for the following pairs: between SME and SNE ($r = 0.45$, $p < 0.001$), between GME and GNE ($r = 0.90$, $p < 0.001$). NM was positively correlated with GME ($r = 0.43$, $p < 0.001$) and GNE ($r = 0.50$, $p < 0.001$). Likewise, SNE was positively correlated with GME ($r = 0.43$, $p < 0.001$) and GNE ($r = 0.52$, $p < 0.001$). The correlations or the interactions among mucus-related genes from the skin, gills and intestine are also reported in Fig. 7. Significant correlation was also detected between histologically analysed mucous cells indices and most of the mucus-related gene data. SNE was positively correlated with skin *cathl1* ($r = 0.32$, $p = 0.007$) and negatively with skin *muc5ac2* ($r = -0.30$, $p = 0.015$). NM was positively correlated with intestinal *cathl1* ($r = 0.45$, $p = 0.001$) and negatively with intestinal *muc2* ($r = -0.38$, $p = 0.004$).

3.5. Short chain fatty acid composition

In total 7 short chain fatty acids were detected in the digesta and the sum of these SCFAs varied from 31 to 60 mmol/L, based on values from fish fed the different diets (Table 6). The total SCFAs were significantly affected by feed ingredient composition. Fish fed BG1 had significantly higher total SCFAs, followed by BG5 and BG2. Fish fed feeds without probiotics had significantly more total SCFAs than those with probiotics. The interaction between the two main factors (FeedIn×ProbTr) was not significantly different for the total SCFAs. Most of the individual SCFAs, except acetoacetic acids were significantly affected only by feed ingredients. Feeding with probiotics resulted in a significant reduction in acetoacetic acid and a tendency towards reduction in succinic acid ($p = 0.051$, Table 6) in digesta compared to those of fish fed diets without probiotics. Fish fed BG1 had more lactic acids, while BG5 feed groups had more acetoacetic acids in the digesta. Irrespective of feed groups, the concentration of butyric acid was the lowest among the determined SCFAs.

4. Discussion

Mucosal surfaces of the skin, gills and intestine with their inherent protecting capacity and arsenal of immune molecules are vital for the

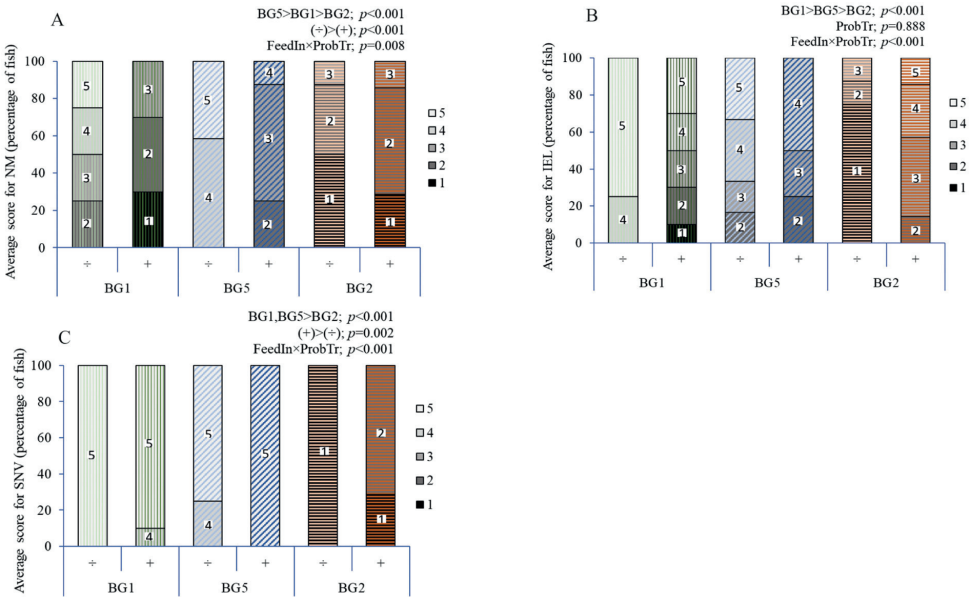

Fig. 3. Scores associated with the cells and cell feature of the distal intestine of Atlantic salmon offered different feeds. (A) NM - number of mucous cells, (B) IEL - number of intraepithelial lymphocytes and (C) SNV - supranuclear vacuoles per villi. BG1, BG5 and BG2 are marine-, plant- and soybean meal-based feeds, respectively. For each feed, light colour bar (left side) represents diet without probiotics (÷) and dark colour (right side) represents diet with probiotics (+). The effects of main factors (FeedIn and ProbTr) and their interaction (FeedIn×ProbTr) were determined by non-parametric two-way ANOVA and *p* values are indicated in the upper right corner. Scores are presented as percentage and legends indicate the scores. The labels on the stacked bar plots are the scores of a particular group.

health of fishes. A damaged mucosal surface in fish fails to effectively carry out its function, thereby making the fish susceptible to infectious diseases. The present study was designed to reveal the efficacy of both plant- or marine-based feeds and probiotics, by assessing the growth performance, morphology of the mucosal surfaces of the skin, gills and distal intestine, mucus-related gene expression in the aforementioned organs and SCFA composition in the digesta of Atlantic salmon. Overall, the present study showed that feed ingredient composition (FeedIn), probiotic treatment (ProbTr) and interaction between feed ingredients and probiotics (FeedIn×ProbTr) significantly affected the parameters of interest.

Use of probiotics isolated either from the GI (Ramesh et al., 2015) or mucus (Tapia-Paniagua et al., 2012) of aquatic animals could be considered as an efficient strategy to ensure sustainable aquaculture. In the present study, a mix of *Lactobacillus plantarum* R2 Biocenol™ (CCM 8674) and *Lactobacillus fermentum* R3 Biocenol™ (CCM 8675) were coated on the feeds. Earlier studies have indicated that a combination of two or more probiotic bacteria, including species from *Lactobacillus,* may improve growth and immune performance of the host aquatic animals (Alishahi et al., 2018; Beck et al., 2015; Foysal et al., 2020; Wang and Gu, 2010; Xu et al., 2012).

The LAB strains used in the present study were isolated from the intestinal content of rainbow trout (*Oncorhynchus mykiss*) and the bacteria were considered as probiotics based on the features, namely tolerance to different pH values, bile, temperature, antagonistic activity against salmonid pathogens such as *Aeromonas salmonicida* subsp. *salmonicida* CCM 1307 and *Yersinia ruckeri* CCM 6093 and the best growth properties *in vitro* (Fečkaninová et al., 2019). These probiotic strains have the potential for use in prevention, intervention or therapy of infections in aquaculture. Our previous study indicated that dietary supplementation with the two LAB strains modulated the composition and interaction of the intestinal microbiota of Atlantic salmon. *L. fermentum* feeding increased the bacterial diversity in the intestinal mucus of the fish (Gupta et al., 2019a). Among the LAB strains isolated from Chinese pickles, *L. fermentum* showed the most effective antibacterial activity against *Staphylococcus aureus* (Song et al., 2021). In a study with common carp, a diet supplemented with *L. fermentum* URLP18 at 2×10^8 CFU/g improved growth performance, non-specific immunity and health status and survival rate during a *Aeromonas hydrophila* challenge (Krishnaveni et al., 2021). Improved disease resistance was also demonstrated in tilapia fed *L. plantarum* prior to infecting the fish with the bacterial fish pathogen *Edwardsiella tarda* (Sherif et al., 2021). In the latter study, there was no difference in mortality between groups fed *L. plantarum* for 2 and 4 weeks prior to the infection with *E. tarda*. *L. plantarum* has also demonstrated a protective role in tilapia exposed to waterborne aluminum (Al) (Yu et al., 2017); the bacteria significantly increased feed utilization and growth performance, decreased the mortality of Al-exposed fish, reduced pathological conditions as well as Al accumulation in tissues. We did not include a challenge experiment as part of this study because our design was intended to investigate if there was any effect of supplementation of the two probiotics *L. fermentum and L. plantarum* in marine- or plant- derived feeds.

4.1. Effect of feed ingredients and probiotics on the growth performance

The 38-day long feeding study did not reveal any significant differences in most of the performance indices of the study groups. The plant-derived ingredients are approximately 3–6 times cheaper than fishmeal (The World Bank, 2021). Hence, our nonsignificant differences in the growth data indicate that cheaper non-marine source derived ingredients can impart the same growth in Atlantic salmon compared to marine-based ingredients. The lower condition factor of BG2 fed fish after 38 days of feeding can be in line with other studies that employed SBM in the diets of Atlantic salmon (Baeverfjord and Krogdahl, 1996;

Table 5
Gene expression in the skin, gills and intestine of Atlantic salmon.

Tissues:		Skin				Gills		Intestine			
Gene type:		AMPs		Mucins		Mucins		AMPs			Mucins
Parameters:		*def1*	*cath1*	*muc5ac1*	*muc5ac2*	*muc5b*	*muc5ac2*	*muc5b*	*def3*	*def4*	*cath1*	*muc2*
Means of main effect:												
FeedIn	BG1	0.63 ± 0.07	0.45 ± 0.06^{A}	0.43 ± 0.05	0.53 ± 0.07^{B}	0.30 ± 0.04	1.08 ± 0.09	0.12 ± 0.02^{AB}	1.05 ± 0.25^{AB}	1.10 ± 0.11^{B}	0.08 ± 0.03^{A}	2.75 ± 0.20^{B}
	BG5	0.79 ± 0.09	0.65 ± 0.06^{B}	0.48 ± 0.06	0.35 ± 0.06^{A}	0.38 ± 0.04	1.02 ± 0.08	0.18 ± 0.03^{B}	1.38 ± 0.34^{B}	0.87 ± 0.14^{A}	0.10 ± 0.04^{AB}	2.53 ± 0.24^{B}
	BG2	0.75 ± 0.06	0.44 ± 0.04^{A}	0.58 ± 0.10	0.35 ± 0.05^{A}	0.32 ± 0.05	0.91 ± 0.09	0.10 ± 0.02^{A}	0.40 ± 0.11^{A}	0.77 ± 0.08^{A}	0.12 ± 0.03^{B}	0.71 ± 0.05^{A}
ProbTr	÷	0.73 ± 0.07	0.50 ± 0.05	0.42 ± 0.06^{X}	0.37 ± 0.05	0.29 ± 0.04^{X}	1.01 ± 0.09	0.13 ± 0.02	0.59 ± 0.12	0.85 ± 0.11	0.04 ± 0.01^{X}	1.99 ± 0.15
	+	0.71 ± 0.07	0.53 ± 0.05	0.58 ± 0.08^{Y}	0.44 ± 0.06	0.37 ± 0.04^{Y}	0.99 ± 0.09	0.13 ± 0.02	1.30 ± 0.34	0.98 ± 0.12	0.16 ± 0.06^{Y}	2.00 ± 0.18
p-values	FeedIn (F)	0.089	<0.001	0.627	0.006	0.169	0.165	0.005	0.001	0.002	0.028	<0.001
	ProbTr (P)	0.803	0.283	0.014	0.159	0.021	0.927	0.458	0.065	0.052	<0.001	0.939
	F × P	0.827	0.005	<0.001	0.636	0.001	0.342	0.353	0.056	0.201	0.311	0.907

BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed; FeedIn, factor feed ingredients; ProbTr, factor probiotics; ÷, without probiotics; +, with probiotics. AMPs, Antimicrobial peptides; *def1, defensin1; def3, defensin3; def4, defensin4; cath1, cathelicidin1*; F × P, Interaction between feed type and probiotics. Significant differences ($p < 0.05$) among feed groups (BG1, BG5 and BG2) are indicated by uppercase superscripts A, B and C (based on post-hoc tests for the group), and between probiotic groups (without, ÷ and with, +) are indicated by the uppercase letters X and Y in each column (based on the probiotic main effect). For interaction effects and post-hoc results for each feed type, please refer to the corresponding figure. Values are presented as means ± SEM, n = 12 per treatment group. Data were analysed by two-way ANOVA followed by Tukey's HSD test.

Knudsen et al., 2007; Krogdahl et al., 2015; Sørensen et al., 2021; Urán et al., 2008b). A previous study showed that fish fed BG2 had morphological changes consistent with soybean meal-induced enteritis (Sørensen et al., 2021). This condition is associated with saponins in full fat soybean meal (Knudsen et al., 2007; Krogdahl et al., 2015). Heat treatment can dampen the action of heat stable antinutritional factors (ANFs) such as saponins, phytate, tannins, oligosaccharides, phytoestrogens (Drew et al., 2007; Liener, 1994). On the other hand, heat labile ANFs, typical proteins such as lectins and protease inhibitors are easily inactivated to safe levels during extrusion (Romarheim et al., 2006). All the experimental feeds were extruded prior to the LAB coating. Hence, we expect that heat labile ANFs in soybean meal were inactivated during extrusion, while the process did not remove the heat stable ANFs in the BG2 diet. Phytate is usually reduced through enzymatic treatment (Storebakken et al., 1998), and was most likely present in BG5. The duration of the experiment was too short to reveal effects of feed composition or probiotics on growth performance, in contrast to other studies that reported the ability of probiotics to improve growth performance, survival rate and health status of fish (Ramos et al., 2017; Wuertz et al., 2021; Xia et al., 2020).

4.2. Effect of feed ingredients and probiotics on histology and gene expression

4.2.1. Dorsal skin mucous cells, mucin and AMP genes

Histological evaluation of salmon skin indicated that the feed ingredients and probiotics evoked changes in the microscopic structure of the epidermis. We observed an increase in the number of skin mucous cells per unit skin epithelium area (SNE) when the fish were fed plant- (BG5) and SBM-based (BG2) feeds. The fish fed marine-based feeds (BG1) had a significantly lower SNE. Based on the findings from the study of the intestine, antinutritional factors present in the plant and SBM-based feeds can cause intestinal inflammation (Krogdahl et al., 2015), which in turn can activate the mucosal immune system of skin, as described in Sørensen et al. (2021). Such a connection between local immune systems has been shown in many cases. In humans, for example, intestinal bowel disease is known to cause disturbances in the host defence system and overstimulate certain immune pathways, and this response can lead to cutaneous disorders such as sub-epidermal blisters (Huang et al., 2012). The increased SNE by probiotics indicate an activation of skin mucosal response as described in Hernandez et al. (2010).

The area of skin mucous cells per unit area of skin epithelium (SME) was not significantly affected by feed ingredients or the probiotic treatment. Nevertheless, we observed a positive correlation between SNE and SME. Marine-based feed groups had less SNE, but apparently larger mucous cells. On the other hand, SBM-based feed groups had higher SNE, so apparently, smaller mucous cells. Mucous cells in the epidermis are essential for the production of mucus (Pittman et al., 2013), and mucus contains mainly mucins, which either bind to outer layer of epidermis and provide additional layer of defence to protect epithelial cells, or create viscous gel that prevents microbial penetration (Dang et al., 2020; Dash et al., 2018). Hyperplasia of skin mucous cells seems to be a general response to unfavourable physiological factors, like stress and low pH (Zuchelkowski et al., 1985, 1981), chemical factors, like high water aluminium levels (Ledy et al., 2003), high water nitrate levels and low dissolved oxygen (Vatsos et al., 2010), or biological factors, like pathogens (van der Marel et al., 2010).

Fishes are constantly in contact with pathogens (opportunistic or obligatory), and when pathogens invade the skin, the mucous cells that are located in the epidermis, will continuously secrete mucus to physically remove the pathogens from the surface (Karlsen et al., 2018; Peatman et al., 2015). In addition, AMPs present in fish mucus kill pathogens by interacting directly and disturbing the osmotic pressure in microbial cells (Mahlapuu et al., 2016; Raju et al., 2020). Administration of probiotics to the plant-based feed tended to downregulate the mRNA levels of AMPs while the SBM-fed group had higher expression of

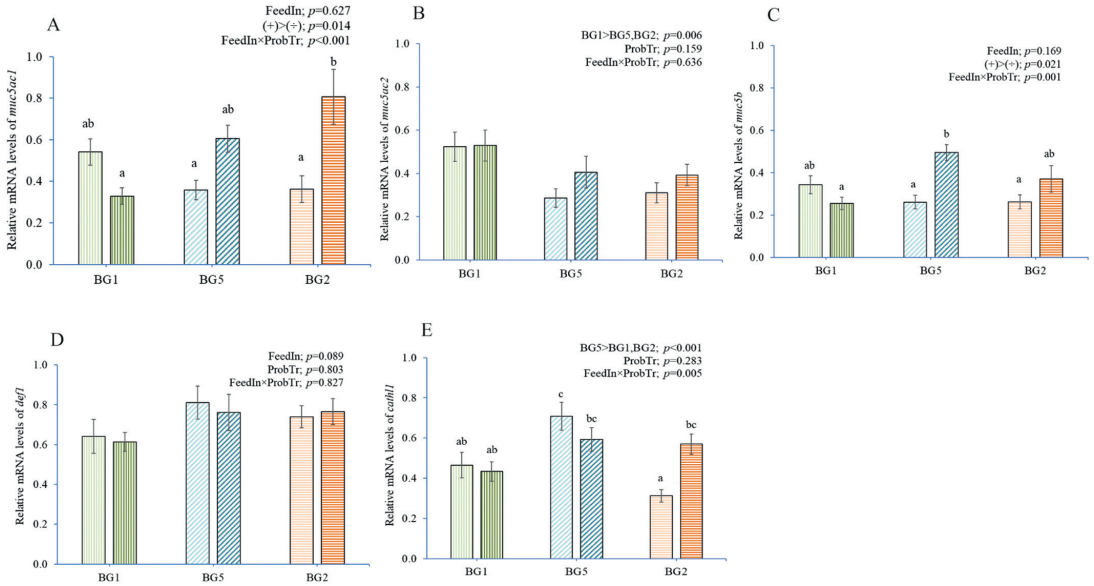

Fig. 4. Relative mRNA levels of mucin and antimicrobial peptide genes in the skin of Atlantic salmon fed different diets. (A) *muc5ac1*, (B) *muc5ac2*, (C) *muc5b*, (D) *defl* (*defensin1*) and (E) *cathl1* (*cathelicidin1*). BG1, BG5 and BG2 are marine-, plant- and soybean meal-based feeds, respectively. For each feed, light colour bar (left side) represents diet without probiotics (÷) and dark colour (right side) represents diet with probiotics (+). The effects of main factors (FeedIn and ProbTr) and their interaction (FeedIn×ProbTr) were determined by two-way ANOVA and *p* values are indicated in the upper right corner. Different lowercase letters denote significant difference ($p < 0.05$) among all diet groups; based on post-hoc (Tukey's HSD) tests. Values are presented as mean ± SEM.

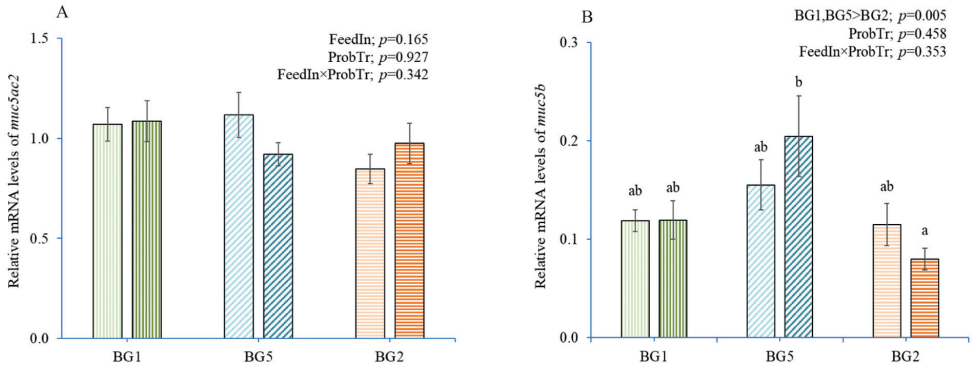

Fig. 5. Relative mRNA levels of mucin genes in the gills of Atlantic salmon fed different diets. (A) *muc5ac2* and (B) *muc5b*. BG1, BG5 and BG2 are marine-, plant- and soybean meal-based feeds, respectively. For each feed, light colour bar (left side) represents diet without probiotics (÷) and dark colour (right side) represents diet with probiotics (+). The effects of main factors (FeedIn and ProbTr) and their interaction (FeedIn×ProbTr) were determined by two-way ANOVA and *p* values are indicated in the upper right corner. Different lowercase letters denote significant difference ($p < 0.05$) among all diet groups; based on post-hoc (Tukey's HSD) tests. Values are presented as mean ± SEM.

AMP genes. Furthermore, the positive correlation between SNE and the skin *cathl1* suggests that probiotics might have influenced both the AMP gene expression and the number of skin mucous cells.

As for the mucin gene, *muc5ac2* it was downregulated in fish fed plant- and SBM-based feeds; this result is not in line with the histological observation of increased number of mucous cells in the skin. Although the probiotic supplementation did not influence the expression of a gel-forming mucin gene, *muc5ac2* in any of the diet groups, the combination of plant-based, or SBM-based feeds and probiotics caused an upregulation of two other gel-forming mucin genes, namely *muc5ac1* and *muc5b*. This observation agrees with the result on increased number of mucous cells observed in skin epidermis.

4.2.2. Gills mucous cells and mucin genes

Pathogens can increase the gill mucous cell number and mucus production (Andrews et al., 2010; Lødemel et al., 2001). Hyperplasia and hypertrophy of gill mucous cells are general responses to external stimuli (Dang et al., 2020, 2019; Haddeland et al., 2020). A correlation

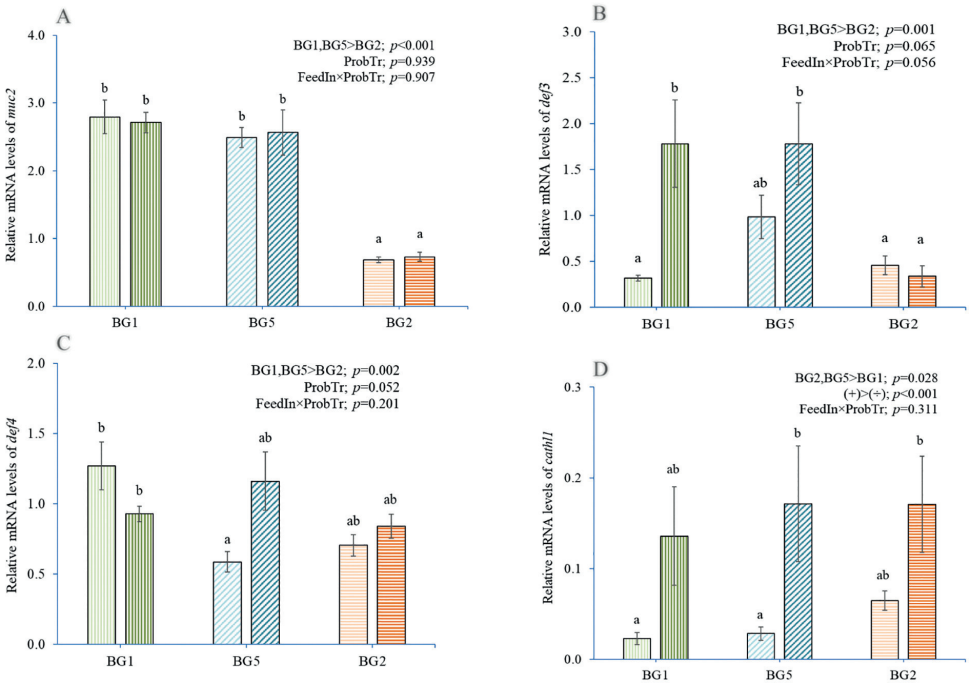

Fig. 6. Relative mRNA levels of mucin and antimicrobial peptides genes in the distal intestine of Atlantic salmon fed different diets. (A) *muc2*, (B) *def3* (*defensin3*), (C) *def4* (*defensin4*) and (D) *cathl1* (*cathelicidin1*). BG1, BG5 and BG2 are marine-, plant- and soybean meal-based feeds, respectively. For each feed, light colour bar (left side) represents diet without probiotics (÷) and dark colour (right side) represents diet with probiotics (+). The effects of main factors (FeedIn and ProbTr) and their interaction (FeedIn×ProbTr) were determined by two-way ANOVA and *p* values are indicated in the upper right corner. Different lowercase letters denote significant difference ($p < 0.05$) among all diet groups; based on post-hoc (Tukey's HSD) tests. Values are presented as mean ± SEM.

between number of mucous cells and the mucus secretion was documented by Bosi et al. (2005).

In the present study, our analyses detected significant effects of both feed ingredients and probiotics on the gill mucous cells. The parameter GME was used to assess the total area of gill mucous cells that cover unit area of gill epithelium. For fish fed marine-based feed without probiotics (BG1÷), it was 0.024, indicating that 100 μm^2 of gill epithelium is covered by 2.4 μm^2 of mucous cells. For the plant-based (BG5÷) and SBM (BG2÷)- based groups, GME values were 0.039 and 0.049, respectively. Probiotic incorporation in all the three feeds - marine-, plant- and SBM-based - significantly increased the GME by 1.7, 1.4 and 1.5 times than their corresponding groups without probiotics (BG1÷, BG5÷ and BG2÷), respectively. Having more mucous cells is linked to better disease resistance in the case of amoebic gill disease (Roberts and Powell, 2005). Therefore, probiotics used in our study have shown their potential to be included among the candidates that can be utilized in aquaculture disease prevention.

Histomorphometric analysis of fish gills showed a similar trend for GNE and GME. Furthermore, there was a positive correlation between GME and GNE, indicating that the GME might have increased due to increased GNE. GNE indicates the number of mucous cells per unit area of gill epithelium. Fish fed feeds without probiotics (÷) had lower value for GNE compared to feed groups with probiotics (+). Fish fed the marine-based feed (BG1÷) had on average 300 mucous cells per mm^2. For fish fed the plant (BG5÷)- and SBM-based feed (BG2÷), the GNE were 2 and 3 times higher compared to marine-based feed (BG1÷) groups, respectively. The feeds BG1+, BG5+ and BG2+ increased the number of gill mucous cells per unit area of gill epithelium (GNE) by 2.3, 1.4 and 1.5 times, respectively, compared to the respective fish groups fed feeds without probiotics. The dietary administration of probiotics might have altered the metabolism in the intestine and the metabolites (bile acids, lipoproteins, amino acids and SCFAs) might have translocated through blood to the gills, thus the increased response (Martin et al., 2007).

The relative mRNA levels of *muc5ac2* in the gills were unaffected by feed ingredients and probiotics. The lower expression of *muc5b* in fish fed BG2 indicates the gill health marker potential of the gene. Dietary administration of probiotics to BG2÷ feed groups further downregulated the expression of the mucin gene *muc5b*. However, an upregulation pattern was observed for fish fed BG5. A study has revealed that the number of goblet cells in the airway epithelium of rats increased and there was a subsequent increase in the expression of the mucin genes *muc5a* and *muc5b* (Kim et al., 2019). The significant positive correlation between gill mucous cell indices (GME and GNE) and other two mucous cell indices in the skin (SNE and SME) indicates the relationship between mucosal tissues in different organs and their response to different feed ingredients and probiotics. However, gill mucin gene expression results did not significantly correlate with histological observations related to gills (Fig. 7).

4.2.3. Distal intestinal morphology, mucin and AMP genes

The height of the simple villi differed among the feed groups and this observation is in line with earlier studies (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2015; Moldal et al., 2014; Sohrabnezhad et al., 2017; Urán et al., 2009). Intact and longer villi are associated with more enterocytes, higher enzyme production, and improved absorption of

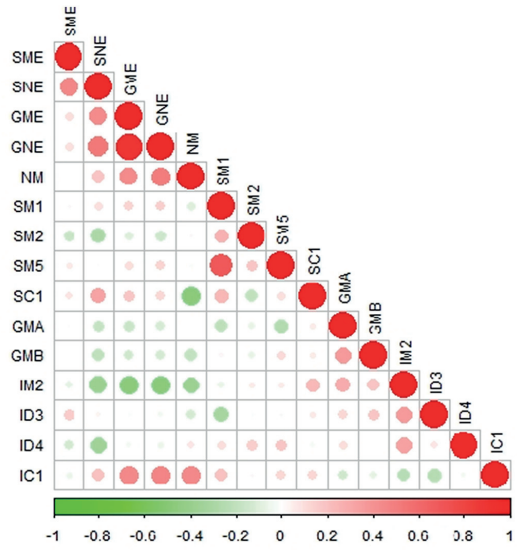

Fig. 7. Plot showing the correlation for all the combinations of histologically evaluated mucous cell parameters and the selected mucus-related genes. Skin *defensin1* is not shown because the correlation is not significant. Significant correlations ($p < 0.05$; Spearman rank correlation test) are shown using circles. Positive correlations are indicated by shades of red, and negative correlations are shown by shades of green. Blank cells indicate non-significant correlations between the variables. SME, the ratio between total area of mucous cells and total area of epithelium in the dorsal skin. SNE, the ratio between number of mucous cells and total area of epithelium in the dorsal skin. GME, the ratio between total area of mucous cells and total area of epithelium in the gills. GNE, the ratio between number of mucous cells and total area of epithelium in the gills. NM, number of intestinal mucous cells. SM1, skin *muc5ac1*. SM2, skin *muc5ac2*. SM5, skin *muc5b*. SC1, skin *cathelicidin1*. GMA, gill *muc5ac2*. GMB, gills *muc5b*. IM2, intestine *muc2*. ID3, intestine *defensin3*. ID4, intestine *defensin4*. IC1, intestine *cathelicidin1*. *(For interpretation of the references to colour in this figure legend, the reader* should*referthe web version of this article.)*

nutrients (Caspary, 1992). Wild caught post-smolt Atlantic salmon weighing 120 g was reported to have villi of height 0.7 mm (Løkka et al., 2013). In the present study, fish fed the marine-based diet had the longest villi (on average 1.1 mm), but SBM-based feed reduced the height to 0.87, due to inflammation. Feeding the fish with probiotics increased the villi height in the SBM fed group. This result is in line with earlier studies that reported improved intestinal structure and immunity in tilapia fed lactic acid bacteria (Pirarat et al., 2011) and increased villi height in rainbow trout fingerlings fed probiotic-supplemented (*Bacillus cereus*) diet (Gisbert et al., 2013). Even in piglets, probiotic feeding increased villi height (Gāliņa et al., 2020). The lactic acid bacteria, *Pediococcus acidilactici* in combination with short chain fructooligosaccharides increased villi height in the anterior intestine of Atlantic salmon reared in sea cages (Abid et al., 2013). The two probiotic strains used in the present experiment might have colonized the intestine, as noted in a previous study (Gupta et al., 2019a) and fermented the oligosaccharides to produce more total SCFAs, thus improving the villi structure. The fish fed marine-based feed had significantly wider villi compared to the other two feed groups, and the administered probiotics did not affect the villi width in Atlantic salmon, as observed in rainbow trout fed probiotic-supplemented (*Bacillus cereus*) feed (Gisbert et al., 2013). Average width of villus was calculated based on measurements taken at 5 different locations of a villus (Supplementary Fig. 3). Although the fish fed SBM-based feed had widened villi, quantitative measurements revealed that the villi width of plant-based feed group was similar to that of SBM-based feed group. Width of the villi was calculated considering both height of enterocytes (two sides) and width of lamina propria. It should be noted that in the fish fed the SBM-based feed, the height of enterocytes decreased while the width of the lamina propria increased. Therefore, we did not observe any significant differences in the overall width in fish fed the plant- and the SBM-based feeds. Nonetheless, studies that assessed SBM-induced enteritis reported widening of villi width (Moldal et al., 2014); based on semi-quantitative scoring. Our findings suggest that width of villi cannot solely be used as an index to quantify the morphological changes in the distal intestine. Other indices like height of the enterocytes and width of the lamina propria should also be included.

The present study has also evaluated the height of the enterocytes (columnar epithelium) in the distal intestine of Atlantic salmon. Marine-based feed in the present study provided essential nutrients including amino acids to the fish so that the columnar epithelium can develop

Table 6
Short-chain fatty acid concentration (mmol/L) in the digesta of the study groups.

Parameters:		Formic acids	Acetoacetic acids	Lactic acids	Succinic acids	Acetic acids	Propionic acids	Butyric acids	Total acids
Means of main effect:									
FeedIn	BG1	4.83 ± 0.27^{C}	10.99 ± 0.59^{A}	16.73 ± 0.65^{C}	9.63 ± 0.52^{B}	12.50 ± 0.72^{B}	3.83 ± 0.42^{B}	2.56 ± 0.60^{B}	59.93 ± 1.55^{C}
	BG5	3.14 ± 0.31^{B}	15.04 ± 0.78^{B}	6.73 ± 0.45^{B}	5.99 ± 0.34^{A}	8.09 ± 0.64^{A}	2.56 ± 0.26^{A}	0.86 ± 0.07^{A}	41.92 ± 1.14^{B}
	BG2	2.42 ± 0.20^{A}	10.04 ± 0.46^{A}	4.59 ± 0.36^{A}	5.52 ± 0.24^{A}	9.40 ± 0.90^{A}	2.32 ± 0.27^{A}	1.12 ± 0.14^{A}	34.56 ± 1.95^{A}
ProbTr	÷	3.58 ± 0.30	12.45 ± 0.79^{Y}	9.09 ± 0.43	7.39 ± 0.38	10.52 ± 0.86	3.06 ± 0.36	1.94 ± 0.40	47.21 ± 1.56^{Y}
	+	3.35 ± 0.22	11.59 ± 0.43^{X}	9.75 ± 0.55	6.71 ± 0.35	9.48 ± 0.65	2.75 ± 0.27	1.08 ± 0.15	43.73 ± 1.53^{X}
Means of interaction effect:									
BG1	÷	5.14 ± 0.43^{d}	10.23 ± 0.62^{b}	16.12 ± 0.68^{d}	10.00 ± 0.40^{b}	12.70 ± 0.87^{b}	4.24 ± 0.52^{b}	3.01 ± 0.89	60.24 ± 1.35^{d}
	+	4.51 ± 0.11^{cd}	11.75 ± 0.55^{b}	17.78 ± 0.63^{d}	9.27 ± 0.64^{b}	12.30 ± 0.57^{b}	3.42 ± 0.32^{ab}	2.10 ± 0.32	59.61 ± 1.75^{d}
BG5	÷	3.53 ± 0.36^{bc}	14.90 ± 1.1^{cd}	6.65 ± 0.36^{bc}	6.19 ± 0.40^{a}	9.03 ± 0.69^{a}	2.59 ± 0.30^{a}	1.72 ± 0.14	44.10 ± 1.41^{c}
	+	2.76 ± 0.27^{ab}	15.18 ± 0.47^{d}	6.82 ± 0.53^{c}	5.80 ± 0.27^{a}	7.15 ± 0.58^{a}	2.54 ± 0.22^{a}	NA	39.74 ± 0.86^{bc}
BG2	÷	2.08 ± 0.12^{a}	12.23 ± 0.66^{bc}	4.51 ± 0.24^{a}	5.99 ± 0.33^{a}	9.81 ± 1.02^{ab}	2.36 ± 0.26^{a}	1.11 ± 0.16	37.29 ± 1.92^{ab}
	+	2.77 ± 0.28^{ab}	7.85 ± 0.26^{a}	4.66 ± 0.48^{ab}	5.06 ± 0.15^{a}	8.99 ± 0.79^{a}	2.29 ± 0.28^{a}	1.14 ± 0.12	31.83 ± 1.98^{a}
p-values	FeedIn(F)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.002
	ProbTr (P)	0.472	0.048	0.240	0.051	0.106	0.276	0.492	0.010
	F × P	0.013	<0.001	0.460	0.214	0.616	0.458	0.383	0.291

BG1, marine-based feed; BG5, plant-based feed; BG2, soybean meal-based feed; FeedIn, factor feed ingredients; ProbTr, factor probiotics; ÷, without probiotics; +, with probiotics. F × P, Interaction between feed type and probiotics. NA, No data available. Valeric acid was tested, but not detected in any of the diet groups. The uppercase letters A, B and C (based on post-hoc tests) represent significant differences ($p < 0.05$) among feed groups (BG1, BG5 and BG2); and the uppercase letters X and Y (based on the probiotic main effect) represent significant differences between the probiotic groups (without, ÷ and with, +). Significant differences ($p < 0.05$) among all groups are indicated by different superscripts (a, b, c, or d; post hoc results for each feed type) in each column. Values are mean ± SEM, n = 10 per treatment group. Data were analysed by two-way ANOVA followed by Tukey's HSD test.

properly without any height-associated defects. When compared to marine-based feed, on average, a nine and 13.5 μm reduction in the height of intestinal epithelium was observed in the fish fed plant- and SBM-based feed, respectively. Indeed, enterocytes of the distal intestine of Atlantic salmon are the first cells that are affected when the fish are fed SBM-based diets (Urán et al., 2008a, 2008c). The present study quantitatively confirmed that the intestinal epithelium height was reduced in fish fed plant- and SBM-based ingredients.

Previous studies have assessed the width of the lamina propria by semi-quantitative scoring (Knudsen et al., 2007). The present study has used a quantitative approach and found a widening of lamina propria in fish fed SBM-incorporated feed. SBM-induced enteritis causes widening of the central stroma of the mucosal folds (Baeverfjord and Krogdahl, 1996). The present study showed that administration of probiotics significantly reduced the width of the lamina propria in fish fed BG1+ and BG2+. Probiotics also reduced the number of intraepithelial lymphocytes in the group fed BG2+ that had intestinal inflammation. In contrast, feeding *P. acidilactici* was found to increase the number of intraepithelial lymphocytes in Atlantic salmon (Vasanth et al., 2015). The present study also suggests that probiotics alleviate the progression of inflammation caused by SBM. A possible mechanism could be that probiotics reduce the lamina propria width possibly by suppressing the influx of the inflammatory cells. Other studies with mammals have also shown improved intestinal tight-junction and barrier function via modulation of protein components (Sultana et al., 2013; Yang et al., 2016). However, further research is needed to assess the inflammatory response markers.

In the present study, acid and neutral goblet cells were found scattered among the intestinal epithelial cells of fish fed BG1 and BG5. Administration of probiotics to these groups further increased the number of mucous cells. This is in line with other studies in fish that reported increased proliferation and differentiation of goblet cells and a consequent increase in mucus secretion in seabream fed *L. fermentum* (Dawood et al., 2015). Furthermore, *L. rhamnosus* or *P. acidilactici* feeding was found to increase the number of mucous cells in tilapia intestine (Pirarat et al., 2011; Standen et al., 2013). Moreover, higher goblet cell density was reported in rainbow trout fed *Spirulina platensis* (Sheikhzadeh et al., 2019). Dietary and oral administration of probiotic was also found to increase the number of goblet cells in the intestine of mice (El Aidy et al., 2013), piglets (Gāliņa et al., 2020; Zhang et al., 2017) and pigs (Desantis et al., 2019).

A possible mode of action of probiotics is that they colonize the mucus and make use of the mucin molecules as carbon, nitrogen and energy sources (Meslin et al., 1999). They release end-products of mucus fermentation, different secretory metabolites, and bioactive factors, which activate diverse signalling cascades and secretory elements that affect goblet cells. Members of microbiota can release proteolytic enzymes like meprin β from the apical membrane of enterocyte. Meprin β helps in the detachment of mucus from goblet cells and the metalloprotease cleaves the N-terminal region of the MUC2 mucin (Derrien et al., 2010; Schütte et al., 2014). Moreover, probiotic structural elements, such as lipopolysaccharides, flagellin A, and lipoteichoic acids or several metabolites (adenosine triphosphate) can regulate mucin gene expression by affecting the host immune responses (Dharmani et al., 2009).

The increased number and aggregated mucous cells in fish fed SBM-based feed (BG2) may be a general response to inflammation. Interestingly and in contrast to the observations in the skin and gill histomorphometry, the group fed the SBM-based diet supplemented with the probiotics (BG2+), did not show a further increase in the number of mucous cells, compared to BG2÷ but a decrease. A possible explanation for this observation, could be that feeding fish with SBM and probiotics, both factors that tend to increase the production of mucus, for a prolonged period of time, could have led to a depletion of the mucous cells. This has been observed in many cases of chronic intestinal inflammation, wherein the initial increased mucus production was markedly decreased after a while (Dharmani et al., 2009; Kim and Ho, 2010). However, both BG5+ and BG2+ had almost similar scores linked to mucous cells. This indicates a potential interaction of the probiotics and the different feed ingredients on the number of mucous cells.

In the present study, the *muc2* expression in the distal intestine was not altered by the administration of probiotics. The upregulation of the AMP genes *def3*, *def4* and *cathl1* with the administration of probiotics suggests increased immune responses (Rakers et al., 2013). Moreover, significant positive correlation between *cathl1* and NM indicates that feed ingredients or probiotics influenced the AMP gene, *cathl1* and increased the mucous cells number in the intestine of Atlantic salmon. Intestinal mucin gene *muc2* was positively correlated with skin *cathl1* ($r = 0.24$, $p = 0.051$) and gill *muc5ac2* ($r = 0.28$, $p = 0.018$), indicating the association of the mucosal areas in different mucosal tissues (Fig. 7). In colonic murine mucosa, *cathelicidin* gene was upregulated by bacterial DNA through Toll-like receptor-mediated pathway (Koon et al., 2011). Intestinal inflammation breaks the mucosal barrier, which in turn gives way for opportunistic bacteria to translocate into the intestinal layers (Vrakas et al., 2017). *Cathelicidin*s were upregulated in the inflamed intestine of Atlantic salmon fed soy saponin (Kiron et al., 2020). It is also stated that gastrointestinal tract disorders can be treated through supplementation of *cathelicidin* peptides (Chow et al., 2013). Hence, probiotic-induced *cathl1* can be considered as a strategy to counteract intestinal inflammation.

Supranuclear vacuoles in the distal intestinal enterocytes of Atlantic salmon appear approximately 54 days post hatch (Sahlmann et al., 2015). Macromolecules like proteins are taken up via pinocytosis in epithelial cells, and some intracellular proteins like ferritin ends up in supranuclear vacuoles (Elbal et al., 2004; He et al., 2012; Rombout et al., 1985). Endocytic vesicles and lysosomes fuse, and subsequently ferritin digestion occurs in the SNVs. Accumulation of SNV in distal intestinal enterocytes of Atlantic salmon has also been reported previously (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2003; Sanden et al., 2005; Urán et al., 2008a). The marine-based feed group in the present study had large SNVs along the entire apical part of the distal intestinal enterocytes. In the present study, fish fed the plant-based feed also had similar characteristics. However, the fish fed SBM-based feed developed enteritis and lacked SNVs. Such anomalies have also been reported by other authors (Bakke-McKellep et al., 2000; Krogdahl et al., 2003; Krogdahl and Bakke-McKellep, 2005; Nordrum et al., 2000; Urán et al., 2009). Disappearance of SNVs is associated with reduced endocytosis or uptake block (Urán et al., 2008c), corroborating with the results on the absence of small SNVs. Probiotic feeding in the present study helped in the reappearance of SNVs in the enterocytes of the fish. Thus, we suggest new modes of action of probiotics on the host health; enhancing endocytosis and aiding in subsequent reappearance of the SNVs.

4.3. Effect of feed ingredients and probiotics on short chain fatty acids

Intestinal microbiota utilizes dietary ingredients especially certain fibres, and by fermenting them they produce SCFAs which are absorbed by the intestinal epithelium of fish. Of these SCFAs, butyrate is utilized by the intestinal epithelial cells as an energy source, propionate is taken up by liver and high levels of acetate can be detected in blood (Louis and Flint, 2017). The SCFAs improve growth and health of the fish because they reduce the luminal pH, avoid infections, strengthen immune system and maintain mucosal integrity (Adorian et al., 2020; Guillon and Champ, 2000; Hoseinifar et al., 2017; Park and Floch, 2007). Supplementation of dietary fibres, which can be utilized by microbiota to produce SCFAs (e.g. acetic and butyric acid) in the digesta, was shown to influence the intestinal mucous cells as well as skin mucus production in fish (Adorian et al., 2020).

In the present study, plant- and SBM-based feeds were expected to provide more fibres than marine-based feed but did not result in more SCFAs. It has been reported that there is no linear correlation between dietary fibre and SCFA concentration in rat cecum (Den Besten et al.,

2013; Levrat et al., 1991). Moreover, administration of probiotics was also expected to influence the microbiota because one of the probiotics (*Lactobacillus*) was found to be a core member in Atlantic salmon (Gupta et al., 2019a). Thus, feed ingredient composition and probiotics were expected to alter the SCFA profile. The level of SCFAs in salmon faeces was in the same range as reported for rats (Campbell et al., 1997). In the rat study, the SCFAs ranged from 36 to 61 mmol/L depending on the intake of fibre, but the ratio between the acetate:propionate:butyrate remained the same. We also observed a significant reduction in total SCFAs in the plant- (BG5) and SBM (BG2)-based feed consumed fish compared to the marine-based feed group. The ratio between acetic: propionic acids was in the range 3 to 4.16; lower in the BG1 fed fish and higher for the BG2 fed group. The ratio between propionic:butyric acids was between 1.41 and 2.1. Marine-based and plant or SBM-based diet can shape the SCFA profile differently; we observed a shift in dominant SCFA (from lactic acid to acetoacetic acid). The plant or SBM-based feeds, BG5 and BG2 contained more long fibres rather than oligosaccharides and type of fibre is known to affect the formation and profile of SCFAs. Fish fed plant or SBM-based ingredients had lower faecal dry matter content (Sørensen et al., 2021), which could explain the lower concentration of SCFAs.

In humans, acetate, propionate and butyrate account for 85–95% of the SCFAs and acetic acid alone accounts for more than 50% (Markowiak-Kopeć and Śliżewska, 2020). Studies of human microbiota have revealed the relationship between SCFAs and microbiota, and intestinal microbiome balance maintenance and microbial metabolite production stimulation by probiotic microorganisms (Markowiak-Kopeć and Śliżewska, 2020; Tsukuda et al., 2021). Acetic acid was a dominant SCFA in the present experiment also, but lactic acid and acetoacetic acid were higher in fish fed BG1 and BG5, respectively. Research with rats has also shown that SCFAs are involved in MUC gene transcription and thickness of mucous layer; feeds that provide more SCFAs, but low proportion of butyrate, help in forming thicker mucous layer in the colon (Hedemann et al., 2009). In line with this, our experiment showed the best gut health in fish fed BG1, producing the highest concentration of total SCFAs, with a rather high concentration of acetic acid. The SCFAs provide energy to the intestinal epithelium cells and stimulates the release of gastrointestinal peptide or growth factors which may affect cell proliferation, thereby increasing villi height (Blottiere et al., 2003; Pelicano et al., 2005). Although we observed only lower concentration of butyric acid in the digesta, the SCFA may still have an important role for intestinal health in Atlantic salmon. In the present study, administration of probiotics had significantly reduced the total SCFAs as well as the acetoacetic acid concentration in digesta. Campbell et al. (1997) observed the lowest concentration of SCFAs in the intestine of rats fed cellulose instead of short chain fibres. A noteworthy observation in the present study was that the probiotic administration tended to reduce the total SCFAs ($p = 0.010$), mainly because of a significant reduction in acetoacetic acids in fish fed the BG2 diet. However, further research should confirm how probiotics could shift the microbiota profile in the host intestine.

5. Conclusion

The present study has shown that number of mucous cells in the dorsal skin, gills and distal intestine were affected by feed ingredient composition and probiotics. Appearance of many mucous cells can be interpreted as an overall immune response to intestinal inflammation. The distal intestinal histomorphology of fish was influenced by the feed ingredient composition. Intestinal indices of fish fed plant-based feed was almost similar to that of fish fed marine-based feed. However, fish fed SBM-based feed developed enteritis. Addition of probiotics to SBM-based feed groups did not completely prevent the development of enteritis. However, positive responses like increased villi height, reduced width of lamina propria, reduced number of intraepithelial lymphocytes and reappearance of supra nuclear vacuoles were observed in Atlantic salmon post-smolts. Expression of mucin and AMP genes were tissue specific and the mRNA levels were affected by feed ingredient composition and probiotics. Correlation between mucous cell histomorphometric indices and gene expression data suggests that feed ingredients or probiotics influence both the mucus cell counts and mucus-related gene expression. Moreover, short chain fatty acid composition was also altered. In order to boost innate immune response and enhance intestinal health, the probiotics employed in the present study can be incorporated in marine- and plant-based feed without compromising fish growth. Although probiotics tended to alleviate the feed induced inflammation, further knowledge should be acquired if these probiotics are to be used as supplements in SBM-based salmon feed.

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.

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Appendix A. Supplementary data

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References

Aas, T.S., Ytrestøyl, T., Åsgård, T., 2019. Utilization of feed resources in the production of Atlantic salmon (*Salmo salar*) in Norway: an update for 2016. Aquac. Rep. 15, 100216. https://doi.org/10.1016/j.aqrep.2019.100216.

Abid, A., Davies, S.J., Waines, P., Emery, M., Castex, M., Gioacchini, G., Merrifield, D.L., 2013. Dietary synbiotic application modulates Atlantic salmon (*Salmo salar*) intestinal microbial communities and intestinal immunity. Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2013.09.039.

Adorian, T.J., Mombach, P.I., Fagundes, M.B., Wagner, R., Pianesso, D., Telles, Y.B., da Silva, L.P., 2020. Linseed fibers modulate the production of short-chain fatty acids and improve performance and plasma and skin mucus parameters of silver catfish (*Rhamdia quelen*). Fish Physiol. Biochem. https://doi.org/10.1007/s10695-020-00885-7.

Alishahi, M., Tulaby Dezfuly, Z., Mohammadian, T., Mesbah, M., 2018. Effects of two probiotics, *Lactobacillus plantarum* and *Lactobacillus bulgaricus* on growth performance and intestinal lactic acid bacteria of *Cyprinus carpio*. Iran. J. Vet. Med. 12, 207–218. https://doi.org/10.22059/ijvm.2018.235444.1004816.

Alonso, S., Carmen Castro, M., Berdasco, M., de la Banda, I.G., Moreno-Ventas, X., de Rojas, A.H., 2019. Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fishes for potential application as probiotics in aquaculture. Probiotics Antimicrob. Proteins 11. https://doi.org/10.1007/s12602-018-9439-2.

Andani, H.R.R., Tukmechi, A., Meshkini, S., Sheikhzadeh, N., 2012. Antagonistic activity of two potential probiotic bacteria from fish intestines and investigation of their effects on growth performance and immune response in rainbow trout (*Oncorhynchus mykiss*). J. Appl. Ichthyol. 28, 728–734. https://doi.org/10.1111/j.1439-0426.2012.01974.x.

Andrews, M., Battaglene, S., Cobcroft, J., Adams, M., Noga, E., Nowak, B., 2010. Host response to the chondracanthid copepod *Chondracanthus goldsmidi*, a gill parasite of the striped trumpeter, *Latris lineata* (Forster), in Tasmania. J. Fish Dis. https://doi.org/10.1111/j.1365-2761.2009.01107.x.

Aranishi, F., Mano, N., 2000. Antibacterial cathepsins in different types of ambicoloured Japanese flounder skin. Fish Shellfish Immunol. 10, 87–89. https://doi.org/10.1006/fsim.1999.0225.

Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison

with the intestines of fasted fish. J. Fish Dis. 19, 375–387. https://doi.org/10.1111/j.1365-2761.1996.tb00376.x.
Bakke-McKellep, A.M., Nordrum, S., Krogdahl, Å., Buddington, R.K., 2000. Absorption of glucose, amino acids, and dipeptides by the intestines of Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. https://doi.org/10.1023/A:1007872929847.
Bakke-McKellep, A.M., Penn, M.H., Salas, P.M., Refstie, S., Sperstad, S., Landsverk, T., Krogdahl, Å., 2007. Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). Br. J. Nutr. 97, 699–713. https://doi.org/10.1017/S0007114507381397.
Bang-Jensen, B., Gu, J., Sindre, H., 2019. The health situation in norwegian aquaculture. Veterinaerinstituttet 37–41.
Bansemer, M.S., Forder, R.E.A., Howarth, G.S., Suitor, G.M., Bowyer, J., Stone, D.A.J., 2015. The effect of dietary soybean meal and soy protein concentrate on the intestinal mucus layer and development of subacute enteritis in Yellowtail Kingfish (*Seriola lalandi*) at suboptimal water temperature. Aquac. Nutr. 21, 300–310. https://doi.org/10.1111/anu.12160.
Beck, B.R., Kim, D., Jeon, J., Lee, S.M., Kim, H.K., Kim, O.J., Song, S.K., 2015. The effects of combined dietary probiotics *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 on innate immunity and disease resistance in olive flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol. 42, 177–183. https://doi.org/10.1016/j.fsi.2014.10.035.
Blottiere, H.M., Buecher, B., Galmiche, J.-P., Cherbut, C., 2003. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. Proc. Nutr. Soc. https://doi.org/10.1079/pns2002215.
Booman, M., Forster, I., Vederas, J.C., Groman, D.B., Jones, S.R.M., 2018. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*). Aquaculture 483, 238–243. https://doi.org/10.1016/j.aquaculture.2017.10.025.
Bosi, G., Arrighi, S., Di Giancamillo, A., Domeneghini, C., 2005. Histochemistry of glycoconjugates in mucous cells of *Salmo trutta* uninfected and naturally parasitized with intestinal helminths. Dis. Aquat. Org. https://doi.org/10.3354/dao064045.
Bridle, A., Nosworthy, E., Polinski, M., Nowak, B., 2011. Evidence of an antimicrobial-immunomodulatory role of Atlantic salmon cathelicidins during infection with *Yersinia ruckeri*. PLoS One 6, e23417. https://doi.org/10.1371/journal.pone.0023417.
Broekman, D.C., Guðmundsson, G.H., Maier, V.H., 2013. Differential regulation of cathelicidin in salmon and cod. Fish Shellfish Immunol. 35, 532–538. https://doi.org/10.1016/j.fsi.2013.05.005.
Brunner, S.R., Varga, J.F.A., Dixon, B., 2020. Antimicrobial peptides of salmonid fish: from form to function. Biology (Basel) 9, 1–17. https://doi.org/10.3390/biology9080233.
Buttle, L.G., Burrells, A.C., Good, J.E., Williams, P.D., Southgate, P.J., Burrells, C., 2001. The binding of soybean agglutinin (SBA) to the intestinal epithelium of Atlantic salmon, *Salmo salar* and Rainbow trout, *Oncorhynchus mykiss*, fed high levels of soybean meal. Vet. Immunol. Immunopathol. https://doi.org/10.1016/S0165-2427(01)00269-0.
Cain, K., Swan, C., 2010. Barrier function and immunology. In: The Multifunctional Gut of Fish, pp. 111–134. https://doi.org/10.1016/S1546-5098(10)03003-7.
Campbell, J.M., Fahey, G.C., Wolf, B.W., 1997. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J. Nutr. 127, 130–136. https://doi.org/10.1093/jn/127.1.130.
Caspary, W.F., 1992. Physiology and pathophysiology of intestinal absorption. In: American Journal of Clinical Nutrition. https://doi.org/10.1093/ajcn/55.1.299s.
Cerezuela, R., Fumanal, M., Tapia-Paniagua, S.T., Meseguer, J., Moriñigo, M. ángel, Esteban, M. ángeles, 2013. Changes in intestinal morphology and microbiota caused by dietary administration of inulin and *Bacillus subtilis* in gilthead sea bream (*Sparus aurata* L.) specimens. Fish Shellfish Immunol. 34, 1063–1070. https://doi.org/10.1016/j.fsi.2013.01.015.
Chang, C.-I., Zhang, Y.-A., Zou, J., Nie, P., Secombes, C.J., 2006. Two cathelicidin genes are present in both rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Antimicrob. Agents Chemother. 50, 185–195. https://doi.org/10.1128/AAC.50.1.185-195.2006.
Chow, J.Y.C., Li, Z.J., Kei, W.K., Cho, C.H., 2013. Cathelicidin a potential therapeutic peptide for gastrointestinal inflammation and cancer. World J. Gastroenterol. 19, 2731–2735. https://doi.org/10.3748/wjg.v19.i18.2731.
Concha, M.I., Molina, S., Oyarzún, C., Villanueva, J., Amthauer, R., 2003. Local expression of apolipoprotein A-I gene and a possible role for HDL in primary defence in the carp skin. Fish Shellfish Immunol. 14, 259–273. https://doi.org/10.1006/fsim.2002.0435.
Dang, M., Pittman, K., Bach, L., Sonne, C., Hansson, S.V., Søndergaard, J., Nowak, B., 2019. Mucous cell responses to contaminants and parasites in shorthorn sculpins (*Myoxocephalus scorpius*) from a former lead-zinc mine in West Greenland. Sci. Total Environ. 678, 207–216. https://doi.org/10.1016/j.scitotenv.2019.04.412.
Dang, M., Pittman, K., Sonne, C., Hansson, S., Bach, L., Søndergaard, J., Nowak, B., 2020. Histological mucous cell quantification and mucosal mapping reveal different aspects of mucous cell responses in gills and skin of shorthorn sculpins (*Myoxocephalus scorpius*). Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2020.03.020.
Dash, S., Das, S.K., Samal, J., Thatoi, H.N., 2018. Epidermal mucus, a major determinant in fish health: a review. Iran. J. Vet. Res. 19, 72–81.
Dawood, M.A.O., Koshio, S., Ishikawa, M., Yokoyama, S., 2015. Effects of heat killed *Lactobacillus plantarum* (LP20) supplemental diets on growth performance, stress resistance and immune response of red sea bream, *Pagrus major*. Aquaculture. https://doi.org/10.1016/j.aquaculture.2015.02.005.
Den Besten, G., Van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.J., Bakker, B.M., 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J. Lipid Res. 54, 2325–2340. https://doi.org/10.1194/jlr.R036012.
Derrien, M., van Passel, M.W.J., van de Bovenkamp, J.H.B., Schipper, R.G., de Vos, W.M., Dekker, J., 2010. Mucin-bacterial interactions in the human oral cavity and digestive tract. Gut Microbes 1, 254–268. https://doi.org/10.4161/gmic.1.4.12778.
Desantis, S., Mastrodonato, M., Accogli, G., Rossi, G., Crovace, A.M., 2019. Effects of a probiotic on the morphology and mucin composition of pig intestine. Histol. Histopathol. https://doi.org/10.14670/HH-18-106.
Dharmani, P., Srivastava, V., Kissoon-Singh, V., Chadee, K., 2009. Role of intestinal mucins in innate host defense mechanisms against pathogens. J. Innate Immun. https://doi.org/10.1159/000163037.
Drew, M.D., Borgeson, T.L., Thiessen, D.L., 2007. A review of processing of feed ingredients to enhance diet digestibility in finfish. Anim. Feed Sci. Technol. https://doi.org/10.1016/j.anifeedsci.2007.06.019.
Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Stanton, C., 2020. Replacing fishmeal with plant protein in Atlantic salmon (*Salmo salar*) diets by supplementation with fish protein hydrolysate. Sci. Rep. 10, 1–16. https://doi.org/10.1038/s41598-020-60325-7.
El Aidy, S., Merrifield, C.A., Derrien, M., Van Baarlen, P., Hooiveld, G., Levenez, F., Kleerebezem, M., 2013. The gut microbiota elicits a profound metabolic reorientation in the mouse jejunal mucosa during conventionalisation. Gut. https://doi.org/10.1136/gutjnl-2011-301955.
Elbal, M.T., García Hernández, M.P., Lozano, M.T., Agulleiro, B., 2004. Development of the digestive tract of gilthead sea bream (*Sparus aurata* L.). Light and electron microscopic studies. Aquaculture. https://doi.org/10.1016/j.aquaculture.2003.11.028.
Farup, P.G., Rudi, K., Hestad, K., 2016. Faecal short-chain fatty acids – a diagnostic biomarker for irritable bowel syndrome? BMC Gastroenterol. 16, 1–7. https://doi.org/10.1186/s12876-016-0446-z.
Fečkaninová, A., Koščová, J., Mudroňová, D., Schusterová, P., Cingeľová Maruščáková, I., Popelka, P., 2019. Characterization of two novel lactic acid bacteria isolated from the intestine of rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Slovakia. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.03.026.
Feys, J., 2016. Nonparametric tests for the interaction in two-way factorial designs using R. R J 8, 367–378. https://doi.org/10.32614/rj-2016-027.
Firth, K.J., Johnson, S.C., Ross, N.W., 2000. Characterization of proteases in the skin mucus of Atlantic salmon (*Salmo salar*) infected with the salmon louse (*Lepeophtheirus salmonis*) and in whole-body louse homogenate. J. Parasitol. https://doi.org/10.1645/0022-3395(2000)086[1199:copits]2.0.co;2.
Foysal, M.J., Fotedar, R., Siddik, M.A.B., Tay, A., 2020. *Lactobacillus acidophilus* and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (*Cherax cainii*). Sci. Rep. 10 https://doi.org/10.1038/s41598-020-62655-y.
Gajardo, K., Jaramillo-Torres, A., Kortner, T.M., Merrifield, D.L., Tinsley, J., Bakke, A.M., Krogdahl, Å., 2017. Alternative protein sources in the diet modulate microbiota and functionality in the distal intestine of Atlantic salmon (*Salmo salar*). Appl. Environ. Microbiol. 83, 1–16. https://doi.org/10.1128/AEM.02615-16.
Galiņa, D., Ansonska, L., Valdovska, A., 2020. Effect of probiotics and herbal products on intestinal histomorphological and immunological development in piglets. Vet. Med. Int. https://doi.org/10.1155/2020/3461768.
Gancarcikova, S., Lauko, S., Hrckova, G., Andrejcakova, Z., Hajduckova, V., Madar, M., Bomba, A., 2020. Innovative animal model of DSS-induced ulcerative colitis in pseudo germ-free mice. Cells. https://doi.org/10.3390/cells9122571.
Gatesoupe, F.J., 2007. Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. J. Mol. Microbiol. Biotechnol. 14 https://doi.org/10.1159/000106089.
Gisbert, E., Castillo, M., Skalli, A., Andree, K.B., Badiola, I., 2013. *Bacillus cereus* var. *toyoi* promotes growth, affects the histological organization and microbiota of the intestinal mucosa in rainbow trout fingerlings1. J. Anim. Sci. 91, 2766–2774. https://doi.org/10.2527/jas.2012-5414.
Gong, Y., Sørensen, S.L., Dahle, D., Nadanasabesan, N., Dias, J., Valente, L.M.P., Kiron, V., 2020. Approaches to improve utilization of *Nannochloropsis oceanica* in plant-based feeds for Atlantic salmon. Aquaculture 522, 735122. https://doi.org/10.1016/j.aquaculture.2020.735122.
Guillon, F., Champ, M., 2000. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. In: Food Research International. https://doi.org/10.1016/S0963-9969(00)00038-7.
Gupta, S., Fečkaninová, A., Lokesh, J., Koščová, J., Sørensen, M., Fernandes, J., Kiron, V., 2019a. *Lactobacillus* dominate in the intestine of Atlantic salmon fed dietary probiotics. Front. Microbiol. 9 https://doi.org/10.3389/fmicb.2018.03247.
Gupta, S., Lokesh, J., Abdelhafiz, Y., Siriyappagouder, P., Pierre, R., Sørensen, M., Kiron, V., 2019b. Macroalga-derived alginate oligosaccharide alters intestinal bacteria of Atlantic salmon. Front. Microbiol. 10 https://doi.org/10.3389/fmicb.2019.02037.
Haddeland, S., Lazado, C.C., Merkin, G.V., Myre, O.J., Okubamichael, M., Pedersen, L.-F., Pittman, K., 2020. Dynamic morphometrics of mucous cells reveal the minimal impact of therapeutic doses of peracetic acid on Atlantic salmon gill health. Aquaculture 534, 736315. https://doi.org/10.1016/j.aquaculture.2020.736315.
Hai, N.V., 2015. The use of probiotics in aquaculture. J. Appl. Microbiol. 119, 917–935. https://doi.org/10.1111/jam.12886.
Hatten, F., Fredriksen, Å., Hordvik, I., Endresen, C., 2001. Presence of IgM in cutaneous mucus, but not in gut mucus of Atlantic salmon, *Salmo salar*. Serum IgM is rapidly degraded when added to gut mucus. Fish Shellfish Immunol. 11, 257–268. https://doi.org/10.1006/fsim.2000.0313.

He, T., Xiao, Z., Liu, Q., Ma, D., Xu, S., Xiao, Y., Li, J., 2012. Ontogeny of the digestive tract and enzymes in rock bream *Oplegnathus fasciatus* (Temminck et Schlegel 1844) larvae. Fish Physiol. Biochem. https://doi.org/10.1007/s10695-011-9507-y.

Hedemann, M.S., Theil, P.K., Bach Knudsen, K.E., 2009. The thickness of the intestinal mucous layer in the colon of rats fed various sources of non-digestible carbohydrates is positively correlated with the pool of SCFA but negatively correlated with the proportion of butyric acid in digesta. Br. J. Nutr. 102, 117–125. https://doi.org/10.1017/S0007114508143549.

Hernandez, L.H.H., Barrera, T.C., Mejia, J.C., Mejia, G.C., Del Carmen, M., Dosta, M., Sotres, J.A.M., 2010. Effects of the commercial probiotic *Lactobacillus casei* on the growth, protein content of skin mucus and stress resistance of juveniles of the Porthole livebearer *Poecilopsis gracilis* (Poecilidae). Aquac. Nutr. 16, 407–411. https://doi.org/10.1111/j.1365-2095.2009.00679.x.

Hoseinifar, S.H., Roosta, Z., Hajimoradloo, A., Vakili, F., 2015. The effects of *Lactobacillus acidophilus* as feed supplement on skin mucosal immune parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (*Xiphophorus helleri*). Fish Shellfish Immunol. 42, 533–538. https://doi.org/10.1016/j.fsi.2014.12.003.

Hoseinifar, S.H., Sun, Y.Z., Caipang, C.M., 2017. Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquac. Res. https://doi.org/10.1111/are.13239.

Hovda, M.B., Lunestad, B.T., Fontanillas, R., Rosnes, J.T., 2007. Molecular characterisation of the intestinal microbiota of farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 272, 581–588. https://doi.org/10.1016/j.aquaculture.2007.08.045.

Huang, B.L., Chandra, S., Shih, D.Q., 2012. Skin manifestations of inflammatory bowel disease. Front. Physiol. 3 (FEB), 1–13. https://doi.org/10.3389/fphys.2012.00013.

Iversen, A., Asche, F., Hermansen, Ø., Nystøyl, R., 2020. Production cost and competitiveness in major salmon farming countries 2003–2018. Aquaculture 522, 735089. https://doi.org/10.1016/j.aquaculture.2020.735089.

Johansson, M.E.V., Phillipson, M., Petersson, J., Velcich, A., Holm, L., Hansson, G.C., 2008. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc. Natl. Acad. Sci. 105, 15064–15069. https://doi.org/10.1073/pnas.0803124105.

Karlsen, C., Ytteborg, E., Timmerhaus, G., Høst, V., Handeland, S., Jørgensen, S.M., Krasnov, A., 2018. Atlantic salmon skin barrier functions gradually enhance after seawater transfer. Sci. Rep. 8, 9510. https://doi.org/10.1038/s41598-018-27818-y.

Kim, Y.S., Ho, S.B., 2010. Intestinal goblet cells and mucins in health and disease: recent insights and progress. Curr. Gastroenterol. Rep. https://doi.org/10.1007/s11894-010-0131-2.

Kim, H.T., Yin, W., Nakamichi, Y., Panza, P., Grohmann, B., Buettner, C., Stainier, D.Y.R., 2019. WNT/RYK signaling restricts goblet cell differentiation during lung development and repair. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1911071116.

Kiron, V., 2012. Fish immune system and its nutritional modulation for preventive health care. Anim. Feed Sci. Technol. 173, 111–133. https://doi.org/10.1016/j.anifeedsci.2011.12.015.

Kiron, V., Sørensen, M., Huntley, M., Vasanth, G.K., Gong, Y., Dahle, D., Palihawadana, A.M., 2016. Defatted biomass of the microalga, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. Front. Mar. Sci. 3 https://doi.org/10.3389/fmars.2016.00067.

Kiron, V., Park, Y., Siriyappagouder, P., Dahle, D., Vasanth, G.K., Dias, J., Trichet, V.V., 2020. Intestinal transcriptome analysis reveals soy derivative-linked changes in Atlantic salmon. Front. Immunol. 11, 1–14. https://doi.org/10.3389/fimmu.2020.596514.

Knudsen, D., Urán, P., Arnous, A., Koppe, W., Frøkiær, H., 2007. Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. J. Agric. Food Chem. https://doi.org/10.1021/jf0626967.

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

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., Bäckhed, F., 2016. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell. https://doi.org/10.1016/j.cell.2016.05.041.

Koon, H.W., Shih, D.Q., Chen, J., Bakirtzi, K., Hing, T.C., Law, I., Pothoulakis, C., 2011. Cathelicidin signaling via the Toll-like receptor protects against colitis in mice. Gastroenterology 141. https://doi.org/10.1053/j.gastro.2011.06.079, 1852-1863.e3.

Kortner, T.M., Skugor, S., Penn, M.H., Mydland, L.T., Djordjevic, B., Hillestad, M., Krogdahl, Å., 2012. Dietary soyasaponin supplementation to pea protein concentrate reveals nutrigenomic interactions underlying enteropathy in Atlantic salmon (*Salmo salar*). BMC Vet. Res. https://doi.org/10.1186/1746-6148-8-101.

Kraus, H., 1961. Kurze mitteilung über das vorkommen von Lactobazillien auf fischen heringen. Lebensmittelhyg 12, 101–102.

Krishnaveni, G., Vignesh, S., Vidhyalakshmi, N., Vijay, V., Ramesh, U., 2021. Effects of dietary supplementation of *Lactobacillus fermentum* URLP18 on growth, innate immunity and survival against *Aeromonas hydrophila* ATCC 7966 challenge in freshwater fish *Cyprinus carpio* (common carp). Aquac. Res. 52, 1160–1176. https://doi.org/10.1111/are.14974.

Krogdahl, Å., Bakke-McKellep, A.M., 2005. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 141, 450–460. https://doi.org/10.1016/j.cbpb.2005.06.002.

Krogdahl, Å., Bakke-Mckellep, A.M., RØed, K.H., Baeverfjord, G., 2000. Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa. Aquac. Nutr. https://doi.org/10.1046/j.1365-2095.2000.00129.x.

Krogdahl, Å., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 9, 361–371. https://doi.org/10.1046/j.1365-2095.2003.00264.x.

Krogdahl, Å., Gajardo, K., Kortner, T.M., Penn, M., Gu, M., Berge, G.M., Bakke, A.M., 2015. Soya saponins induce enteritis in Atlantic salmon (*Salmo salar* L.). J. Agric. Food Chem. 63, 3887–3902. https://doi.org/10.1021/jf506242t.

Lauzon, H.L., Ringø, E., 2011. Prevalence and application of lactic acid bacteria in aquatic environments. In: Lactic Acid Bacteria: Microbiological and Functional Aspects, Fourth edition. https://doi.org/10.1201/b11503-32.

Ledy, K., Giambérini, L., Pihan, J.C., 2003. Mucous cell responses in gill and skin of brown trout *Salmo trutta fario* in acidic, aluminium-containing stream water. Dis. Aquat. Org. https://doi.org/10.3354/dao056235.

Levrat, M.-A., Rémésy, C., Demigné, C., 1991. High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. J. Nutr. 121, 1730–1737. https://doi.org/10.1093/jn/121.11.1730.

Liener, I.E., 1994. Implications of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nutr. 34, 31–67. https://doi.org/10.1080/10408399409527649.

Lødemel, J.B., Mayhew, T.M., Myklebust, R., Olsen, R.E., Espelid, S., Ringø, E., 2001. Effect of three dietary oils on disease susceptibility in Arctic charr (*Salvelinus alpinus* L.) during cohabitant challenge with *Aeromonas salmonicida* ssp. *salmonicida*. Aquac. Res. https://doi.org/10.1046/j.1365-2109.2001.00621.x.

Løkka, G., Austbø, L., Falk, K., Bjerkås, I., Koppang, E.O., 2013. Intestinal morphology of the wild Atlantic salmon (*Salmo salar*). J. Morphol. 274, 859–876. https://doi.org/10.1002/jmor.20142.

Louis, P., Flint, H.J., 2017. Formation of propionate and butyrate by the human colonic microbiota. Environ. Microbiol. 19, 29–41. https://doi.org/10.1111/1462-2920.13589.

Louis, P., Hold, G.L., Flint, H.J., 2014. The gut microbiota, bacterial metabolites and colorectal cancer. Nat. Rev. Microbiol. https://doi.org/10.1038/nrmicro3344.

Lowrey, L., Woodhams, D.C., Tacchi, L., Salinas, I., 2015. Topographical mapping of the rainbow trout (*Oncorhynchus mykiss*) microbiome reveals a diverse bacterial community with antifungal properties in the skin. Appl. Environ. Microbiol. 81, 6915–6925. https://doi.org/10.1128/AEM.01826-15.

Magnadóttir, B., 2006. Innate immunity of fish (overview). Fish Shellfish Immunol. 20, 137–151. https://doi.org/10.1016/j.fsi.2004.09.006.

Mahlapuu, M., Håkansson, J., Ringstad, L., Björn, C., 2016. Antimicrobial peptides: An emerging category of therapeutic agents. Front. Cell. Infect. Microbiol. https://doi.org/10.3389/fcimb.2016.00194.

Marcos-López, M., Calduch-Giner, J.A., Mirimin, L., MacCarthy, E., Rodger, H.D., O'Connor, I., Piazzon, M.C., 2018. Gene expression analysis of Atlantic salmon gills reveals mucin 5 and interleukin 4/13 as key molecules during amoebic gill disease. Sci. Rep. 8, 1–15. https://doi.org/10.1038/s41598-018-32019-8.

Markowiak-Kopeć, P., Śliżewska, K., 2020. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients 12. https://doi.org/10.3390/nu12041107.

Martin, F.P.J., Dumas, M.E., Wang, Y., Legido-Quigley, C., Yap, I.K.S., Tang, H., Nicholson, J.K., 2007. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. Mol. Syst. Biol. 3 https://doi.org/10.1038/msb4100153.

Merrifield, D.L., Dimitroglou, A., Bradley, G., Baker, R.T.M., Davies, S.J., 2010. Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. Aquac. Nutr. 16, 504–510. https://doi.org/10.1111/j.1365-2095.2009.00689.x.

Merrifield, D.L., Balcázar, J.L., Daniels, C., Zhou, Z., Carnevali, O., Sun, Y.Z., Ringø, E., 2014. Indigenous lactic acid bacteria in fish and crustaceans. In: Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics. https://doi.org/10.1002/9781118897263.ch6.

Meslin, J.C., Fontaine, N., Andrieux, C., 1999. Variation of mucin distribution in the rat intestine, caecum and colon: effect of the bacterial flora. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. https://doi.org/10.1016/S1095-6433(99)00056-2.

Minniti, G., Rød Sandve, S., Padra, J.T., Heldal Hagen, L., Lindén, S., Pope, P.B.Ø., Vaaje-Kolstad, G., 2019. The farmed Atlantic salmon (*Salmo salar*) skin–mucus proteome and its nutrient potential for the resident bacterial community. Genes (Basel) 10, 515. https://doi.org/10.3390/genes10070515.

Moldal, T., Løkka, G., Wiik-Nielsen, J., Austbø, L., Torstensen, B.E., Rosenlund, G., Koppang, E.O., 2014. Substitution of dietary fish oil with plant oils is associated with shortened mid intestinal folds in Atlantic salmon (*Salmo salar*). BMC Vet. Res. 10, 60. https://doi.org/10.1186/1746-6148-10-60.

Morrison, D.J., Preston, T., 2016. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes. https://doi.org/10.1080/19490976.2015.1134082.

Nayak, S.K., 2010. Probiotics and immunity: A fish perspective. Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2010.02.017.

Nordrum, S., Bakke-McKellep, A., Krogdahl, Å., Buddington, R., 2000. Effects of soybean meal and salinity on intestinal transport of nutrients in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 125, 317–335. https://doi.org/10.1016/S0305-0491(99)00190-X.

Núñez-Acuña, G., Marambio, J.P., Valenzuela, T., Wadsworth, S., Gallardo-Escárate, C., 2016. Antimicrobial peptides from *Salmo salar* skin induce frontal filament development and olfactory/cuticle-related genes in the sea louse *Caligus rogercresseyi*. Aquaculture 464, 171–177. https://doi.org/10.1016/j.aquaculture.2016.06.023.

Núñez-Acuña, G., Gallardo-Escárate, C., Fields, D.M., Shema, S., Skiftesvik, A.B., Ormazábal, I., Browman, H.I., 2018. The Atlantic salmon (*Salmo salar*) antimicrobial peptide cathelicidin-2 is a molecular host-associated cue for the salmon louse (*Lepeophtheirus salmonis*). Sci. Rep. 8, 13738. https://doi.org/10.1038/s41598-018-31885-6.

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., Skrede, A., 2009. Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)-Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture. https://doi.org/10.1016/j.aquaculture.2008.12.012.

Park, J., Floch, M.H., 2007. Prebiotics, probiotics, and dietary fiber in gastrointestinal disease. Gastroenterol. Clin. N. Am. https://doi.org/10.1016/j.gtc.2007.03.001.

Peatman, E., Lange, M., Zhao, H., Beck, B.H., 2015. Physiology and immunology of mucosal barriers in catfish (*Ictalurus* spp.). Tissue Barriers. https://doi.org/10.1080/21688370.2015.1068907.

Pelicano, E., Souza, P., Souza, H., Figueiredo, D., Boiago, M., Carvalho, S., Bordon, V., 2005. Intestinal mucosa development in broiler chickens fed natural growth promoters. Rev. Bras. Ciência Avícola. https://doi.org/10.1590/s1516-635x2005000400005.

Pérez-Sánchez, T., Ruiz-Zarzuela, I., de Blas, I., Balcázar, J.L., 2014. Probiotics in aquaculture: a current assessment. Rev. Aquac. https://doi.org/10.1111/raq.12033.

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., Maita, M., 2011. Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Res. Vet. Sci. 91, e92–e97. https://doi.org/10.1016/j.rvsc.2011.02.014.

Pittman, K., Sourd, P., Ravnøy, B., Espeland, Ø., Fiksdal, I.U., Oen, T., Sweetman, J., 2011. Novel method for quantifying salmonid mucous cells. J. Fish Dis. 34, 931–936. https://doi.org/10.1111/j.1365-2761.2011.01308.x.

Pittman, K., Pittman, A., Karlson, S., Cieplinska, T., Sourd, P., Redmond, K., Sweetman, E., 2013. Body site matters: an evaluation and application of a novel histological methodology on the quantification of mucous cells in the skin of Atlantic salmon, *Salmo salar* L. J. Fish Dis. 36, 115–127. https://doi.org/10.1111/jfd.12002.

Raju, S.V., Sarkar, P., Kumar, P., Arockiaraj, J., 2020. Piscidin, fish antimicrobial peptide: structure, classification, properties, mechanism, gene regulation and therapeutical importance. Int. J. Pept. Res. Ther. https://doi.org/10.1007/s10989-020-10068-w.

Rakers, S., Niklasson, L., Steinhagen, D., Kruse, C., Schauber, J., Sundell, K., Paus, R., 2013. Antimicrobial peptides (AMPs) from fish epidermis: perspectives for investigative dermatology. J. Invest. Dermatol. 133, 1140–1149. https://doi.org/10.1038/jid.2012.503.

Ramesh, D., Vinothkanna, A., Rai, A.K., Vignesh, V.S., 2015. Isolation of potential probiotic *Bacillus* spp. and assessment of their subcellular components to induce immune responses in *Labeo rohita* against *Aeromonas hydrophila*. Fish Shellfish Immunol. 45. https://doi.org/10.1016/j.fsi.2015.04.018.

Ramos, M.A., Batista, S., Pires, M.A., Silva, A.P., Pereira, L.F., Saavedra, M.J., Rema, P., 2017. Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. Animal 11, 1259–1269. https://doi.org/10.1017/S1751731116002792.

Refstie, S., Korsøen, Ø.J., Storebakken, T., Baeverfjord, G., Lein, I., Roem, A.J., 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture. https://doi.org/10.1016/S0044-8486(00)00382-3.

Reyes-Becerril, M., Guardiola, F., Rojas, M., Ascencio-Valle, F., Esteban, M.Á., 2013. Dietary administration of microalgae *Navicula* sp. affects immune status and gene expression of gilthead seabream (*Sparus aurata*). Fish Shellfish Immunol. 35, 883–889. https://doi.org/10.1016/j.fsi.2013.06.026.

Richards, J.L., Yap, Y.A., McLeod, K.H., MacKay, C.R., Marinō, E., 2016. Dietary metabolites and the gut microbiota: an alternative approach to control inflammatory and autoimmune diseases. Clin. Transl. Immunol. https://doi.org/10.1038/cti.2016.29.

Ringø, E., 2004. Lactic acid bacteria in fish and fish farming. In: Lactic Acid Bacteria, pp. 581–610. https://doi.org/10.1201/9780824752033.ch21.

Ringø, E., Gatesoupe, F.J., 1998. Lactic acid bacteria in fish: a review. Aquaculture 160. https://doi.org/10.1016/S0044-8486(97)00299-8.

Ringø, E., Schillinger, U., Holzapfel, W., 2005. Antimicrobial activity of lactic acid bacteria isolated from aquatic animals and the use of lactic acid bacteria in aquaculture. In: Biology of Growing Animals, pp. 418–453. https://doi.org/10.1016/S1877-1823(09)70051-7.

Ringø, E., Hoseinifar, S.H., Ghosh, K., Van Doan, H., Beck, B.R., Song, S.K., 2018. Lactic acid bacteria in finfish-An update. Front. Microbiol. 9, 1–37. https://doi.org/10.3389/fmicb.2018.01818.

Roberts, S.D., Powell, M.D., 2005. The viscosity and glycoprotein biochemistry of salmonid mucus varies with species, salinity and the presence of amoebic gill disease. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. https://doi.org/10.1007/s00360-004-0453-1.

Romarheim, O.H., Skrede, A., Gao, Y., Krogdahl, Å., Denstadli, V., Lilleeng, E., Storebakken, T., 2006. Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 256, 354–364. https://doi.org/10.1016/j.aquaculture.2006.02.006.

Rombout, J.H.W.M., Lamers, C.H.J., Helfrich, M.H., Dekker, A., Taverne-Thiele, J.J., 1985. Uptake and transport of intact macromolecules in the intestinal epithelium of carp (*Cyprinus carpio* L.) and the possible immunological implications. Cell Tissue Res. https://doi.org/10.1007/BF00219230.

Sahlmann, C., Gu, J., Kortner, T.M., Lein, I., Krogdahl, Å., Bakke, A.M., 2015. Ontogeny of the digestive system of Atlantic Salmon (*Salmo salar* L.) and effects of soybean meal from start-feeding. PLoS One 10. https://doi.org/10.1371/journal.pone.0124179 e0124179.

Sanden, M., Olsvik, P.A., 2009. Intestinal cellular localization of PCNA protein and CYP1A mRNA in Atlantic salmon *Salmo salar* L. exposed to a model toxicant. BMC Physiol. 9, 1–11. https://doi.org/10.1186/1472-6793-9-3.

Sanden, M., Berntssen, M.H.G., Krogdahl, Å., Hemre, G.I., Bakke-McKellep, A.M., 2005. An examination of the intestinal tract of Atlantic salmon, *Salmo salar* L., parr fed different varieties of soy and maize. J. Fish Dis. https://doi.org/10.1111/j.1365-2761.2005.00618.x.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods. https://doi.org/10.1038/nmeth.2089.

Schütte, A., Ermund, A., Becker-Pauly, C., Johansson, M.E.V., Rodriguez-Pineiro, A.M., Bäckhed, F., Hansson, G.C., 2014. Microbial-induced meprin β cleavage in MUC2 mucin and a functional CFTR channel are required to release anchored small intestinal mucus. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1407597111.

Sheikhzadeh, N., Mousavi, S., Hamidian, G., Firouzamandi, M., Khani Oushani, A., Mardani, K., 2019. Role of dietary *Spirulina platensis* in improving mucosal immune responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.05.009.

Sherif, A.H., Gouda, M.Y., Al-Sokary, E.T., Elseify, M.M., 2021. *Lactobacillus plantarum* enhances immunity of Nile tilapia *Oreochromis niloticus* challenged with *Edwardsiella tarda*. Aquac. Res. 52 https://doi.org/10.1111/are.14955.

Silva, P.F., McGurk, C., Knudsen, D.L., Adams, A., Thompson, K.D., Bron, J.E., 2015. Histological evaluation of soya bean-induced enteritis in Atlantic salmon (*Salmo salar* L.): quantitative image analysis vs. semi-quantitative visual scoring. Aquaculture 445, 42–56. https://doi.org/10.1016/j.aquaculture.2015.04.002.

Sohrabnezhad, M., Sudagar, M., Mazandarani, M., 2017. Effect of dietary soybean meal and multienzyme on intestine histology of beluga sturgeon (*Huso huso*). Int. Aquat. Res. https://doi.org/10.1007/s40071-017-0172-3.

Song, J., Peng, S., Yang, J., Zhou, F., Suo, H., 2021. Isolation and identification of novel antibacterial peptides produced by *Lactobacillus fermentum* SHY10 in Chinese pickles. Food Chem. 348, 129097. https://doi.org/10.1016/j.foodchem.2021.129097.

Sørensen, M., Penn, M., El-Mowafi, A., Storebakken, T., Chunfang, C., Øverland, M., Krogdahl, Å., 2011. Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L). Aquaculture 314, 145–152. https://doi.org/10.1016/j.aquaculture.2011.02.013.

Sørensen, S.L., Park, Y., Gong, Y., Vasanth, G.K., Dahle, D., Korsnes, K., Sørensen, M., 2021. Nutrient digestibility, growth, mucosal barrier status, and activity of leucocytes from head kidney of Atlantic salmon fed marine- or plant-derived protein and lipid sources. Front. Immunol. 11 https://doi.org/10.3389/fimmu.2020.623726.

Sprague, M., Dick, J.R., Tocher, D.R., 2016. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006-2015. Sci. Rep. https://doi.org/10.1038/srep21892.

Standen, B.T., Rawling, M.D., Davies, S.J., Castex, M., Foey, A., Gioacchini, G., Merrifield, D.L., 2013. Probiotic *Pediococcus acidilactici* modulates both localised intestinal- and peripheral-immunity in tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 35, 1097–1104. https://doi.org/10.1016/j.fsi.2013.07.018.

Storebakken, T., Shearer, K.D., Roem, A.J., 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture. https://doi.org/10.1016/S0044-8486(97)00284-6.

Sultana, R., McBain, A.J., O'Neill, C.A., 2013. Strain-dependent augmentation of tight-junction barrier function in human primary epidermal keratinocytes by *Lactobacillus* and *Bifidobacterium* Lysates. Appl. Environ. Microbiol. 79, 4887–4894. https://doi.org/10.1128/AEM.00982-13.

Sundell, K.S., Sundh, H., 2012. Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. Front. Physiol. 3 (SEP), 1–13. https://doi.org/10.3389/fphys.2012.00388.

Sveen, L.R., Grammes, F.T., Ytteborg, E., Takle, H., Jørgensen, S.M., 2017. Genome-wide analysis of Atlantic salmon (*Salmo salar*) mucin genes and their role as biomarkers. PLoS One. https://doi.org/10.1371/journal.pone.0189103.

Tapia-Paniagua, S.T., Díaz-Rosales, P., León-Rubio, J.M., de la Banda, I.G., Lobo, C., Alarcón, F.J., Moriñigo, M.A., 2012. Use of the probiotic *Shewanella putrefaciens* Pdp11 on the culture of Senegalese sole (*Solea senegalensis*, Kaup 1858) and gilthead seabream (*Sparus aurata* L.). Aquac. Int. 20 https://doi.org/10.1007/s10499-012-9509-5.

The World Bank, 2021. Commodity Markets [WWW Document]. URL. https://www.worldbank.org/en/research/commodity-markets.

Torrecillas, S., Caballero, M.J., Mompel, D., Montero, D., Zamorano, M.J., Robaina, L., Izquierdo, M., 2017. Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets. Fish Shellfish Immunol. 67, 302–311. https://doi.org/10.1016/j.fsi.2017.06.022.

Tsukuda, N., Yahagi, K., Hara, T., Watanabe, Y., Matsumoto, H., Mori, H., Matsuki, T., 2021. Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. ISME J. https://doi.org/10.1038/s41396-021-00937-7.

Urán, P.A., Schrama, J.W., Rombout, J.H.W.M., Obach, A., Jensen, L., Koppe, W., Verreth, J.A.J., 2008a. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar* L.) at different temperatures. Aquac. Nutr. 14, 324–330. https://doi.org/10.1111/j.1365-2095.2007.00534.x.

Urán, P.A., Gonçalves, A.A., Taverne-Thiele, J.J., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M., 2008b. Soybean meal induces intestinal inflammation in

common carp (*Cyprinus carpio* L.). Fish Shellfish Immunol. 25, 751–760. https://doi.org/10.1016/j.fsi.2008.02.013.

Urán, P.A., Aydin, R., Schrama, J.W., Verreth, J.A.J., Rombout, J.H.W.M., 2008c. Soybean meal-induced uptake block in Atlantic salmon *Salmo salar* distal enterocytes. J. Fish Biol. https://doi.org/10.1111/j.1095-8649.2008.02091.x.

Urán, P.A., Schrama, J.W., Rombout, J.H.W.M., Taverne-Thiele, J.J., Obach, A., Koppe, W., Verreth, J.A.J., 2009. Time-related changes of the intestinal morphology of Atlantic salmon, *Salmo salar* L., at two different soybean meal inclusion levels. J. Fish Dis. 32, 733–744. https://doi.org/10.1111/j.1365-2761.2009.01049.x.

van Baarlen, P., Wells, J.M., Kleerebezem, M., 2013. Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. Trends Immunol. 34, 208–215. https://doi.org/10.1016/j.it.2013.01.005.

Van Den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J.J., Hendriks, H.G.C.J.M., Koninkx, J.G.J.F., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. Aquaculture. https://doi.org/10.1016/0044-8486(91)90174-6.

Van Den Ingh, T.S.G.A.M., Olli, J.J., Krogdahl, Å., 1996. Alcohol-soluble components in soybeans cause morphological changes in the distal intestine of Atlantic salmon, *Salmo salar* L. J. Fish Dis. https://doi.org/10.1111/j.1365-2761.1996.tb00119.x.

van der Marel, M., Caspari, N., Neuhaus, H., Meyer, W., Enss, M.L., Steinhagen, D., 2010. Changes in skin mucus of common carp, *Cyprinus carpio* L., after exposure to water with a high bacterial load. J. Fish Dis. https://doi.org/10.1111/j.1365-2761.2010.01140.x.

Vasanth, G.K., Kiron, V., Kulkarni, A., Dahle, D., Lokesh, J., Kitani, Y., 2015. A microbial feed additive abates intestinal inflammation in Atlantic salmon. Front. Immunol. 6 https://doi.org/10.3389/fimmu.2015.00409.

Vatsos, I.N., Kotzamanis, Y., Henry, M., Angelidis, P., Alexis, M., 2010. Monitoring stress in fish by applying image analysis to their skin mucous cells. Eur. J. Histochem. 54, 22. https://doi.org/10.4081/ejh.2010.e22.

Vrakas, S., Mountzouris, K.C., Michalopoulos, G., Karamanolis, G., Papatheodoridis, G., Tzathas, C., Gazouli, M., 2017. Intestinal bacteria composition and translocation of bacteria in inflammatory bowel disease. PLoS One 12, 1–11. https://doi.org/10.1371/journal.pone.0170034.

Wang, Y., Gu, Q., 2010. Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. Mar. Biol. Res. 6, 327–332. https://doi.org/10.1080/17451000903300893.

Wang, S., Wang, Y., Ma, J., Ding, Y., Zhang, S., 2011. Phosvitin plays a critical role in the immunity of zebrafish embryos via acting as a pattern recognition receptor and an antimicrobial effector. J. Biol. Chem. https://doi.org/10.1074/jbc.M111.247635.

Wobbrock, J.O., Findlater, L., Gergle, D., Higgins, J.J., 2011. The Aligned Rank Transform for nonparametric factorial analyses using only ANOVA procedures. In: Conf. Hum. Factors Comput. Syst. - Proc., pp. 143–146. https://doi.org/10.1145/1978942.1978963.

Wuertz, S., Schroeder, A., Wanka, K.M., 2021. Probiotics in fish nutrition—Long-standing household remedy or native nutraceuticals? Water 13, 1348. https://doi.org/10.3390/w13101348.

Xia, Y., Wang, M., Gao, F., Lu, M., Chen, G., 2020. Effects of dietary probiotic supplementation on the growth, gut health and disease resistance of juvenile Nile tilapia (*Oreochromis niloticus*). Anim. Nutr. 6 https://doi.org/10.1016/j.aninu.2019.07.002.

Xu, Q.Y., Wang, C.A., Zhao, Z.G., Luo, L., 2012. Effects of replacement of fish meal by soy protein isolate on the growth, digestive enzyme activity and serum biochemical parameters for juvenile amur sturgeon (*Acipenser schrenckii*). Asian-Australas. J. Anim. Sci. https://doi.org/10.5713/ajas.2012.12192.

Yang, G.Y., Zhu, Y.H., Zhang, W., Zhou, D., Zhai, C.C., Wang, J.F., 2016. Influence of orally fed a select mixture of *Bacillus* probiotics on intestinal T-cell migration in weaned MUC4 resistant pigs following *Escherichia coli* challenge. Vet. Res. https://doi.org/10.1186/s13567-016-0355-8.

Ytrestøyl, T., Aas, T.S., Åsgård, T., 2015. Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. Aquaculture 448, 365–374. https://doi.org/10.1016/j.aquaculture.2015.06.023.

Yu, L., Zhai, Q., Zhu, J., Zhang, C., Li, T., Liu, X., Chen, W., 2017. Dietary *Lactobacillus plantarum* supplementation enhances growth performance and alleviates aluminum toxicity in tilapia. Ecotoxicol. Environ. Saf. 143 https://doi.org/10.1016/j.ecoenv.2017.05.023.

Zhang, W., Zhu, Y.-H., Zhou, D., Wu, Q., Song, D., Dicksved, J., Wang, J.-F., 2017. Oral administration of a select mixture of *Bacillus* probiotics affects the gut microbiota and goblet cell function following *Escherichia coli* challenge in newly weaned pigs of genotype MUC4 that are supposed to be enterotoxigenic *E. coli* F4ab/ac receptor. Appl. Environ. Microbiol. 83 https://doi.org/10.1128/AEM.02747-16.

Zuchelkowski, E.M., Lantz, R.C., Hinton, D.E., 1981. Effects of acid-stress on epidermal mucous cells of the brown bullhead *Ictalurus nebulosus* (LeSeur): a morphometric study. Anat. Rec. 200, 33–39. https://doi.org/10.1002/ar.1092000104.

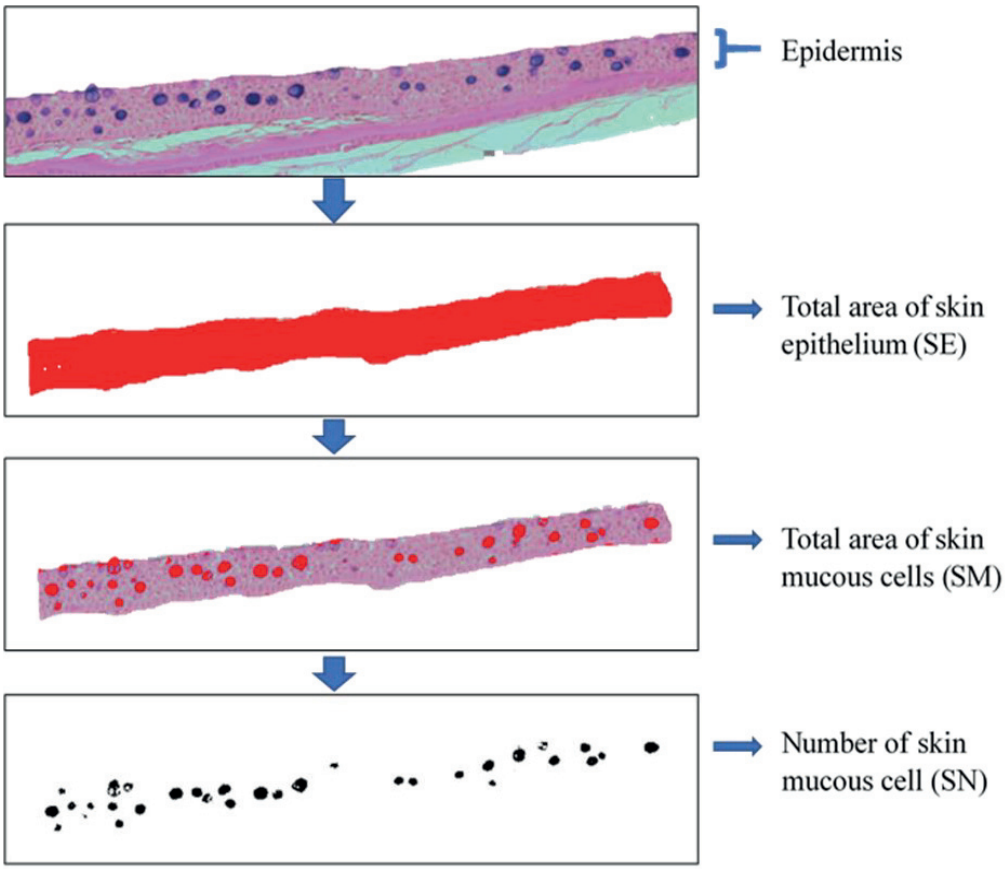
Zuchelkowski, E.M., Pinkstaff, C.A., Hinton, D.E., 1985. Mucosubstance histochemistry in control and acid-stressed epidermis of brown bullhead catfish, *Ictalurus nebulosus* (LeSueur). Anat. Rec. https://doi.org/10.1002/ar.1092120402.

Supplementary table 1

Details of the parameters and scores that were considered for the semi-quantitative scoring of the distal intestine of Atlantic salmon.

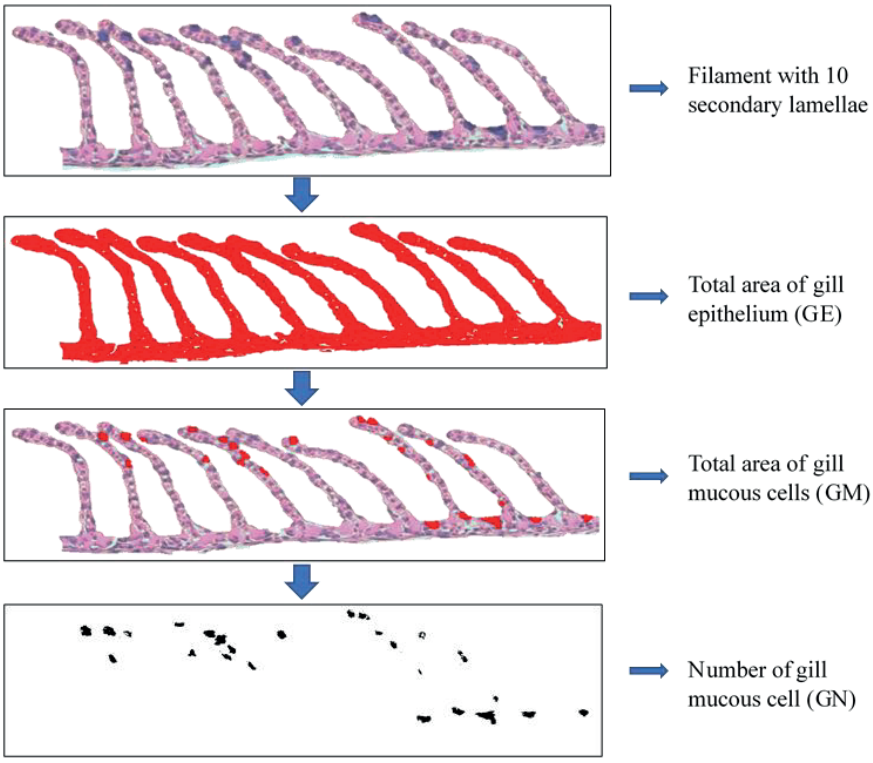
Parameter	Score	Description
NM	1	Above 31 NM per simple villi, densely distributed small mucous cells throughout the complex villi
	2	26 to 31 NM per simple villi, many of small and large mucous cells throughout the complex villi
	3	21 to 26 NM per simple villi, many small and few large mucous cells throughout the complex villi
	4	16 to 21 NM per simple villi, many large and few small mucous cells throughout the complex villi
	5	Below 16 NM per simple villi, large mucous cells evenly distributed throughout the complex villi
IEL	1	Above 15 IEL per simple villi, densely distributed in the complex villi
	2	13 to 15 IEL per simple villi
	3	11 to 13 IEL per simple villi
	4	9 to 11 IEL per simple villi
	5	Below 9 IEL per simple villi, evenly distributed in the complex villi
SNV	1	Completely absent or no vacuoles
	2	Scattered, tiny few vacuoles present at least in some part of enterocytes
	3	Obviously reduced, few small vacuoles present in many enterocytes
	4	Mildly reduced, more medium-sized vacuoles present almost half of the enterocytes
	5	Highly vacuolated, large vacuoles present almost along the entire apical part of enterocytes

Number of mucous cells (NM), number of intraepithelial lymphocytes (IEL) and presence of supranuclear vacuoles in enterocytes (SNV) per villus.



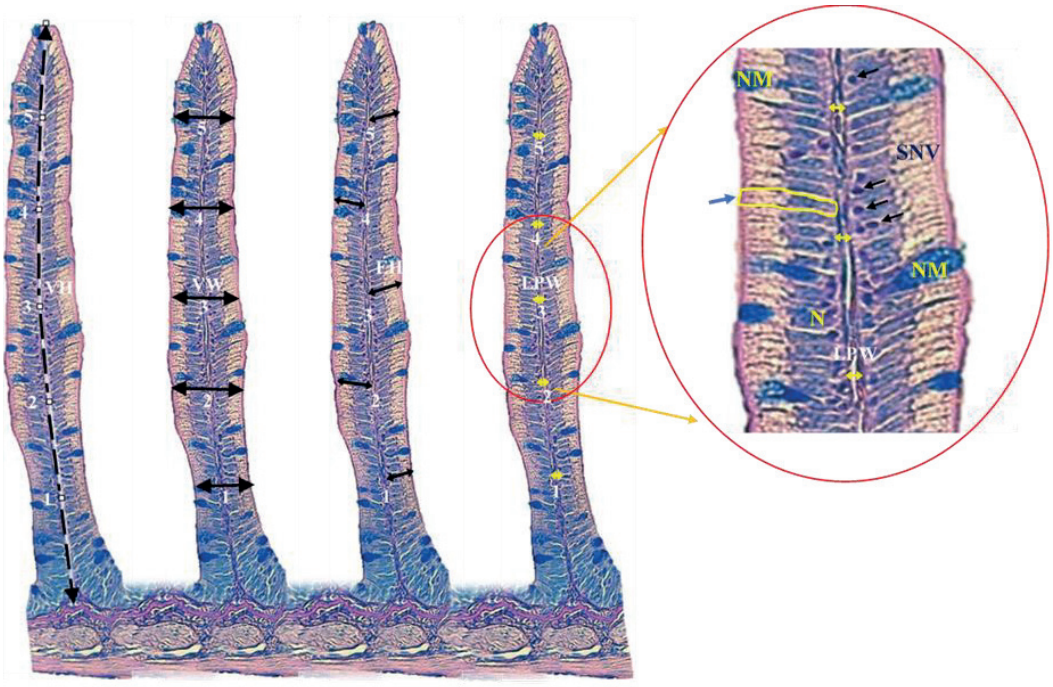
Supplementary fig. 1.

Quantitative image analysis technique used to evaluate skin mucous cells.



Supplementary fig. 2.

Quantitative image analysis technique used to evaluate gill mucous cells.



Supplementary fig. 3.

Quantitative and semi-quantitative image analysis technique used to evaluate the distal intestine of Atlantic salmon. Indices; VH – height of villi, VW – width of villi, EH – height of enterocytes, LPW – width of lamina propria, NM – number of mucous cells, black arrows (see inset) – intraepithelial lymphocytes (IEL) and SNV – supranuclear vacuoles. Average of 5 values per villus were registered for VW, EH and LPW. The letter "N" (see inset) denotes the nucleus of the enterocyte. Yellow box and blue arrow indicate a columnar epithelial cell.

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The mucosal surfaces are efficient defense sites of fishes, and these barriers have important physiological and immune functions. Plant-based ingredients can compromise the health of Atlantic salmon and hence, different feed additives or novel ingredients can be exploited to improve the health of mucosal surfaces. The current salmon feeds contain ingredients from legumes that have antinutritional factors including saponins. These compounds are known to impart adverse effects on nutrient digestion, utilization, and health of Atlantic salmon. In the present thesis, experiments were conducted to investigate the ability of probiotics, Ca-butyrate, and microalgae to improve the mucosal health of salmon fed marine- or plant-based diets. The results revealed that mucosal health of the fish fed marine-based diet was better than those fed plant-based diets. The findings indicated that lactic acid bacteria or Ca-butyrate can be used to improve mucosal health i.e., to prevent the soybean meal or soyasaponin-induced enteritis. Feed additives improved the intestine micromorphological features, by preserving the villi and enterocyte height, and supranuclear vacuoles in the enterocytes. Inclusion of microalgae such as *Nannochloropsis* sp. (7.5% of the feed), in plant-based diets also improved the mucosal health. This research adds new knowledge to the understanding of the interplay between certain ingredients/additives in salmon feeds and mucosal health of salmon. The findings are expected to bolster the sustainability aspect of the Atlantic salmon aquaculture in Norway.

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