A comparison of digestive strategies for fishes with different feeding habits: digestive enzyme activities, intestinal morphology and gut microbiota

Fang Jiao[1], Lei Zhang[2], Samwel Mchele Limbu[3], and hua Rong[2]

[1]South China Agricultural University
[2]Xiangyang Polytechnic
[3]University of Dar es Salaam

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Abstract

Feeding habit determines the digestive tract structure and intestinal microflora. However, the relationship among feeding habit, digestive physiology intestinal, and microbial diversity of omnivorous, herbivorous, filter-feeder and carnivorous fish reared in the same pond has not been compared. This study compared the digestive enzyme activities, intestinal morphology and intestinal microflora of omnivorous (Carassius auratus), herbivorous (Ctenopharyngodon idellus), carnivorous (Siniperca chuatsi) and filter-feeder (Shizothorax grahami) and predicted the potential functions of specific microflora on different nutrients. Twelve intestine samples were collected from each of the four fishes from Dianchi Lake. The composition and diversity of microbial communities were determined by using high throughput sequencing of 16S rDNA. The results showed that the filter-feeder fish had significantly higher protease but lower amylase activities in the intestine than herbivorous. The carnivorous fish intestine had more microvilli branches and complex structures than other fish species in the order carnivorous > herbivorous > filter-feeder > omnivorous. The diversity of intestinal microflora was higher in omnivorous and followed the order omnivorous > herbivorous > filter-feeder > carnivorous. Acinetobacter species and Bacteroides species were the most dominant flora in carnivorous and herbivorous fish, respectively. Acinetobacter johnsonii, Acinetobacter lwoffii and Pseudomonas stutzeri might help the host to digest protein, while Bacteroidetes species may help the host to digest cellulose. Taken together, feeding habit determines the digestive enzyme activities, intestinal tissue morphology and differential colonization of fish intestinal flora. The knowledge obtained is useful in designing appropriate approaches for feed formulation and feeding practices in for fish.

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Fang Jiao[1], Lei Zhang[2], Samwel Mchele Limbu[3], Hua Rong[2,*]

[1]College of Marine Sciences, South China Agricultural University, Guangzhou 510642, China

[2]Xiangyang Polytechnic, Xiangyang, 441050, China

[3]Department of Aquaculture Technology, School of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, P. O. Box 60091, Dar es Salaam, Tanzania

*Corresponding author.

E-mail address : *huarong@scau.edu.cn* (H. Rong)

Abstract

Feeding habit determines the digestive tract structure and intestinal microflora. However, the relationship among feeding habit, digestive physiology intestinal, and microbial diversity of omnivorous, herbivorous, filter-feeder and carnivorous fish reared in the same pond has not been compared. This study compared the digestive enzyme activities, intestinal morphology and intestinal microflora of omnivorous (*Carassius auratus*), herbivorous (*Ctenopharyngodon idellus*), carnivorous (*Siniperca chuatsi*) and filter-feeder (*Shizothorax grahami*) and predicted the potential functions of specific microflora on different nutrients. Twelve intestine samples were collected from each of the four fishes from Dianchi Lake. The composition and diversity of microbial communities were determined by using high throughput sequencing of 16S rDNA. The results showed that the filter-feeder fish had significantly higher protease but lower amylase activities in the intestine than herbivorous. The carnivorous fish intestine had more microvilli branches and complex structures than other fish species in the order carnivorous > herbivorous > filter-feeder > omnivorous. The diversity of intestinal microflora was higher in omnivorous and followed the order omnivorous > herbivorous > filter-feeder > carnivorous. *Acinetobacter* species and *Bacteroides* species were the most dominant flora in carnivorous and herbivorous fish, respectively. *Acinetobacter johnsonii* , *Acinetobacter lwoffii* and *Pseudomonas stutzeri* might help the host to digest protein, while *Bacteroidetes* species may help the host to digest cellulose. Taken together, feeding habit determines the digestive enzyme activities, intestinal tissue morphology and differential colonization of fish intestinal flora. The knowledge obtained is useful in designing appropriate approaches for feed formulation and feeding practices in for fish.

KEYWORDS feeding habit; intestinal microbiota; digestive enzymes; 16S rDNA

1 | INTRODUCTION

The feeding habits of fish are reflected by their digestive organ, mainly the intestine. Scholars generally classify fish feeding habits as herbivorous, carnivorous, omnivorous and filter-feeders according to feeding method and food content. The intestine tract is the main site for digestion and nutritional uptake, which has been regarded as a key organ in fish nutrition (Kumar et al., 2005; Wang et al., 2018; Zhou et al., 2021). The fish digestive enzymes activities are closely related to the diet consumed and the ability of fish to digest and absorb different nutrients (Bakke et al., 2010; Liu et al., 2021). Evidently, previous studies found herbivorous fish such as Roho labeo (*Labeo rohita*) and Japanese eel (*Anguilla japonica*) had stronger amylase activity compared to carnivorous fish such as Great white catfish (*Wallago attu*) (Agrawal et al., 1975) and rainbow trout (*Oncorhynchus mykiss*) (Hidalgo et al., 1999). Therefore, the influence of feeding habits on digestive enzymes activities is beyond doubt.

The feeding habits of fish also affect digestive tract structure and intestinal microorganisms (Li et al., 2019; Meng et al., 2014; Valdes et al., 2018). Interference with intestinal morphology such as muscularis thickness and villi width affect nutrients absorption and intestinal microbiota (Limbu et al., 2018). Fish gut microbiota contribute to digestion and affect gastrointestinal tract development and overall growth of fish (Clements et al., 2014; Ghanbari et al., 2015). However, feeding habits (Larsen et al., 2014; Meng & Nie, 2019; Roeselers et al., 2011), which determines the feed composition consumed (Benson et al., 2010; Spor et al., 2011; Sullam et al., 2012) has been reported to shape microbial communities in fish (Larsen et al., 2014; Meng & Nie, 2019; Roeselers et al., 2011). Accordingly, diet has been reported as a dominant source of variation in the microbiota composition of rainbow trout (Desai et al., 2012; Ingerslev et al., 2014). The disruption in intestinal microbiota induced by feeding habit via diet usually affect digestive functions of the host through disturbance in bacterial digestive enzyme production (Ghanbari et al., 2015). Certain gut microbiota such as the cellulolytic enzyme-producing bacterial community, which were isolated from the intestinal tract of herbivorous fish species are known to metabolize a remarkable variety of substrates (Li et al., 2016) improving growth performance. Therefore, several studies have explored the manipulation of gut microbiota through diet to improve fish growth (Fan et al., 2021; Li et al., 2019; Pan et al., 2021). However, studies exploring the relationship between feeding habits, digestive enzyme activity, intestinal structure and gut microbial composition in fish are currently limited. Such a knowledge gap limits our knowledge on designing appropriate approaches for feed formulation and feeding practices in aquaculture.

China is currently the largest producer and consumer of cultured fish (FAO, 2022), including Grass carp

(*Ctenopharyngodon idellus*), a herbivorous fish (Liu et al., 2017) and mandarin fish (*Siniperca chuatsi*), a carnivorous fish (Shen et al., 2021). Aquaculture production in China also include species such as Dianchi high-back crucian carp (*Carassius auratus*), an omnivorous fish (Shi et al., 2017) and Kunming Schizothoracin (*Schizothorax grahami*), a filter-feeding fish, which are endemic to Yunnan, China. These species are the main economic fish produced in Yunnan because of their nutritional value (Zheng et al., 2016). To ensure continued production of these species, knowledge on the influence of feeding habits on digestive enzymes, intestinal morphology and microbiota composition is needed for effective feed formulation. The present study compared the relationship between feeding habits, and digestive enzyme, intestinal morphology and intestinal microbiota of *C. idella, S. chuatsi* ,*C. auratus* and *S. grahami* as herbivorous, carnivorous, omnivorous and filter-feeding fish representative species, respectively. The results obtained provide a scientific basis for development of appropriate formulation of compound fish feeds.

2 | MATERIALS AND METHODS

2.1 | Fish sampling

Ten individual fish for each species (*Ctenopharyngodon idellus* ,*Siniperca chuatsi* , *Carassius auratus* and*Schizothorax grahami*) were caught by trolling boats in the Dianchi Lake, Kunming, Yunan, China. The fish were transported live in plastic bags provided with dissolved oxygen by car to the Aquaculture Laboratory of Yunnan Agricultural University, where they were euthanized by immersing them into 40 mg/L eugenol (Shanghai Reagent, China). The average weights of the sampled fish were determined by using a weighing scale as 1323.60 ± 40.20 g for *C. idellus* , 471.10 ± 23.94 for *S. grahami* , 841.30 ± 34.54 g for *S. chuatsi* and 350.4 ± 25.98 g for *C. auratus* .

2.2 | Determination of digestive enzymes activities

Three fish for each species were carefully dissected and intestine and hepatopancreas were sampled and transferred into an Eppendorf tube and immediately placed into liquid nitrogen. The tubes containing the samples were stored at -80 degC until needed for analysis of enzymes activities. On analysis day, the hepatopancreas and intestine samples were weighed and mixed with 9 times phosphate buffer saline (PBS) (w:v = 1:9), then homogenized by using an electric homogenizer (Ningbo Scientz Biotechnology) in ice bath for 15 s. The resulting homogenate was carefully pipetted and centrifuged at 12,000 rpm at 4 degC for 20 min. Finally, the liquid supernatant was removed for digestive enzymes analysis. The digestive enzymes activities including pepsin, trypsin, lipase and amylase, and total protein concentration of hepatopancreas and intestine were determined by using specific commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) based on instructions from the manufacturer.

2.3 | Intestinal morphology analysis

Three fish tissues from midgut for each feeding habit was collected and prepared for intestinal morphology analysis as described previously (Limbu et al., 2018). The tissues were fixed with 4% paraformaldehyde for 24 h, then dehydrated with 75% absolute ethanol. The tissues were then transferred into xylene twice for transparent, immersed into paraffin wax three times, embedded and cooled. The intestine tissues were sliced transversely into pieces with approximately 5 to 6 μm, dried and stained by using hematoxylin and eosin (H & E). The slides were finally examined by using electronic biological microscope KOPPACE at 40x to 1600x (Kopace Technology Co., LTD, Shenzhen, China). Villi height (VH), villi width (VW) and muscularis thickness (MT) were measured from at least 30 segments for each feeding habit by using Case Viewer software. Villi height index (VHI), villi width index (VWI) and muscularis thickness index (MTI) were calculated as VH, VW and MT divided by individual fish body weight$^{1/3}$.

2.4 | DNA extraction and high-throughput sequencing analysis of intestinal microbiota

We sterilized the scalpels, tweezers, and scissors by heating at 180 °C for 2h before using them for DNA extraction. We also wiped the fish surface, the desktop and instruments used by using 75% alcohol to disinfect them. Afterwards, we collected the gut contents of the remaining four fish and placed them into sterile tubes under sterile conditions. The tubes containing the samples were immediately placed into liquid

nitrogen and then stored at -80 degC until DNA extraction. The four intestine samples for herbivorous fish*C. idellus* were abbreviated as HE, filter feeder *S. chuatsi* as FI, omnivorous *C. auratus* as OM and carnivorous *S. grahami* as CA for convenient reporting. These samples were subjected to DNA extraction using the PowerFood Microbial DNA isolation kit (QIAGEN Srl, Milan, Italy) following manufacturer's instructions. The quality and quantity of the DNA were checked by using gel electrophoresis and a Qubit 4 Fluorometer (Thermo Fisher Scientific, USA). Primers 341F: ACTCCTACGGGAGGCAGCAG and 806R: GGACTACHVGGGTATCTAAT were used to generate the PCR amplicons for the 16S rRNA gene V3 - V4 region on Illumina sequencing platform (HiSeq 2500, Beijing igeneCode Biotech Co., Ltd., Beijing, China).

2.5 | Bioinformatics analysis

To obtain high-quality clean reads, raw reads were demultiplexed and filtered for quality based on the methods developed by Fadrosh et al. (2014). Cleaned tags were obtained by FASTP (Chen et al., 2018). Sequences were assigned to operational taxonomic units (OTUs) based on a similarity cut-off of 97% by using UPARSE (v7.0.1090) (Edgar, 2013). The representative sequences from each OTU were then taxonomically classified using the Ribosomal Database Project (RDP) Classifier (v2.2) with confidence threshold of 80%.

2.6 | Prediction of microbiome functions by using bioinformatics analysis

We predicted the gut microbiome functions by using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to elucidate the physiological features and metabolism capability during the process of dietary conversion. To compare the functional categories of microbiota among the four groups (HE, FI, OM and CA) by PICRUSt analyses, functional profile heatmaps based on 423 categories (KEGG level-3) were constructed, which showed marked differences among feeding habits.

2.7 | Statistical analyses

All the data for enzymes activities and intestinal morphology were tested for normality and homogeneity of variances by using Shapiro-Wilk and Levene's tests, respectively. Afterwards, one-way analysis of variance (ANOVA) was used to test for statistical differences on the data for enzymes activities and intestinal morphology among the four feeding habits. Tukey multiple comparisons test was used to compare for significant differences among the four feeding habits when ANOVA indicated statistical differences. The analysis of enzymes activities and intestinal morphology data was conducted by using SPSS 20.0 (SPSS, Inc., Chicago, IL, USA). Results with P [?] 0.05 were considered significant different. The results obtained are expressed as mean +- standard error of the mean (SEM).

The differences in bacterial phylotype distribution was assessed by using principal component analysis (PCA). The alpha-diversity indices (the abundance coverage-based Estimator - ACE, Chao1, Shannon and Simpson indices) were generated by using Mothur v1.31.2 (http://www.mothur.org/wiki/Calculators). Abundance of microbiota was analyzed by using ACE and Chao1 indices while microbiota species diversity was assessed by using Shannon and Simpson indices. Linear discriminant analysis effect size (LEfSe) was analyzed by using the R statistical package (v3.1.1).

3 RESULTS

3.1 | Comparative analysis of digestive enzymes activity

The feeding habits affected differently the digestive enzymes activities of the four fish species in the hepatopancreas and intestine ($p<$ .05; Table 1). The carnivorous fish (*S. chuatsi*) had higher trypsin and pancrelipase activities in the hepatopancreas and enteropeptidase in the intestine than herbivorous (*C. idella*), omnivorous (*C. auratus*) and the filter-feeder fish (*S. grahami*) ($p <$ .05). Moreover, the filter-feeder fish had higher trypsin and pancrelipase activities in the hepatopancreas but lower entero-amylase in the intestine than the herbivorous and omnivorous fishes ($p <$ .05). The omnivorous fish had significantly higher enteropeptidase activity in the intestine than herbivorous and filter-feeder fishes ($p <$ .05). However, the herbivorous and omnivorous fish species had no significant difference in trypsin and pancrelipase activities in

the hapatopancreas ($p > .05$). Similarly, the herbivorous and filter-feeder fishes had no significant difference in enteropeptidase activity in the intestine ($p > .05$).

Interestingly, the carnivorous fish (*S. chuatsi*) had significantly lower amylopsin activity in the hapatopancreas and entero-amylase in the intestine than the herbivorous, omnivorous and filter-feeder fishes ($p < .05$). Similarly, the omnivorous fish had significantly lower amylopsin activity than herbivorous and filter feeding fishes in the hepatopancreas ($p< .05$). The herbivorous and filter feeding fishes had no significant difference in amylopsin activity in the hepatopancreas ($p > .05$). Equally, herbivorous and filter-feeder fishes had no significant difference in enteropeptidase in the intestine ($p > .05$). The herbivorous and omnivorous had significantly higher intestinal lipase activity than filter feeder and carnivorous fish species ($p < .05$). Similarly, the carnivorous fish had significantly higher intestinal lipase than the filter-feeder fish ($p < .05$). The filter-feeder fish had significantly lower entero-amylase activity than herbivorous and omnivorous fishes in the intestine ($p < .05$). However, the herbivorous and omnivorous fish species had no significant differences in intestinal lipase and entero-amylase activities ($p> .05$).

3.2 | Intestinal tissue morphology

The VH, VW and MT differed significantly among the four feeding habits of the fish species (Figure 1; $p < .05$). The intestinal microvilli of carnivorous fish had many branches and complex structures. The order of microvilli complexity was carnivorous > herbivorous > filter-feeder > omnivorous. Herbivorous fish had significantly higher VH and VHI than filter-feeder, carnivorous and omnivorous fish species ($p < .05$). Similarly, carnivorous fish had significantly higher VH and VHI than filter-feeder and omnivorous fish species ($p < .05$). Likewise, filter-feeder fish had significantly higher VH and VHI than omnivorous fish species ($p < .05$). On the contrary, carnivorous fish had significantly higher VW, MT, VWI and MTI than herbivorous, filter-feeder and omnivorous fish species ($p < .05$). Similarly, herbivorous fish had significantly higher VW, MT, VWI and MTI than filter-feeder and omnivorous fish species ($p < .05$). Likewise, omnivorous fish had significantly higher MT, VWI and MTI than filter-feeder fish species ($p < .05$). However, filter-feeder and omnivorous fish species had no significant difference in VW ($p > .05$).

3.3 | Microbial complexity of fish gut flora

A total of 2,300 OTU were obtained for all the four fishes. A total of 223 OTUs were shared by all species (9.7%), while 332 (223 + 42 + 47 + 20) OTUs (14.4%) were shared by herbivorous and carnivorous (Figure 2). The herbivorous fish had relatively higher number of unique OTUs (467) equivalent to 20.3% followed by omnivorous fish (251 OTUs) representing 10.9%, while filter-feeder fish had 113 OTUs equivalent to 4.9% and carnivorous fish had only 43 OTUs making up 1.9%.

3.4 | Microbiota abundance and diversity

The carnivorous species had significantly lower number of microbiota species than omnivorous species (Figure 3A; $p < .05$). However, carnivorous, herbivorous and filter-feeder had no significant number of microbiota species ($p > .05$). Similarly, carnivorous and filter-feeder fish species had no significant difference in number of microbiota species ($p > .05$). The carnivorous species also had significantly lower abundance as reflected by Chao1 (Figure 3B) and ACE (Figure 3C) than omnivorous and herbivorous fish species ($p < .05$). The filter-feeder species also had significantly lower Chao1 than omnivorous species ($p < .05$). However, omnivorous, herbivorous and filter-feeder species had statistically no difference in Chao1 while herbivorous and filter-feeder species had no significant difference in ACE ($p > .05$). Similarly, carnivorous and filter-feeder fish species had no significant difference in in Chao1 and ACE ($p > .05$). The carnivorous fish had significantly lower Shannon diversity index (Figure 3D), but higher Simpson's diversity index (Figure 3E). However, omnivorous, herbivorous and filter-feeder species had no significant difference in Shannon diversity index and Simpson's diversity index ($p > .05$). Similarly, carnivorous, herbivorous and filter-feeder fish species had no significant difference in Shannon diversity index and Simpson's diversity index ($p > .05$). The community diversity of the four fish followed the order omnivorous > herbivorous > filter-feeder > carnivorous.

3.5 | Abundance and composition of microbiota at phyla, genera and species levels

A total of 37 phyla were obtained from all fish species (Supplementary Figure 1S). We then selected the most abundant phyla with above 5%. We obtained nine phyla classified as Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria phyla with relatively high abundance, representing 66.60%, 9.82%, 9.04% and 5.18%, respectively (Figure 4). Herbivorous fish species had significantly lower abundance of Proteobacteria phylum than carnivorous and filter-feeding species ($p < .05$). On the contrary, herbivorous fish species had significantly higher abundance of Firmicutes and Bacteroides phyla than omnivorous, filter-feeder and carnivorous species ($p< .05$). The omnivorous fish species had significantly higher abundance of *Verrucomicrobia* phylum than omnivorous, filter-feeder and herbivorous species ($p < .05$).

A total of 324 bacterial genera were obtained. We then removed unidentified bacterial genera, and others and selected only those with more than 0.5% abundance. The results on composition showed 45 bacterial genera were obtained (Supplementary Figure 2S). The four fish species with different feeding habits had distinct microbiota composition at genera for the nine phyla. Carnivorous and filter-feeder species were dominated with *Limnobacter* species (25.05% and 23.56%) and *Pseudomonas* species (26.07% and 8.16%), respectively. The microbiota of omnivorous species was mainly composed of *Rhodobacter* species (14.99%), *Zymomonas* species (10.63%), *Clavibacter* species (8.57%) and *Luteolibacter*species (6.68%). The herbivorous fish species was mainly composed of*Bacteroides* species (22.56%) and *Citrobacter* species (9.43%).

We obtained 14 genera with significant differences in abundance of microbiota (Table 3). The carnivorous fish species had significant higher *Acinetobacter* species than the other three fish species ($p < .05$). The omnivorous fish species had higher abundance of *Anaerospora* , *Arenimonas* ,*Dechloromonas* , *Deefgea* , *Luteolibacter* and*Zymomonas* genera than herbivorous and carnivorous fish species ($p < .05$). The herbivorous fish species had significantly higher *Bacteroides* genus than omnivorous, carnivorous and filter feeder fish species ($p < .05$).*Escherichia* , *Limnobacter* and *Mycoplana* genera were abundant flora of filter-feeder and carnivorous fish species, while*Pseudomonas* genus was abundant flora of filter-feeder and omnivorous fish species.

A total of 2048 species of bacteria were obtained. *Pseudomonas stutzeri* , *Escherichia coli* and *Acinetobacter lwoffii* were the most abundant species (Figure 5). The *Acinetobacter johnsonii* , *Acinetobacter lwoffii* , *Escherichia coli* and*Pseudomonas stutzeri* had significant different abundance among omnivorous, herbivorous, filter-feeder and carnivorous fish species ($p < .05$). The carnivorous fish species had significantly higher *Acinetobacter johnsonii* , *Acinetobacter lwoffii* and *Pseudomonas stutzeri* than the other three fish species ($p < .05$). The filter-feeder and carnivorous fish species had significantly higher *Escherichia coli* than omnivorous and herbivorous fish species.

3.6 | LEfSe analysis of significantly enriched microbial communities

Linear discriminant analysis effect size (LEfSe) was used to characterize enriched microbial communities (Figure 6A). There were 48 differences among the four fish species with different feeding habits, classified from phylum to genus. The Proteobacteria phylum was common to all the four fish species. The omnivorous species had significantly higher enriched gut microbiota species than the other three species. Indeed, omnivorous species had significantly enriched Fusobacteria, Actinobacteria and Verrucomicrobia phyla compared to carnivorous, herbivorous and filter-feeder fish species. The most enriched bacteria in the gut of the four fish species followed the trend omnivorous (25) > carnivorous (10) > filter-feeder (8) > herbivorous (5) (Figure 6B). The omnivorous species enriched *Fusobacteriales, Fusobacteriaceae, Fusobacteriia, Fusobacteria, Dechloromonas, Saprospirales, Saprospirae, Chitinophagaceae, Paucibacter, Microbacteriaceae, Luteolibacter, Clavibacter, Actinomycetales, Actinobacteria, Actinobacteria, Zymomonas, Verrucomicrobiales, Verrucomicrobiae, Verrucomicrobia, Verrucomicrobiaceae, Rhodobacter, Rhodobacterales* and*Rhodobacteraceae* . The herbivorous fish species enriched*Aeromonadales, Aeromonadaceae, Lachnospiraceae, Clostridia* and*Clostridiales* . Moreover, carnivorous fish species enriched*Acinetobacter, Moraxellaceae, Hyphomonadaceae, Mycoplana, Pseudomonas, Pseudomonadaceae, Pseudomonadales, Caulobacteraceae, Caulobacterales* and *Proteobacteria* . The filter-feeder fish enriched *Anaerolineae, Neisseriales, Neisseriaceae, Escherichia, Comamonadaceae, Burkholderiales, Betaproteobacteria* and*Limnobacter* .

3.7 | Predicted gut microflora functions

A total of 423 metabolism pathways (Kyoto Encyclopedia of Genes and Genomes, KEGG level-3) were constructed. The four fish species showed marked differences in functional profile (Supplementary Figure 3S). The microbial functions among the four fishes with different feeding habits showed that, 39 pathways related to digestion were identified, including those associated with carbohydrate, protein and amino acid, energy and lipid metabolism (Figure 7). Of all the pathways identified, 27 pathways were significantly changed (Figure 7). The herbivorous fish species had higher carbohydrate metabolism than carnivorous fish species ($p< .05$). Moreover, herbivorous fish species increased pathways related to carbohydrate metabolism [i.e. glycolysis III (from glucose), galactose degradation I (Leloir pathway), superpathway of D-glucarate and D-galactarate degradation, reductive TCA cycle I and incomplete reductive TCA cycle] than in carnivorous fish species. Interestingly, carnivorous had more enriched protein and amino acid metabolism pathways [superpathway of ornithine degradation, superpathway of L-arginine and L-ornithine degradation and L-arginine degradation II (AST pathway)] and lipid metabolism (fatty acid salvage) than herbivorous and omnivorous fish species.

4 | DISCUSSION

Feeding habit reflects directly the digestive ability of fish to different nutrient components. The ability of fish to digest and utilize different nutrients in feed is affected by the structure of digestive tract, the secretion of digestive enzymes and the composition and diversity of intestinal microbes. This study explored the relationship among feeding habits of omnivorous, carnivorous, herbivorous and filter-feeder fish species and digestive physiology, intestinal morphology, and intestinal microbial diversity. We also predicted the functions of microbiome from the four fishes. We found clear differences in the enzymes activities of the four fish species depending on their feeding habits. Evidently, the carnivorous fish (*S. chuatsi*) had higher trypsin and pancrelipase activities in the hepatopancreas and enteropeptidase in the intestine than herbivorous (*C. idella*), omnivorous (*C. auratus*) and the filter-feeder fish (*S. grahami*). The variations in digestive enzymes are caused by the different feeding habits (Xu et al., 2011). Accordingly, the activities of trypsin, intestinal protease and pancreatic lipase were roughly carnivorous > omnivorous > herbivorous, reflecting the feeding on animal materials with high protein and lipid requiring secretions of related enzymes to digest them. Similarly, Liu et al. (2014) reported higher protease activity in carnivorous fish than omnivorous and herbivorous fish.

However, the intestinal lipase activity in this study was higher in herbivorous and omnivorous fish species than filter-feeder and carnivorous fish species. Contrary, Parrizas et al. (1994) reported higher lipase activity in carnivorous fish than herbivorous and omnivorous fish. The higher lipase activity in stomachless fish is probably due to the relationship between intestinal tissue structure and digestive enzymes (Pan et al., 1996). In our study, the mandarin fish (*S. chuatsi*) represents a carnivorous fish with a stomach, grass carp (*C. idellus*) and Dianchi high-back crucian carp (*C. auratus*) are typical herbivorous and omnivorous species, respectively, without stomach and, Kunming Schizothoracin (*S. grahami*) is a filter-feeder fish with an enlarged sac between eosophagus and intestinal tract, which can secrete digestive fluid and perform some stomach functions. Therefore, the higher intestinal lipase activity in the stomachless fish was due to secretions of the enzyme from the intestine, which performs some functions of the stomach. Interestingly, this study found higher pancreatic amylase and intestinal amylase activities in herbivorous than carnivorous species. Similarly, Li et al. (2012) found higher amylase in herbivorous than omnivorous and Liu and Zhang (2001) reported higher amylase activity in omnivorous fish than carnivorous fish. The higher amylase activity in herbivorous fish is related to the utilization of carbohydrates in the feed. Similarly, omnivorous fish can use higher carbohydrate levels than carnivorous fish (Li et al., 2015a).

This study found significant differences in intestinal villi height, villi width and muscularis thickness among the four fish. These variations are due to the morphological and structural characteristics of the fish gut reflected by the different feeding habits (Liu & Zhang, 2001; Zeng & Ye, 1998). The intestinal microvilli of carnivorous fish had many branches and complex structures. The complexity of the microvilli (villi length and villi width) and muscularis thickness, was in the order carnivorous > herbivorous > omnivorous. The

intestinal structure accommodates, transports and digests feed and absorbs nutrients. The height, width and complexity of intestinal microvilli increase the surface area for digestion and absorption of digested nutrients (Sun et al., 2019). The muscularis thickness is composed of smooth muscle, which promotes the movement of food in the intestine through rhythmic relaxation and contraction. The thickness of the muscle layer directly reflects the contraction and peristalsis ability of the intestine. Accordingly, strengthening intestinal contraction and peristalsis is an effective means to increase feed digestion and reduces chyme circulation (Bian et al., 2021). Generally, carnivorous fish have shorter intestines (Day et al., 2014). Accordingly, carnivorous increase the complexity of the intestinal structure to reduce circulation rate of chyme and enhance absorption of nutrients. Therefore, the complexity of the intestinal structure is adapted to the feeding habits of fish so as to achieve fully absorption of nutrients.

The intestinal microflora of vertebrates plays an important role in host nutrition, (Liu et al., 2016; Valdes et al., 2018). Many studies have shown that dietary feeding habits (Miyake et al., 2015; Zhou et al., 2021) and host species (Li et al., 2019; Youngblut et al., 2019) are the main factors affecting the gut microbiota of fish. This study also found that the diversity of fish gut microbiota was significantly affected by feeding habits and host species. The diversity of gut microbiota species and abundance determine the stability of the host gut microbiota associated with host nutrition (Kuang et al., 2020). The community diversity of the four fish species followed the order omnivorous > herbivorous > filter-feeder > carnivorous. Contrary, Li et al. (2015b) reported higher bacterial diversity in the gut of the filter-feeding than herbivorous. It has been reported that the higher the Shannon index, the better the stability of the bacterial community and the better the digestion of nutrients (Zhang et al., 2019).

The results of this study showed Proteobacteria and Firmicutes phyla as typical dominant flora in the gut of the four fish species. Proteobacteria and Firmicutes are two phyla typical dominant flora in the intestine of many fish, such as *Oncorhynchus mykiss*(Ingerslev et al., 2014), *Nibea coibor* and *Nibea diacanthus* (Li et al., 2019), *Megalobrama terminalis* (Liu et al., 2021), *Micropterus salmoides* (Zhou et al., 2021) and*Symphysodon haraldi* (Zhang et al., 2021). The four fish species had variations in abundant of microbiota species. The different symbiotic bacteria carried by fish species may be caused by the selective enrichment of different microorganisms due to variations in feeding and host species. A previous study indicated that during evolution, hosts tend to acquire suitable environmental bacteria by recognizing adhesion mechanisms on the cell surface (McFall-Ngai, 2015). In addition, the differential enrichment of specific flora under different feeding conditions may also adapt to the function of the flora, for example, *Bacillus* species and *Cetacea* species are potential candidates for probiotics (Larsen et al., 2014),*Pseudomonas* species produces vitamin B_{12}, and*Fusobacterium* species produces butyrate (Zhou et al., 2019). Accordingly, the carnivorous gut was dominated by *A. johnsonii* ,*A. luckii* and *P. stutzeri* bacteria species, which may contribute to the digestion of proteins, while *Bacteroides*species were dominant in herbivorous fish gut may help the host to digest cellulose. The presence of these microbiome in the fish species are useful in host nutrition. Indeed, the intestinal microbes of the carnivorous (*S. chuatsi*) showed higher protein digestion potential and lower carbohydrate digestion potential, while gut microbes of herbivorous fish (*C. idellus*) showed lower protein and high carbohydrate digestion, consistent with their feeding habits.

5 | CONCLUSION

Taken together, the digestive enzymes activities, intestinal morphology, and intestinal microbiome composition and diversity of fish are significantly affected by feeding habits. Accordingly, carnivorous fish possess higher trypsin and lipase activities related to their higher feeding habit on protein and lipid. On the contrary, herbivorous fish species utilizes plant materials related to the higher amylase enzyme activity. The intestinal microvilli of carnivorous fish had many branches and complex structures to increase surface area for digestion and absorption of digested nutrients as an adaption to the short intestine. The feeding habits led to various adaptations of microbiota related to the selective colonization for various biological functions. The results provide an understanding of the different digestive strategies of omnivorous, carnivorous, herbivorous and filter-feeder fish to improve feed formulation for better feed utilization and digestibility in order to enhance nutrient absorption for promoting growth performance.

AUTHOR CONTRIBUTION

Conceived and designed the experiments: Hua Rong; Performed the experiments: Fang Jiao, Lei Zhang; Analyzed the data: Fang Jiao, Lei Zhang; Wrote the paper: Fang Jiao, Hua Rong; Review and editing: Hua Rong and Samwel Mchele Limbu. All authors read the manuscript and approved it in its final version.

CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in [Rong, Hua (2022), "Analysis of intestinal microbes in fish", Mendeley Data, V1, doi: 10.17632/5zmmwbfhhv.1]

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REFERENCES

Agrawal, V. P., Sastry, K. V., & Kaushab, S. K. (1975). Digestive enzymes of three teleost fishes. *Acta physiologica Academiae Scientiarum Hungaricae, 46* (2), 93-98.

Bakke, A. M., Glover, C., & Krogdahl, A. (2010). 2 - Feeding, digestion and absorption of nutrients. In M. Grosell, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 30, pp. 57-110): Academic Press.

Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., Zhang, M., Oh, P. L., Nehrenberg, D., Hua, K., Kachman, S. D., Moriyama, E. N., Walter, J., Peterson, D. A., & Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences, 107* (44), 18933-18938. doi:*https://doi.org/10.1073/pnas.1007028107*

Bian, Y. H., Xu, X. Y., & Duan, Z. P. (2021). Effects of replacing fish meal with yeast culture on growth performance, serum biochemical indices and intestinal morphology of largemouth bass (*Micropterus salmoides*). *Chinese Journal of Animal Nutrition, 33* (9), 5182-5192.

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics, 34* (17), i884-i890. doi:*https://doi.org/10.1093/bioinformatics/bty560*

Clements, K. D., Angert, E. R., Montgomery, W. L., & Choat, J. H. (2014). Intestinal microbiota in fishes: what's known and what's not.*Molecular Ecology, 23* (8), 1891-1898. doi:*https://doi.org/10.1111/mec.12699*

Day, R. D., Tibbetts, I. R., & Secor, S. M. (2014). Physiological responses to short-term fasting among herbivorous, omnivorous, and carnivorous fishes. *Journal of Comparative Physiology B, 184* (4), 497-512. doi:10.1007/s00360-014-0813-4

Desai, A. R., Links, M. G., Collins, S. A., Mansfield, G. S., Drew, M. D., Van Kessel, A. G., & Hill, J. E. (2012). Effects of plant-based diets on the distal gut microbiome of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 350-353* , 134-142. doi:*https://doi.org/10.1016/j.aquaculture.2012.04.005*

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods, 10* (10), 996-998. doi:*https://doi.org/10.1038/nmeth.2604*

Fadrosh, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R. M., & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform.*Microbiome, 2* (1), 6. doi:*https://doi.org/10.1186/2049-2618-2-6*

Fan, Y., Wang, X., Wang, Y., Liu, H., Yu, X., Li, L., Ye, H., Wang, S., Gai, C., Xu, L., Diao, J., & Guo, P. (2021). Potential effects of dietary probiotics with Chinese herb polysaccharides on the growth performance, immunity, disease resistance, and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). *Journal of the World Aquaculture Society, 52* (6), 1194-1208. doi:*https://doi.org/10.1111/jwas.12757*

FAO. (2022). *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation* . Retrieved from Rome, Italy:

Ghanbari, M., Kneifel, W., & Domig, K. J. (2015). A new view of the fish gut microbiome: Advances from next-generation sequencing.*Aquaculture, 448* , 464-475. doi:*https://doi.org/10.1016/j.aquaculture.2015.06.033*

Hidalgo, M. C., Urea, E., & Sanz, A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture, 170* (3), 267-283. doi:*https://doi.org/10.1016/S0044-8486(98)00413-X*

Ingerslev, H. C., von Gersdorff Jorgensen, L., Lenz Strube, M., Larsen, N., Dalsgaard, I., Boye, M., & Madsen, L. (2014). The development of the gut microbiota in rainbow trout (*Oncorhynchus mykiss*) is affected by first feeding and diet type. *Aquaculture, 424-425* , 24-34. doi:*https://doi.org/10.1016/j.aquaculture.2013.12.032*

Kuang, T., He, A., Lin, Y., Huang, X., Liu, L., & Zhou, L. (2020). Comparative analysis of microbial communities associated with the gill, gut, and habitat of two filter-feeding fish. *Aquaculture Reports, 18* , 100501. doi:*https://doi.org/10.1016/j.aqrep.2020.100501*

Kumar, S., Sahu, N. P., Pal, A. K., Choudhury, D., Yengkokpam, S., & Mukherjee, S. C. (2005). Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita*juveniles. *Fish & Shellfish Immunology, 19* (4), 331-344. doi:*https://doi.org/10.1016/j.fsi.2005.03.001*

Larsen, A. M., Mohammed, H. H., & Arias, C. R. (2014). Characterization of the gut microbiota of three commercially valuable warmwater fish species. *Journal of Applied Microbiology, 116* (6), 1396-1404. doi:*https://doi.org/10.1111/jam.12475*

Li, H., Wu, S., Wirth, S., Hao, Y., Wang, W., Zou, H., Li, W., & Wang, G. (2016). Diversity and activity of cellulolytic bacteria, isolated from the gut contents of grass carp (*Ctenopharyngodon idellus*) (Valenciennes) fed on Sudan grass (*Sorghum sudanense*) or artificial feedstuffs. *Aquaculture Research, 47* (1), 153-164. doi:*https://doi.org/10.1111/are.12478*

Li, S., Liu, H., Tan, B., Dong, X., Yang, Q., Chi, S., & Zhang, S. (2015a). Effects of dietary carbohydrate levels on the gene expression and the activity of PEPCK in marine fishes with different food habits.*Acta Hydrobiologica Sinica, 39* (1), 80-89.

Li, T., Long, M., Gatesoupe, F.-J., Zhang, Q., Li, A., & Gong, X. (2015b). Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. *Microbial Ecology, 69* (1), 25-36. doi:*https://doi.org/10.1007/s00248-014-0480-8*

Li, X. S., Zhu, X. M., & Han, D. (2012). *Comparative studies on carbohydrate utilization by three fishes of different food habits. Abstracts of Papers Presented at the Annual Conference of Chinese Fisheries Association* .

Li, Z., Zhang, X., Aweya, J. J., Wang, S., Hu, Z., Li, S., & Wen, X. (2019). Formulated diet alters gut microbiota compositions in marine fish *Nibea coibor* and *Nibea diacanthus* . *Aquaculture Research, 50* (1), 126-138. doi:*https://doi.org/10.1111/are.13874*

Limbu, S. M., Zhou, L., Sun, S.-X., Zhang, M.-L., & Du, Z.-Y. (2018). Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. *Environment International, 115* , 205-219. doi:*https://doi.org/10.1016/j.envint.2018.03.034*

Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F., & Wang, W. (2016). The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Scientific Reports, 6* (1), 24340. doi:*https://doi.org/10.1038/srep24340*

Liu, H., & Zhang, Y. (2001). The anatomy on the digestive system of*Silurus meridionalis* . *Journal of Quanzhou Normal College, 19* (6), 75-79.

Liu, M., Guo, W., Wu, F., Qu, Q., Tan, Q., & Gong, W. (2017). Dietary supplementation of sodium butyrate may benefit growth performance and intestinal function in juvenile grass carp (*Ctenopharyngodon idellus*). *Aquaculture Research, 48* (8), 4102-4111. doi:*https://doi.org/10.1111/are.13230*

Liu, Y., Li, X., Li, J., & Chen, W. (2021). The gut microbiome composition and degradation enzymes activity of black Amur bream (*Megalobrama terminalis*) in response to breeding migratory behavior. *Ecology and Evolution, 11* (10), 5150-5163. doi:*https://doi.org/10.1002/ece3.7407*

Liu, Y. Z., He, G., & Zhou, H. H. (2014). Substitution system of animal and plant protein source in aquatic feed and its relationship with feeding habits of cultured fish. *Hebei Fisheries, 8* , 54-57.

McFall-Ngai, M. J. (2015). The development of cooperative associations between animals and bacteria: Establishing detente among domains1.*American Zoologist, 38* (4), 593-608. doi:*https://doi.org/10.1093/icb/38.4.593*

Meng, H., Zhang, Y., Zhao, L., Zhao, W., He, C., Honaker, C. F., Zhai, Z., Sun, Z., & Siegel, P. B. (2014). Body weight selection affects quantitative genetic correlated responses in gut microbiota. *PLoS ONE, 9* (3), e89862. doi:*https://doi.org/10.1371/journal.pone.0089862*

Meng, X., & Nie, G. (2019). Advances of intestinal microbiota and lipid metabolism of fish. *Journal of Fishery Sciences of China, 26* , 1221–1229.

Miyake, S., Ngugi, D. K., & Stingl, U. (2015). Diet strongly influences the gut microbiota of surgeonfishes. *Molecular Ecology, 24* (3), 656-672. doi:*https://doi.org/10.1111/mec.13050*

Pan, Q., Guo, G., Fang, Z., & Li, Z. (1996). The comparative anatomy studies on digestive system of 6 fish species of stomach-containing teleost in freshwater. *Journal Huazhong Agricultural University, 15* (5), 463-469.

Pan, W., Qin, C., Zuo, T., Yu, G., Zhu, W., Ma, H., & Xi, S. (2021). Is metagenomic analysis an effective way to analyze fish feeding habits? A case of the yellowfin sea bream *Acanthopagrus latus* (Houttuyn) in Daya Bay. *Frontiers in Marine Science, 8* . doi:*https://doi.org/10.3389/fmars.2021.634651*

Parrizas, M., Planas, J., Plisetskaya, E. M., & Gutierrez, J. (1994). Insulin binding and receptor tyrosine kinase activity in skeletal muscle of carnivorous and omnivorous fish. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 266* (6), R1944-R1950. doi:*https://doi.org/10.1152/ajpregu.1994.266.6.R1944*

Roeselers, G., Mittge, E. K., Stephens, W. Z., Parichy, D. M., Cavanaugh, C. M., Guillemin, K., & Rawls, J. F. (2011). Evidence for a core gut microbiota in the zebrafish. *The ISME journal, 5* (10), 1595-1608. doi:*https://doi.org/10.1038/ismej.2011.38*

Shen, Y., Li, H., Zhao, J., Tang, S., Zhao, Y., Bi, Y., & Chen, X. (2021). The digestive system of mandarin fish (*Siniperca chuatsi*) can adapt to domestication by feeding with artificial diet.*Aquaculture, 538* , 736546. doi:*https://doi.org/10.1016/j.aquaculture.2021.736546*

Shi, X., Luo, Z., Chen, F., Wei, C.-C., Wu, K., Zhu, X.-M., & Liu, X. (2017). Effect of fish meal replacement by Chlorella meal with dietary cellulase addition on growth performance, digestive enzymatic activities, histology and myogenic genes' expression for crucian carp*Carassius auratus* . *Aquaculture Research, 48* (6), 3244-3256. doi:*https://doi.org/10.1111/are.13154*

Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology, 9* (4), 279-290. doi:*https://doi.org/10.1038/nrmicro2540*

Sullam, K. E., Essinger, S. D., Lozupone, C. A., O'connor, M. P., Rosen, G. L., Knight, R., Kilham, S. S., & Russell, J. A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Molecular Ecology, 21* (13), 3363-3378. doi:*https://doi.org/10.1111/j.1365-294X.2012.05552.x*

Sun, Y. X., Dong, H. B., & Duan, Y. F. (2019). Progresses in stress damage and protection studies on fish intestine. *Transactions of Oceanology and Limnology, 3* , 174-183.

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ, 361* , k2179. doi:*https://doi.org/10.1136/bmj.k2179*

Wang, A. R., Ran, C., Ringo, E., & Zhou, Z. G. (2018). Progress in fish gastrointestinal microbiota research. *Reviews in Aquaculture, 10* (3), 626-640. doi:*https://doi.org/10.1111/raq.12191*

Xu, B. H., Wang, Y. N., & Xiao, D. Y. (2011). The comparative histology studies on digestive system of four species of economic freshwater fishes. *Chinese Agricultural Science Bulletin, 27* (32), 47-55.

Youngblut, N. D., Reischer, G. H., Walters, W., Schuster, N., Walzer, C., Stalder, G., Ley, R. E., & Farnleitner, A. H. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications, 10* (1), 2200. doi:*https://doi.org/10.1038/s41467-019-10191-3*

Zeng, D., & Ye, Y. (1998). Studies on digestive system and different feeding habits of some fishes in freshwater. *Journal of Southwest Agricultural University, 20* (4), 361-364.

Zhang, C., Zheng, X., Ren, X., Li, Y., & Wang, Y. (2019). Bacterial diversity in gut of large yellow croaker *Larimichthys crocea* and black sea bream *Sparus macrocephalus* reared in an inshore net pen. *Fisheries Science, 85* (6), 1027-1036. doi:*https://doi.org/10.1007/s12562-019-01349-5*

Zhang, Y., Wen, B., Meng, L.-J., Gao, J.-Z., & Chen, Z.-Z. (2021). Dynamic changes of gut microbiota of discus fish (*Symphysodon haraldi*) at different feeding stages. *Aquaculture, 531* , 735912. doi:*https://doi.org/10.1016/j.aquaculture.2020.735912*

Zheng, Z., Yu, E., Li, Z., Ou, H., & Wang, G. (2016). The complete mitochondrial genome of *Shizothorax grahami* (Cypriniformes: Cyprinidae). *Mitochondrial DNA Part B, 1* (1), 775-776. doi:*https://doi.org/10.1080/23802359.2016.1238757*

Zhou, L., Lin, K.-T., Gan, L., Sun, J.-J., Guo, C.-J., Liu, L., & Huang, X.-D. (2019). Intestinal microbiota of grass carp fed faba beans: A comparative study. *Microorganisms, 7* (10), 465. doi:*https://doi.org/10.3390/microorganisms7100465*

Zhou, Y.-L., He, G.-L., Jin, T., Chen, Y.-J., Dai, F.-Y., Luo, L., & Lin, S.-M. (2021). High dietary starch impairs intestinal health and microbiota of largemouth bass, *Micropterus salmoides* .*Aquaculture, 534* , 736261. doi:*https://doi.org/10.1016/j.aquaculture.2020.736261*

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