The supplementation of a prebiotic improves the microbial community in the gut and the skin of Atlantic salmon (Salmo salar)

Baumgärtner, Simon; James, Jack; Ellison, Amy

Aquaculture Reports

DOI:
10.1016/j.aqrep.2022.101204

Published: 01/08/2022

Publisher's PDF, also known as Version of record

Aquaculture Reports 25 (2022) 101204

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

The supplementation of a prebiotic improves the microbial community in the gut and the skin of Atlantic salmon (*Salmo salar)*

Simon Baumgärtner [a,*], Jack James [b], Amy Ellison [a]

[a] Bangor University, School of Natural Sciences, Bangor LL57 2UW, UK
[b] Pontus Research Ltd, Unit E, Hirwaun Industrial Estate, Aberdare CF449UP, UK

ARTICLE INFO

Keywords:
Microbiome
Prebiotics
Atlantic salmon
Fish health
Sustainable nutrition
Novel feeding strategies
16S rRNA microbiome profiling

ABSTRACT

Aquaculture growth is hindered by an increasing number of challenges, primarily infectious diseases and inappropriate or unsustainable fish nutrition. Hence it is critical to develop novel prevention strategies to minimise infectious diseases and pharmaceutical interventions. Nutritional challenges and the health of the fish could be improved by managing their microbial communities. Microbiomes can play a crucial role in fish physiology, particularly in digestion, by metabolizing largely indigestible feed components for the host or synthesis essential micronutrients. Beyond their nutritional role, microbiomes are considered the first line of defence against pathogens. In this study, a novel prebiotic mix (Selectovit), composed of 1,3/1,6-beta glucans, mannan-oligosaccharides, nucleic acids, nucleotides, medium chain fatty acids and single chain fatty acids, was tested at different inclusion levels (0.0; 0.5; 1.0; 2.0 g/kg) in the diet of Atlantic salmon (*Salmo salar*). Using experimental feed trials and 16 S rRNA microbiome profiling, the impact of the prebiotic blend on fish growth and microbial community within both the gastrointestinal tract and the skin was assessed. Overall, the supplement showed no significant impact on growth. However, we clearly demonstrate that the prebiotic can significantly manipulate the microbial community of the distal intestine and the skin. Several potential beneficial bacteria such as *Bacillus* and *Mycoplasma spp.* were significantly more abundant in the prebiotic-fed groups compared to the control. In contrast, putative pathogenic bacteria were less abundant in the salmon fed the prebiotic blend. Interestingly, the supplement induced more changes in the skin than in the gut. There is growing evidence in fish for highly complex interactions between the microbial communities of the digestive system and external mucosa, and with the host immune system. Further research in this field could lead to the creation of novel bacterial biomarkers and new non-invasive strategies for fish digestive health monitoring.

1. Introduction

In the last decade, aquaculture production has been the fastest-growing animal food production sector (FAO, 2020). Despite steady growth, future expansion is hindered by an increasing number of challenges, primarily: (1) infectious diseases and (2) inappropriate or unsustainable fish nutrition (Shinn et al., 2018). Infectious diseases are considered the greatest threat to sustainable aquaculture production development. High mortalities and losses in production volume, resulting from disease outbreaks, are a substantial risk for global food security and represent an increasing challenge for maintaining animal welfare (Bank, 2014; Assefa and Abunna, 2018). Hence, it is critical to develop prevention strategies to minimise infectious diseases, in addition to treating diseased fish. Vaccinations have been one of the most efficient strategies for some pathogens (Ma et al., 2019). However, due to the involved costs, the application of vaccines is limited in many countries. In addition, vaccines are not available yet for all diseases and/or fish species (Adams, 2019). Therefore, alternative preventative strategies are urgently needed to promote high animal welfare standards on farms.

The second major challenge to the aquaculture industry is sustainable nutrition (Hasan and Halwart, 2009). Feeds typically represent the single greatest production cost to fish farms (Asche and Oglend, 2016). Due to the rapid growth of aquaculture production, and widely exploited wild fish stocks, marine-derived fishmeal and fish oils are limited and increasingly expensive feed ingredients (Hodar et al., 2019). Plant-based feed components are often used as substitutes, but their use is constrained due to differing nutritional profiles to fish-based ingredients

* Corresponding author.
E-mail address: simon.baumgaertner@t-online.de (S. Baumgärtner).

https://doi.org/10.1016/j.aqrep.2022.101204
Received 17 March 2022; Received in revised form 1 June 2022; Accepted 1 June 2022
Available online 16 June 2022

(Francis, Makkar and Becker, 2001). Fish have specific nutritional requirements, and as such inappropriate nutrition and plant-derived anti-nutritional factors can negatively impact growth performance, health, and welfare of the fish (Refstie et al., 2000; Pratoomyot et al., 2010; Al-Thobaiti et al., 2018).

Nutritional challenges and the health of the fish could be improved by managing their microbial communities. Microbial communities including bacteria, archaea and fungi – "microbiomes" – which colonise all mucosal surfaces of fish that are in contact with the environment, including the skin, gills and digestive tract (Merrifield and Rodiles, 2015). Genetic origin, environmental factors and diet are the main drivers of the microbial composition in fish (de Bruijn et al., 2018). Microbiomes can play a crucial role in fish physiology, particularly in digestion. For example, biosynthesis of exogenous enzymes by microbiota in the gastrointestinal tract increases digestion efficiency (Ray, Ghosh and Ringø, 2012). Moreover, certain bacteria and other microbes can metabolise plant starches and cellulose, that are largely indigestible to the host especially for carnivorous fish (Ni et al., 2014). In addition, bacteria can synthesise essential vitamins and other compounds, such as eicosapentaenoic acid (EPA), that contribute to the overall health of the host (Dailey et al., 2016; Yang et al., 2020). Therefore, there is rapidly growing interest in how fish microbiomes can be managed and enhanced for the benefit of aquaculture productivity (Kuebutornye et al., 2020).

Beyond their nutritional role, microbiomes contribute to host immune responses and disease resistance. Epithelial mucus, rich in microbes, is considered the first line of defence against invading pathogens. In fish and other vertebrates, the host immune system (both innate and adaptive pathways) can regulate the abundance of commensal and pathogenic bacteria and actively shape the microbiota composition (Merrifield and Rodiles, 2015; Chen, Liu and Hu, 2019; Zheng, Liwinski and Elinav, 2020). Conversely, metabolites produced by bacteria (e.g., Polysaccharide A (PSA), Short Chain Fatty Acids SCFAs) can travel through the circulatory system of the host, act on immune cells and subsequently induce tissue specific immune responses, which may in turn contribute to resistance against invading pathogens (Merrifield and Rodiles, 2015; Xiong, Nie and Chen, 2019; Zheng, Liwinski and Elinav, 2020). In addition, commensal microbes can compete with pathogens through niche exclusion, competition of essential resources, and/or the production of antimicrobial compounds also known as antibiosis (Kamada et al., 2013; de Bruijn et al., 2018). Healthy fish are typically characterized by a stable and highly diverse microbial community (Li et al., 2017). In contrast, diseased fish often show a loss of diversity - known as dysbiosis - and a high abundance of pathogenic bacteria (Stecher, Maier and Hardt, 2013; de Bruijn et al., 2018). As our understanding of the crucial roles of microbiomes for fish increases, there is a growing interest to maintain and beneficially manipulate fish microbiomes to improve productivity, health and disease resistance in aquaculture.

Recent strategies to manage the microbial community include the alteration of diet composition (e.g., lipid and protein sources), and the supplementation of probiotics and prebiotic in diets (Egerton et al., 2018). Probiotics involve the application of live organisms, such as *Bacillus* or *Lactococcus* spp., to improve the balance between beneficial and harmful bacteria (Perumal, Thirunavukkarasu and Pachiappan, 2015). Their inclusion in diet has been shown to improve welfare (e.g., improved digestion, upregulation of immune genes, increased disease resistance) and growth performance in various fish species (El-Haroun, Goda and Kabir Chowdhury, 2006). In contrast, prebiotics comprise specific feed materials or feed additives that enhance the growth of specific microorganisms (Merrifield et al., 2010). A broad range of prebiotics have been tested in fish, including yeast cell wall derived components (e.g., 1,3/1,6-beta-glucans, mannan-oligosaccharides), yeast cytoplasm derived components (nucleic acids and nucleotides) or short- and medium-chain fatty acids.

1,3/1,6-beta-glucans and mannan-oligosaccharides have been widely tested in cultured fish species with supplementation typically improving growth performance, feed utilisation and immune activity (Abid et al., 2013; Selim and Reda, 2015; Jami et al., 2019; Staykov et al., 2007; Jami et al. 2019). In addition, mannan-oligosaccharides may reduce the multiplication of specific bacteria in the intestinal tract (Momeni-Moghaddam et al., 2015; Teng and Kim, 2018; Gainza and Romero, 2020). Crucially, fish fed with 1,3/1,6-beta-glucans and mannan oligosaccharides showed higher survival when challenged with *Yersinia ruckeri* (Selim and Reda, 2015). Nucleic acids and nucleotides are another promising group of micronutrients for enhancing fish health and improving digestive capacities. These have been shown to increase lysozyme activity, suggesting raised non-specific as well as specific immune response (Akhter et al., 2015; Xu et al., 2015). In rainbow trout, nucleotide supplementation resulted in higher survival when exposed to *Vibrio anguillarum* (Burrells, Williams and Forno, 2001). Other prebiotics such as the supplementation of medium-chain fatty acids and single chain fatty acids actively shaped the microbial community by increasing the abundance of several beneficial and health-promoting bacteria (Rimoldi et al., 2018). Most research studies focus on the interaction between supplements and the gut microbial community, and few studies have analysed the impact of feed prebiotics on microbiota outside the gastrointestinal tract. These non-gut mucosal microbiomes – such as on the skin and gills – can play a critical role in fish disease resistance (Boutin et al., 2013; Zhang et al., 2018). Moreover, many existing and emerging disease issues in aquaculture result in skin and/or gill pathologies (Schmidt, Thompson and Padrós, 2018). Therefore, novel management strategies to enhance these mucosal microbial communities may play an important role in aquaculture health.

The relative success of prebiotics varies and depends on a range of factors including dose, time of supplementation, production methods, culture conditions and fish species, but they hold promise as strategies to take control of the microbial community of cultured fish (Merrifield et al., 2010; Akhter et al., 2015). However, studies of their effects in combination, and/or the impact on microbial communities outside the digestive systems remains understudied. In the following study, a novel prebiotic mix, composed of 1,3/1,6-beta glucans, mannan-oligosaccharides, nucleic acids, nucleotides, medium chain fatty acids and single chain fatty acids, is tested at different inclusion levels in Atlantic salmon (*Salmo salar*). The main goal of the study was to assess, using experimental feed trials and 16 S rRNA microbiome profiling, how the prebiotic blend impacts fish performance and the overall microbial community of the host, within both the gastrointestinal tract and the skin.

2. Methods

2.1. Feeding trials and sampling procedures

Nutrition fish trials were carried out at the Pontus research facility (Aberdare, Wales), using Atlantic salmon parr (*Salmo salar*), supplied by Cooke Aquaculture (average weight 32 g). 384 salmon were acclimatised for two weeks in a recirculating aquaculture system (RAS) prior to start of feeding experiment. The system consisted of 12 × 200 L tanks, with flow rates 400 L/hr per tank, and 10 % water changes to maintain water chemistry parameters (see below). The RAS was equipped with a drum filter and filter bag for mechanical filtering, a biological moving bed filter, an ultraviolet disinfection unit, as well as a degassing tower, with oxygen supplementation via an oxygen cone. Animal handling procedures were approved by Pontus animal ethics committee.

At the start of trials, fish were randomly distributed into 12 tanks (200 L), with triplicate tanks per treatment group. Fish were raised for 12 weeks on one of four experimental diets with different inclusion levels (0.0, 0.5, 1.0, 2.0 g/kg) of a blend of potential health stimulants and prebiotics. The feed was formulated and produced in cooperation with Sparos and Chemoforma AG (Augst, Switzerland), and composed of yeast derived mannans and,1,3/1,6-beta glucans, nucleic acids and nucleotides, medium chain fatty acids as well as single chain fatty acids.

The soy protein concentrate (SPC) and wheat were replaced as the inclusion of the prebiotic blend increases (Table 1). All diets were formulated to meet commercial standards and the principle nutritional requirements of Atlantic salmon parr (Table 1). The quality and nutritional profile of the feed was assessed by a proximate composition analysis (Stirling University). Fish were fed to satiation by hand, four times a day. Feed intake was monitored daily. Throughout the trial, tanks were exposed to an 8:16 h light: dark regime. Water parameters in the RAS system were maintained at 12.0 °C (± 1 °C), > 80 % oxygen saturation, pH 7.25 (± 0.15), < 0.03 mg/l ammonia, < 0.6 mg/l nitrite and < 75 mg/l nitrate, in accordance with optimal welfare conditions for salmon.

For the assessment of growth performance, batch weights (total biomass per tank) were taken at week 0, 6 and 12 of the trials. Fish were starved for 24 h prior to weighing. At the end of trial, ten random fish from each tank were individually weighed and measured for standard length. Growth performance was assessed using specific growth rate (SGR); percentage body weight gain per day. In addition, feed intake (FI) was calculated as percentage of body weight per day. Moreover, feed conversion ratio (FCR) was calculated as the ratio of feed intake to weight gain. Mortalities were recorded and summarized as overall survival (%). For microbiome analysis, five random fish from every tank were sampled (15 per treatment group). Fish were anaesthetized with MS222 (Methansulfonate, 200 mg/L) and killed by the destruction of the brain. Microbiome swab samples were taken from the skin and from the distal intestine. Skin swabs were run along the entire lateral body surface six times. For intestine samples, the whole intestine was removed using a sterile dissection kit. A 1 cm long piece of the distal intestine was cut, opened, and faecal residues removed using sterile distilled water, followed by rubbing the mucosal surface with a swab. All swab samples were immediately frozen and stored at - 80 °C until DNA extraction.

2.2. DNA extraction, 16 S rRNA amplification and sequencing

Total DNA was extracted from each skin and intestine sample using the Qiamp DNA mini kit, following manufacturer instructions. Extracted DNA was stored immediately at − 20 °C. A subset of the samples was quantified using the Qubit BR DNA assays to verify successful DNA extraction. PCR amplification and library preparation were performed by 2-step PCR targeting of the V1-V2 region of the 16 S rRNA gene. First round of PCR amplification used 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 338 R (5'- TCTGCTGCCTCCCGTAGGAGT −3') primers with addition of universal tails (Bohmann et al., 2021) and were performed in triplicates for each extraction sample. The PCR reaction volume was 25 ul, including 12.5 ul PCR mix (NEB Q5 Hotstart High fidelity PCR master mix), 0.5 ul of each primer (10 μM), 10.5 ul H_2O and 1 ul of DNA. The cycling protocol was as follows: 98 °C for 30 s, 35 cycles of 98 °C for 10 s, 55 °C for 30 s, 72 °C for 30 s and final elongation for 10 min. PCR products were visualised by agarose gel electrophoresis to ensure successful amplification. Negative controls for DNA extractions and PCRs, and a mock community as a positive control, were included for sequencing. PCR round 1 triplicates were pooled and cleaned using Agencourt AMPure XP beads according to manufacturer instructions. The second round of PCR introduced Illumina adapter sequences and unique indexes for samples identification (Bohmann et al., 2021). PCR round 2 conditions were as above using 15 cycles. Final PCR products were individually bead cleaned as above and quantified using Qubit BR DNA assays. Cleaned libraries were pooled equimolarly and sequenced using an Illumina MiSeq v2 2 × 250 bp run at Bangor University Centre for Environmental Biotechnology. Raw sequence data are available at the NCBI Short Read Archive (SRA) under accession PRJNA800661.

Table 1
Feed formulation based on nutritional requirement of the parr stage of Atlantic salmon [SPC=Soy Protein Concentrate, MCP=Monocalcium phosphate].

Diet	1 [Control]	2 [0.5 g/kg]	3 [1 g/kg]	4 [2 g/kg]
Fishmeal [g/kg]	300.0	300.0	300.0	300.0
Maize gluten [g/kg]	200.0	200.0	200.0	200.0
SPC [g/kg]	133.0	133.5	133.5	133.5
Sunflower meal [g/kg]	100.0	100.0	100.0	100.0
Wheat [g/kg]	68.8	67.8	67.3	66.3
Fish oil [g/kg]	159.5	159.5	159.5	159.5
Rapeseed oil [g/kg]	16.0	16.0	16.0	16.0
Experimental Blend [g/kg]	0	0.5	1	2
Premix [g/kg]	10.0	10.0	10.0	10.0
MCP [g/kg]	12.7	12.7	12.7	12.7
Total	1000.0	1000.0	1000.0	1000

2.3. Bioinformatics & statistics

Paired end demultiplexed sequencing reads were imported into Quantitative Insights Into Microbial Ecology 2 (QIIME2). Sequences were then quality filtered, trimmed, dereplicated, chimeras rejected, and pair-end reads merged in QIIME2 using DADA2 with standard settings. Reads were clustered by 99 % identity using the de-novo function. Classification of Amplicon Sequence Variants (ASVs) was performed using a scikit-learn naive Bayes machine-learning classifier trained using sequences representing the bacterial V1 – V2 rRNA region available from the SILVA database (https://www.arb-silva.de/download/archive/qiime;Silva_132), and taxonomic classifications were based on the q2-feature classifier in QIIME2. The classifier then assigned taxonomic information to representative sequences of each ASV. The QIIME2 output was further processed in RStudio (Version 4.0.3) with the package "phyloseq"(McMurdie and Holmes, 2013). Rarefaction analysis was used to determine sufficient read depth, samples with less than 10 000 sequences were excluded. Subsequent filtering excluded taxa with less than 100 reads and taxa found in only one sample. R was used to analyse significant differences in alpha (Pairwise Wilcoxon signed-rank test) and beta (Pairwise Adonis) diversity measures. Significant differential abundance of ASVs between fish fed the prebiotic blend and the control (no prebiotic) was determined using DESeq2 (FDR-corrected $p < 0.05$). In addition, significant differential abundances of all taxonomic levels (non-parametric Wilcoxon test, FDR-corrected $p < 0.05$) were assigned and visualised using the Microbiome Analyst heat trees(Chong et al., 2020). Growth performance indicators were tested in R for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). If normality and homogeneity was confirmed, significant ($p < 0.05$) differences between treatment groups were determined using a Tukey pairwise pos hoc analysis of the Anova results.

3. Results

Over the 12-week feeding trial period, survival was greater than 95 % in all treatments with no significant difference among groups (1 = 95 %, 2 = 98 %, 3 = 97 %, 4 = 98 %; Table 2). Specific growth rate (SGR) was marginally lower in the two highest inclusion level diet treatments; however, these differences were non-significant (Table 2). Feed intake

Table 2
Summary of average growth performance indicators of salmon parr on experimental diets. SGR = Specific Growth Rate, FI=Feed Intake, FCR=Feed Conversion Ratio, Standard deviation added.

Diet	1 [Control]	2 [0.5 g/kg]	3 [1 g/kg]	4 [2 g/kg]
SGR (% bw d-1)	0.92 ± 0.04	0.90 ± 0.07	0.83 ± 0.05	0.87 ± 0.03
FI (% bw d-1)	0.77 ± 0.01	0.76 ± 0.03	0.74 ± 0.03	0.73 ± 0.03
FCR (kg feed/kg gain)	0.83 ± 0.01	0.86 ± 0.06	0.91 ± 0.02	0.86 ± 0.07
End weight (g)	98.95 ± 1.07	95.04 ± 5.95	91.37 ± 1.66	91.70 ± 2.11
Survival (%)	95.83 ± 1.80	98.96 ± 1.47	97.92 ± 1.47	98.96 ± 1.47

and food conversion ratios were also non-significant among treatments.

A total of 9.5 million raw read pairs were produced from the 115 sequenced samples. After filtering and data pre-processing, a total of 7 million reads (average reads per sample 65 371, range = 12 820 – 119 551) were retained. Rarefaction curves determined samples with less than 10 000 reads should be removed due to insufficient read depth (all samples passed this criteria). For diversity tests, skin samples were rarefied to 12 820 reads and gut samples were rarefied to 22 247 reads per sample. In total, 266 ASVs for the gut and 330 ASVs for the skin were retained for further analysis.

In the skin, Shannon diversity of the third treatment (inclusion 1 g/kg) was significantly lower compared to all other experimental groups, (Fig. 1 A). No significant differences were observed between the other three groups. No differences could be determined for Chao 1 richness. In gut samples, we found no significant difference in either diversity measures, (Fig. 1 B).

Test of beta diversity of the distal intestine samples indicated significant group segregation ($p < 0.05$). This was largely driven by differences in the 1 g/kg inclusion group, (Fig. 1 D). The communities of this group were significantly different to the control group ($p = 0.003$) and the lowest inclusion level of the prebiotic ($p = 0.003$). Similar to the gut, within the skin samples the third treatment group was the biggest driver of group differences, although not significant (Fig. 1 C).

Proteobacteria were found to be the dominant phylum of microbial communities in both the gut and the skin (Fig. 2). At genus level, the skin microbial community is largely dominated by *Acinetobacter (38 %)*, followed by *Pseudomonas (15 %)* and *Alkanindiges (5 %,* Fig. 2 B), whereas the gut has a more evenly distributed community (Fig. 2 A), with *Pseudomonas (22 %)*, *Cupriavidus (9 %)* & *Janthinobacterium (8 %)* the most abundant taxa.

In comparison to the control group, the feed supplement induced more differences in the microbial community for the skin compared to the gut using both differential abundance tests (gut: 27 ASVs differentially abundant vs. skin: 44 ASVs, Figs. 3 & 4). In the skin samples, Proteobacteria and Bacteroidetes were significantly less abundant in fish fed the prebiotic blend, while Actinobacteria and Firmicutes were more abundant (Fig. 3 D-F). In contrast, no significant differences were found at phylum level in gut samples (Fig. 3 A-C). At genus level, *Bacillus*, *Mycetocola* and *Salinocola* were consistently more abundant in the gut of all prebiotic treatment groups compared to controls, whilst *Mobiluncus* and *Paraperlucidibaca* were less abundant in prebiotic-fed fish. The intermediate inclusion level (1 g/kg) showed the greatest impact on abundance of microbial taxa compared to the control group in the distal intestine, with genera such including *Corynebacterium* and *Paraperlucidibaca* less abundant compared to the control (Fig. 3 A-C). Similar to gut samples, *Bacillus* is among the genera that were increased in the skin of fish fed the prebiotic blend. Towards a higher inclusion level, the number of ASVs in the skin from the phylum of Bacteroidetes rise,

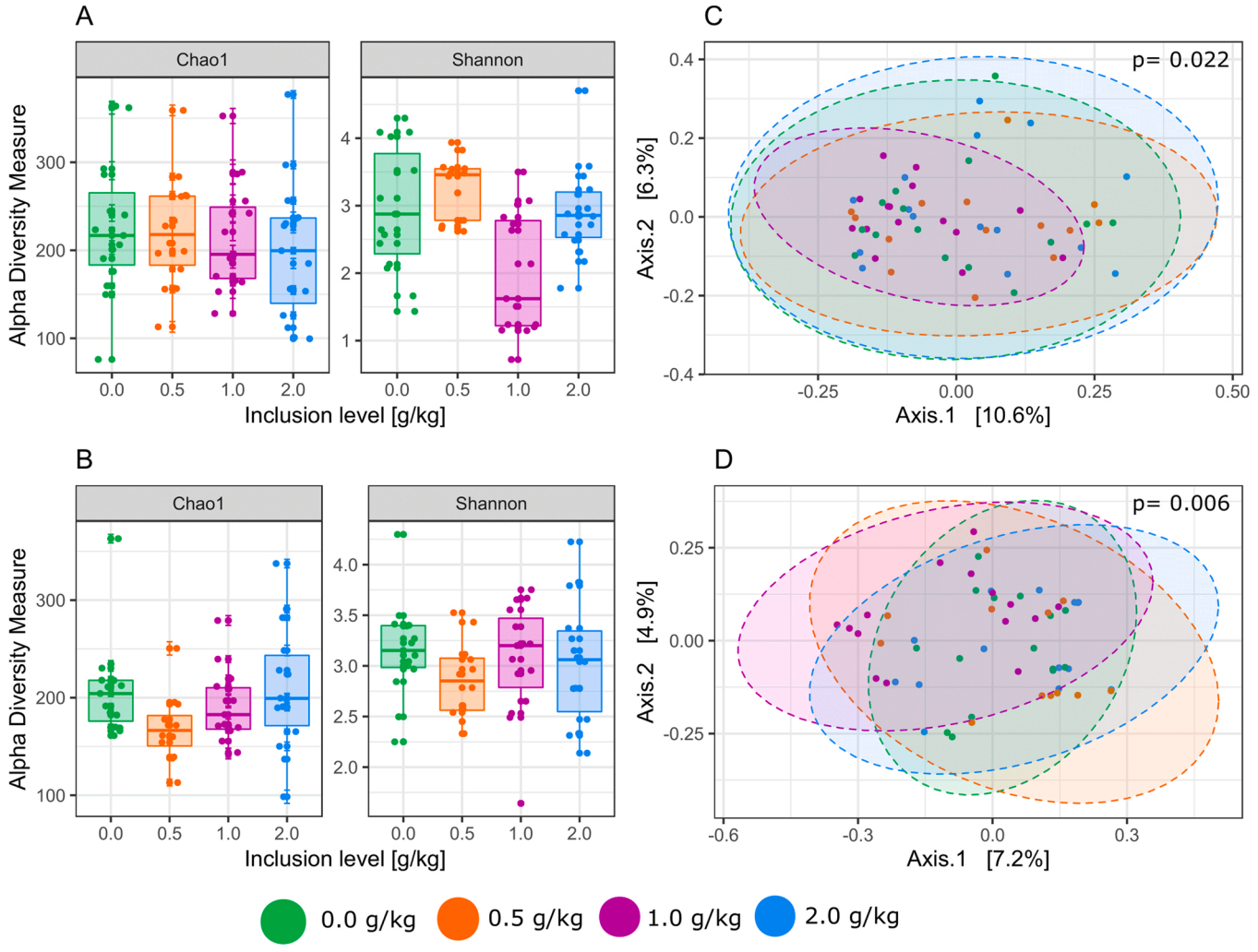

Fig. 1. Diversity of the microbial community in skin and the gut under prebiotic inclusion levels (green = 0.0 [control], orange = 0.5 g/kg, purple = 1.0 g/kg, blue = 2.0 g/kg), Alpha diversity measured by Chao1 and Shannon indices in the A) skin and B) gut. NMDS ordination of Beta diversity of C) skin and D) gut communities. Ellipses indicate 95 % confidence.

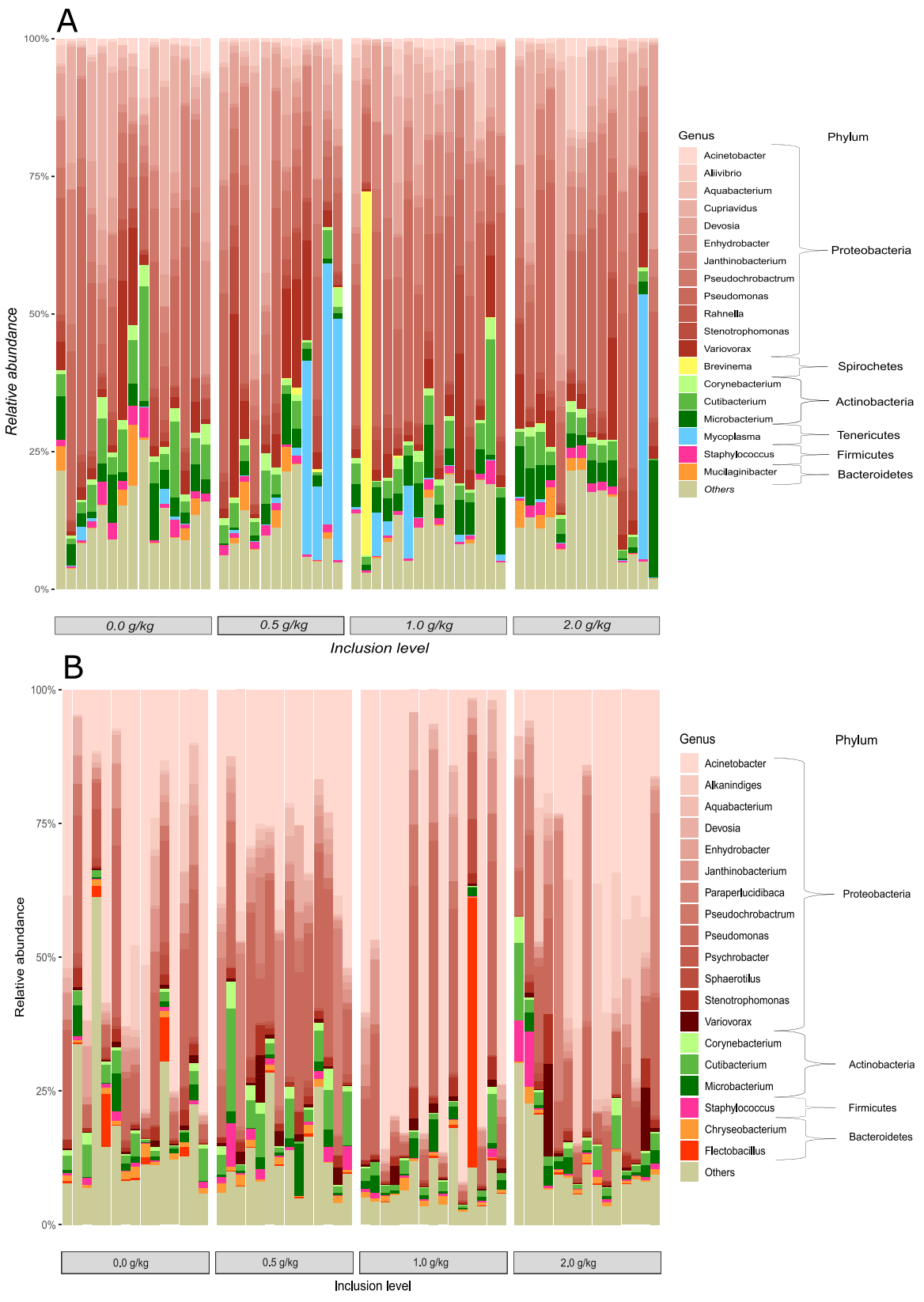

Fig. 2. Relative abundance of the top 20 genera of the microbial community in the A) gut and B) skin, colour shades separate taxa at Phylum level.

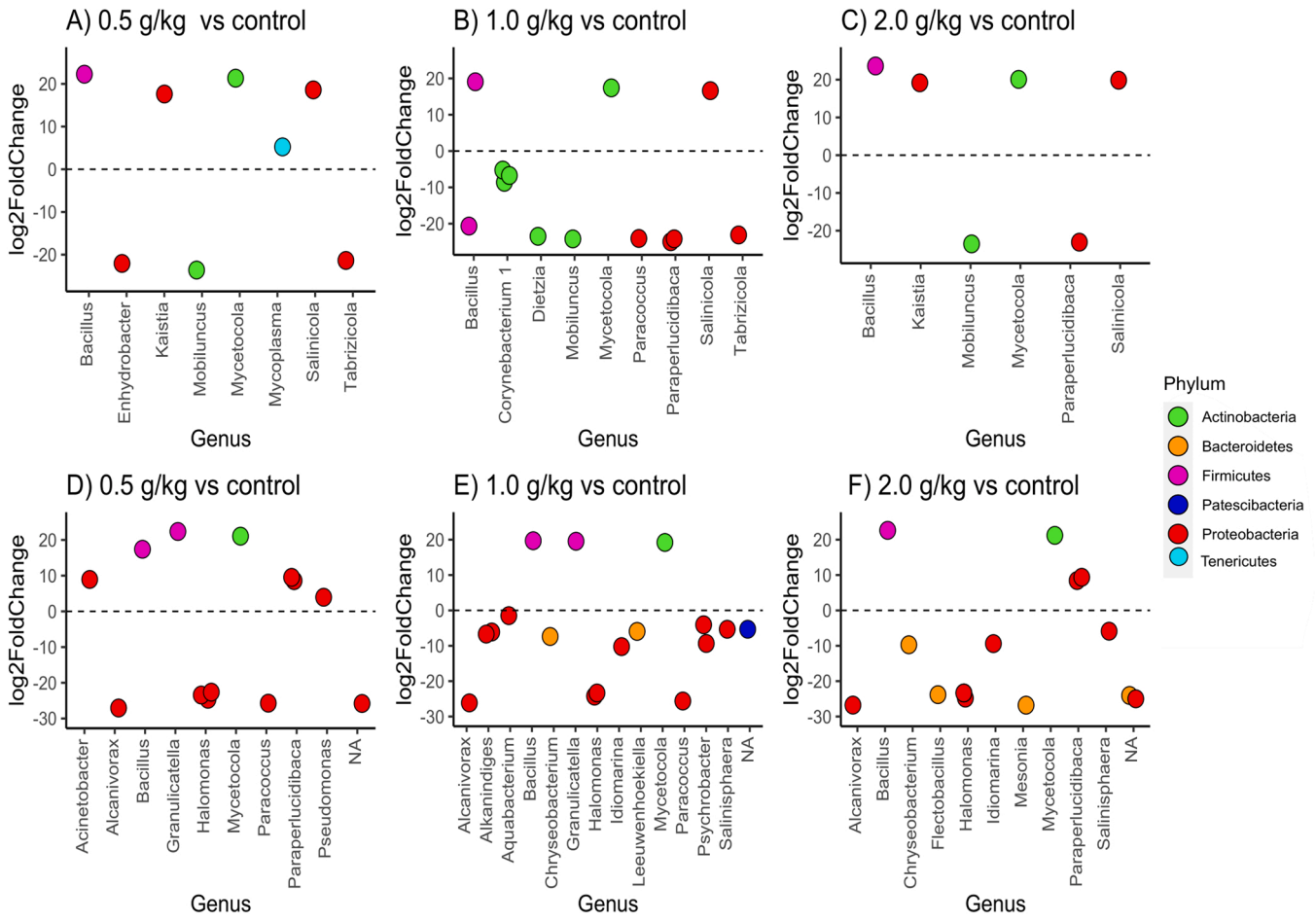

Fig. 3. Differential abundance analysis with Deseq2 for the gut (A, B, C) and the skin (D, E, F). Significant different (p ¡ 0.05) ASVs summarized to Genus level, differences in colours distinguish in Phylum levels.

including *Chryseobacterium*, *Flectobacillus* and *Mesonia* (Fig. 3 D-F).

4. Discussion

The present study compared the effects of different inclusion levels of Selectovit, a prebiotic blend (mannans and,1,3/1,6-beta glucans, nucleic acids and nucleotides, medium-chain fatty acids, single chain fatty acids), on growth performance indicators and the microbial composition of the skin and the distal intestine of Atlantic salmon. We demonstrate that the prebiotic supplementation, although has negligible effects on growth, induces significant changes in the mucosal microbiomes. Interestingly, we observed greater changes in the skin microbiomes, illustrating potentially important interactions between the use of nutritional prebiotic supplements and wider host health. Further interrogation of these interactions could lead to the development of specific bacterial biomarkers that reveal new insights into the overall health status of the fish and could result in novel strategies of health monitoring (O'Neill et al., 2016; Zheng, Liwinski and Elinav, 2020; Pessemier et al., 2021).

Supplementation of Atlantic salmon diet with Selectovit resulted in no significant effects on any growth performance indicators (SGR, FI, FCR, End weight and survival, Table 2). To our knowledge, the individual components of Selectovit have not been tested so far as a combined blend. However, the single components of the prebiotic blend (mannans and,1,3/1,6-beta glucans, nucleic acids and nucleotides, medium-chain fatty acids, single chain fatty acids) have been used widely in different cultured fish species (Selim and Reda, 2015; Torrecillas et al., 2015; Hisano et al., 2018). Interestingly, mannans and 1, 3/1,6-beta-glucans have been shown to have variable impacts on growth performance in salmon and trout. Whilst some studies indicate reduced growth performance (Grisdale-Helland, Helland and Gatlin, 2008; Refstie et al., 2010), others show no effect (Grisdale-Helland, Helland and Gatlin, 2008; Refstie et al., 2010; Dimitroglou et al., 2011) or improved growth (Staykov et al., 2007; Jami et al., 2019). Similarly varying growth results were found using nucleotides as a single prebiotic supplement in feeds (Xu et al., 2015; Bowyer et al., 2019). Recent findings suggest that the optimal dosage for best growth results using nucleotides stands at around 0.1 % and 0.2 % inclusion, however, the optimal dosage for mannans and glucans is highly variable and needs further investigation (Ringø et al., 2011; Akhter et al., 2015). The variation in growth performance could be correlated to differences in product composition. Mannans, 1,3/1,6-beta-glucans and nucleotides are typically derived from yeasts, that may vary in their properties and/or quality due to differences in processing, culture conditions or the genetic origin of the yeast (Klis et al., 2002; Kwiatkowski and Edgar, 2012; Orlean, 2012; Liu et al., 2021).

Although prebiotic supplementation regimes may not always affect growth, several studies have revealed their positive impacts on fish gut morphology (Ringø et al., 2011). Nucleotides, mannans and 1,3/1, 6-beta-glucans have all been shown to induce significant increases in microvilli length, thus increased the absorptive surface in the gut (Dimitroglou et al., 2011; Selim and Reda, 2015; Guo et al., 2017). As immunostimulants, prebiotics can enhance the activity of non-specific defence mechanisms and improve resistance to infectious diseases (Burrells, Williams and Forno, 2001; Akhter et al., 2015). For example, Pattern recognition receptors (PRRs) such as 1,3/1,6-beta-glucan

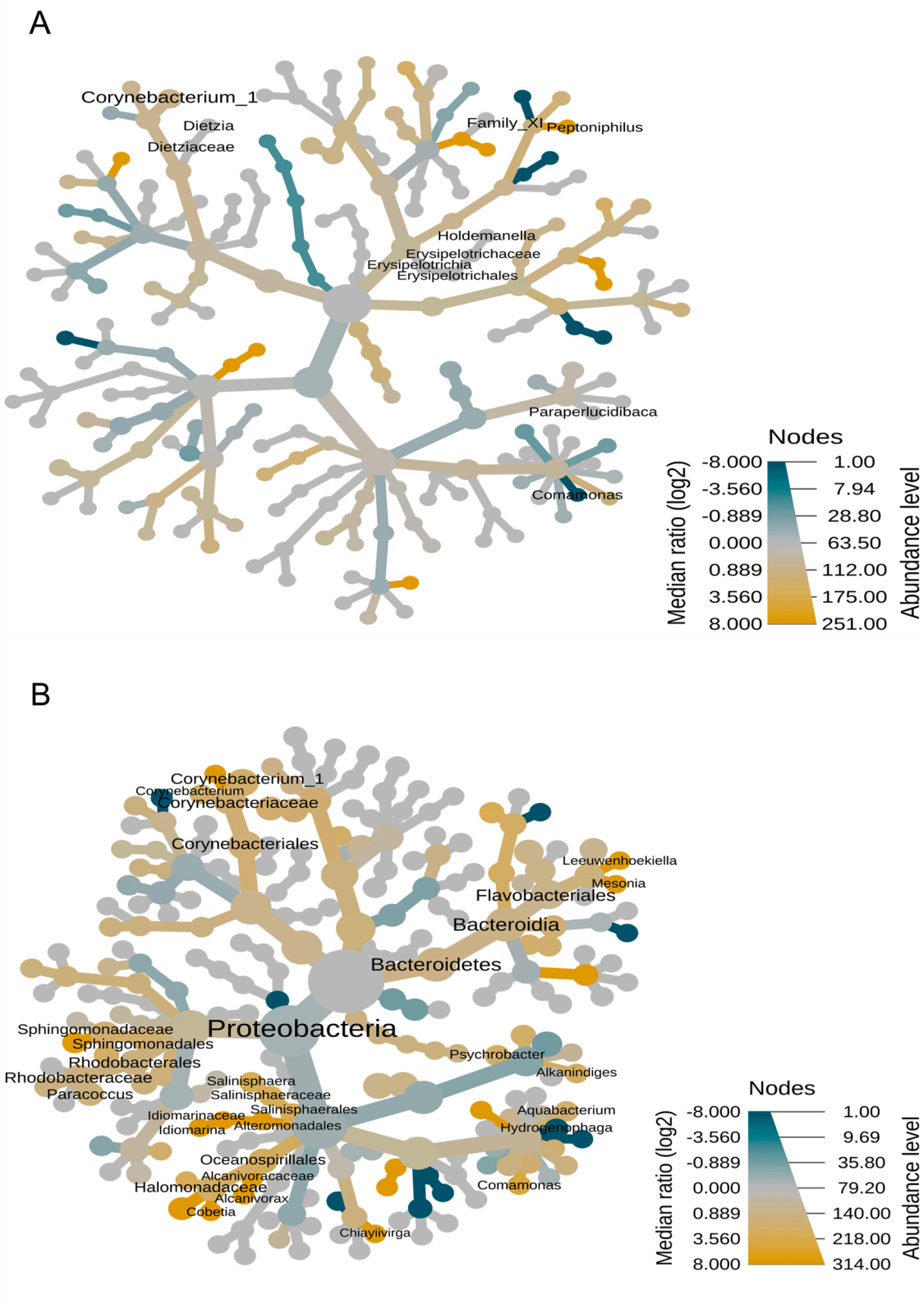

Fig. 4. Heat tree analysis Control vs third treatment (1 g/kg) for the A) gut and B) skin, significant different features are printed, yellow indicates higher abundance in the control, while green indicates greater abundance in the treatment group.

receptors expressed by macrophages, directly interact with 1,3/1, 6-beta-glucan supplements, resulting in their immunomodulatory activity (Akhter et al., 2015; Zheng, Liwinski and Elinav, 2020). Prebiotic-driven alterations in intestine morphology and/or host immune interactions can subsequently affect the microbial composition in the gut (Xu et al., 2015; Guo et al., 2017; Yang et al., 2020; Hu et al., 2021). The microbial composition of mucosal surfaces plays a vital role fish health, and thus is considered an important factor to ensure successful aquaculture production (de Bruijn et al., 2018; Wang et al., 2018; Bozzi et al., 2021).

In the present study, the prebiotic blend demonstrated no clear trend of overall microbial diversity in the gut and the skin (Fig. 1). The overall structure of abundant Phyla in the gut and the skin are similar to findings observed in recent studies of farmed Atlantic salmon, indicating Proteobacteria and Actinobacteria as the predominant groups (Wang et al., 2018, 2020; Uren Webster et al., 2021). However, prebiotic supplementation resulted in significant alterations in the relative abundance of certain bacteria taxa in both tissues (Figs. 3 & 4).

At genus level, *Bacillus* ASVs were consistently more abundant in the microbial community of the skin and the gut in fish fed the prebiotic blend at all inclusion levels (Fig. 3). Various species of this genus are considered being beneficial for the digestion and the health of the host. *Bacillus spp*. can synthesize various enzymes such as cellulase, amylase, protease and chitinase and thus can increase the overall digestibility of feeds. Moreover, cellulase and chitinase can break down feed components that are largely indigestible for carnivorous species (Ray, Ghosh and Ringø, 2012; Hodar et al., 2019). This can be beneficial for the aquaculture industry that is facing nutritional challenges induced by the increasing substitution of a marine-based product by alternative plant or insect-based sources (Refstie et al., 2000; Pratoomyot et al., 2010; Al-Thobaiti et al., 2018). As a health benefit, *Bacillus spp*. produce antimicrobial compounds that inhibit the growth of pathogenic bacteria and reduce the risk of disease outbreaks and mortalities. For example, increased survival and growth has been demonstrated in fish fed *Bacillus spp*. as a feed supplement (e.g. *Bacillus subtilis* and *Bacillus licheniformis*), including rainbow trout, challenged with *Yersinia ruckeri*, a common fish pathogen (Raida et al., 2003). In addition to the health and growth benefits, *Bacillus spp*. are spore-forming and can colonize the fish gut that enable its use as a probiotic. Due to their beneficial properties, *Bacillus spp*. are becoming widely used probiotic additives in the aquaculture industry (Rahman et al., 2021). In addition, *Mycoplasma* was more prevalent in the distal intestine of the treatment groups, and significantly more abundant in individuals fed 0.5 g/kg of the prebiotic blend (Fig. 2 A & 3A). *Mycoplasma* is typically found in the gut of healthy and faster-growing individuals of farmed Atlantic salmon and therefore considered a potential biomarker for health assessment (Bozzi et al., 2021; Cheaib et al., 2020).

In contrast, species of the genus *Chryseobacterium* are considered emerging fish pathogens in aquaculture production. For example, *Chryseobacterium pisciola* has caused mass mortalities in farmed rainbow trout and Atlantic salmon (Zamora et al., 2012). In this study, *Chryseobacterium* ASVs were significantly less abundant on the skin at higher inclusion levels of the prebiotic (Fig. 3 E & F), suggesting a positive effect of the prebiotic blend. Reduced growth of pathogenic bacteria can be explained by various pathways. Bacteria growth may be suppressed by antimicrobial compounds or niche exclusion by other commensal bacteria such as *Bacillus,* or improved host immune response (Kamada et al., 2013; de Bruijn et al., 2018; Zheng, Liwinski and Elinav, 2020). Indeed, a number of components of the tested prebiotic (e.g., 1,3/1, 6-beta-glucans, mannans) are known immunomodulators (Abid et al., 2013; Selim and Reda, 2015; Jami et al., 2019). Future studies to investigate the relative contribution of enhanced anti-pathogenic microbiome community members and/or beneficial immune stimulation via Selectovit supplementation are needed.

Interestingly, we found that the dietary supplementation induced greater changes in the microbial community in the skin than in the intestine (Figs. 3 & 4). This suggests a link between the skin microbiome and dietary changes. In zebrafish, a dietary probiotic containing *Lactobacillus rhamnosus* induced greater changes in the skin microbiome along with enhanced immune functions showing improved lysozyme activity, immunoglobulin concentrations and peroxidase activity (Hu et al., 2021). A previous study in Yellowtail Kingfish on the effect of gut enteritis on the gut and skin microbiota found diseased fish exhibited more microbial dysbiosis in the skin compared to the gut (Legrand et al., 2020). Further, multiple metabolic and immune pathways were up-regulated mostly in the skin, suggesting the high sensitivity of the skin mucosal surface to the host health (Legrand et al., 2020). In humans, the interaction between the gut and the skin is far more understood. There is evidence, that metabolic products such as short-chain fatty acids (SCFAs) from fibre fermentation in the gut have a crucial role in determining the microbial composition, thus subsequently influencing cutaneous immune defence mechanisms (Egawa, Honda and Kabashima, 2017; Salem et al., 2018). Metabolites produced by pathogenic bacteria are now considered biomarkers of human intestinal dysbiosis (O'Neill et al., 2016; Salem et al., 2018). These gastrointestinal-derived metabolites can circulate the host via the blood and subsequently accumulate in the skin, resulting in impaired epidermal differentiation and skin barrier integrity in humans (O'Neill et al., 2016; Pessemier et al., 2021). In addition, host pattern recognition receptors (e.g., toll-like receptors, TLRs) can sense gut derived microbial signals and induce skin-specific host immune response against pathogens but also shape the skin microbial composition (Akhter et al., 2015; Zheng, Liwinski and Elinav, 2020). Further investigation of the interactions between the gut health status and skin microbiota in fish could be highly beneficial to health monitoring in aquaculture. Specific changes in the skin microbiome – which can be assessed non-destructively - could be used as biomarkers to diagnose diseases and health issues in the gut (Xiong, Nie and Chen, 2019).

5. Conclusion

Overall, the inclusion of Selectovit as a prebiotic supplement for Atlantic salmon showed no significant impact on growth. However, we clearly demonstrate that the prebiotic can significantly manipulate the microbial community of the distal intestine and the skin. Several potential beneficial bacteria such as *Bacillus* and *Mycoplasma spp.* were significantly more abundant in the prebiotic-fed groups compared to the control. In contrast, pathogenic bacteria were less abundant in the fish fed the prebiotic blend. This suggests a positive effect of the prebiotic blend on the microbial composition along with several potential crucial health benefits that can be provided to the host. Findings support the use of prebiotics as a promising method to take control of the microbial communities in fish. Interestingly, the supplement induced more changes in the skin than in the gut. There is growing evidence in fish for highly complex interactions between the microbial communities of the digestive system and external mucosa, and with the host immune system. Further research in this field could lead to the creation of novel bacterial biomarkers and new non-invasive strategies for fish digestive health monitoring. In the future, it will be beneficial to investigate host gene expression, to assess the effect of prebiotics and the microbial community on host metabolic and immune pathways.

CRediT authorship contribution statement

Simon Baumgärtner: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Project administration. **Jack James**: Conceptualization, Supervision, Resources, Project administration. **Amy Ellison**: Supervision, Resources, Writing – review & editing, Project administration.

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.

Acknowledgments

This study was funded by the European Regional Development Fund through the Welsh Government (KESS2 East), Chemoforma AG (Switzerland) and The Fishmongers' Company (UK). AE was additionally supported by a Biotechnology and Biological Sciences Research

Council (UK) Future Leader Fellowship (BB/R010609/1). We thank Klaus Hoffmann (Chemoforma AG) and John Williamson (JW Nutrition Ltd) to provide us the prebiotic supplement (Selectovit) and the concept for the experiment. We thank staff at Bangor University Centre for Environmental Biotechnology for their assistance in generating sequence data. We thank members of Pontus Research for their assistance in performing the feed trial. Bioinformatic analyses were supported by the Supercomputing Wales project, which is part-funded by the European Regional Development Fund (ERDF) via Welsh Government. The mention of proprietary products does not constitute an endorsement of the product by Bangor University and does not imply its approval to the exclusion of other products that may also be suitable.

References

Abid, A., Davies, S.J., Waines, P., Emery, M., Castex, M., Gioacchini, G., Carnevali, O., Bickerdike, R., Romero, J., Merrifield, D.L., 2013. Dietary synbiotic application modulates Atlantic salmon (Salmo salar) intestinal microbial communities and intestinal immunity. Fish. Shellfish Immunol. 35 (6), 1948–1956. https://doi.org/10.1016/j.fsi.2013.09.039.

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

Akhter, N., et al., 2015. Probiotics and prebiotics associated with aquaculture: a review. In: Fish and Shellfish Immunology, 45. Elsevier Ltd, pp. 733–741. https://doi.org/10.1016/j.fsi.2015.05.038.

Al-Thobaiti, A., Al-Ghanim, K., Ahmed, Z., Suliman, E.M., Mahboob, S., 2018. Impact of replacing fish meal by a mixture of different plant protein sources on the growth performance in nile tilapia (Oreochromis niloticus L.) diets. Braz. J. Biol. 78 (3), 525–534. https://doi.org/10.1590/1519-6984.172230.

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 (1), 35–47. https://doi.org/10.1016/j.jcomm.2015.11.001.

Assefa, A., Abunna, F., 2018. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. Vet. Med. Int. 2018, 2018. https://doi.org/10.1155/2018/5432497.

Bank, T.W. (2014) 'Reducing Disease Risk In Aquaculture', *The World Bank. Agriculture and Environmental Services*, (88257), p. 119. Available at: ⟨http://documents.worldbank.org/curated/en/110681468054563438/Reducing-disease-risk-in-aquaculture⟩.

Bohmann, K., Elbrecht, V., Carøe, C., Bista, I., Leese, F., Bunce, M., Yu, D.W., Seymour, M., Dumbrell, A.J., Creer, S., 2021. Strategies for sample labelling and library preparation in DNA metabarcoding studies. Mol. Ecol. Resour. September, 1–16. https://doi.org/10.1111/1755-0998.13512.

Boutin, S., Bernatchez, L., Audet, C., Derôme, N., 2013. Network analysis highlights complex interactions between pathogen, host and commensal microbiota. PLoS ONE 8 (12), 1–16. https://doi.org/10.1371/journal.pone.0084772.

Bowyer, P.H., El-Haroun, E.R., Hassaan, M., Salim, H., Davies, S.J., 2019. Dietary nucleotides enhance growth performance, feed efficiency and intestinal functional topography in European Seabass (Dicentrarchus labrax). Aquac. Res. 50 (7), 1921–1930. https://doi.org/10.1111/are.14078.

Bozzi, D., Rasmussen, J.A., Carøe, C., Sveier, H., Nordøy, K., Gilbert, M., Limborg, M.T., 2021. Salmon gut microbiota correlates with disease infection status: potential for monitoring health in farmed animals. Anim. Micro. 3 (1), 30. https://doi.org/10.1186/s42523-021-00096-2.

de Bruijn, I., Liu, Y., Wiegertjes, G.F., Raaijmakers, J.M., 2018. Exploring fish microbial communities to mitigate emerging diseases in aquaculture. FEMS Microbiol. Ecol. 94 (1), 1–12. https://doi.org/10.1093/femsec/fix161.

Burrells, C., Williams, P.D., Forno, P.F., 2001. Dietary nucleotides: a novel supplement in fish feeds: 1. Effects on resistance to disease in salmonids. Aquaculture 199 (1–2), 159–169. https://doi.org/10.1016/S0044-8486(01)00577-4.

Cheaib, B. et al. (2020) 'Unpicking the mysterious symbiosis of Mycoplasma in salmonids'. doi: 10.1101/2020.07.17.209767.

Chen, S.W., Liu, C.H., Hu, S.Y., 2019. Dietary administration of probiotic Paenibacillus ehimensis NPUST1 with bacteriocin-like activity improves growth performance and immunity against Aeromonas hydrophila and streptococcus iniae in Nile tilapia (Oreochromis niloticus). In: Fish and Shellfish Immunology, 84. Elsevier, pp. 695–703. https://doi.org/10.1016/j.fsi.2018.10.059.

Chong, J., et al., 2020. Using MicrobiomeAnalyst for Comprehensive Statistical, Functional, and Meta-analysis of Microbiome Data. In: Nature Protocols, 15. Springer, US, pp. 799–821. https://doi.org/10.1038/s41596-019-0264-1.

Dailey, F.E., McGraw, J.E., Jensen, B.J., Bishop, S.S., Lokken, J.P., Dorff, K.J., Ripley, M. P., Munro, J.B., 2016. The microbiota of freshwater fish and freshwater niches contain omega-3 fatty acid-producing Shewanella species. Appl. Environ. Microbiol. 82 (1), 218–231. https://doi.org/10.1128/AEM.02266-15.

De Pessemier, B., Grine, L., Debaere, M., Maes, A., Paetzold, B., Callewaert, C., 2021. Gut–skin axis: Current knowledge of the interrelationship between microbial dysbiosis and skin conditions. Microorganisms 9 (2), 1–33. https://doi.org/10.3390/microorganisms9020353.

Dimitroglou, A., Reynolds, P., Ravnoy, B., Johnsen, F., 2011. The effect of mannan oligosaccharide supplementation on atlantic salmon smolts (Salmo salar L.) fed diets with high levels of plant proteins. J. Aquac. Res. Dev. s1 (SPEC. ISSUE 1), 1–6. https://doi.org/10.4172/2155-9546.S1-011.

Egawa, G., Honda, T., Kabashima, K., 2017. SCFAs control skin immune responses via increasing tregs. J. Invest. Dermatol. 137 (4), 800–801. https://doi.org/10.1016/j.jid.2016.12.022.

Egerton, S., et al., 2018. The gut microbiota of marine fish. Front. Microbiol. 9, 1–17. https://doi.org/10.3389/fmicb.2018.00873.

El-Haroun, E.R., Goda, A.M.A.S., Kabir Chowdhury, M.A., 2006. Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia Oreochromis niloticus (L.). Aquac. Res. 37 (14), 1473–1480. https://doi.org/10.1111/j.1365-2109.2006.01584.x.

FAO (2020) *The State of World Fisheries and Aquaculture 2020*.

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 199, 197–227. https://doi.org/10.1016/S0044-8486(01)00526-9.

Gainza, O., Romero, J., 2020. Effect of mannan oligosaccharides on the microbiota and productivity parameters of Litopenaeus vannamei shrimp under intensive cultivation in Ecuador. Sci. Rep. 10 (1), 1–12. https://doi.org/10.1038/s41598-020-59587-y.

Grisdale-Helland, B., Helland, S.J., Gatlin, D.M., 2008. The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (Salmo salar). Aquaculture 283 (1–4), 163–167. https://doi.org/10.1016/j.aquaculture.2008.07.012.

Guo, X., Ran, C., Zhang, Z., He, S., Jin, M., Zhou, Z., 2017. The growth-promoting effect of dietary nucleotides in fish is associated with an intestinal microbiota-mediated reduction in energy expenditure. J. Nutr. 147 (5), 781–788. https://doi.org/10.3945/jn.116.245506.

Hasan, M., Halwart, M., 2009. Fish as feed inputs for aquaculture: Practices, sustainability and implications. Pract., Sustain. Implic. 518, 407 (Available at). ⟨http://docs.niwa.co.nz/library/public/FAOfatp518.pdf⟩.

Hisano, H., Soares, M.P., Luiggi, F.G., Arena, A.C., 2018. Dietary β-glucans and mannanoligosaccharides improve growth performance and intestinal morphology of juvenile pacu Piaractus mesopotamicus (Holmberg, 1887). Aquac. Int. Aquac. Int. 26 (1), 213–223. https://doi.org/10.1007/s10499-017-0210-6.

Hodar, A.R., et al., 2019. Fishmeal and fish oil replacement for aquafeed formulation by using alternative sources - a review. J. Exp. Zool., India 23 (1), 13–21.

Hu, C., et al., 2021. 'Shift in skin microbiota and immune functions of zebrafish after combined exposure to perfluorobutanesulfonate and probiotic Lactobacillus rhamnosus'. In: Ecotoxicology and Environmental Safety, 218. Elsevier Inc,. https://doi.org/10.1016/j.ecoenv.2021.112310.

Jami, M.J., et al., 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). In: Fish and Shellfish Immunology. Elsevier,, pp. 202–208. https://doi.org/10.1016/j.fsi.2019.05.024.

Kamada, N., Chen, G.Y., Inohara, N., Núñez, G., 2013. Control of pathogens and pathobionts by the gut microbiota. Nat. Immunol. 14 (7), 685–690. https://doi.org/10.1038/ni.2608.

Klis, F.M., et al., 2002. Dynamics of cell wall structure in Saccharomyces cerevisiae. FEMS Microbiol. Rev. 26 (3), 239–256. https://doi.org/10.1016/S0168-6445(02)00087-6.

Kuebutornye, F., Abarike, E.D., Lu, Y., Hlordzi, V., Sakyi, M.E., Afriyie, G., Wang, Z., Li, Y., Xie, C.X., 2020. Mechanisms and the role of probiotic Bacillus in mitigating fish pathogens in aquaculture. Fish. Physiol. Biochem. 46 (3), 819–841. https://doi.org/10.1007/s10695-019-00754-y.

Kwiatkowski, S., Edgar, S., 2012. Yeast (saccharomyces cerevisiae) glucan polysaccharides – occurrence, separation and application in food, feed and health industries. Complex World Polysacch. https://doi.org/10.5772/48100.

Legrand, T.P.R.A., Wynne, J.W., Weyrich, L.S., Oxley, A.P.A., 2020. Investigating both mucosal immunity and microbiota in response to gut enteritis in yellowtail kingfish. Microorganisms 8 (9), 1–16. https://doi.org/10.3390/microorganisms8091267.

Li, T., Li, H., Gatesoupe, F.J., She, R., Lin, Q., Yan, X., Li, J., Li, X., 2017. Bacterial signatures of "red-operculum" disease in the gut of crucian carp (Carassius auratus). Microb. Ecol. 74 (3), 510–521. https://doi.org/10.1007/s00248-017-0967-1.

Liu, Y., et al., 2021. Structure, preparation, modification, and bioactivities of β-glucan and mannan from yeast cell wall: a review. Int. J. Biol. Macromol. 173, 445–456. https://doi.org/10.1016/j.ijbiomac.2021.01.125.

Ma, J., Bruce, T.J., Jones, E.M., Cain, K.D., 2019. A review of fish vaccine development strategies: conventional methods and modern biotechnological approaches. Microorganisms 7 (11). https://doi.org/10.3390/microorganisms7110569.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8 (4), 61217. https://doi.org/10.1371/journal.pone.0061217.

Merrifield, D.L., Rodiles, A., 2015. Mucosal Health in Aquaculture The fish microbiome and its interactions with mucosal tissues. Mucosal Health in Aquaculture. Elsevier Inc. https://doi.org/10.1016/B978-0-12-417186-2/00010-8.

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bøgwald, J., Castex, M., Ringø, E., 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302 (1–2), 1–18. https://doi.org/10.1016/j.aquaculture.2010.02.007.

Momeni-Moghaddam, P., Keyvanshokooh, S., Ziaei-Nejad, S., Parviz Salati, A., Pasha-Zanoosi, H., 2015. Effects of mannan oligosaccharide supplementation on growth, some immune responses and gut lactic acid bacteria of common carp (Cyprinus carpio) fingerlings. Vet. Res. Forum.: Int. Q. J. 6 (3), 239–244 (Available at). ⟨http://www.ncbi.nlm.nih.gov/pubmed/26893815%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4611979⟩.

Ni, J., Yan, Q., Yu, Y., Zhang, T., 2014. Factors influencing the grass carp gut microbiome and its effect on metabolism. FEMS Microbiol. Ecol. 87 (3), 704–714. https://doi.org/10.1111/1574-6941.12256.

O'neill, C.A., Monteleone, G., McLaughlin, J.T., Paus, R., 2016. The gut-skin axis in health and disease: a paradigm with therapeutic implications. BioEssays 38 (11), 1167–1176. https://doi.org/10.1002/bies.201600008.

Orlean, P., 2012. Architecture and biosynthesis of the Saccharomyces cerevisiae cell wall. Genetics 192 (3), 775–818. https://doi.org/10.1534/genetics.112.144485.

Perumal, S., Thirunavukkarasu, A.R., Pachiappan, P., 2015. Advances in marine and brackishwater aquaculture. Adv. Mar. Brackishwater Aquacult. 1–262. https://doi.org/10.1007/978-81-322-2271-2.

Pratoomyot, J., et al., 2010. Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (Salmo salar L.). In: Aquaculture, 305. Elsevier B.V, pp. 124–132. https://doi.org/10.1016/j.aquaculture.2010.04.019.

Rahman, A. et al. (2021) 'Effects of Probiotic Bacillus on Fish Growth Performance, Immune Response and Disease Resistance in Aquaculture', (March). doi: 10.20944/preprints202103.0075.v1.

Raida, M.K., Larsen, J.L., Nielsen, M.E., Buchmann, K., 2003. Enhanced resistance of rainbow trout, Oncorhynchus mykiss (Walbaum), against Yersinia ruckeri challenge following oral administration of Bacillus subtilis and B. licheniformis (BioPlus2B). J. Fish. Dis. 26 (8), 495–498. https://doi.org/10.1046/j.1365-2761.2003.00480.x.

Ray, A.K., Ghosh, K., Ringø, E., 2012. Enzyme-producing bacteria isolated from fish gut: a review. Aquac. Nutr. 18 (5), 465–492. https://doi.org/10.1111/j.1365-2095.2012.00943.x.

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 190 (1–2), 49–63. https://doi.org/10.1016/S0044-8486(00)00382-3.

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

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 (7), 1–27. https://doi.org/10.7717/peerj.5355.

Ringø, E., et al., 2011. Use of immunostimulants and nucleotides in aquaculture: a review. J. Mar. Sci.: Res. Dev. 02 (01) https://doi.org/10.4172/2155-9910.1000104.

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 (JUL), 1–14. https://doi.org/10.3389/fmicb.2018.01459.

Schmidt, J.G., Thompson, K.D., Padrós, R., 2018. Emerging skin diseases in aquaculture. Bull. Eur. Assoc. Fish. Pathol. 38 (3), 122–129.

Selim, K.M., Reda, R.M., 2015. Beta-Glucans and mannan oligosaccharides enhance growth and immunity in Nile Tilapia. North Am. J. Aquac. 77 (1), 22–30. https://doi.org/10.1080/15222055.2014.951812.

Shinn, A.P., Pratoomyot, J., Metselaar, M., Gomes, G.B. (2018) 'Diseases in Aquaculture - Counting the costs of the Top 100', *World Nutrition Forum S.C.O.P.E.*, (October), pp. 227–262.

Staykov, Y., Spring, P., Denev, S., Sweetman, J., 2007. Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (Oncorhynchus mykiss). Aquac. Int. 15 (2), 153–161. https://doi.org/10.1007/s10499-007-9096-z.

Stecher, B., Maier, L., Hardt, W.D., 2013. "Blooming" in the gut: How dysbiosis might contribute to pathogen evolution. In: Nature Reviews Microbiology, 11. Nature Publishing Group, pp. 277–284. https://doi.org/10.1038/nrmicro2989.

Teng, P.Y., Kim, W.K., 2018. Review: Roles of prebiotics in intestinal ecosystem of broilers. Front. Vet. Sci. 5 (OCT), 1–18. https://doi.org/10.3389/fvets.2018.00245.

Torrecillas, S., Montero, D., Caballero, M.J., Pittman, K.A., Custódio, M., Campo, A., Sweetman, J., Izquierdo, M., 2015. Dietary mannan oligosaccharides: counteracting the side effects of soybean oil inclusion on European sea bass (Dicentrarchus labrax) gut health? Front. Immunol. 6 (JUL), 1–15. https://doi.org/10.3389/fimmu.2015.00397.

Uren Webster, T.M., Consuegra, S., Garcia de Leaniz, C., 2021. Early life stress causes persistent impacts on the microbiome of Atlantic salmon. In: Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics, 40. Elsevier Inc. https://doi.org/10.1016/j.cbd.2021.100888

Wang, C., Sun, G., Li, S., Li, X., Liu, Y., 2018. Intestinal microbiota of healthy and unhealthy Atlantic salmon Salmo salar L. in a recirculating aquaculture system. J. Oceanol. Limnol. 36 (2), 414–426. https://doi.org/10.1007/s00343-017-6203-5.

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

Xiong, J.B., Nie, L., Chen, J., 2019. Current understanding on the roles of gut microbiota in fish disease and immunity. Zool. Res. 40 (2), 70–76. https://doi.org/10.24272/j.issn.2095-8137.2018.069.

Xu, L., Ran, C., He, S., Zhang, J., Hu, J., Yang, Y., Du, Z., Yang, Y., Zhou, Z., 2015. Effects of dietary yeast nucleotides on growth, non-specific immunity, intestine growth and intestinal microbiota of juvenile hybrid tilapia Oreochromis niloticus ♀ × Oreochromis aureus ♂. Anim. Nutr. 1 (3), 244–251. https://doi.org/10.1016/j.aninu.2015.08.006.

Yang, Q., Liang, Q., Balakrishnan, B., Belobrajdic, D.P., Feng, Q.J., Zhang, W., 2020. Role of dietary nutrients in the modulation of gut microbiota: a narrative review. Nutrients 12 (2), 1–57. https://doi.org/10.3390/nu12020381.

Zamora, L., Vela, A.I., Palacios, M.A., Domínguez, L., Fernández-Garayzábal, J.F., 2012. First isolation and characterization of Chryseobacterium shigense from rainbow trout. BMC Vet. Res. 8, 1–5. https://doi.org/10.1186/1746-6148-8-77.

Zhang, X., Ding, L., Yu, Y., Kong, W., Yin, Y., Huang, Z., Zhang, X., Xu, Z., 2018. The change of teleost skin commensal microbiota is associated with skin mucosal transcriptomic responses during parasitic infection by ichthyophthirius multifillis. Front. Immunol. 9 (December), 2972. https://doi.org/10.3389/fimmu.2018.02972.

Zheng, D., Liwinski, T., Elinav, E., 2020. Interaction between microbiota and immunity in health and disease. In: Cell Research. Springer, US, pp. 492–506. https://doi.org/10.1038/s41422-020-0332-7.