RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 11: 2077-2088 (November 2026)

Comparative Metagenomic Profiling of Gut Microbiome Remodeling Induced by Dietary *Chlorella* and *Porphyra* in Goldfish (*Carassius auratus*)

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10.18805/IJAR.B-5915

ABSTRACT

Background: Dietary algae have gained considerable attention as natural feed additives in aquaculture owing to their rich nutritional composition and bioactive compounds that can beneficially modulate the intestinal microbiome and improve fish health. However, information regarding the comparative effects of microalgae and macroalgae on the gut microbial ecology of ornamental fish remains limited. Therefore, the present study evaluated the influence of dietary *Chlorella* sp. and *Porphyra* sp. supplementation on the gut microbiota of goldfish (*Carassius auratus*).

Methods: Two parallel feeding experiments were conducted for 75 days using seven isonitrogenous diets comprising a control diet and diets supplemented with *Chlorella* sp. (5, 7.5 and 10%) and *Porphyra* sp. (5, 10 and 15%). Gut microbial communities were characterized using 16S rRNA gene-based metagenomic sequencing to assess changes in microbial diversity and taxonomic composition following dietary supplementation.

Result: Metagenomic analysis revealed significant treatment-dependent alterations in gut microbial diversity and community structure. Algal-supplemented groups exhibited greater species richness and alpha diversity than the control, with the highest diversity observed in the 10% *Chlorella* (FVCT3) and 10% *Porphyra* (FVPT2) groups. Dietary algae also enriched beneficial bacterial genera, *Cetobacterium* and other genera like *Saccharothrix*, *Luteolibacter* and *Pseudoxanthomonas*, which are associated with nutrient metabolism, vitamin synthesis and intestinal homeostasis. These findings indicate that dietary *Chlorella* sp. and *Porphyra* sp. effectively reshape the gut microbiota of *C. auratus*, highlighting their potential as sustainable functional feed additives for improving intestinal health in ornamental aquaculture.

Key words: 16S rRNA sequencing, *Carassius auratus*, *Chlorella*, Metagenomics, *Porphyra*.

INTRODUCTION

The ornamental fish industry constitutes one of the most rapidly expanding sectors of global aquaculture and contributes substantially to international trade, employment generation and recreational fisheries. The global ornamental fish market is valued at over USD 15 billion annually, with billions of live fish traded worldwide (Raja *et al*., 2019). Among freshwater ornamental species, goldfish (*Carassius auratus*) occupies a prominent position owing to its remarkable phenotypic diversity, vibrant pigmentation, ease of culture and widespread consumer acceptance. In ornamental aquaculture, economic value is determined not only by growth and survival but also by external appearance, physiological fitness, stress tolerance and disease resistance. Consequently, the development of nutritionally balanced and health-promoting dietary interventions has emerged as a major priority for sustainable ornamental fish production.

The increasing concerns associated with the indiscriminate use of synthetic chemicals and antibiotic growth promoters have intensified the search for natural and environmentally sustainable feed additives in aquaculture. In this regard, algae have attracted considerable interest because of their exceptional nutritional profile and abundance of biologically active compounds (Mohammed and Al-Khamis, 2024). Microalgae and marine macro algae are rich sources of proteins, essential amino acids, vitamins, minerals,

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How to cite this article: Vala, F.D., Joshi, N.H. and Solanki, V.K. (2026). Comparative Metagenomic Profiling of Gut Microbiome Remodeling Induced by Dietary *Chlorella* and *Porphyra* in Goldfish (*Carassius auratus*). *Indian Journal of Animal Research*. **60(11):** 2077-2088. doi: 10.18805/IJAR.B-5915.

Submitted: 16-07-2026 **Accepted:** 13-08-2026 **Online:** 13-10-2026

polyunsaturated fatty acids, pigments, antioxidants and complex polysaccharides that exert beneficial effects on fish growth, metabolism and immune responses (Awad and Awaad, 2017; Holdt and Kraan, 2011). Among microalgae, *Chlorella* sp. has received particular attention owing to its high protein content and the presence of chlorophyll, carotenoids, vitamins and chlorella growth factor (CGF), which possess antioxidant, immunostimulatory and growth-promoting properties (Becker, 2007). *Chlorella* spp. characterized by high protein content, balanced essential amino acid profiles, polyunsaturated fatty acids, vitamins, pigments and antioxidant compounds, making them effective dietary supplements for improving growth performance, feed utilization, immune responses, antioxidant status and gut microbial composition in cultured fish (Ahmad *et al*., 2020). In contrast, macroalgae such as *Porphyra* spp. a marine red macro alga commonly known as nori contain comparatively lower protein levels but are rich in sulfated polysaccharides such as porphyran, minerals, vitamins, carotenoids and phenolic compounds that contribute to enhanced digestive health, antioxidant capacity, immunomodulation and disease resistance (Kim *et al*., 2014; Brawley *et al*., 2017) which exhibit antioxidant, antimicrobial and immunomodulatory activities.

Previous feeding studies have demonstrated that both microalgae and macroalgae can be effectively utilized in aquafeeds, although their nutritional roles differ. Microalgae such as *Chlorella vulgaris* (*Chlorophyta*) have consistently improved growth performance, feed efficiency, antioxidant capacity and immune responses in fish because of their high protein, essential fatty acids and pigment contents (Batista *et al*., 2020; Ahmad *et al*., 2023). In contrast, macroalgae such as *Porphyra dioica* (*Rhodophyta*), *Ulva rigida* (*Chlorophyta*) and *Gracilaria vermiculophylla* (*Rhodophyta*) have primarily enhanced intestinal health, antioxidant status, immune function and disease resistance through their abundance of polysaccharides, minerals and phenolic compounds, with comparatively smaller effects on growth performance (Silva *et al*., 2015; Peixoto *et al*., 2016). However, these studies evaluated microalgae and macroalgae independently under different experimental conditions and direct comparisons between representative microalgal and macroalgal species remain limited.

Beyond their nutritional significance, algal bioactive compounds have recently gained considerable attention for their ability to modulate the intestinal microbiome. The gastrointestinal tract of fish harbors a highly complex and dynamic microbial ecosystem that performs essential functions in digestion, nutrient metabolism, biosynthesis of vitamins, maintenance of epithelial integrity, immune regulation and protection against pathogenic microorganisms (Ghanbari *et al*., 2015). The composition and functionality of these microbial communities are strongly influenced by dietary composition, making nutrition one of the most effective approaches for manipulating gut microbial homeostasis and improving host health.

The advent of high-throughput sequencing technologies, particularly 16S rRNA gene sequencing, has revolutionized the understanding of fish gut microbial ecology. Unlike conventional culture-dependent methods, metagenomics approaches permit comprehensive characterization of both cultivable and uncultivable microorganisms inhabiting the gastrointestinal tract and provide detailed information on microbial diversity, taxonomic composition and community dynamics (Romero *et al*., 2014). Dietary modulation of the intestinal microbiota has been associated with enhanced nutrient assimilation, improved immune competence and increased resistance to environmental stress and infectious diseases in cultured fish species.

Previous investigations have demonstrated that dietary algae can improve growth performance, feed utilization, antioxidant status and immune responses in several aquaculture species (Hoseinifar *et al*., 2018; Sivakumar *et al*., 2018; Priyatharshni *et al*., 2024). Nevertheless, comparative information regarding the effects of microalgae and macroalgae on the intestinal microbial ecology of ornamental fishes remains limited. In particular, studies evaluating the influence of *Chlorella* sp. and *Porphyra* sp. on the gut microbiome of goldfish (*Carassius auratus*) are scarce. Therefore, the present study was undertaken to comparatively investigate the effects of dietary supplementation with *Chlorella* sp. and *Porphyra* sp. on the gut microbial community structure of *C. auratus* using 16S rRNA metagenomics analysis and to assess their potential as sustainable dietary interventions for improving intestinal microbial balance and promoting gut health in ornamental aquaculture.

MATERIALS AND METHODS

Experimental site and duration

The feeding experiment was conducted during 2024-2025 at the Wet Laboratory, Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India. Metagenomics sequencing and downstream bioinformatics analyses were performed at the Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat, India.

Experimental fish and husbandry conditions

Healthy juvenile goldfish (*Carassius auratus*) with an initial body weight of 1.85±0.003 g were procured and acclimatized to laboratory conditions before the commencement of the experiment. The fish were maintained in glass aquaria under continuous aeration and fed a basal diet during the acclimatization period. Subsequently, fish were randomly distributed into experimental units at a stocking density of ten fish per aquarium and reared under standard husbandry conditions throughout the experimental period.

Experimental design and dietary treatments

The experiment was conducted in a completely randomized design (CRD) using twenty-eight glass aquaria (35 L

capacity) for a period of 75 days. Seven isonitrogenous diets containing approximately 30% crude protein were formulated. The dietary treatments comprised a control diet without algal supplementation (FVT0), three diets supplemented with *Chlorella* sp. at inclusion levels of 5% (FVCT1), 7.5% (FVCT2) and 10% (FVCT3) and three diets supplemented with *Porphyra* sp. at inclusion levels of 5% (FVPT1), 10% (FVPT2) and 15% (FVPT3). Fish were fed twice daily at a feeding rate equivalent to 5% of body weight.

Hindgut sampling and sample preparation

At the end of the feeding trial, fish from all replicate aquaria within each treatment were pooled to obtain a single representative composite sample per treatment for DNA extraction and sequencing. Pooling was performed to reduce individual and tank-specific variation and minimize sampling bias. Fish were euthanized and dissected aseptically under a laminar airflow cabinet using sterile surgical instruments to prevent cross-contamination. The hindgut region was carefully excised and pooled intestinal samples from each treatment were collected in sterile microcentrifuge tubes and preserved at -80°C until further molecular analyses.

Metagenomic DNA extraction and quality assessment

Metagenomic DNA was extracted from hindgut samples using the QIAamp® DNA Stool Mini Kit (QIAGEN, Canada) according to the manufacturer's instructions with minor modifications. The concentration and purity of the extracted DNA were determined using a QIAxpert spectrophotometer (QIAGEN, Germany) and further quantified using the Qubit® dsDNA Broad Range Assay Kit (Thermo Fisher Scientific, USA). DNA integrity was evaluated by electrophoresis on 1% agarose gel. The quality of extracted DNA was further verified through amplification of the universal bacterial 16S rRNA gene using primers 27F (5′-AGAGTTTG ATCCTG GCTCAG-3′) and 1492R (5′-GGTTACCT TGTTACGACTT-3′) following the method described by Miller *et al*. (2013).

Amplification of the 16S rRNA gene and metagenomic sequencing

Amplicon libraries were prepared according to the standard Illumina protocol for 16S metagenomic library preparation. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′). The amplified libraries were validated using a bioanalyzer (Agilent Technologies, USA) and quantified with a Qubit Fluorometer v4.0 and dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, USA). Sequencing was performed on the Illumina NovaSeq 6000 platform using a 500-cycle paired-end chemistry (2 × 250 bp) on an SP flow cell.

Bioinformatic processing and statistical analyses

Raw sequencing data were processed using the Illumina BaseSpace 16S Metagenomics pipeline. The number of Illumina passing-filter (PF) reads per sample ranged from 197,627 to 291,413 and was used for downstream bioinformatics and microbial community analyses. Sequence read quality was assessed using FastQC. RefSeq Ribosomal Database Project (RDP) reference databases were used for taxonomic assignment. The microbial taxonomy was studied for statistical differences ($p<0.05$) between the studied groups and the core microbiota, Rarefaction analysis, alpha-diversity indices and relative abundance was compared using microbiome analyst. Statistical analysis of metagenomic profiles (STAMP) version 2.0.0 and PAST version 3.25 were employed for comparative analyses of microbial communities and diversity indices by performing the Kruskal-Wallis test. The results are presented as mean values and statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Experiment I: Effects of dietary *Chlorella* supplementation on the gut microbiome of goldfish (*Carassius auratus*)

Sequencing coverage and microbial richness

Rarefaction curves gradually approached saturation at higher sequence counts, indicating adequate sequencing depth and comprehensive representation of intestinal bacterial communities across all treatments (Fig 1). Among the dietary groups, FVCT3 exhibited the highest species richness, followed by FVCT2 and FVCT1, whereas the control (FVT0) showed the lowest richness. The distinct separation of rarefaction curves indicated treatment-specific differences in microbial community composition.

Alpha-diversity responses

Alpha-diversity analysis revealed progressive increases in microbial diversity with increasing levels of dietary *Chlorella* supplementation (Fig 2 and Table 1). FVCT3 exhibited the highest microbial diversity, followed by FVCT2 and FVCT1, while the control group displayed the lowest diversity indices.

Phylum-level taxonomic composition

Dietary supplementation with *Chlorella* induced marked alterations in phylum-level bacterial composition (Fig 3). FVCT2 showed relatively greater abundances of Nitrospirota, Thermomicrobiota, Actinomycetota and Gemmatimonadota. FVCT3 was characterized by increased abundance of Cyanobacteriota, Bacillota, Mycoplasmatota and Campylobacterota. In contrast, the control group was predominantly composed of Pseudomonadota, Acidobacteriota, Verrucomicrobiota and Chloroflexota. FVCT1 exhibited higher abundances of Bacteroidota, Fusobacteriota and Spirochaetota.

Genus-level microbial community composition

Genus-level analysis demonstrated pronounced treatment-dependent differences in microbial community structure (Fig 4 and Table 2). FVCT3 exhibited increased abundances of *Cetobacterium*, *Luteolibacter*, *Pseudoxanthomonas* and

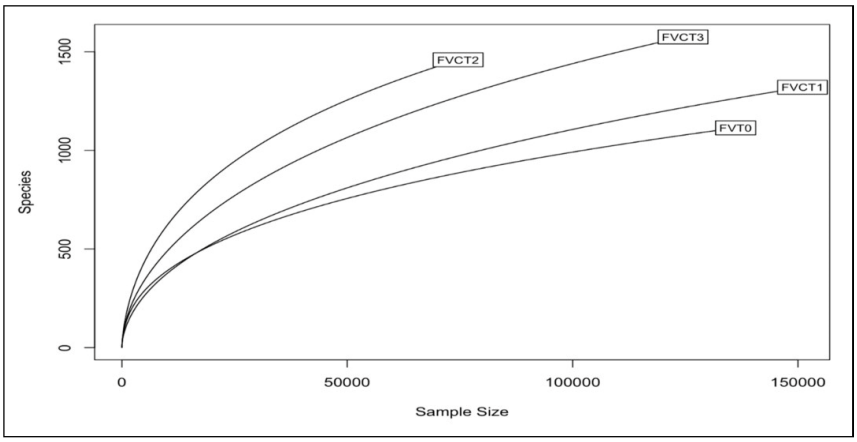


Fig 1: Rarefaction curves illustrating sequencing depth and observed species richness of the intestinal bacterial communities in goldfish (*Carassius auratus*) fed diets supplemented with different levels of *Chlorella* sp.

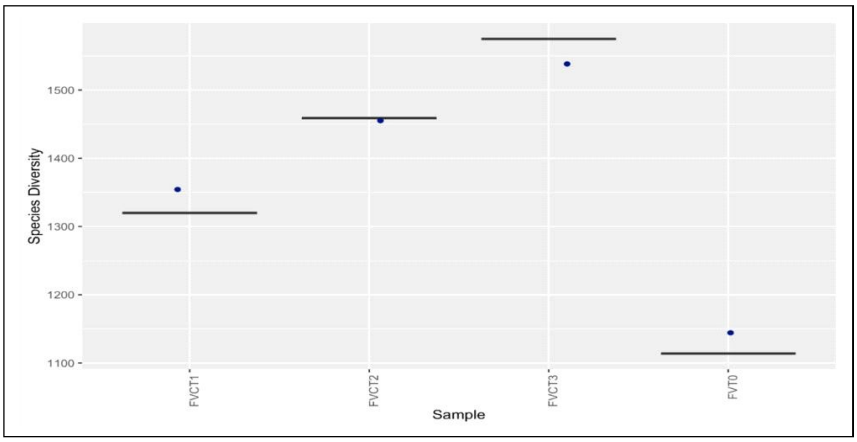


Fig 2: Alpha diversity (Observed species richness) of the intestinal bacterial communities in goldfish (*Carassius auratus*) fed diets supplemented with different levels of *Chlorella* sp., with statistical differences among treatments determined using the kruskal-wallis test (P<0.05).

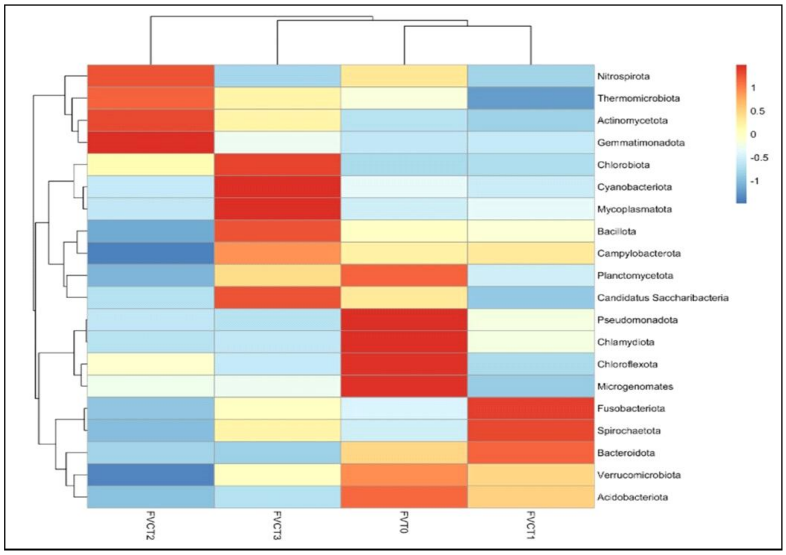


Fig 3: Comparative phylum-level taxonomic profiles of the gut bacterial communities in goldfish (*Carassius auratus*) following dietary *Chlorella* sp. supplementation.

Saccharothrix. The control group showed comparatively lower abundances of these genera and was dominated by fewer bacterial taxa.

Species-level microbial composition

The intestinal microbiota across all treatments was predominantly composed of *Cetobacterium somerae*, *Luteolibacter gellanilyticus*, *Pseudoclavibacter soli* and *Pseudoxanthomonas mexicana* (Fig 5). FVCT1 showed enrichment of *Roseicitreum antarcticum*, *Flavihumibacter cheonanensis*, *Roseomonas oryzicola* and *Rhodobacter flagellatus*. FVCT2 exhibited increased abundance of *Uruburuella testudinis*, *Dielma fastidiosa* and *Bdellovibrio bacteriovorus*. FVCT3 was characterized by enrichment of *Luteolibacter algae*, *Terrimicrobium sacchariphilum*, *Porphyromonas pogonae*, *Planctomicrobium piriforme*, *Novipirellula aureliae*, *Rhizobium qilianense* and *Thermoclostridium caenicola*. The control group was

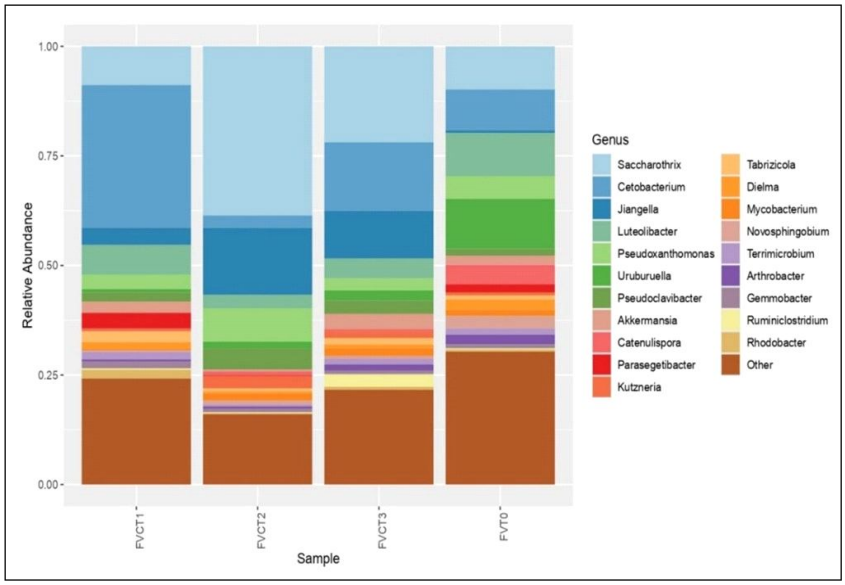


Fig 4: Genus-level taxonomic composition and relative abundance of the intestinal bacterial communities in goldfish (*Carassius auratus*) fed diets supplemented with different inclusion levels of *Chlorella* sp., illustrating treatment-dependent changes in gut microbiota composition.

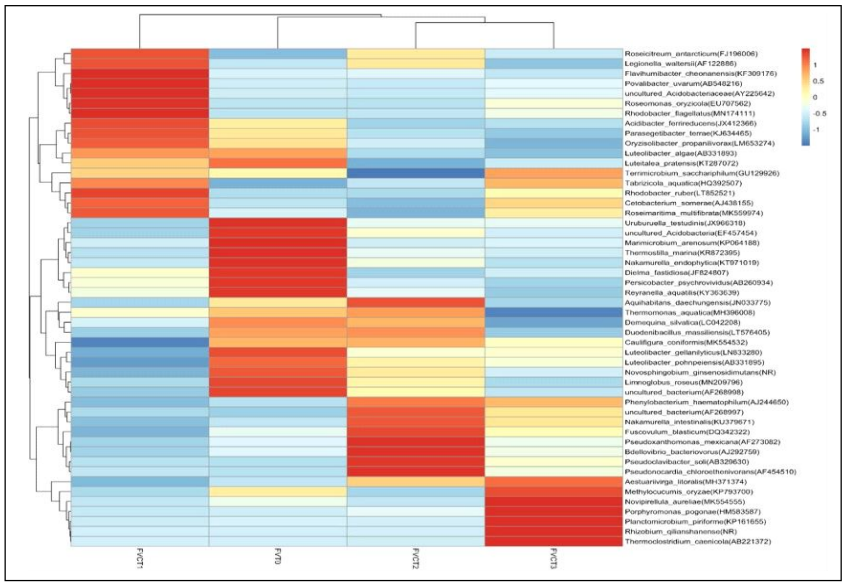


Fig 5: Heatmap showing the species-level microbial signatures and relative abundance patterns of the intestinal microbiota in goldfish (*Carassius auratus*) following dietary supplementation with different levels of *Chlorella* sp., highlighting treatment-specific shifts in bacterial community composition.

enriched with *Rhodobacter ruber* and *Povalibacter uvarum*.

Experiment II: Effects of dietary *Porphyra* supplementation on the gut microbiome of goldfish (*Carassius auratus*)

Sequencing coverage and microbial richness

Rarefaction curves gradually reached saturation, indicating adequate sequencing coverage and reliable characterization of intestinal bacterial communities (Fig 6). FVPT2 consistently exhibited the highest species richness, followed by FVPT3 and FVPT1. In contrast, the control group displayed the lowest richness.

Alpha-diversity responses

Alpha-diversity analysis demonstrated significant treatment-dependent differences in microbial diversity (Fig 7 and Table 1). FVPT2 exhibited the highest microbial diversity and species evenness, followed by FVPT3 and FVPT1, while the control group consistently showed the lowest diversity indices.

Phylum-level taxonomic composition

Dietary supplementation with *Porphyra* substantially altered phylum-level microbial composition (Fig 8). FVPT2 showed increased abundances of Fusobacteriota, Actinomycetota and Bacteroidota, whereas FVPT3 exhibited greater abundance of Bacillota and Pseudomonadota. The control group showed comparatively lower taxonomic diversity and was dominated by fewer bacterial phyla.

Genus-level microbial community composition

Genus-level analysis revealed pronounced restructuring of the intestinal microbiota following *Porphyra* supplementation (Fig 9 and Table 2). FVPT2 exhibited increased abundances of *Cetobacterium*, *Luteolibacter*, *Pseudoxanthomonas* and several polysaccharide-utilizing bacterial genera.

Species-level microbial composition

Cetobacterium somerae remained consistently abundant across all treatments, with the highest abundance observed

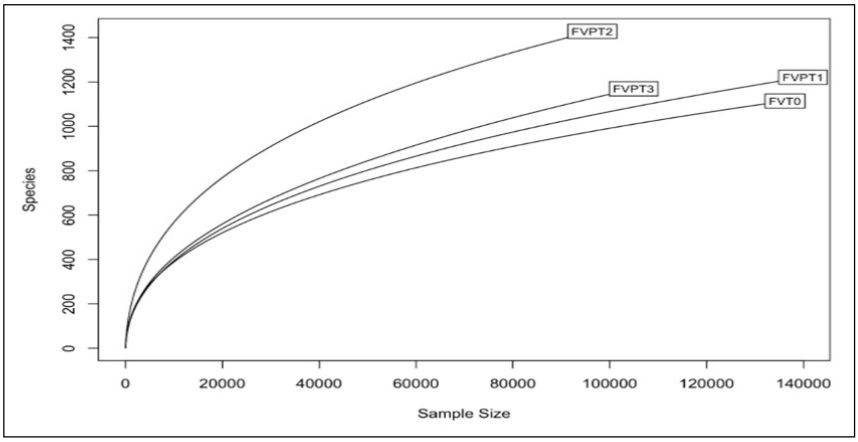


Fig 6: Rarefaction analysis demonstrating sequencing coverage and microbial species richness in the gut microbiota of goldfish (*Carassius auratus*) receiving *Porphyra* sp.-supplemented diets.

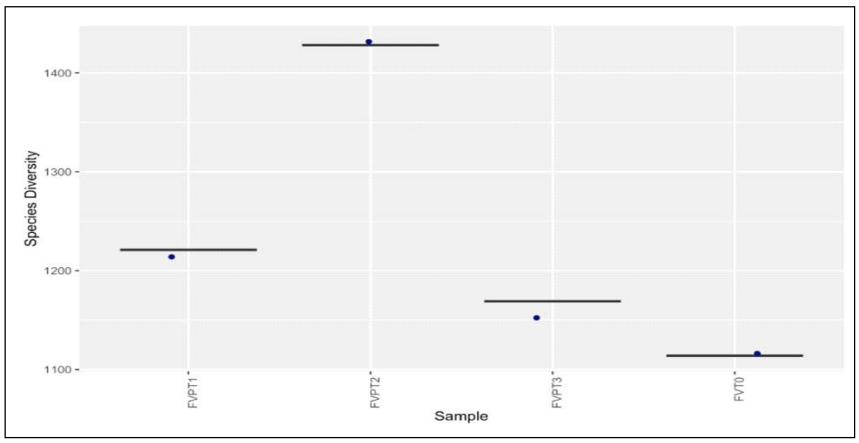


Fig 7: Alpha-diversity indices depicting gut microbial community structure in goldfish (*Carassius auratus*) fed experimental diets containing *Porphyra* sp (P<0.01).

in FVPT3 (Fig 10). Core bacterial species, including *Luteolibacter gellanilyticus*, *Terrimicrobium sacchariphilum*, *Pseudoclavibacter soli*, *Pseudoxanthomonas mexicana* and *Novosphingobium ginsenosidimutans*, were detected in all treatments. FVPT2 was enriched with *Dielma fastidiosa*, *Uruburuella testudinis*, *Rhodobacter flagellatus*, *Roseimaritima multifibrata*, *Tabrizicola aquatica* and *Thermomonas aquatica*. FVPT1 exhibited greater abundance of *Noviherbaspirillum aurantiacum*, *Roseicitreum antarcticum* and *Terrimicrobium sacchariphilum*. FVPT3 was characterized by enrichment of *Luteolibacter algae*, *Aquihabitans daechungensis*, *Methylococcus oryzae* and *Thermoclostridium caenicola*. The control group showed enrichment of *Rhodobacter ruber* and *Reyranella aquatilis*.

Table 1: Shannon diversity index and observed species richness of intestinal microbial communities in goldfish (*Carassius auratus*) fed experimental diets.

Sample ID	Shannon species diversity	Number of species identified
FVT0	2.787	1,114
FVPT2	2.596	1,320
FVCT2	2.706	1,459
FVPT3	2.232	1,170
FVPT1	2.683	1,221
FVCT3	2.163	1,575
FVCT1	1.687	1,429

Table 2: Name of the most abundant genus in each sample.

Sample ID	Name of genus
FVT0	*Uruburuella*
FVPT2	*Cetobacterium*
FVPT1	*Saccharothrix*
FVPT3	*Saccharothrix*
FVCT1	*Saccharothrix*
FVCT2	*Saccharothrix*
FVCT3	*Saccharothrix*

Core microbiota and treatment-specific bacterial signatures

Distinct bacterial signatures were observed among the control and macroalgae-supplemented groups, indicating that dietary supplementation with *Chlorella* and *Porphyra* altered the composition of the intestinal microbiota by enriching treatment-specific bacterial taxa (Table 3).

In the *Chlorella*-supplemented groups, the control (FVT0) was characterized by the presence of *Marimicrobium arenosum*, *Thermostilla marina*, *Dielma fastidiosa*, *Persicobacter psychrovividus* and *Reyranella aquatilis*. Supplementation with 5% *Chlorella* (FVCT1) resulted in the enrichment of *Roseicitreum antarcticum*, *Flavihumibacter cheonanensis*, *Povalibacter uvarum*, *Roseomonas oryzicola*, *Rhodobacter flagellatus* and *Acidibacter ferrireducens*. The 7.5% *Chlorella* treatment (FVCT2) was distinguished by the occurrence of *Phenylobacterium haematophilum*, *Fuscoavulum blasticum* and the predatory bacterium *Bdellovibrio bacteriovorus*. In the 10% *Chlorella* group (FVCT3), unique bacterial signatures included *Porphyromonas pogonae*, *Planctomicrobium piriforme*, *Novipirellula aureliae*, *Rhizobium qilianense*, *Methylococcus oryzae* and *Thermoclostridium caenicola*, indicating a marked shift in microbial community composition at the highest supplementation level.

Similarly, dietary *Porphyra* supplementation produced distinct bacterial assemblages. The control group (FVT0)

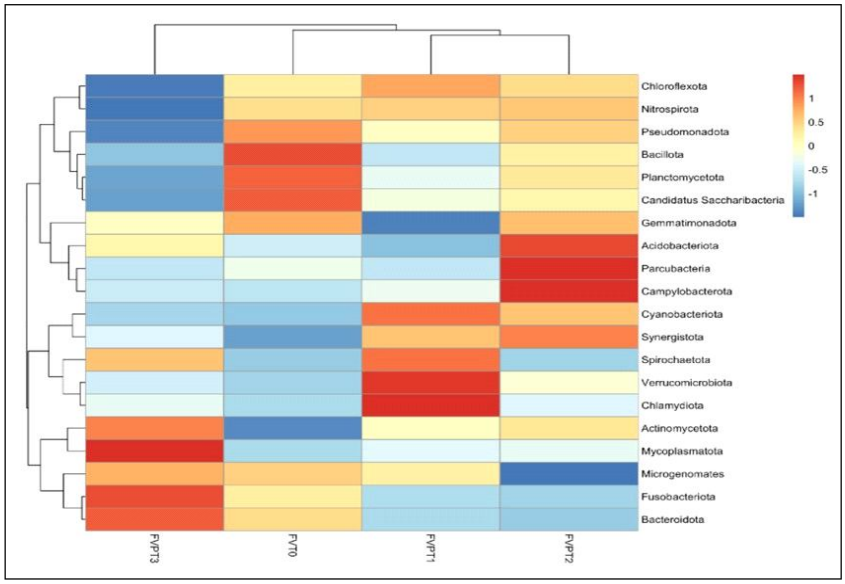


Fig 8: Taxonomic composition and relative abundance of gut bacterial phyla in goldfish (*Carassius auratus*) receiving *Porphyra* sp.-supplemented diets.

contained *Marimicrobium arenosum*, *Limnoglobus roseus*, *Thermostilla marina* and *Persicobacter psychrovividus* as representative taxa. The 5% *Porphyra* group (FVPT1) showed enrichment of *Noviherbaspirillum aurantiacum*, *Roseicitreum antarcticum*, *Reyranella aquatilis*, *Povalibacter uvarum* and *Terrimicrobium sacchariphilum*. The 10% *Porphyra* treatment (FVPT2) was characterized by the presence of *Rhodobacter flagellatus*, *Roseimaritima multifibrata*, *Tabrizicola aquatica*, *Thermomonas aquatica* and *Legionella septentrionalis*. In contrast, the 15% *Porphyra* group (FVPT3) exhibited unique enrichment of *Cetobacterium somerae*, *Pseudoxanthomonas mexicana*, *Aquihabitans daechungensis*, *Methylococcus oryzae* and *Thermoclostridium caenicola*.

Overall, both *Chlorella* and *Porphyra* supplementation promoted treatment-specific bacterial communities compared with the control. While several taxa such as *Marimicrobium arenosum*, *Thermostilla marina*, *Reyranella aquatilis*, *Povalibacter uvarum*, *Methylococcus oryzae* and *Thermoclostridium caenicola* were shared across treatments, each supplementation level was associated with a distinct set of bacterial species, suggested that

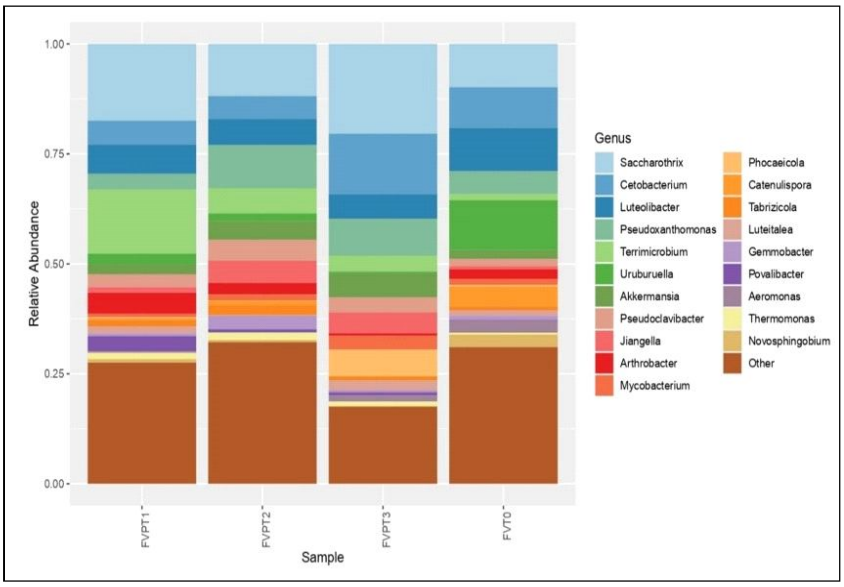


Fig 9: Comparative analysis of the genus-level gut microbiota in goldfish (*Carassius auratus*) fed experimental diets containing *Porphyra* sp.

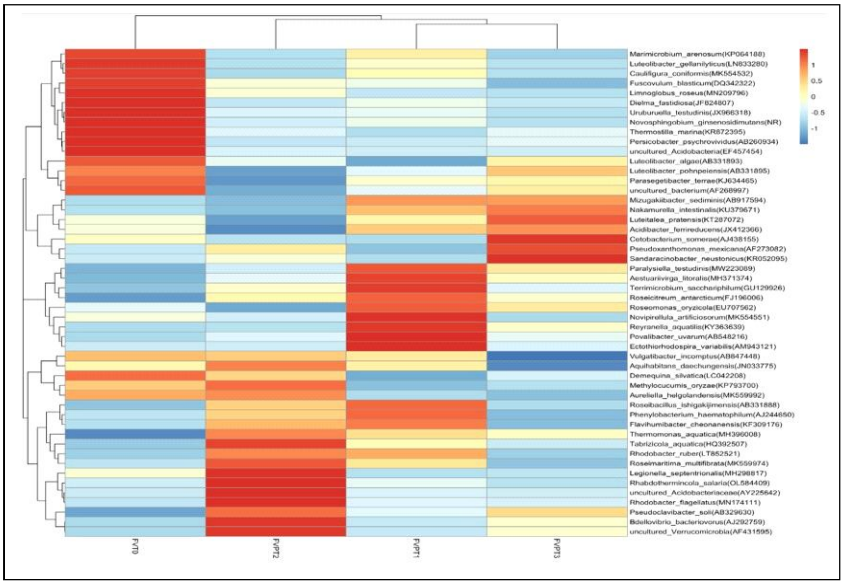


Fig 10: Species-level heatmap revealing the composition and distribution of the gut microbiota in goldfish (*Carassius auratus*) following dietary supplementation with *Porphyra* sp.

dietary macroalgae modulated the gut microbial composition in a dose-dependent manner. The most pronounced bacterial diversification was observed in the highest *Chlorella* supplementation (FVCT3), whereas *Porphyra* supplementation resulted in progressive replacement of control-associated taxa with treatment-specific bacterial signatures, particularly in FVPT2 and FVPT3.

Dietary supplementation with *Chlorella* substantially modified the intestinal microbial ecology of goldfish by increasing microbial richness, diversity and taxonomic complexity. The higher alpha diversity observed in FVCT2 and FVCT3 indicates the establishment of a more complex and functionally resilient intestinal ecosystem. Increased microbial diversity is generally associated with enhanced metabolic flexibility, improved functional redundancy and greater resistance to environmental disturbances and pathogen invasion (Egerton *et al*., 2018; Ringø *et al*., 2018). The enrichment of Actinomycetota, Bacillota, Bacteroidota and Fusobacteriota suggests enhanced capacities for nutrient degradation, fermentation and maintenance of intestinal homeostasis. Members of Actinomycetota are known producers of extracellular enzymes and antimicrobial compounds (Ringø *et al.,* 2016), while Bacillota encompass numerous probiotic bacteria involved in digestive enzyme production and immune modulation (Barka *et al*., 2016; Hai, 2015). Likewise, Bacteroidota and Fusobacteriota contribute to polysaccharide degradation, vitamin B12 biosynthesis and short-chain fatty acid production (Tsuchiya *et al*., 2008; Li *et al*., 2015). Cyanobacteriota may also indicate dietary transfer of algae-associated microbiota and microbial interactions stimulated by algal bioactive compounds (Paerl and Otten, 2013).

At the genus and species levels, the enrichment of *Cetobacterium*, *Luteolibacter*, *Pseudoxanthomonas* and *Saccharothrix* indicates enhanced microbial functionality and metabolic versatility. In particular, *Cetobacterium somerae* is a dominant freshwater fish commensal involved in vitamin B12 synthesis and short-chain fatty acid production, thereby contributing to intestinal homeostasis (Tsuchiya *et al.,* 2008). The occurrence of *Bdellovibrio bacteriovorus* in FVCT2 further suggests enhanced microbial regulation through suppression of opportunistic bacteria (Dashiff and Kadouri, 2011). Similarly, enrichment of *Terrimicrobium sacchariphilum*, *Luteolibacter algae* and *Thermoclostridium caenicola* indicates increased carbohydrate fermentation and production of beneficial microbial metabolites.

The observed microbiome remodeling is likely attributable to the rich nutritional composition of *Chlorella*, including proteins, chlorophyll, carotenoids, vitamins and complex polysaccharides, which may function as prebiotic substrates for beneficial intestinal microorganisms (Becker, 2007; Gouveia *et al*., 2012). Collectively, these findings demonstrate that dietary *Chlorella*, particularly at the 10% inclusion level, effectively promotes microbial

Table 3: Core microbiota and treatment-specific bacterial signatures in *Chlorella* and *Porphyra* supplemented Group.

FVT0	FVCT1	FVCT2	FVCT3	FVPT1	FVPT2	FVPT3
Marimicrobium arenosum	*Roseicitreum antarcticum*	*Phenylobacterium haemmatophilum*	*Porphyromonas pogonae*	*Noviherbaspirillum aurantiacum*	*Rhodobacter flagellates*	*Cetobacterium somerae*
Thermostilla marina	*Flavihumibacter cheonanensis*	*Fuscoavulum blasticum*	*Planctomicrobium piriforme*	*Roseitreum antarcticum*	*Roseimaritima multifibrata*	*Pseudoxanthomonas multifibrata*
Dielma fastidiosa	*Povalibacter uvarum*	*Bdellovibrio bacteriovorus*	*Novipirellula aureliae*	*Reyranella aquatilis*	*Tabrizicola aquatica*	*Aquihabitans daechungensis*
Persicobacter psychrovirus	*Roseomonas oryzicola*		*Rhizobium qilianense*	*Povalibacter uvarum*	*Thermomonas aquatica*	*Methylococcus oryzae*
Reyranella aquatilis	*Rhodobacter flagellatus*		*Methylococcus oryzae*	*Terrimicrobium sacchariphilum*	*Legionella septentrionalis*	*Thermoclostridium caenicola*
	Acidibacter		*Thermoclostridium*			

diversification and enriches bacterial populations associated with nutrient metabolism and intestinal health in *Carassius auratus*.

Dietary supplementation with *Porphyra* markedly influenced the intestinal microbiota of goldfish by promoting microbial richness, diversity and taxonomic restructuring. The highest microbial diversity and species evenness observed in FVPT2 suggest that moderate inclusion of *Porphyra* promoted the establishment of a balanced and metabolically diverse intestinal ecosystem. Increased microbial diversity is generally considered indicative of enhanced ecological stability, broader metabolic capabilities and improved resistance to pathogen colonization (Egerton *et al*., 2018; Ringø *et al*., 2018).

The enrichment of Fusobacteriota, Bacteroidota, Actinomycetota and Bacillota suggests improved capacities for polysaccharide degradation, nutrient assimilation and production of beneficial microbial metabolites (Thomas *et al*., 2011; Gajardo *et al*., 2016). At the genus level, increased abundance of *Cetobacterium*, *Luteolibacter* and *Pseudoxanthomonas* indicates enhanced microbial-mediated nutrient transformation and intestinal functionality (Tsuchiya *et al*., 2008).

The persistent dominance of *Cetobacterium somerae*, particularly in FVPT3, suggests improved vitamin B12 biosynthesis, amino acid metabolism and short-chain fatty acid production (Tsuchiya *et al.,* 2008; Xia *et al.,* 2014). Enrichment of *Terrimicrobium sacchariphilum*, *Luteolibacter algae* and *Thermoclostridium caenicola* further indicates enhanced carbohydrate fermentation and anaerobic metabolism (Newton *et al*., 2011), whereas the occurrence of *Rhodobacter flagellatus*, *Roseimaritima multifibrata* and *Tabrizicola aquatica* suggests improved nutrient cycling and production of bioactive metabolites that may support intestinal homeostasis (Gómez and Balcázar, 2008).

The observed microbiome remodeling is likely attributable to the abundance of bioactive compounds in *Porphyra*, including sulfated polysaccharides such as porphyran, phycobiliproteins, minerals and antioxidant compounds. These constituents may act as prebiotic substrates that selectively stimulate microorganisms and promote microbial interactions that improve digestive efficiency and intestinal health. Overall, the findings demonstrate that dietary *Porphyra*, particularly at the 10% inclusion level, effectively modulates gut microbial communities and promotes the establishment of a functionally robust intestinal ecosystem in *Carassius auratus*.

CONCLUSION

Dietary supplementation with *Chlorella* and *Porphyra* significantly modulated the intestinal microbiome of goldfish (*Carassius auratus*) in a treatment-dependent manner. Both algal supplements enhanced microbial richness and diversity and promoted the enrichment of bacterial taxa associated with nutrient metabolism, vitamin biosynthesis and intestinal homeostasis. Among the dietary treatments, supplementation with 10% *Chlorella* and 10% *Porphyra* produced the most pronounced effects on microbial diversification and community restructuring. These findings indicate that algal supplementation can beneficially modulate the gut microbial community and promote a more diverse and functionally robust intestinal microbiome. The observed taxonomic shifts suggest the potential of *Chlorella* and *Porphyra* as sustainable functional feed ingredients for ornamental aquaculture; however, their direct effects on intestinal health require further validation through physiological and histological investigations

The present study has several limitations that should be considered when interpreting the findings. Intestinal samples from replicate aquaria were pooled to obtain a representative treatment-level sample for sequencing; therefore, within-treatment biological variation in the gut microbiome could not be assessed. In addition, the use of 16S rRNA gene sequencing provided taxonomic profiles of the microbial communities but did not directly evaluate their functional activity. Furthermore, conclusions regarding intestinal health were inferred from changes in microbial composition rather than supported by direct physiological, histological, or molecular assessments of intestinal function. Future studies incorporating individual biological replicates, shotgun metagenomics or metatranscriptomics and complementary intestinal health analyses will provide a more comprehensive understanding of the effects of *Chlorella* and *Porphyra* supplementation on the gut ecosystem of goldfish.

ACKNOWLEDGEMENT

The authors are grateful to the College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India, for providing the necessary facilities and infrastructural support to carry out this research. The author sincerely acknowledges the invaluable guidance, constant encouragement and insightful suggestions provided by the Major Guide throughout this research work. The authors also acknowledge the Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat, India, for providing sequencing and bioinformatics facilities for the metagenomic analyses.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

The research conducted in this study involved fish specimens and ethical approval was obtained from the research committee (Letter No. KU/DR/PG/Synopsis/Fish-VRL/186/2024) of Kamdhenu University.

Data availability statement

The raw metagenomics sequences of the samples have been deposited to the NCBI SRA under the BioProject PRJNA1508771.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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