frontiers in Marine Science

ORIGINAL RESEARCH
published: 20 May 2021
doi: 10.3389/fmars.2021.665576

Dietary Lipid Effects on Gut Microbiota of First Feeding Atlantic Salmon (*Salmo salar* L.)

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Specialty section:
This article was submitted to Marine Fisheries, Aquaculture and Living Resources, a section of the journal Frontiers in Marine Science

Received: *08 February 2021*
Accepted: *21 April 2021*
Published: *20 May 2021*

Citation:
Nikouli E, Kormas KA, Jin Y, Olsen Y, Bakke I and Vadstein O (2021) Dietary Lipid Effects on Gut Microbiota of First Feeding Atlantic Salmon (Salmo salar L.). Front. Mar. Sci. 8:665576. doi: 10.3389/fmars.2021.665576

Decline in fish oil and fish meal availability has forced the aquaculture sector to investigate alternative and sustainable aquafeed ingredients. Despite that several studies have evaluated the effect of fish oil replacement in aquaculture fish species, there is a knowledge gap on the effects of alternative dietary lipid sources on the gut microbiota in early life stages of *Salmo salar*. The present study evaluated the influence of dietary administration of two different lipid sources (fish oil and vegetable oil) on the intestinal microbiota of first feeding Atlantic salmon (*S. salar*) up to 93 days post first feeding (dpff). The two diets used in this study, FD (fish oil diet) and VD (blend of rapeseed, linseed and palm oils diet), were formulated to cover the fish nutritional requirements. Apart from the lipid source, the rest of the feed components were identical in the two diets. Hindgut samples were collected at 0, 35, 65, and 93 dpff. Moreover, fertilized eggs, yolk sac larvae, rearing water and feed were also collected in order to assess a possible contribution of their microbiota to the colonization and bacterial succession of the fish intestines. To analyze the bacterial communities, amplicon sequencing was used targeting the V3–V4 region of the 16S rRNA gene. The findings indicate that feeding on either fish oil or vegetable oil-based diet, fish growth variables (mean wet weight and total length) did not differ significantly during the experiment ($p > 0.05$). No significant differences were also found between the two dietary groups, regarding their gut bacteria composition, after the analysis of the 16S rRNA sequencing data. Instead, gut microbiota changed with age, and each stage was characterized by different dominant bacteria. These operational taxonomic units (OTUs) were related to species that provide different functions and have been isolated from a variety of environments. The results also show little OTUs overlap between the host and rearing environment microbiota. Overall, this study revealed the occurrence of a core microbiota in early life of Atlantic salmon independent of the feed-contained oil origin.

Keywords: *Salmo salar*, larvae, gut microbiota, dietary intervention, fish oil replacement

INTRODUCTION

Fishmeal and fish oil have been the main ingredients in diets for farmed carnivorous fish species, providing the fed fish with the necessary proteins and lipids for high growth performance and resulting in a nutritionally rich final product (International Fishmeal and Oil Manufacturers Association [IFOMA], 2001; Turchini et al., 2010). Due to the declining availability of fishmeal and fish oil, their contents in feed are reduced (Hardy, 2010) and substituted by a variety of alternative feed ingredients. As changes in fish diet ingredients can alter the gut microbiota of fish species, it is important to evaluate the impact of these new diets with lower fish-meal and -oil contents on the composition of the gut microbial communities for reared fish species (for a review see Ringø et al., 2016).

In Atlantic salmon (*S. salar*), a carnivorous fish with significant economic value in European aquaculture (FAO, 2004), the effect of the alternative aquafeed ingredients on the gut microbiota have been evaluated previously and, in some cases, it was revealed that changes associated with intestinal disorders and slower growth performance, were related to the fishmeal diets (e.g., Green et al., 2013; Navarrete et al., 2013; Schmidt et al., 2016; Gajardo et al., 2017; Booman et al., 2018; Egerton et al., 2020). These studies, however, have focused mainly on alternative protein sources and on juveniles and adult stages.

Although feed is considered as the main factor that affect the gut bacterial communities in fish species, data from previous studies have also shown variations in gut microbial communities across development stages which seem to be affected not only by the provided feed but also from the microbial communities of the rearing environment (Bakke et al., 2013, 2015; Stephens et al., 2016; Dehler et al., 2017; Egerton et al., 2018). For example, recent work by Minich et al. (2020) recognizes the strong association between the build environment, i.e., tank biofilm and water from the hatchery installation, and Atlantic salmon mucosal microbiota. In a different salmonid species (rainbow trout), gut microbiota was detectable before first feeding commenced, potentially due to contact with the surrounding water and yok sac digestion, indicating that gut microbiota establishment initiates at first feeding and that diet-type affect the bacterial composition (Ingerslev et al., 2014a,b).

Moreover, it has been reported, that fish egg fragments are consumed from the newly hatched larvae, and their microbiome can affect gut microbiota colonization in fish species (Olafsen, 1984; Beveridge et al., 1991; Nikouli et al., 2019). The significant stage of the mouth opening, in aspect of larval microbiota manipulation, have been also recognized in shrimp larvae by Wang et al. (2020) in aquaculture conditions. In addition, evidence also suggest that as early life stages are more prone in environmental/climate changes, then probably is more crucial to study the microbiome shaping on these stages (Lowe et al., 2021). On the other hand, studies have shown that host development considered to had greater effect than hatching environment on the gut microbiota colonization and succession (Califano et al., 2017; Nikouli et al., 2019; Xiao et al., 2021).

Apart from a few studies, which have investigated the gut microbial communities in early life stages of Atlantic salmon (Llewellyn et al., 2016; Dehler et al., 2017; Lokesh et al., 2019), there is a knowledge gap on the effects of a different dietary lipid source on the gut microbiota in early life stages of this fish species, as only Clarkson et al. (2017) have partially investigated the impact of fish oil replacement by vegetable oils during a dietary experiment in diploid and triploid populations of Atlantic salmon. The objective of the present study was to evaluate the influence of total replacement of fish oil with a blend of terrestrial alternative oils (rapeseed, linseed and palm oils) on the intestinal microbiota of first feeding Atlantic salmon. We also characterized the bacterial communities of the rearing environment to determine their contribution in the early colonization and the succession of the fish intestines.

MATERIALS AND METHODS

Experimental Design and Sampling

The study was carried out within the Norwegian animal welfare act guidelines, in accordance with EU regulation (EC Directive 2010/63/EU), approved by the Animal Ethics and Welfare Committee of the Norwegian University of Science and Technology (case number 16/10070). The experiment was conducted at the Ervik hatchery (Frøya, Norway) as described previously in Jin et al. (2019). Briefly, a fast-growing Atlantic salmon aquaculture strain was cultivated from fertilized eggs until 93 days post first feeding (dpff). The two diets used in this study, FD (fish oil diet) and VD (blend of rapeseed, linseed and palm oils diet), were formulated to cover the fish nutritional requirements. Apart from the lipid source, the rest of the feed components were identical in the two diets (see **Supplementary Table 1**).

Each dietary treatment was tested in duplicated groups of 200 Atlantic salmon individuals (0.23 g $\pm$ 0.03/fish). On sampling days (0, 35, 65 and 93 dpff) 10 fish from each tank were randomly collected and sacrificed by immersion in 40 mg/L Benzocaine (BENZOAK VET, ACD Pharmaceuticals AS, Oslo, Norway). Furthermore, duplicate samples of rearing water (100 ml/tank) were collected and filtered through 0.2 μm membrane filters (GTTP, Millipore, United States) using a low (<1,500 mmHg) vacuum apparatus. For gut microbiota analysis, hindguts were removed by aseptic dissection and rinsed with ultra-pure water. Moreover, 10 fertilized eggs (EG), 10 yolk sac larvae (YS), and 0.25 g of the provided feeds were sampled in order to assess the contribution of their microbiota on the colonization of Atlantic salmon gut.

DNA Extraction and Sequencing

DNA was isolated from Atlantic salmon (eggs/yolk sac larvae/hindguts) and environmental (water/diets) samples by using the QIAGEN QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol "DNA Purification from Tissues." Bacterial communities were characterized by 16S rRNA amplicon sequencing. All samples analyzed individually and pooled prior the 16S rRNA analysis as follow: (a) DNA extracts from 5 individual fish samples (eggs, yolk sac larvae and hindguts) were pooled, resulting in two

pooled samples from each time point/fish tank (**Supplementary Table 2**) and (b) the DNA from the rearing water samples were pooled, resulting in 1 water sample per replicate tank ("STW"—initial stock tank, "FW"—rearing water from fish oil (FD) group and "VW"—rearing water from vegetable oil (VD) treatment.

PCR amplification and sequencing was performed at MRDNA Ltd.[1] (Shallowater, TX, United States) facilities on a MiSeq using paired end reads (2 × 300 bp) following the manufacturer's guidelines. A total of 37 samples (representing 30 pooled fish samples, 5 pooled water samples and 2 feed samples) was used in the final amplicon library. The 16S rRNA gene V3-V4 variable region PCR primers S-D-Bact-0341-b-S-17 and S-D-Bact-115 0785-a-A-21 (Klindworth et al., 2013) with barcodes on the forward primer (**Supplementary Table 3**) were used in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, United States) under the following conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. After that, the samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR product was used to prepare illumina DNA library.

Data Analysis

Sequencing raw data were processed with the MOTHUR software (version 1.40.5) (Schloss et al., 2009, 2011) and the operational taxonomic units (OTUs) were classified with the SILVA database release 132 (Quast et al., 2013; Yilmaz et al., 2014) following the methodology described in Nikouli et al. (2018). Identification of closest relative of the Most abundant OTUs was performed with Nucleotide Blast[2]. Raw sequence data from this study have been submitted to the Sequence Read Archive[3] with BioProject accession number PRJNA520982. Statistical analysis and graphical illustrations were performed in the Palaeontological STudies (PAST) software (Hammer et al., 2001) and in the R Studio platform Version 1.1.419 (RStudio Team, 2020), with 3.4.3 R version and enveomics.R package, Version 1.2.0 (Rodriguez-R and Konstantinidis, 2016).

RESULTS

Fish Growth Performance

The growth performance of the fish was evaluated throughout the experiment (see **Supplementary Table 4** and **Supplementary Figure 1**) and at none of the sampling points the mean wet weight or total length differed significantly across replicate tanks or between dietary treatments (FD and VD) ($p > 0.05$; **Supplementary Figure 1**). The initial (D0) mean weight was 0.23 ± 0.03 g (±SD). After 93 days (D93) the final mean weight was 4.58 ± 1.74 g for the group FD and 4.54 ± 1.78 g for the group VD. Regarding total mean length, the initial (D0) was 29.9 ± 1.6 cm which increased to 76.0 ± 8.9 cm and 73.8 ± 9.2 cm at D93 for the groups FD and VD, respectively.

Bacterial Diversity

The analysis of the 16S rRNA sequencing data revealed a total of 4,548 unique OTUs, with the rarefaction curves (**Supplementary Figure 2**) and the OTUs richness coverage based on the Chao1 index (**Supplementary Table 5**) indicating satisfactory sequencing depth. Diversity was considerable higher for rearing water (STW, FW, VW) than gut and diet samples, both in terms of OTU richness (**Table 1**) and evenness (**Supplementary Table 5**).

Taxonomic classification showed the presence of 21 bacterial phyla (**Figure 1** and **Supplementary Figure 3**). OTUs which were not classified to known bacterial phyla were only 3.0% of the relative abundance and are assigned as "Bacteria_unclassified." Proteobacteria (37.2%), Firmicutes (23.9%), Actinobacteria (18.8%), and Bacteroidetes (12.8%) were the dominant bacterial phyla in the dataset. The remaining 18 phyla (Planctomycetes, Verrucomicrobia, Patescibacteria, Dependentiae, Acidobacteria, Gemmatimonadetes, Fusobacteria, Cyanobacteria, Deinococcus-Thermus, Fibrobacteres, Armatimonadetes, Nitrospirae, Spirochaetes, Elusimicrobia, Omnitrophicaeota, Tenericutes, Chloroflexi, and Kiritimatiellaeota) were present with relative abundance ≤ 2%.

S. salar Microbiota

Comparing Atlantic salmon microbiota between the different life stages, fertilized eggs (EG) had the highest observed and estimated (Chao1) OTU richness (172 ± 100 and 222 ± 114, respectively). At the yolk sac stage (YS), the OTU richness decreased to 87 ± 0.7 and increased again at first feeding (D0). After that, OTU richness was on the same level until D93 when it decreased (**Table 1**). Proteobacteria was the dominant bacterial phylum in the samples, mainly due to γ- and β-Proteobacteria (**Supplementary Figure 4**). β-Proteobacteria was the dominant subphylum in pre-feeding stages (EG, YS, D0), with representatives mainly from the Burkholderiaceae and Chitinibacteraceae families (**Supplementary Figure 5**). However, in fertilized eggs (EG), OTUs representing β-Proteobacteriales were classified only at class level (44.1% of the total reads). γ-Proteobacteria dominated the period with active feeding (D35—D93) in both dietary treatments, with Pseudomonadaceae, Xanthomonadaceae, Vibrionaceae, Enterobacteriaceae, Moraxellaceae, and Aeromonadaceae as the most abundant families. However, their relative abundances differed between the two dietary treatments (**Supplementary Figure 6**). Actinobacteria, the dominant bacterial phylum at the late stages (D35 and D65) in vegetable oil dietary group (VD), was due to the high relative abundance of mainly Propionibacteriales, Corynebacteriales, and Micrococcales representatives. The presence of Firmicutes and Bacteroidetes was due to the classes Bacilli and Bacteroidia.

[1] www.mrdnalab.com

[2] https://blast.ncbi.nlm.nih.gov/Blast.cgi

[3] https://www.ncbi.nlm.nih.gov/sra/

TABLE 1 | Amplicon sequencing results of 16S rRNA gene diversity reported in all sample categories.

Samples	Reads	Observed OTUs richness	No. of the most dominant OTUs (cumulative relative dominance ≥ 80%)	Most abundant OTU (% of total reads) and closest relative (≥97%)
EG	22,151 ± 7168.6 N = 2	172 ± 99.7	16	SOTU0011 (23.9%)—*Methylotenera versatilis*
YS	14,382 ± 3186.2 N = 2	87 ± 0.7	10	SOTU0013 (19.4%)—*Delftia acidovorans*
D0	21,081 ± 1712.6 N = 2	132 ± 26.2	14	SOUT0009 (32.3%)—*Iodobacter fluviatilis*
D35F	7,658 ± 5011.0 N = 4	121 ± 64.8	46	SOTU0017 (9.3%)—*Pseudomonas viridiflava*
D65F	2,735 ± 1660.5 N = 4	110 ± 29.9	56	SOTU0070 (7.9%)—*Janthinobacterium agaricidamnosum*
D93F	2,003 ± 637.1 N = 4	93 ± 6.4	51	SOTU0005 (11.5%)—*Cloacibacterium normanense*
D35V	25,175 ± 27875.9 N = 4	135 ± 46.2	33	SOTU0005 (10.4%)—*Cloacibacterium normanense*
D65V	4,812 ± 1975.0 N = 3	132 ± 11.7	37	SOTU0005 (11.1%)—*Cloacibacterium normanense*
D93V	1,170 ± 608.3 N = 4	79 ± 25.3	46	SOTU0004 (7.0%)—*Weissella cibaria*
FD	21,022 N = 1	259	7	SOTU0004 (38.6%)—*Weissella cibaria*
VD	20,699 N = 1	216	8	SOTU0004 (37.8%)—*Weissella cibaria*
STW	53,280 N = 1	2,422	259	SOTU0001 (9.4%)—*Polynucleobacter necessaries*
FW	76,806 ± 11852.5 N = 2	1,683 ± 183.8	52	SOTU0001 (14.5%)—*Polynucleobacter necessaries*
VW	53,618 ± 8553.9 N = 2	1,100 ± 137.2	35	SOTU0001 (20.8%)—*Polynucleobacter necessaries*

Pre-feeding S. salar life stages: Eggs (EG), yolk sac larvae (YS) and "D0." Feeding stages of (a) fish oil group (FD): D35F, D65F, and D93F and (b) vegetable oil group (VD): D35V, D65V, and D93V. Rearing water: Pre-feeding tank (STW), fish oil group (FW) and vegetable oil group (VW). Feed: vegetable oil (VD) and fish oil feed (FD). N, Number of biological replicates analyzed; D, Day.

Microbial Communities in Diets and Rearing Water

The bacterial communities in feed samples, consisted almost entirely of Firmicutes (relative abundance of 84.2 and 82.1% in FD and VD, respectively; **Figure 1**). The Firmicutes were affiliated to the Lactobacillaceae (38.5 and 36.6% in FD and VD, respectively) and Leuconostocaceae families (37.9 and 38.8% in FD and VD, respectively). The rearing water samples (VW, FW, WST) contained mainly Proteobacteria, Actinobacteria and Bacteroidetes species, with Burkholderiaceae (β-Proteobacteria), Sporichthyaceae (Actinobacteria) and Chitinophagaceae (Bacteroidetes) as the most abundant families (**Figure 1**). In contrast to the experimental diets, Firmicutes in water samples were detected in relative abundance ≤ 1%.

Similarities Between Bacterial Communities

Statistical analysis revealed no significant differences (Tukey's test, $p > 0.05$, **Supplementary Table 6**) in the bacterial community composition of the Atlantic salmon samples between the pre-feeding stages (EG, YS, D0). However, EG and D0 samples differed significantly from those taken during the feeding period (D35—D93) in both dietary treatments, with stage D35 in VD group as the only exception. YS bacterial communities differed significantly ($p < 0.05$) with the bacterial communities only at D93 in both dietary groups (FD and VD). The gut microbiota of the host did not reveal significant differences between the two dietary groups for the different stages ($p > 0.05$), again with stage D35 in VD group as the only exception (**Supplementary Table 6**).

Further comparison of the bacterial community composition of Atlantic salmon hindguts, based on a Bray–Curtis distance matrix (**Figure 2**), showed a clear separation between bacterial communities in gut and bacterial communities of the rearing environment (water and diets). Moreover, the bacterial communities of the host were more similar with respect to life stages than to the diet treatments (**Figure 2** and **Supplementary Figure 7**), and this is also indicated through the similarity percentages analysis (SIMPER) based on Bray–Curtis distance. According to the results of the analysis the average dissimilarity among the groups of the same life stages was 76.0%, whereas the average dissimilarity within groups of the same dietary treatment was 78.5% (FD) and 83.6% (VD).

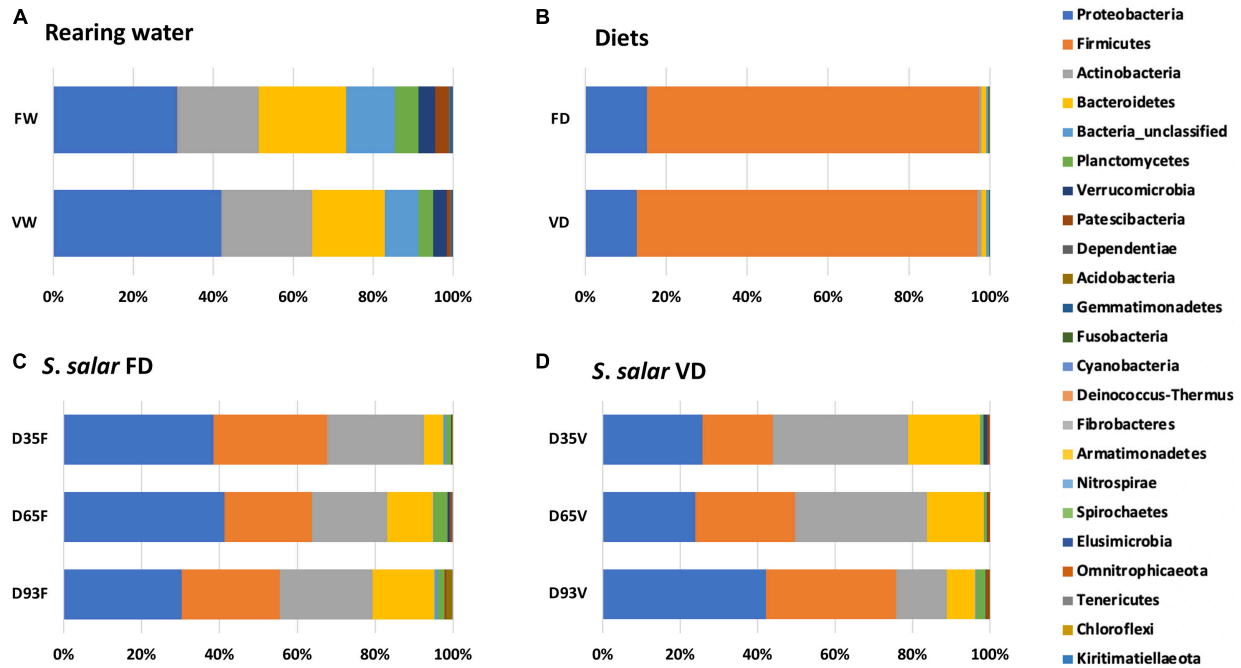

FIGURE 1 | Phylum composition of microbiota from rearing water **(A)**, diets **(B)** and hindgut of *S. salar* samples in Fish oil "FD" **(C)** and vegetable oil "VD" **(D)** dietary treatments, at D35, D65, and D93 post first feeding.

Common and Unique OTUs

Overall, only 2.3% of the OTUs were found in all sample types (rearing water, diets, pre- and after first feeding hindguts). 75.4% of OTUs occurred only in water samples (**Figure 3**). From the 1,004 OTUs detected in total in Atlantic salmon samples, 423 OTUs (9.3% of the OTUs) were unique in that type of samples. The majority of them (343 OTUs) were unique in the host at the active feeding stages, whereas 13 OTUs were shared among all samples independent of life stage or diet treatment.

DISCUSSION

In the present study, we evaluated the influence of dietary administration of two different lipid sources (fish oil and vegetable oil) on the gut bacterial communities of first feeding Atlantic salmon. Moreover, we characterized the bacterial communities from the rearing environment (rearing water and feeds) and the epibiotas of fertilized eggs and yolk sac larvae to determine their contribution in the bacterial colonization and succession of the gut. Previous studies suggest that the bacterial communities of the rearing environment, mainly from the rearing water and the feed, are important sources for community assembly of the intestinal microbiota of fish (Hansen and Olafsen, 1999; Nayak, 2010; McDonald et al., 2012; Scott et al., 2013; Bolnick et al., 2014; Eichmiller et al., 2016; Kashinskaya et al., 2018). For example, Schmidt et al. (2016), reported a significant effect on intestinal microbial communities in postsmolt Atlantic salmon following replacement of dietary fishmeal with plant ingredients. However, the results in the present study suggest that substitution of fish oil by vegetable oils did not significantly affect the composition of intestinal microbial communities in the same host species.

Furthermore, the results of the present study indicate little overlap between the bacterial communities of the host with that of the rearing environment (water and feed), whereas the life stage appeared to be the main factor affecting the structure of gut microbiota. These results are in agreement with previous findings from Llewellyn et al. (2016), who studied 96 wild-caught individuals of Atlantic salmon with different age and habitats and observed grouping of their intestinal bacterial communities based on the lifecycle stage. In addition, Lokesh et al. (2019), reported stage specific microbial enrichment in intestinal mucosa of the same host species (samples from embryonic stages up to 80 weeks post hatch). Similar stage specific signatures have also been reported across development in *Sparus aurata* (Nikouli et al., 2019), *Danio rerio* (Stephens et al., 2016), and *Gadus morhua* (Bakke et al., 2015) suporting further that the life stage seems to be the primary force shaping gut microbiota in juveniles' stages of fish. The change in microbiota with life stage can be due to both host-microbe (e.g., development in morphology and immune system) and microbe-microbe interactions (mutualism, commensalism and competition). The significance of these factors is, however, still not known.

Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were the dominant bacterial phyla detected in the host samples for both dietary treatments in our study. These bacterial phyla seem to characterize the bacterial communities in individuals of Atlantic salmon at the freshwater life cycle stages (Llewellyn et al., 2016). These bacterial phyla are also commonly found in

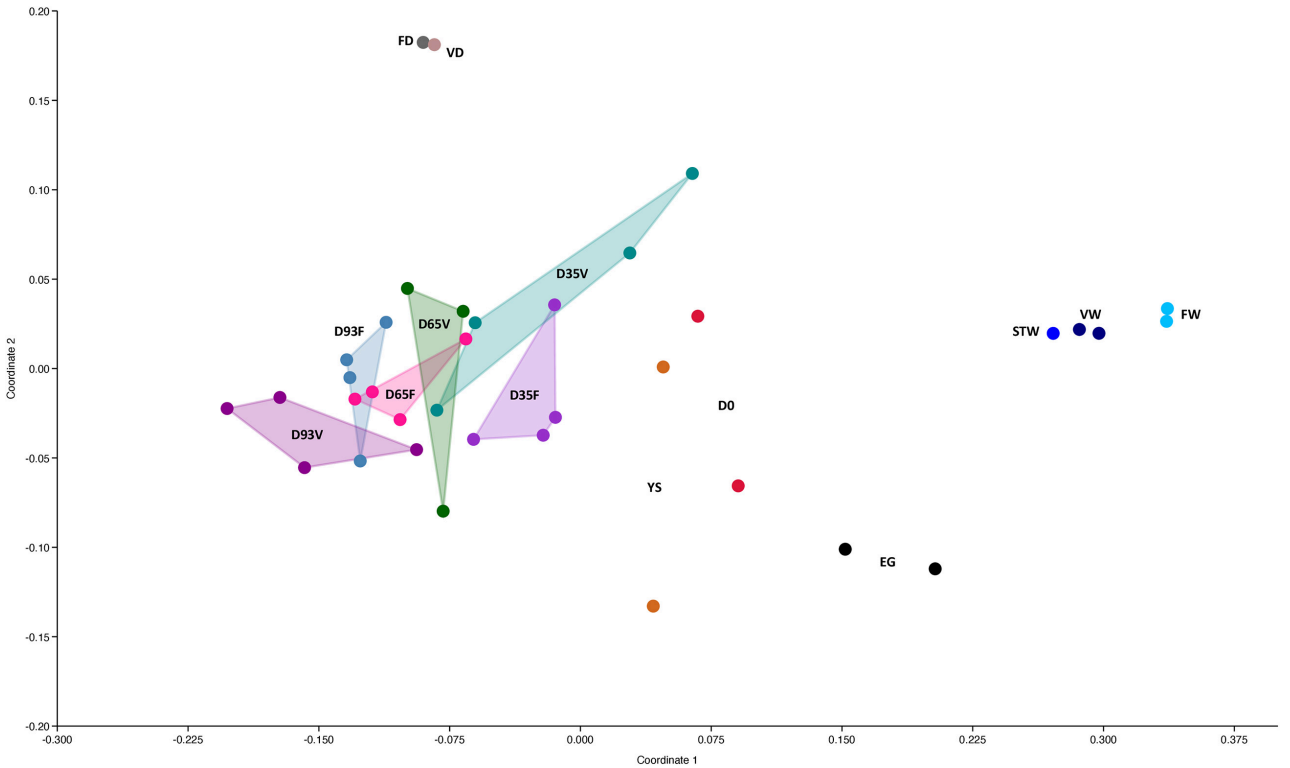

FIGURE 2 | Non-metric multidimensional scaling (nMDS) plot for all the bacterial communities of all sample categories based on Bray-Curtis distances. Pre-feding stages: Eggs (EG), yolk sac larvae (YS) and "D0". Feeding stages of (a) fish oil group (FD): D35F, D65F, and D93F and (b) vegetable oil group (VD): D35V, D65V, and D93V. Rearing water: Pre-feeding tank water (STW), Fish oil group rearing water (FW), vegetable oil group (VW). Feed: vegetable oil feed (VD) and fish oil feed (FD). D, Day.

the gut bacterial communities of both saltwater and freshwater fish species (Hansen and Olafsen, 1999; Nayak, 2010; McDonald et al., 2012; Navarrete et al., 2013; Bolnick et al., 2014; Kormas et al., 2014; Llewellyn et al., 2016; Stephens et al., 2016; Dehler et al., 2017; Tarnecki et al., 2017; Booman et al., 2018; Lokesh et al., 2019; Nikouli et al., 2018, 2019).

Despite the fact that the two experimental feeds contained almost entirely Firmicutes, the increase in relative abundance of Firmicutes in samples after the onset of feeding was not solely due to feed specific OTUs. It should also be noted that 26.4% of the bacterial representatives detected on fertilized eggs (EG) were not detected in the water of the incubation tank (WST). This support the view that the microbial communities of fish eggs may be vertically transmitted from their parents or horizontally from their breading tank (Hansen and Olafsen, 1989; Nikouli et al., 2019).

In agreement with previous studies (Schmidt et al., 2016; Lokesh et al., 2019; Nikouli et al., 2019) the observed species richness in water samples was always an order of magnitude higher than the richness of the host samples. Bacterial communities in rearing water did not show major shifts during the experiment. OTU0001 dominated at all time points, with closest relative the bacterial species *Polynucleobacter necessaries*. This species is commonly found in freshwater samples and it can contribute to the catabolism of urea and reduction of nitrate (Boscaro et al., 2013). The dominant bacterial species in Atlantic salmon samples are related with bacterial species from various habitats. The dominant OTU on fertilized eggs (OTU0011) was classified within the *Methylotenera* genus (β-Proteobacteria) and has previously been detected in fertilized eggs of the same host species by Lokesh et al. (2019). This genus consists of methylotrophic species that use methylamine as sole carbon, energy and nitrogen source (Kalyuzhnaya et al., 2006) and seem to be associated with RAS systems (Minich et al., 2020). The dominant OTU at the YS stage (OTU0013), seems to be related with *Delftia acidovorans* (β-Proteobacteria). Species of the genus *Delftia* are obligate anaerobes, organotrophic and non-fermentative organisms (Wen et al., 1999). They have previously been detected in the gut of healthy individuals of *Epinephelus coioides* (Sun et al., 2009), *Oncorhynchus mykiss* (Navarrete et al., 2012) and *Sparus aurata* (Kormas et al., 2014; Nikouli et al., 2018) and *S. salar* (Gajardo et al., 2016).

Just before onset on feeding (D0), the dominant OTU (OTU0009) showed similarities with the species *Iodobacter fluviatilis* of the Chitinibacteraceae (β-Proteobacteria) family. Species of this genus have been recorded mainly in sediment and water samples (Ryall and Moss, 1975; Wynn-Williams, 1983; Logan, 1989). Their presence on fish skin (*Oncorhynchus mykiss* and *Salmo trutta*) has been associated with skin lesions (Carbajal-González et al., 2011). However, they have previously been

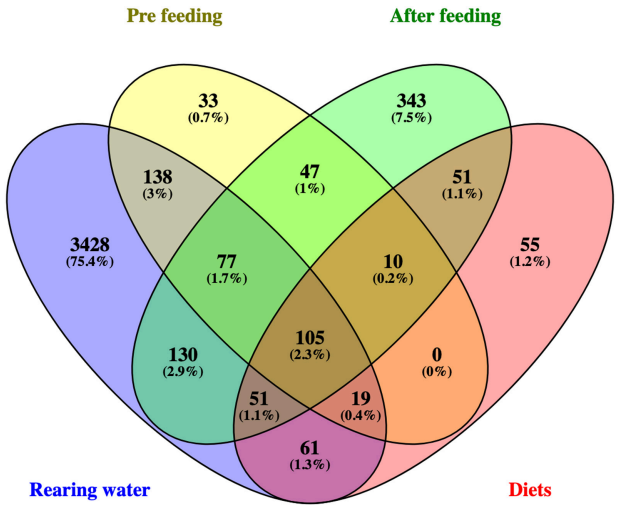

FIGURE 3 | Venn diagram demonstrating the number of the shared and unique operational taxonomic units (OTU) and their percentage of the total library, between S. salar samples [pre-feeding (EG,YS, D0) and post first feeding stages (D35, D65, D93 in both diets)], diets (FD and VD) and rearing water sample (STW, FW, VW).

detected in high relative abundance in healthy *Coreius guichenoti* individuals (Li et al., 2016) whereas the present study reports the presence of this bacterial species in Atlantic salmon gut microbiota for the first time.

After first feeding, although not statistically significant differences were found between the bacterial communities in the hindgut samples of the different life stages, each stage was characterized by different dominant OTUs. Moreover, gut bacterial communities differed also between dietary treatments regarding their dominant bacterial species (OTU). Chitinibacteraceae, the dominant bacterial family on D0 (with relative abundance 32.3%), was detected in ∼50× lower relative abundance (≤0.6%) in the rest of the samples. At D35 and D65 in FD treatment, the dominant OTUs (OTU0017 and OTU0070, classified as *Pseudomonas viridiflava* and *Janthinobacterium agaricidamnosum,* respectively), are described as plant (Alivizatos, 1986; Alimi et al., 2011; Taylor et al., 2011; Sarris et al., 2012) and mushroom pathogens (Lincoln et al., 1999; Graupner et al., 2015). According to recent findings, *Janthinobacterium lividum* (β-Proteobacteria) exhibits antimicrobial activity against multidrug resistant bacteria of clinical and environmental origin, such as Enterococci and Enterobacteriaceae (Baricz et al., 2018). Its presence in the gastrointestinal bacterial communities of Atlantic salmon, may have probiotic activity.

At D35 and D65, samples from the VD dietary treatment, were dominated by OTU0005, with closest relative *Cloacibacterium normanense* (Bacteroidetes). This OTU was also dominant at D93 in FD treatment. According to the literature, this species is frequently present in sewage treatment plants (Benedict and Carlson, 1971; Güde, 1980) where it contributes in the decomposition of complex organic compounds (Bernardet et al., 2002). Similar processes may take place in the intestinal system of Atlantic salmon at D35V, D65V, and D93F. The dominant OTU at D93 (OTU0004), also dominant in both provided feeds (FD, VD), was affiliated with *Weissella cibaria* (Firmicutes). This bacterial species belongs to the lactic acid bacteria, and has antimicrobial activity in the intestinal system of other fish species (Mouriño et al., 2016). Other *Weissella* spp. have been found in gut of *Oncorynchus mykiss* (Lyons et al., 2017; Mortezaei et al., 2020) and Atlantic salmon (Reveco et al., 2014; Godoy et al., 2015; Lokesh et al., 2019). It is worth noting that beside OTU0004, also OTU0013 and OTU0017 are associated with probiotic bacterial species (detected in all time points studied here, from EG to D93, independently of the dietary treatment). This observation suggests a co-evolutionary relationship of these bacterial species with the host studied here, and a possible specialized function in the hosts intestinal system.

CONCLUSION

The present study evaluated the effect of total fish oil replacement by a blend of terrestrial vegetable oils (rapeseed, linseed and palm oils) in the feed on the colonization and the bacterial succession in first feeding of Atlantic salmon, up to 93 days dpff. We demonstrated that feeding on either fish oil or terrestrial vegetable oil diets, did not result in significant differences in the intestinal gut microbiota and growth performance parameters (wet weight and total length). On the contrary, the composition of gut microbiota changed with age, and each stage was characterized by different dominant bacteria. These OTUs are related to species that may have probiotic activity to the host. Finally, this study revealed the occurrence of a core microbiota independent of the studied life stages and diet. These findings indicate that total fish oil replacement by terrestrial vegetable oils is feasible and can lead in low cost formulated feeds. Future work should aim on understanding the functional role of the detected core community which could lead in further feed, growth performance and host health optimization.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA520982.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics and Welfare Committee of the Norwegian University of Science and Technology (case no. 16/10070). The study was carried out within the Norwegian Animal Welfare Act guidelines, in accordance with EU regulation (EC Directive 2010/63/EU).

AUTHOR CONTRIBUTIONS

EN, KK, YO, IB, and OV: methodology. EN: formal analysis. EN and KK: data curation and writing—original draft preparation. EN, KK, YJ, YO, IB, and OV: writing—review and editing. OV: supervision. All authors contributed to the article and approved the submitted version.

FUNDING

EN was awarded with a visiting Ph.D. student fellowship by State Scholarships Foundation/IKY-Greece under the program "Scholarships of IKY in the Marine and Inland Management of Water Resources" and was co-funded by EEA Grants—Financial Mechanism 2009–2014 (85%) and the General Secretariat for Investments and Development (15%). YJ internal IBI funding, China.

ACKNOWLEDGMENTS

We would like to thank Jostein Ervik for rearing the fish, AquaGen AS for providing the fish, and Mari-Ann Østensen for assistance with the sampling and for her valuable suggestions during the planning and development of this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.665576/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.