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Assessing the Effects of Dietary Tea Polyphenols on the Gut Microbiota of Loaches (*Paramisgurnus dabryanus*) under Chronic Ammonia Nitrogen Stress

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Abstract: This study examined the impact of tea polyphenols (TPs) on the intestinal flora of loaches (*Paramisgurnus dabryanus*) under chronic ammonia nitrogen stress using high-throughput sequencing. Two groups of 600 loaches were studied over one month, and they were separated into a control group and tea polyphenol group. Alpha and beta diversity analyses showed diverse bacterial communities, with significant differences in the abundance and uniformity observed initially but not between sampling time points. Cluster analyses revealed distinct differences in microbial communities between groups. A predictive function analysis indicated enrichment in pathways related to amino acid and nucleotide biosynthesis. These findings offer initial insights into how tea polyphenols may affect intestinal microbial communities in loaches under ammonia nitrogen stress.

Keywords: ammonia nitrogen stress; tea polyphenols; loach; gut microbiota; high-throughput sequencing

Key Contribution: Ammonia stress is a common issue in modern aquaculture, and in this study, TPs were found to have a potential positive effect on the gut microbial community of loaches under chronic ammonia stress.

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1. Introduction

Aquaculture systems often experience a sharp increase in the ammonia nitrogen content of water from the decomposition of animal excrement, residual feed, and plankton debris [1,2]. Ammonia nitrogen is mainly introduced via the decomposition of nitrogen-containing organic matter and exists in the forms of NH4+ (ionic ammonia) and NH3 (non-ionized ammonia). When ammonia concentrations in water are elevated, ammonia molecules can enter the animal's body through the skin and gill epithelium, leading to an increase in ammonia nitrogen in the animal [3]. If ammonia nitrogen exposure lasts longer than the organism's tolerance limit and exceeds the body's regulatory threshold, it may harm or damage the antioxidant and immune systems and increase the risk of disease in the animal [4–7]. To prevent this damage, fish nutritionists usually add synthetic antioxidants to their diets; however, these synthetic antioxidants have side effects [8]. Therefore, natural additives are in high demand [8,9].

Tea polyphenols (TPs) are bioactive components found in tea that dissolve easily in water and various organic solvents, such as methanol, ethanol, and acetone. TPs are stable in acidic environments but are susceptible to oxidation under alkaline conditions [10]. The beneficial effects of TPs are primarily attributed to their polyphenolic compounds, particularly catechins, which comprise approximately 75–80% of TPs. Catechins have been associated with several biological benefits, including antioxidant and antitumor properties,

a reduced risk of cardiovascular diseases, and immune enhancement [11–13]. Among the polyphenolic compounds found in green tea, catechin is the primary compound, along with epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), and epicatechin (EC) as the other main constituents [14]. The most abundant catechin is gallate (EGCG), which is the most effective polyphenol component in conferring the above-mentioned beneficial biological properties [15].

The loach (*Paramisgurnus dabryanus*) is a small freshwater benthic fish that is widely distributed throughout East Asia. In recent years, loaches have been cultivated primarily in paddy fields in China [16,17], where the release of fertilizers can lead to significant fluctuations in the ammonia nitrogen levels in the water body. Consequently, this environmental shift adversely affects various physiological parameters of the loach, resulting in negative impacts on the overall well-being [18,19]. Many studies have shown that a TP feed can have beneficial effects on ammonia resistance in fish [20]. This study aimed to investigate the effects of TPs on the gut microbiota structure of large-scale loaches under chronic ammonia nitrogen stress. The aim of this study is to provide insights into the potential of TPs as a safe, effective, and natural antioxidant without any side effects. These findings may offer valuable directions for enhancing the resilience of loaches in challenging environments and may contribute to a broader understanding of the effects of TPs on the gut microbiota of aquatic organisms.

2. Materials and Methods

Our study did not involve endangered or protected species. All efforts were made to minimize animal suffering and discomfort. The experimental protocol was approved by the Animal Ethics Committee of Shenyang Agriculture University (permit number 2021041601). All animal surveys were carried out in accordance with the approved guidelines of Shenyang Agriculture University Experimental Animal Management Committee.

2.1. Experimental Fish

The experimental loach specimens were purchased from a local market in China and transported to the Aquaculture Laboratory of Shenyang Agricultural University in October 2022 for a two-week period of temporary rearing. At the end of the staging period, a month-long trial was conducted. Healthy loaches with an initial weight of 8.41 g/tail were randomly distributed into six water barrels measuring 92.5 cm × 92 cm × 90 cm, with a volume of 100 L. They were named according to the control group (CK1, CK2, and CK3) or TP group (EG1, EG2, and EG3), and 100 loaches were placed in each barrel. The control group was fed basic feed without TPs, whereas the experimental group was given feed with TPs (>98% purity; Zhong Chen Biotechnology Co., Ltd., Henan, China) at a content of 0.01% [21] (Table 1). Two daily feedings at 8:00 A.M. and 4:00 P.M. amounted to 2–3% of the fish's body weight. Ammonia nitrogen mother liquor was prepared by diluting analytically pure NH4Cl (purity > 99%; Shenyang Xilong Chemical Co., Ltd., Shenyang, China) to a concentration of 20 g/L. Subsequently, 10 L of the solution was prepared daily, stored in a static state, and then diluted proportionally to the required experimental concentration when in use.

Table 1. Feed formula of *Paramisgurnus dabryanus* (%).

Ingredient	No Tea Polyphenols	Contains Tea Polyphenols
Fish meal	20	20
Dextrin	5	5
Soybean meal	32	31.99
Flour	10	10
Wheat bran	11	11
Corn gluten powder	15	15
Fish oil	5	5

Table 1. *Cont.*

Ingredient	No Tea Polyphenols	Contains Tea Polyphenols
Calcium phosphate	1	1
Vitamin premix	1	1
Tea polyphenols	0	0.01
Total ratio	100	100

Note: Vitamin premix (mg/kg): riboflavin, 20; thiamin, 20; pyridoxine, 20; vitamin B12, 2; vitamin D, 0.5; vitamin A, 1.83; vitamin K, 10; vitamin E, 10; folic acid, 5; Ca-pantothenate, 50; inositol, 100. 2 Mineral premix (g/kg): monocalcium phosphate.

2.2. Ammonia Stress Test

After the temporary rearing period, ammonia nitrogen was introduced by diluting the NH4Cl to a concentration of 50 mg/L per barrel, which was maintained for 30 days. The water temperature during the test was maintained at (15 ± 3) °C. The experimental container's water was changed twice a day, starting one hour after feeding, with approximately 25% of the water changed each time and 50% of the water changed in a day. To maintain the concentration of ammonia nitrogen in the water, the total ammonia nitrogen concentration was determined by applying a T6 series UV–visible spectrophotometer (Beijing Puxi General Instrument Co., Ltd., Beijing, China) after water exchange. Adjustments were made as necessary using a master batch [22]. The lighting conditions used in the experiment were natural light cycles.

2.3. Sampling

The growth and mortality of the loaches were monitored during the culture period. Sampling was conducted 10, 20, and 30 days after the initiation of the experiment. Loaches from control and TP groups were selected for dissection, and intestinal samples were collected. A total of 18 samples, each weighing approximately 1–1.5 g, were collected from the six groups and placed in sterile centrifuge tubes with a unique identifier. Subsequently, the samples were frozen in liquid nitrogen and stored at –80 °C for subsequent sequencing analysis.

2.4. Growth Performance Measurement

During the experiment, the loaches were weighed every 10 d. The weight gain (WG, Equation (1)), specific growth rate (SGR, Equation (2)), and survival rate (SR, Equation (3)) for all surviving loaches were calculated at the end of the experiment using the following formulas:

$$WG = 100 \times (W2 - W1)/W1(g) \quad (1)$$

$$SGR = 100 \times (W2 - W1)/T \quad (2)$$

$$SR = 100 \times M/N \quad (3)$$

where W1 is the initial body weight (g), W2 is final body weight (g), TF is the total feed consumption (g), T is the number of feeding days, M is the number of surviving individuals, and N is the total number of fish in the experiment.

2.5. DNA Extraction and 16S rRNA High-Throughput Sequencing

DNA extraction and PCR amplification were performed as follows: gut genomic DNA was extracted from six sample groups using the Qubit 2.0 DNA Assay Kit (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. The quality of the extracted DNA was assessed using 1.0% agarose gel electrophoresis and spectrophotometry, and the optical density was measured at a 260 nm/280 nm ratio. All extracted DNA samples were then stored at −20 °C for subsequent analysis. To investigate the structure and composition of the bacterial communities in the loach gut, high-throughput sequencing of the 16S rRNA gene was conducted. The V3–V4 variable region of the bacterial 16S rRNA gene was amplified using the universal forward 338/806 primers (338F: ACTCCTACGGGAGGCAGCCA and 806R GGACTACVSGGGTATCTAAT) [23]. Raw data were

screened using quantitative insights into microbial ecology (QIIME) based on barcode and primer sequences [24].

2.6. Bioinformatics

To ensure the accuracy of the information analysis results, the raw data were spliced and filtered to obtain valid data [25,26]. The sequence was further pruned using the DADA2 method [27], which included deprivation, mass filtering, denoising, splicing, and chimera removal. An amplifying sequence variant (ASV) approach was applied, resulting in 100% similarity clustering after applying DADA2 quality control. Moreover, representative sequences were selected for each ASV, and taxonomic data were assigned to these representative sequences using the classification sklearn algorithm [28]. For sequence alignment, the Green Genes database (version 13·8, http://greengenes.secondgenome.com/, accessed on 6 January 2023) was used as a reference database [29]. For alpha-diversity analysis, the QIIME program was employed to calculate indices such as CHAO1 and observed species richness estimators as well as Shannon and Simpson diversity. The Bray–Curtis distance measurement method was utilized to analyze beta diversity, whereas principal coordinate analysis (PCOA), nonmetric multidimensional scaling (NMDS), and the non-weighted pair group method with arithmetic mean were employed to visualize the changes in microbial community structure among different samples [30]. To examine shared and unique ASVs in samples or groups, Venn diagrams were generated using the R package (https://www.r-project.org/ accessed on 6 January 2023) "Venn Diagram" regardless of their relative abundance [31]. Based on the high-quality sequences, the PICRUST2 method was used to predict microbial function by reconstructing unobserved states. The identified genes and their corresponding functions were cross-referenced with databases such as KEGG (http://www.kegg.jp/, accessed on 6 January 2023) for comparison and validation.

2.7. Statistical Analysis

SPSS Statistical software 26.0 was used to conduct a *t*-test comparing the means of the two groups for independent samples of growth data. Data are expressed as "mean ± standard deviation", and $p < 0.05$ indicates statistically significant differences.

3. Results

3.1. Effects of Tea Polyphenols on Growth Performance

As shown in Table 2, we found that WG and SGR were significantly lower in the TP group than in the control group ($p < 0.05$).

Table 2. Growth performance of loaches fed diets supplemented with tea polyphenols and their combination for 30 days.

	Control Group	Tea Polyphenol Group	*p*-Values
Initial body weight/g	8.29 ± 0.05	8.53 ± 0.32	0.284
Day 10 weight/g	9.68 ± 1.08	9.06 ± 0.12	0.38
Day 20 weight/g	9.11 ± 0.89	9.67 ± 0.25	0.358
Final weight/g	10.8 ± 0.53	9.93 ± 0.13	0.054
Weight gain/%	30.24 ± 5.98 a	16.59 ± 5.67 b	0.045
Specific growth rate/%	8.36 ± 1.68 a	4.68 ± 1.45 b	0.046
Survival/%	71.7	83.3	0.206

Note: Peer data shoulder labels that do not contain the same lowercase letter indicate a significant difference ($p < 0.05$), and those that contain the same letter or no letter indicate a non-significant difference ($p > 0.05$).

3.2. Pyrosequencing of the Gut Bacterial Community

A total of 2,486,325 raw sequence reads were generated from the intestinal samples. After undergoing mass filtering and denoising, 2,018,687 valid sequences were obtained (Supplementary Table S1). Across different classification levels, we identified a total of

4734 amplifying subsequence variants (ASVs), including 270 at the phylum level, 1652 at the genus level, and 768 at the species level (Supplementary Table S2).

The dominant bacterial communities remained consistent across the different sampling times in both groups, although their relative abundances varied. Proteobacteria dominated the gut microbiota at the phylum level in groups CK1, EG1, CK2, EG2, and CK3, representing 45.3%, 48.51%, 40.92%, 33.79%, and 25.71% of the total bacterial community, respectively. Tenercutes exhibited the highest proportions in groups CK1, EG2, CK3, and EG3, accounting for 25.57%, 25.76%, 33.69%, and 27.14% of the microbiota, respectively. Firmicutes were more prevalent in the CK1, EG1, and EG3 groups, with proportions of 21.39%, 26.04%, and 34.13%, respectively. Notably, the EG1 group showed a 15.09% higher content of Bacteroidetes than the CK1 group (Figure 1A).

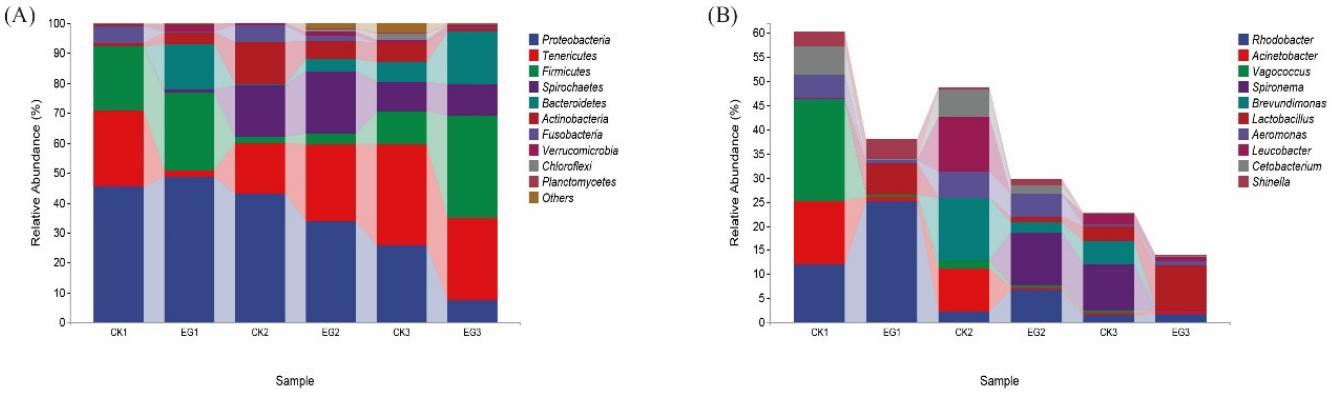

Figure 1. The relative abundance of OTUs: (**A**) phylum levels. (**B**) genera levels.

The dominant genera (with a relative abundance of >5% in at least one sample) included *Rhodobacter*, *Acinetobacter*, *Vagococcus*, *Spironema*, *Brevundimonas*, *Lactobacillus*, *Aeromonas*, *Leucobacter*, *Cetobacterium*, and *Shinella* (Figure 1B). Compared to the control group, the TP feed group exhibited higher levels of *Rhodobacter* and *Lactobacillus*, whereas *Acinetobacter*, *Vagocochus*, *Brevundimonas*, and *Cetobacterium* were more abundant in the control group.

3.3. Alpha Diversity Reveals Alterations in Gut Bacterial Community Structure

We compared the alpha-diversity indices between the control group and the TP feed group to assess changes in the gut bacterial community structure (Table 3). The Chao1 index, which reflects microflora abundance, was significantly higher in the EG1 group than in the control group, whereas no significant differences were observed among the other groups ($p < 0.05$). A high intestinal coverage rate (>0.997) confirmed the accuracy of the sequencing results (Figure 2).

Table 3. Gut microbiota richness and alpha-diversity index statistics in *Paramisgurnus dabryanus*.

Sample	Chao	Coverage	Species	Shannon	Simpson	Group
CK10_1	234.588	0.999705	226.6	3.68927	0.817233	CK1
CK10_2	184.037	0.999535	165.8	2.12276	0.603577	CK1
CK10_3	411.753	0.999451	386.0	5.26191	0.936348	CK1
EG10_1	909.117	0.999194	884.3	6.83122	0.973744	EG1
EG10_2	503.742	0.999483	487.4	4.48923	0.881454	EG1
EG10_3	1354.28	0.998181	1288.9	7.62177	0.978703	EG1
CK20_1	240.27	0.999409	219.5	2.86509	0.753720	CK2
CK20_2	390.41	0.999178	363.9	3.07832	0.742806	CK2
CK20_3	398.164	0.999338	374.8	3.79994	0.785523	CK2

Table 3. *Cont.*

Sample	Chao	Coverage	Species	Shannon	Simpson	Group
EG20_1	511.733	0.999210	494.5	6.19355	0.967188	EG2
EG20_2	695.119	0.998515	649.4	3.48330	0.644694	EG2
EG20_3	243.425	0.999840	241.6	3.82030	0.819175	EG2
CK30_1	387.626	0.999210	362.7	2.45514	0.661230	CK3
CK30_2	1292.57	0.997923	1225.6	6.86703	0.966195	CK3
CK30_3	2402.26	0.996557	2324.1	6.54731	0.827503	CK3
EG30_1	1550.90	0.997167	1493.7	5.90112	0.885300	EG3
EG30_2	858.088	0.999107	828.8	4.03164	0.634363	EG3
EG30_3	1896.01	0.997834	1868.3	7.73055	0.971686	EG3

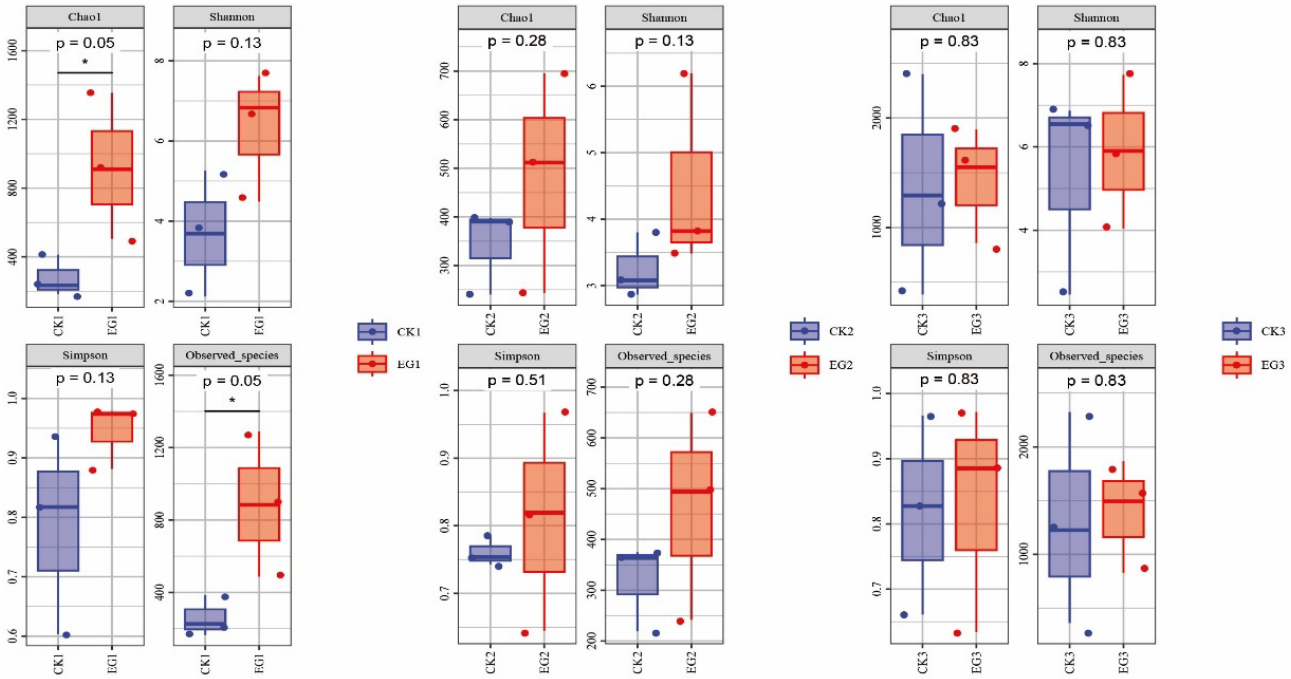

Figure 2. A comparison of alpha-diversity indices (Chao1, Observed species, Shannon, Simpson) of the gut microbial community in *Paramisgurnus dabryanus* performing at 10 days (CK1 vs. EG1), 20 days (CK2 vs. EG2) and 30 days (CK3 vs. EG3). Box plots depict the medians (central horizontal lines), inter-quartile ranges (boxes), and 95% confidence intervals (whiskers). The *p*-values are from the Kruskal–Wallis test. Asterisks indicate statistically significant differences between pairs of values (* $p < 0.05$).

3.4. Beta-Diversity Analysis Reveals Shifts in Gut Bacterial Community Structure

The PCoA scatterplot revealed a significant difference in population composition between the CK1 and EG1 groups, whereas no significant differences were found among the other groups (Figure 3). The two primary coordinates explained 24.9% and 17% of the total variation, respectively. Notably, samples from the CK3 and EG3 groups were clustered below the midline of the vertical axis, whereas samples from the other groups were clustered above the midline. Additionally, the NMDS analysis confirmed a significant difference between the CK1 and EG1 groups but showed no significant differences among the other sample groups.

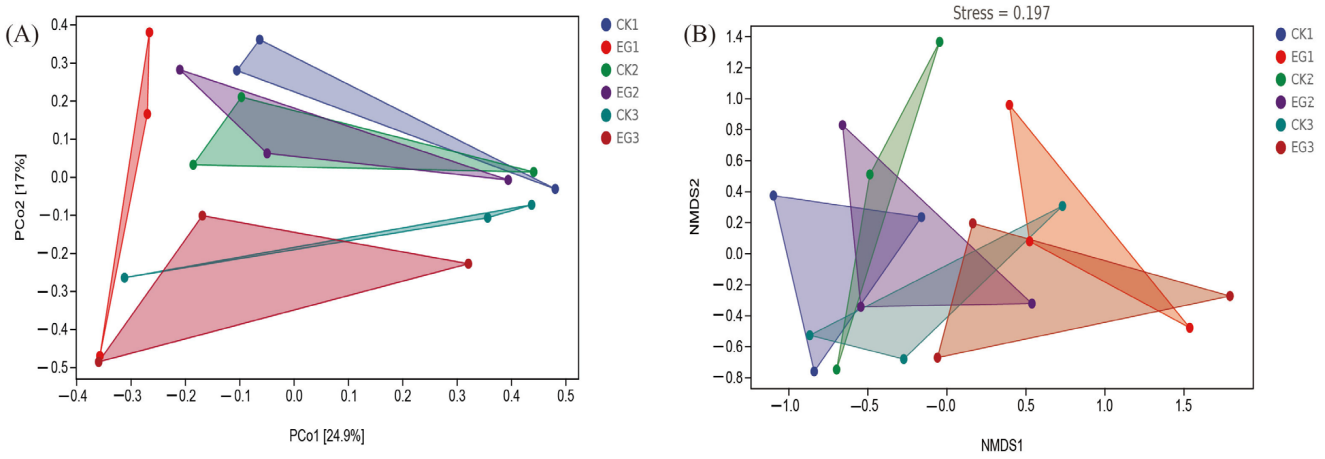

Figure 3. (**A**) Principal coordinate analysis (PCoA) of the gut bacterial community. (**B**) Non-metric multidimensional scaling (NMDS) of gut bacterial community. Taxonomic composition of microbial communities across eight group. Phylum level relative abundance of intestinal microbiome of each group averaged across individual.

3.5. Venn Diagram Analysis

Venn diagrams were used to visualize the shared and unique amplifying sequence variants (ASVs) among the different gut sample groups, representing the core gut microbiota. As shown in Figure 4, all samples shared 101 ASVs. The CK1, CK2, and CK3 groups had 264, 356, and 2597 distinct ASVs, respectively. In contrast, the EG1, EG2, and EG3 groups had 913, 730, and 2238 unique ASVs, respectively.

Figure 4. Unique and shared amplicon sequence variants (ASVs) in different groups. The Venn diagram displays the number of shared and specific ASVs among six groups.

3.6. Taxonomic Differences and Marker Species

To explore the genus-level similarities and differences between samples, we generated a hierarchical clustering heatmap (Figure 5). In the CK2 group, the dominant genera included *Shewanella*, *Brevundimonas*, *Leucobacter*, and *Vibrio*. Specific genera, such as *Vagococcus*, *Luteolibacter*, and *Elizabethkingia*, were observed in the CK1, EG1, and EG2 groups, respectively.

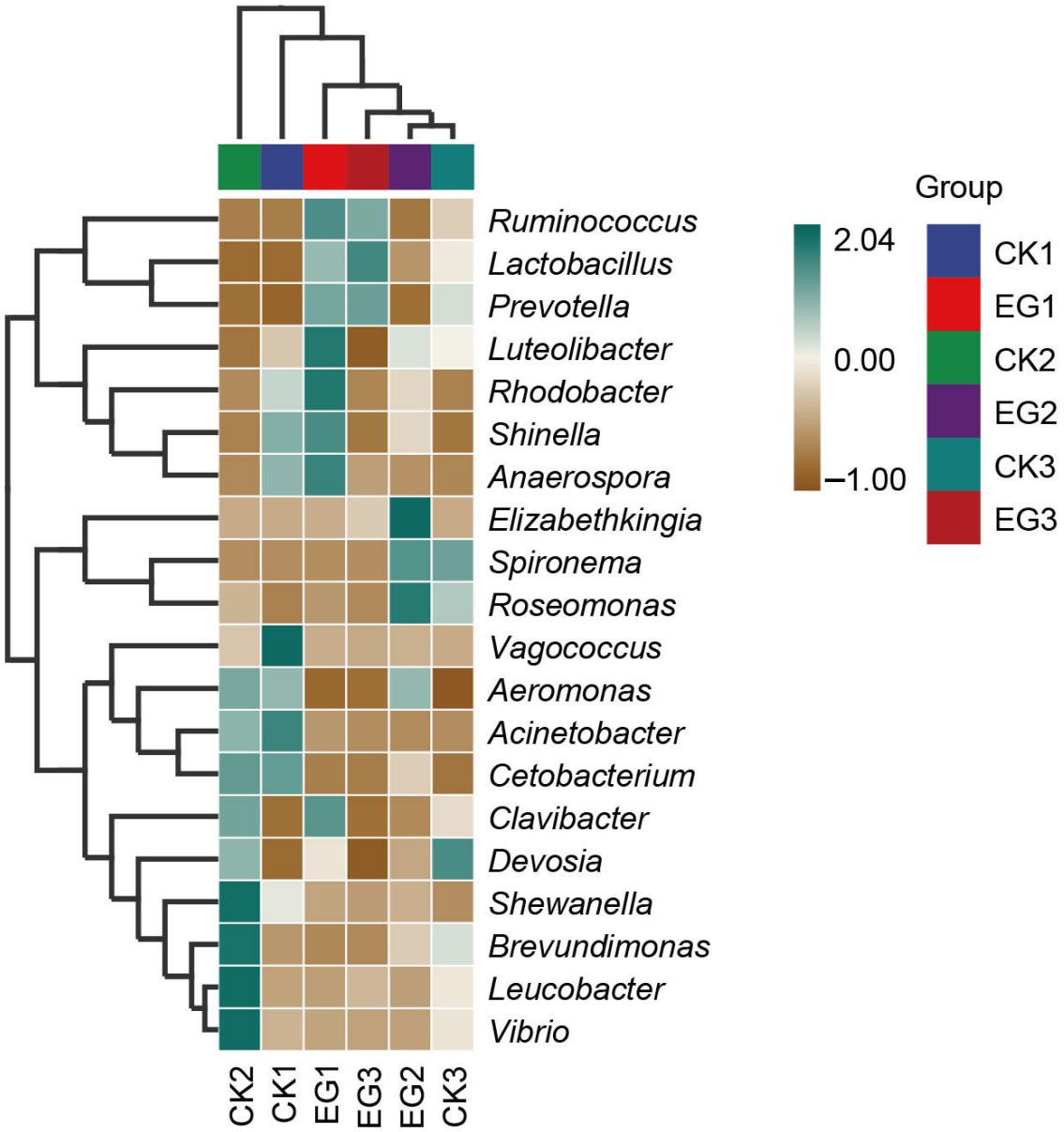

Figure 5. The hierarchical clustering heatmap of the gut bacteria at the genus level.

3.7. Functional Prediction of Intestinal Microbiota

We determined the abundance of active metabolic pathways in the intestinal microbiota by reviewing various metabolic pathway databases. Amino acid biosynthesis pathways were the most abundant among the biosynthetic pathways, followed by pathways related to cofactors; prosthetic groups; electron carriers; and the biosynthesis of vitamins, fatty acids, lipids, nucleosides, and nucleotides (Figure 6).

Figure 6. Relative abundance of metabolic pathways. The horizontal coordinate is the abundance of the functional pathway, the vertical coordinate is the functional pathway at the second classification level of KEGG, and the right most is the first level pathway to which the pathway belongs.

4. Discussion

Ammonia nitrogen poses a serious threat to various aquatic organisms, including fish, shellfish, and aquatic plants [32–37]. The digestive tract plays a crucial role in the transmission and entry of pathogens, making it highly susceptible to diseases. Probiotic additives have been shown to enhance gut performance and improve the overall well-being of fish by bolstering their immune systems [38]. Hence, understanding the role of the gut microbiota in fish immunity and digestive systems is crucial for predicting and treating fish diseases. As a small demersal fish, gaining a better understanding of the immune and digestive systems of loaches through gut microbiota is a fundamental step in predicting and managing fish diseases.

Owing to its extensive biological activity, TPs have been used as a promising natural feed additive for aquaculture species; however, the effects of dietary TPs on the growth performance of loaches remain unclear. It is noteworthy that our findings were consistent

with others that have shown that supplementation with TPs decreased WG in rainbow trout (*Oncorhynchus mykiss*) [39] and black rockfish (*Sebastes schlegeli*) [40]. The significant differences in the WG and SGR of the TP group relative to the control group may have been related to the species and nutritional status of the fish as well as the composition and concentration of polyphenolic compounds in their diet [41].

To the best of our knowledge, few studies have explored the effects of dietary supplementation on the gut bacterial community in loaches under chronic ammonia nitrogen stress. This study investigated the dynamics of the intestinal bacterial community in loaches under ammonia nitrogen stress when TPs were added to their diet. As supported by previous research, the dominant phyla in the gut microbiota of fish typically include Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, with Proteobacteria often being the most prominent [42]. This may be partly attributed to the disproportionately large number of Proteobacteria among all prokaryotes.

In this study, the dominant bacterial genera identified in loaches encompassed five phyla: Proteobacteria, Tenericutes, Firmicutes, Spirochaetes, and Bacteroidetes [43]. These dominant microbiota have also been recognized as significant components of the gut microbiota of cobia and rainbow trout [44,45]. Interestingly, except at the initial sampling point, where Proteobacteria were higher in the TP feed group than in the control group, the opposite trend was observed in subsequent samples, with a gradual decrease in the relative abundance of Proteobacteria. Previous studies have suggested that the substantial enrichment of Proteobacteria signifies an unbalanced and unstable microbial community structure or a diseased state within the host. Therefore, the gradual decrease in Proteobacteria levels suggests a positive modulatory effect of tea polyphenols on beneficial flora [46].

Furthermore, the TP feed group exhibited higher levels of Bacteroidetes and Firmicutes than the control group. This outcome suggests a mutually reinforcing symbiotic relationship between Bacteroidetes and Firmicutes, which jointly promote host energy absorption and storage [47]. Moreover, a comparison of the gut microflora at the phylum level revealed that the dominant microflora remained unchanged between the TP feed and control groups, implying that TPs did not alter the emergence of the dominant flora but rather influenced their relative proportions.

At the genus level, we found that the abundances of *Rhodobacter*, *Lactobacillus*, and *Shinella* were significantly higher in the TP feed group than in the control group, indicating that TPs increase the proportion of beneficial bacteria, such as *Rhodobacter*, while effectively inhibiting pathogenic bacteria, such as *Acinetobacter* [48,49]. The enhanced presence of *Lactobacillus*, which is commonly found in the gut microbiota of fish and involved in crucial biological processes, including digestion, stress responses, and reproduction, further highlights the potential benefits of TPs in promoting gut health and overall fish well-being [50].

Alpha and beta diversity are essential parameters for evaluating the structural characteristics of the microbiota. In this study, we assessed α diversity using various indices, such as Shannon, Simpson, and Chao 1, as well as observed species and Good's coverage values. High Good's coverage values (>0.99) for each sample indicated high sequencing accuracy. In addition, the microbial abundance (Chao 1 and observed species values) was significantly higher in the EG1 group than in the CK1 group, whereas the changes were not significant in the other groups. In agreement with other studies, this difference revealed that the short-term consumption of TPs significantly affects the gut microbiota of loaches [20].

PCoA, NMDS, and heatmap clustering analyses clearly demonstrated distinct characteristics of the gut bacterial community of loaches under the two feeding conditions on day 10. This divergence in microbial composition may be attributed to the abundance of digestive enzymes and symbiotic microorganisms within the gut, whereas TPs are prone to metabolic transformations facilitated by these enzymes or microorganisms [51]. The metabolism of TPs by the gut microbiota involves multiple pathways, leading to the production of diverse metabolites with distinct structures, such as pyrogallol, 5-(3′,4′-dihydroxyphenyl)-, or 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone. Hence, intestinal flora

plays a pivotal role in facilitating the transformation of TPs into biologically active substances [52,53].

The Venn diagram cluster analysis revealed that 101 ASVs were shared among all the samples, shedding light on their unique distribution patterns. The unique bacterial core associated with the intestinal contents primarily comprised Proteobacteria, Tenericutes, and Firmicutes, which is consistent with previous studies on loaches raised in low-temperature environments. The impact of gut microflora on health and metabolic processes is widely recognized [54], and these amino acids play crucial roles in catabolism and fermentation in the gut [55]. According to Zhou et al., TPs can enhance the biosynthesis of many amino acids and derivatives, such as threonine, aspartic acid, and leucine [56]. Our sequencing results indicated a significant enrichment of bacterial genes associated with amino acid, nucleoside, and nucleotide biosynthesis pathways, which is consistent with the results of previous studies [57]. Moreover, a previous study reported that ammonia stress can elevate the levels of various amino acids in the body, including proline, arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, valine, alanine, glutamic acid, tyrosine, and aspartic acid [55]. Additionally, numerous studies have highlighted the role of amino acid synthesis in promoting ammonia nitrogen excretion, thereby reducing ammonia accumulation in the body [58,59].

A comparison of the hierarchical clustering heatmaps showed that CK2 contained several other specific microbial genera, including *Shewanella*, *Brevundimonas*, *Leucobacter*, and *Vibrio*. Of these, *Shewanella* contains strains that are resistant to a wide range of toxic compounds and can survive and colonize synthetic community habitats in polluted areas. In addition, some of these bacteria actively attenuate the harmful effects of toxic products, thereby increasing ecosystem resistance and resilience to pollution. The genus *Brevundimonas* consists of non-fermenting Gram-negative bacteria that are of minor clinical importance, as they can cause many types of infections. The genus *Leucobacter*, which belongs to the phylum Actinobacteria, are Gram-positive and aerobic, and they even show resistance and/or biosorption characteristics to heavy metals [60]. The genus *Vibrio* consists of Gram-negative bacteria that are often recognized as an opportunistic pathogen of fish and other animals that may lead to aquaculture mortality or economic loss. The specific genus of the microorganisms identified in EG2 was *Elizabethkingia*. Microorganisms in this genus, which are Gram-negative, have become a major cause of life-threatening infections in many countries and are often detected in immunocompromised patients. Only one pathogenic bacterium was found in the group fed with TPs, suggesting that TPs may effectively reduce pathogenic bacteria.

As a typical representative of bioactive substances, TPs are an excellent natural antioxidant [61]. Their antioxidant [11], antibacterial [12], and anticancer [13] properties have led to wide application in various fields. Therefore, TPs, as natural and environmentally friendly feeds, have become the key to healthy aquacultures.

This study had certain limitations, particularly a lack of clarity regarding the appropriate dosage of TPs in different environments. Therefore, future studies should consider a wider range of environmental conditions or include more in-depth mechanistic studies exploring the effects of feeding different concentrations of TPs to loaches to fully understand the role of TPs in their gut microbial community composition. In conclusion, our findings support the potentially positive impacts of TPs on the gut microbial community of loaches under ammonia nitrogen stress.

5. Conclusions

Our study indicates that dietary supplementation with tea polyphenols (TPs) alters the gut microbiota composition in loaches, resulting in decreased Proteobacteria and increased Bacteroidetes and Firmicutes levels. This shift towards a more beneficial microbial profile suggests a potential role for TPs in enhancing gut health and overall well-being in loaches. Additionally, TPs appear to modulate pathways related to amino acid and nucleotide biosynthesis, potentially improving metabolic processes associated with ammonia nitrogen

stress tolerance. These findings highlight the potential of TPs as a natural feed additive to improve gut microbial composition and resilience in loaches under ammonia nitrogen stress conditions. Further research is needed to determine optimal dosage regimens and elucidate underlying mechanisms, particularly under varied environmental conditions, to fully exploit the benefits of TPs in aquaculture practices.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9050180/s1, Table S1. Pyrosequencing data of *Paramisgurnus dabryanus* gut bacterial community. Table S2. The number of bacterial operational taxonomic units (OTUs) over all samples.

Author Contributions: Y.C. and Y.L. designed this study. Y.C. collected specimens and performed the experiments. Y.C. and S.S. analyzed the data. Y.C. and Y.L. drafted the manuscript. S.S. and Y.L. reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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