Science of the Total Environment 851 (2022) 158260

Contents lists available at ScienceDirect

Science of the Total Environment

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

Probiotics ameliorate growth retardation of glyphosate by regulating intestinal microbiota and metabolites in crucian carp (*Carassius auratus*)

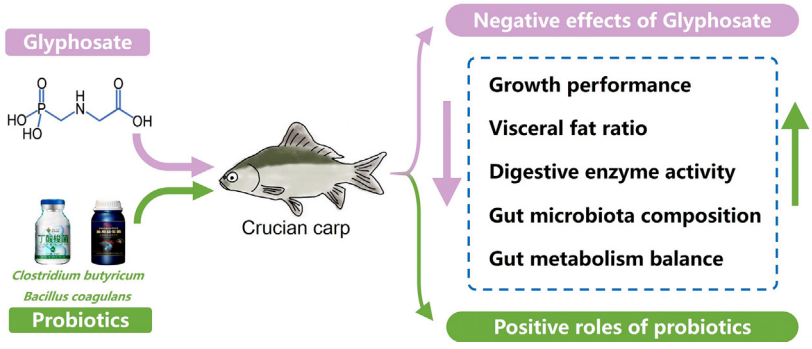
Biao Yan [a,b,c,1], Jian Han [a,1], Yumiao Sun [a,b], Lei Lei [a,b], Jing Yuan [a], Zhixian Qiao [d], Jun Men [d], Xin Wang [d], Yongyong Guo [a], Qidong Wang [a,*], Bingsheng Zhou [a]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academic of Sciences, Wuhan 430072, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] Xianning Engineering Research Center for Healthy Environment, Xianning Medical College, Hubei University of Science and Technology, Xianning 437100, PR China
[d] The Analysis and Testing Center of Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China

HIGHLIGHTS

- Exposure to glyphosate (GLY) caused growth retardation in juvenile crucian carp.
- GLY reduced lipase activity in intestine and visceral fat deposition.
- Gut microbial dysbiosis and metabolic disorder contributed to health hazard in host.
- Probiotics attenuated the health risks of GLY by restoring gut health.
- Here provides new clue for eliminating health risks of GLY in aquatic environment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Henner Hollert

Keywords:
Glyphosate
Growth retardation
Probiotics
Gut microbial metabolomics
Crucian carp

ABSTRACT

Glyphosate (GLY) contamination widely occurred in aquatic environments including aquaculture systems and raised hazard to aquatic organisms such as fish. Probiotics have been reported to alleviate contaminants-induced toxicity. However, whether probiotics could reduce the health risk of GLY to fish remain unknown. Here we investigated the impacts of GLY on crucian carp (*Carassius auratus*) by focusing on the protective roles of two commonly used aquaculture probiotics, *Bacillus coagulans* (BC) and *Clostridium butyricum* (CB). Exposure to GLY significantly caused growth retardation and reduced visceral fat and intestinal lipase activity in crucian carp. 16S rRNA sequencing indicated that dysbiosis of Bacteroidetes at phylum level and *Flavobacterium* at genus level might be primarily responsible for GLY-induced negative growth performance. High throughput targeted quantification for metabolites revealed that GLY changed intestinal metabolites profiles, especially the reduced bile acids and short-chain fatty acids. However, the addition of BC or CB effectively attenuated the adverse effects above by remodeling the gut microbiota composition and

Abbreviations: GLY, Glyphosate; BC, *Bacillus coagulans*; CB, *Clostridium butyricum*; UPLC-MS/MS, Ultra-performance liquid chromatography tandem mass spectrometry; Micro-CT, Micro computed tomography; TAT, Total adipose tissue volume; TV, Tissue volume; WGR, Weight gain rate; SGR, Specific growth rate; MSI, Muscle -somatic index; CF, Condition factor; AMS, α-amylase; LPS, Lipase; TRY, Trypsin; TCA, Taurocholic acide; TCDCA, Taurochenodeoxycholic acid; SCFAs, Short-chain fatty acids; OTU, Operational taxonomic unit; RDA, Redundancy analysis; PcoA, Principal coordinates analysis; PLS-DA, Partial least squares discriminant analysis; PICRUSt2, Phylogenetic investigation of communities by reconstruction of unobserved states; KEGG, Kyoto encyclopedia of genes and genomes.

* Corresponding author at: Institute of Hydrobiology, Chinese Academic of Sciences, No. 7 Donghu South Road, Wuchang District, Wuhan 430072, China.
E-mail addresses: yanbiao@ihb.ac.cn (B. Yan), hanjian@ihb.ac.cn (J. Han), sunyumiao@ihb.ac.cn (Y. Sun), leilei@ihb.ac.cn (L. Lei), yuanj@ihb.ac.cn (J. Yuan), qzhxian@ihb.ac.cn (Z. Qiao), menjun@ihb.ac.cn (J. Men), wangxin@ihb.ac.cn (X. Wang), yyguo@ihb.ac.cn (Y. Guo), wangqd@ihb.ac.cn (Q. Wang), bszhou@ihb.ac.cn (B. Zhou).
[1] Co-first authors.

http://dx.doi.org/10.1016/j.scitotenv.2022.158260
Received 6 June 2022; Received in revised form 18 August 2022; Accepted 20 August 2022
Available online xxxx

improving microbial metabolism. The present study provides novel evidence for ameliorating the harmful effects of GLY on fish species by adding probiotics, which highlights the potential application of probiotics in reducing the health risks of GLY in aquatic environment.

1. Introduction

Glyphosate (GLY) is the most frequently used broad-spectrum organophosphorus herbicide worldwide (Van Bruggen et al., 2018). Although the GLY is most extensively used in agriculture, some is also used pervasively for controlling submerged aquatic weeds and algae in ponds (Annett et al., 2014; Solomon and Thompson, 2011). Studies have shown that GLY can contaminate aquatic environment through multiple routes including spray drift, surface runoff and soil leaching, and even resulting in potential threatens to non-target aquatic organisms like fish species (Brovini et al., 2021; de Brito Rodrigues et al., 2019; Zhang et al., 2017). GLY has been widely occurred in aquatic environment at concentrations generally vary from 3 to 700 μg/L, and as high as 1.2 mg/L was found in freshwater fish ponds (Ding et al., 2021). GLY can also be detected in freshwater aquaculture products (Yan et al., 2022). Mounting evidence has documented that GLY inhibited the growth of fish (Salbego et al., 2010; Giaquinto et al., 2017) and crayfish (*Cherax quadricarinatus*) (Frontera et al., 2011), induced lipid metabolism disorder and hepatotoxicity in juvenile common carp (Liu et al., 2021a), as well as altered gut microbiome and intestinal histomorphology in zebrafish (Ding et al., 2021). The freshwater aquaculture plays vital roles in fishery of China. It accounts for 54 % of the total aquaculture production (Fisheries and Fisheries Administration Bureau of the Ministry of Agriculture and Rural Areas et al., 2018). The hazard of GLY to cultured fish has also aroused great concerns. Although the GLY was commonly found in many aquatic ecosystems and detrimental to fish species (Faria et al., 2021; Folmar et al., 1979), how to reduce or even eliminate its negative effects has not been well studied.

In recent years, probiotics have been widely used in aquaculture due to their effectiveness on production, safety and environmental friendliness (Hai, 2015; López Nadal et al., 2020). For instance, *Bacillus coagulans* (BC) and *Clostridium butyricum* (CB) are two commonly used probiotics in aquaculture. The BC is a non-intestinal inherent facultative anaerobe with the advantages of both bacillus and lactic acid bacteria (Kuebutornye et al., 2019). The CB is an extensively studied non-inherent anaerobic gram-positive bacillus (Chen et al., 2021). As effective feed additives and an alternative to antibiotics for disease prevention, these probiotics have been proven to act as growth promoters for the individual growth, survival and health of farmed species (Hai, 2015; Wang et al., 2019). The gut microbiome is beneficial for the detoxification of the environmental pollutants by altering the physiological conditions, intestinal permeability and enhancing enzymes activity, etc. (Arun et al., 2021; Qi et al., 2021). Probiotics, however, also confer several benefits to modulate gut microbiome and play important roles in attenuating the toxic effects of pollutants in aquaculture (EI-Saadony et al., 2021; Feng et al., 2018). Recent studies have shown that probiotic supplementation mitigated the gut microbial dysbiosis, lipid metabolism disorders, developmental toxicity and growth retardation caused by perfluorobutane sulfonate (PFBS) in zebrafish (Chen et al., 2020; Sun et al., 2021a; Sun et al., 2021b). In addition, probiotics could also modulate metabolism of gut (Islam et al., 2021; Jahan et al., 2021). Metabolites derived from gut microbes are associated with host health and disease. As a result of their biochemical actions, probiotics are predicted to optimize the metabolite profiles of intestinal microbiota (Kitada et al., 2018). For instance, dietary supplementation of *Bacillus cereus* promoted the growth, elevated the immunity and antioxidant status of Pengze crucian carp (Yang et al., 2019a). Moreover, probiotics enhanced the growth performance of rohu (*Labeo rohita*) through upgrading hematology, intestinal microbiota and morphology (Jahan et al., 2021). Thus, it is reasonable to hypothesize that the probiotics could attenuate the GLY-induced adverse effects by restoring intestinal health.

Taking all above into consideration, our present study intends to investigate the potential roles of aquaculture probiotics (such as the BC and CB) in eliminating the adverse effects of GLY on the widely cultivated commercial freshwater species, crucian carp (*Carassius auratus*), which is also commonly used as a model organism in aquatic toxicology (Pang et al., 2018; Sula et al., 2020). In this study, we mainly focused on the alterations in growth performance, visceral fat accumulation, activities of digestive enzymes, gut microbiota composition and metabolism after exposure to GLY with or without probiotics. Multiple experimental approaches, such as micro computed tomography (Micro-CT), 16S rRNA sequencing and targeted quantification metabolomics were applied. Our present study provided novel evidence for the potential application of probiotics in reducing the health risks of GLY in freshwater environment.

2. Materials and methods

2.1. Chemicals and kits

GLY (purity 99.0 %, CAS: 1071-83-6) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Isotopically-labeled internal standard of ^{13}C, ^{15}N-glyphosate (99.2 %, CAS: 1185107-63-4) was supplied by Tortonto Research Chemicals Inc. (North York, Canada). 9-fluorenymethylchloroformate (FMOC-Cl, 98 %, CAS: 28920-43-6) was obtained from Aladdin (Shanghai, China). Individual stock solutions of GLY were dissolved with double distilled water. Commercial kits for detecting activities of α-amylase (AMS; cat#C016-1-1), lipase (LPS; cat#A054-2-1) and trypsin (TRY; cat#A080-2) were purchased from Jiancheng Bio-Engineering Institute (Nanjing, Jiangsu, China). Other reagents used in the present study were of analytic grade.

2.2. Exposure and sampling

Twenty-day-old juvenile crucian carp (*Carassius auratus*) (0.654 ± 0.0376 g, mean body weight), were acclimatized under the laboratory condition (24 ± 1 °C, with a photoperiod of 12 h light /12 h dark) for 2 weeks in glass tanks each containing 350 L water with the provision of continuous aeration (Wang et al., 2022). During this period the crucian carp were fed commercial diets and thereafter randomly distributed in the experimental tanks. In this study, crucian carp were exposed to 0, 1 μg/L (1 ppb) and 1 mg/L (1 ppm) GLY for 14 days, and were concomitantly fed commercial diets twice daily, supplemented with or without CB (Firstv Technology Co., Ltd., China) and BC (Henan Jiushan Technology Co., Ltd., China) at the same dose 1.0×10^6 cfu/g (Fan et al., 2021), respectively. Exposure period was followed by a previous study (Ding et al., 2021), and similar to its half-life (DT50) of about 10 days reported by Fogliatto et al. (2020). Here, 1 μg/L of GLY was selected as environment relevant concentration, and 1 mg/L of GLY was chosen according to the report of Zhang et al. (2017) that GLY's toxic effects were well characterized at the mg/L level.

The crucian carp were randomly allocated into 7 groups (n = 60 per group) including Control, GLY 1 μg/L (GLY 1 ppb), GLY 1 mg/L (GLY 1 ppm), GLY 1 μg/L + CB (GLY 1ppb_CB), GLY 1 mg/L + CB (GLY 1ppm_CB), GLY 1 μg/L + BC (GLY 1ppb_BC), GLY 1 mg/L + BC (GLY 1ppm_BC) (Fig. S1). Each group contained three independent replicate tanks (n = 20 per replicate) in each with 20 L of exposure solution (40 × 25 × 30 cm). The water in all tanks was renewed daily to maintain the appropriate exposure concentrations. After 14 days of exposure, crucian carp were washed with ultrapure water and anesthetized (0.3 % MS-222,

300 mg/L, Sigma-Aldrich) for growth measurement. Then, the individuals or dissected tissues were set for subsequent analysis.

2.3. Measurement of GLY concentration

Analysis of GLY was performed on a Waters Acquity™ UPLC/ Xevo TQ MS (Milford, MA, USA), coupled to a Waters Acquity C18 column (2.1 mm × 50 mm × 1.7 μm, Milford, MA, USA), as previously published protocols (Yan et al., 2022). Briefly, the intestine, brain and muscle tissue samples (n = 3) were freeze-dried, weighed and then placed into 2.0 mL Eppendorf centrifuge tubes with 1 mL acetonitrile, which followed by 90 s of homogenization and10 min of centrifuge at 10000 rpm. 0.5 mL supernatant was treated with 0.5 mL sodium borate buffer and 0.5 mL FMOC-Cl. The mixture was placed on a horizontal oscillator and derivatized at 40 °C for 1 h. Finally, the supernatant was filtered through a 0.45 μm filter prior to UPLC-MS/MS detection. The GLY in procedural blanks was below the detection limit (0.3 ng/g), and the concentrations of GLY in exposure media at 0 h and 24 h are shown in Table S1.

2.4. Growth performance measurement

Before the start and the end of the test, initial average weight (g), final average weight (g), initial average length (cm), final average length (cm), weight gain rate (WGR, %), specific growth rate (SGR, %/d), muscle - somatic index (MSI, %) and condition factor (CF, g/cm^3) of crucian carp in each group were detected (Lee et al., 2018). The above indicators were calculated as follows: initial average weight (g) = initial total weight / initial number; final average weight (g) = final total weight / final number; initial average length (cm) = initial total length / initial number; final average length (cm) = final total length / final number; WGR (%) = (final total weight − initial total weight) / initial total weight × 100; SGR (%/d) = [ln(final average weight) − ln(initial average weight)] / exposure days × 100; MSI (%) = final muscle weight / final weight × 100; CF (g/cm^3) = final weight × length^{-3} × 100.

2.5. Micro-CT analysis

Three crucian carp were randomly selected from each group. The visceral fat from each anesthetized individual after fixation was scanned by a Micro-CT Skyscan 1276 scanner (Bruker, Belgium) following parameters: power 8 W, source voltage 45 kV, current 200 μA, rotation 220°, scaled image pixel size 13 μm, filter 0.54 mm Alpha (Zhou et al., 2021). The fat substance in the whole fish was observed, photographed and reconstructed 3D volume - rendered images using SkyScan CT Vox software (Version 2.3.0) (Sakashita et al., 2019). Finally, total adipose tissue volume (TAT), tissue volume (TV) and percent visceral fat (fat ratio, %) = TAT/TV × 100 were calculated respectively.

2.6. Histological observation

According to the method described by Ding et al. (2021), three intestines of crucian carp from each group were fixed overnight in 10 % formalin at room temperature. The fixed organs were transferred to 70 % ethanol and dehydrated in graded ethanol. Then the dehydrated samples were cleared in xylene, infiltrated, and embedded in paraffin. Serial section of each sample was sliced at 5 μm thickness which followed by hematoxylin-eosin (H&E) staining, and then sealed for pathological observation by using the Aperio ImageScope (Leica, version 12.4).

2.7. Digestive enzymes' activities

Six crucian carp were randomly selected from each group for the analysis of digestive enzymes' activities. The blood was collected from the caudal vein, and the supernatant serum obtained by centrifugation 10 min with 2500 rpm was detected. In addition, the intestine was homogenized at w/v = 1:9 with 4 °C saline (pH = 7.4) to determine the enzyme activity by a microplate reader (Infinite® F50, Tecan). The activity of AMS was determined by the iodine-starch colorimetric method, and calculated as 10 mg starch hydrolyzed per 30 min, per 100 mL of serum or per mg of tissue protein. LPS activity was measured by the methyl resorcinol substrate method, and calculated as the amount of red methyl resorufin produced per hour, per mL of serum or per mg of tissue protein. TRY activity was calculated as the absorbance changed 0.003 per 2 min, per mL of serum or per mg of tissue protein (Zhang et al., 2022). The protein concentrations of all samples were measured by enhanced BCA protein assay kit (cat#P0009, Beyotime, Shanghai, China).

2.8. Intestinal 16S rRNA sequencing and analysis

Microbial diversity in intestine of crucian carp was analyzed by Illumina MiSeq-PE250 sequencing of 16S rRNA V3 - V4 region (338F: 5′- ACTCCTACGGGAGGCAGCAG-3′ and 806R: 5′- GGAC TACHVGGGTWTCTAAT-3′) (Zhou et al., 2018). Three intestines from each tank were pooled as one replicate sample (n = 6). Data analysis were provided by using the Tutools platform (https://www.cloudtutu.com). Sequences were stepwise clustered into operational taxonomic units (OTUs) at a similarity cut-off value of 97 % by using the QIIME2 feature-table rarefy function. The biochemical pathways predicted based on the sequence abundance using Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2). STAMP 2.0 software was used to analyze the difference of Kyoto Encyclopedia of Genes and Genomes (KEGG) function.

2.9. Intestinal metabolite extraction and analysis

High throughput targeted quantification for metabolites (HM350 metabolome) could absolutely quantify 350+ metabolites such as bile acids, fatty acids, indoles, etc. Metabolite extraction, separation, identification and quantification were performed on intestines (three from each group pooled as one replicate sample, n = 3) from Control, GLY 1 ppm, GLY 1ppm_CB and GLY 1ppm_BC, as described previously (Giommi et al., 2022). Metabolites were analyzed by LC-MS QTRAP 6500+ (SCIEX), with a chromatographic column BEH C18 (2.1 mm × 10 cm, 1.7 μm, waters). Sample preprocess, LC-MS/MS detection and metabolomics data analysis acquired by BGI (Shenzhen, China) were shown in Supplementary Text S1.

2.10. Statistical analysis

Statistical graphs were generated using GraphPad Prism 8.02 (San Diego CA, USA). Values were presented as mean ± standard error (Mean ± SEM). The normal distribution and homogeneity of variance were determined by Shapiro-Wilks and Levene's tests, respectively. Significant difference among GLY alone exposure groups was analyzed by one-way ANOVA and post hoc pairwise comparisons. The statistical differences between the two treatments under same GLY exposure level (with or without probiotics) were analyzed by unpaired two-tailed Student's t-test. Gut microbiota sequencing data was performed using the two-tailed Wilcoxon rank-sum test. A probability <0.05 was considered as significant. Spearman analysis and Redundancy analysis (RDA) were employed to detect the interactions among intestinal microbiota, growth variables and differential metabolites among different treatment groups.

3. Results

3.1. Concentrations of GLY in main tissues

The measured concentrations/contents of GLY in exposure solution and fish were shown in the Table S1 and S2, respectively. The GLY of control group, either in exposure media or fish organs, were all under the LOD. The determined concentrations of GLY in both 1 ppb and 1 ppm groups were close to the nominal levels. GLY was not detected in the three organs

from control group. For the 1 ppb of GLY groups (with or without probiotics), it was determined with similar levels between the intestine (5.41–6.92 ng/g dry weight) and the muscle (4.69–5.68 ng/g dry weight), but under the LOQ in the brain. In 1 ppm GLY group, intestine accumulated the highest levels of GLY (303 ng/g dry weight) than the brain (52.6 ng/g dry weight) or muscle (54.1 ng/g dry weight). However, the addition of CB or BC decreased the contents of GLY in the intestine (60.6–63.3 ng/g dry weight), brain (43.4–44.8 ng/g dry weight) and muscle (44.6–46.2 ng/g dry weight).

3.2. Growth performance

Table 1 presented the growth performance of crucian carp in different treatments. The survival rate of crucian carp from each group was >92.1 % during the exposure period, and there were no obvious deformities. There was no difference in initial length and initial weight of crucian carp. At the end of the exposure, the final weights were all increased among all groups. However, when compared with the final weight of the control, there were 16.0 %, 41.0 %, 5.97 %, 22.1 %, 13.0 % and 29.5 % decreases in 1 ppb, 1 ppm, 1 ppb_CB, 1 ppm_CB, 1 ppb_BC and 1 ppm_BC groups, respectively. Compared with the Control, the final average weight, SGR and MSI of crucian carp significantly decreased in GLY 1 ppm group ($p < 0.05$), while increased to some extent after adding probiotics. CF in GLY 1ppb_CB group was significantly higher than that in GLY 1 ppb group ($p < 0.05$), SGR and MSI in GLY 1ppm_CB group were significantly higher than those in GLY_1ppm group ($p < 0.05$), MSI and CF in GLY 1ppm_BC group were markedly higher than those in GLY 1 ppm group ($p < 0.05$).

3.3. Visceral fat deposition

Fig. 1A presented the visceral fat microstructure of crucian carp in different treatments, showing that exposure to GLY reduced the deposition of visceral fat in a dose-dependent manner, while the addition of BC or CB partially rescued this situation. As shown in Fig. 1B, the two GLY groups displayed lower visceral fat ratios than that of the Control ($p < 0.01$). However, the visceral fat ratios in CB-treated groups were significantly higher than those of corresponding GLY groups ($p < 0.01$ and $p < 0.05$ for 1 ppb and 1 ppm groups, respectively).

3.4. Activities of digestive enzymes

Fig. 2A showed that AMS, LPS and TRY activities in serum of GLY 1 ppm group were significantly higher than those in the Control ($p < 0.01$). Compared with the GLY 1 ppm group, AMS, LPS and TRY activities in serum of GLY 1 ppm + CB group significantly reduced ($p < 0.05$), LPS and TRY activities in serum of GLY 1ppm_CB group significantly reduced ($p < 0.01$). In intestine, compared with the Control, the three indexes in GLY exposure groups showed decreasing trends, and the activity of LPS decreased significantly in the 1 ppm group ($p < 0.05$). The addition of BC or CB reduced these decreases but not significantly. (Fig. 2B). Moreover, histological results showed minor damage in intestinal tissue, such as basement membrane damage (Fig. S2).

3.5. Intestinal microbiota diversity and function

OTU results of Alpha diversity analysis showed that the order of OTU value was GLY_1ppm (2779) > GLY_1ppb (814) > control (695). However, with the addition of probiotics BC and CB, the OTU value of GLY 1ppm_BC and GLY 1ppm_CB decreased to 1907 and 1796, respectively (Fig. S3). These indicators, the Chao1, Shannon and Simpson index, provide a general representation of the changes in intestinal microbial species richness and community diversity. The Chao1, Shannon and Simpson index were higher in GLY groups than in control. The addition of BC or CB increased the Chao 1, but decreased the Shannon and Simpson index in the 1 ppb GLY group. When compared with the 1 ppm GLY group, these three indices were decreased by the BC. However, the CB reduced the Chao 1, but increased the Shannon and Simpson index (Fig. S4A(a, b and c)). PCoA showed that only GLY 1 ppb group was concentrated in the second quadrant, and GLY 1 ppm group was mainly concentrated in the first quadrant. GLY 1ppm_BC group and GLY 1ppm_CB group were mainly concentrated in the fourth quadrant, indicating that their intestinal microbial community composition is relatively similar (Fig. S4B(a)). PLS-DA showed that the microbiota samples from different treatment groups were well separated, also indicating that the microbiota of GLY 1ppb_BC group and GLY 1ppb_CB group was similar, and GLY 1 ppm group was obviously different from other groups (Fig. S4B(b)). The results suggested that the community composition of GLY 1 ppm group changed after the addition of probiotics.

At phylum level, Proteobacteria was the dominant flora in different treatment groups (Fig. S5A). At genus level, *Aeromonas* and *Dechloromonas* were the dominant flora in different treatment groups (Fig. S5B). The dominant flora of GLY 1 ppm group at phylum level were Proteobacteria, Actinobacteriota, Bacteroidetes, and at genus level were *Dechloromonas*, *Aeromonas*, *Flavobacterium*. *Aeromonas* relative abundance increased in GLY 1 ppm group, whereas the relative abundance of *Aeromonas* and *Rhodobacter* decreased in GLY 1ppm_CB group. Proteobacteria relative abundance decreased and Bacteroidetes relative abundance increased in GLY 1 ppm group at phylum level, whereas the relative abundance of Bacteroidetes decreased in GLY 1ppm_BC / GLY 1ppm_CB group (Fig. S5). Fig. 3A showed the differences of the dominant flora in each group at phylum level. There was significant increase in Bacteroidetes, Spirochaetota and Aenigmarchaeota between GLY 1 ppm group and other groups. Compared with GLY 1 ppm group, the increase flora of GLY 1ppm_CB group was Actinobacteriota, Acidobacteriota, Desulfobacterota, Gemmatimonadota, Cyanobacteria and Dadabacteria. Fig. 3B showed the differences of the dominant flora in each group at genus level. There was little difference in genera between GLY 1 ppb, GLY 1ppb_BC, GLY 1ppb_CB and the Control. Notably, *Clostridium_sensu_stricto_1* genera and *Bacillus* genera were abundant in Gly_1ppb_CB, Gly_1ppb_BC and Gly_1ppm_BC group. There was significant increase in *Pseudomonas*,

Table 1
Growth performance of crucian carp in different treatments (Mean ± SEM).

Parameters	Control	GLY_1ppb	GLY_1ppm	GLY 1ppb_CB	GLY 1ppm_CB	GLY 1ppb_BC	GLY 1ppm_BC
Survival rate (SR, %)	100 ± 0	97.8 ± 2.23	94.8 ± 2.68	100 ± 0	97.8 ± 2.23	100 ± 0	98.5 ± 1.50
Initial average weight (g)	1.36 ± 0.217	1.36 ± 0.043	1.35 ± 0.078	1.37 ± 0.108	1.31 ± 0.158	1.36 ± 0.117	1.34 ± 0.0780
Final average weight (g)	2.40 ± 0.446	2.02 ± 0.228	1.417 ± 0.0990*	2.26 ± 0.257	1.87 ± 0.157	2.09 ± 0.221	1.69 ± 0.129
Initial average length (cm)	4.08 ± 0.0930	4.10 ± 0.0770	4.12 ± 0.194	4.15 ± 0.0980	3.98 ± 0.103	4.09 ± 0.125	4.13 ± 0.0650
Final average length (cm)	5.23 ± 0.315	4.86 ± 0.162	4.62 ± 0.135	4.87 ± 0.260	4.65 ± 0.163	4.84 ± 0.175	4.70 ± 0.114
Weight gain rate (WGR, %)	23.5 ± 1.66	3.49 ± 0.183	−14.9 ± 0.560	14.8 ± 2.25	−3.46 ± 0.450	9.35 ± 0.135	−9.59 ± 0.199
Specific growth rate (SGR, %/d)	3.94 ± 0.274	3.23 ± 0.815	1.22 ± 0.442**	4.29 ± 1.250	3.48 ± 1.380[b]	4.35 ± 0.884	2.64 ± 0.537
Muscle -somatic index (MSI, %)	0.887 ± 0.148	0.840 ± 0.165	0.415 ± 0.0671*	1.09 ± 0.147	0.680 ± 0.0944[b]	0.888 ± 0.116	0.759 ± 0.113[b]
Condition factor (CF, g/cm^3)	1.77 ± 0.0670	1.71 ± 0.0800	1.59 ± 0.0450	1.84 ± 0.283[a]	1.78 ± 0.0830	1.73 ± 0.0520	1.76 ± 0.210[b]

* Compare with Control, $p < 0.05$.
[a] Compare with GLY_1ppb, $p < 0.05$.
[b] Compare with GLY_1ppm, $p < 0.05$.

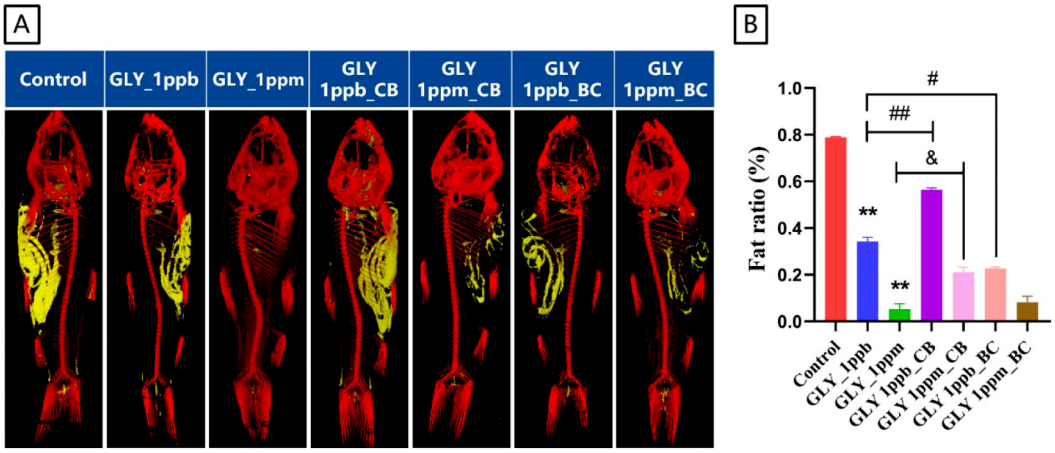


Fig. 1. Micro-CT analyses of crucian carp in different treatments. A. Representative 3D reconstructed images from each treatment group; red for fish bone skeleton and yellow for fat. B. Fat ratio (%), $n = 3$, $^{**}p < 0.01$, compare with Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compare with GLY_1ppb; $^{\&}p < 0.05$, compare with GLY_1ppm.

Flavobacterium, *Brevinema* and *Stenotrophomonas* between GLY 1 ppm group and other groups. Compared with the GLY 1 ppm group, the increased genera of GLY 1ppm_BC group was *Chitinibacter*, *Rubellimicrobium*, *Fimbriiglobus*, and the increased genera of GLY 1ppm_CB group was *Mycobacterium*, *Microbacterium*, *Nocardia*, *Gemmata* and *Blastopirellula*.

PICRUSt2 results showed functional composition of KEGG at Class 2 level in different treatment groups was metabolism (Fig. S6A). Metabolic pathways with a significant difference in mean proportions were identified, including primary bile acid biosynthesis, butanoate metabolism, bile secretion, etc. (Fig. S6B).

3.6. Intestinal metabolic signatures

The contents (10^{-3} μmol/g) of identified metabolites in the gut of crucian carp can be found in Table S3. Given that gut microbiota often modulates host metabolic pathways, targeted metabolomics was employed to compare the metabolic profiles of gut among different treatment groups. 221 metabolites and 18 classes of compounds were identified in this study. Among them, 30 metabolites mainly related to bile acid and butanoate metabolism including 20 bile acids, 4 short-chain fatty acids (SCFAs), 4 indoles and 2 organic acids (Table S2). Fig. 4 showed that

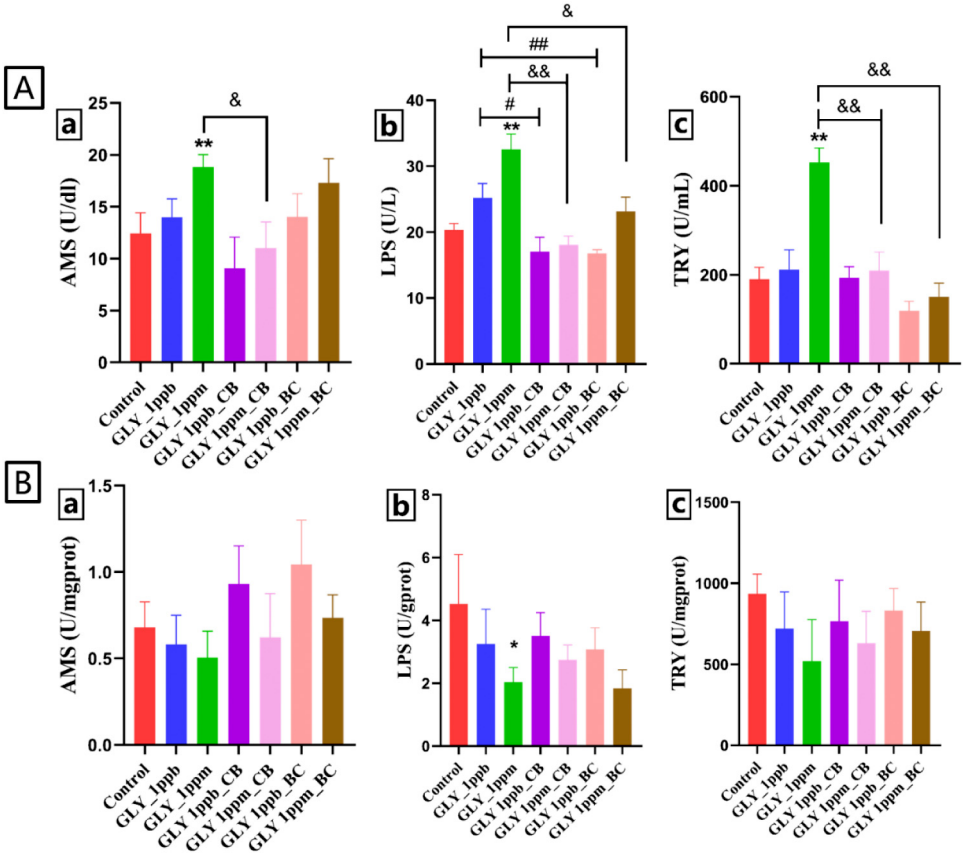


Fig. 2. Activities of digestive enzymes in crucian carp in different treatments. A. a: AMS (α-amylase), b: (lipase), c: TRY (trypsin) in serum. B. a: AMS, b: LPS, c: TRY in intestine. $n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$, compare with Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compare with GLY_1ppb; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, compare with GLY_1ppm.

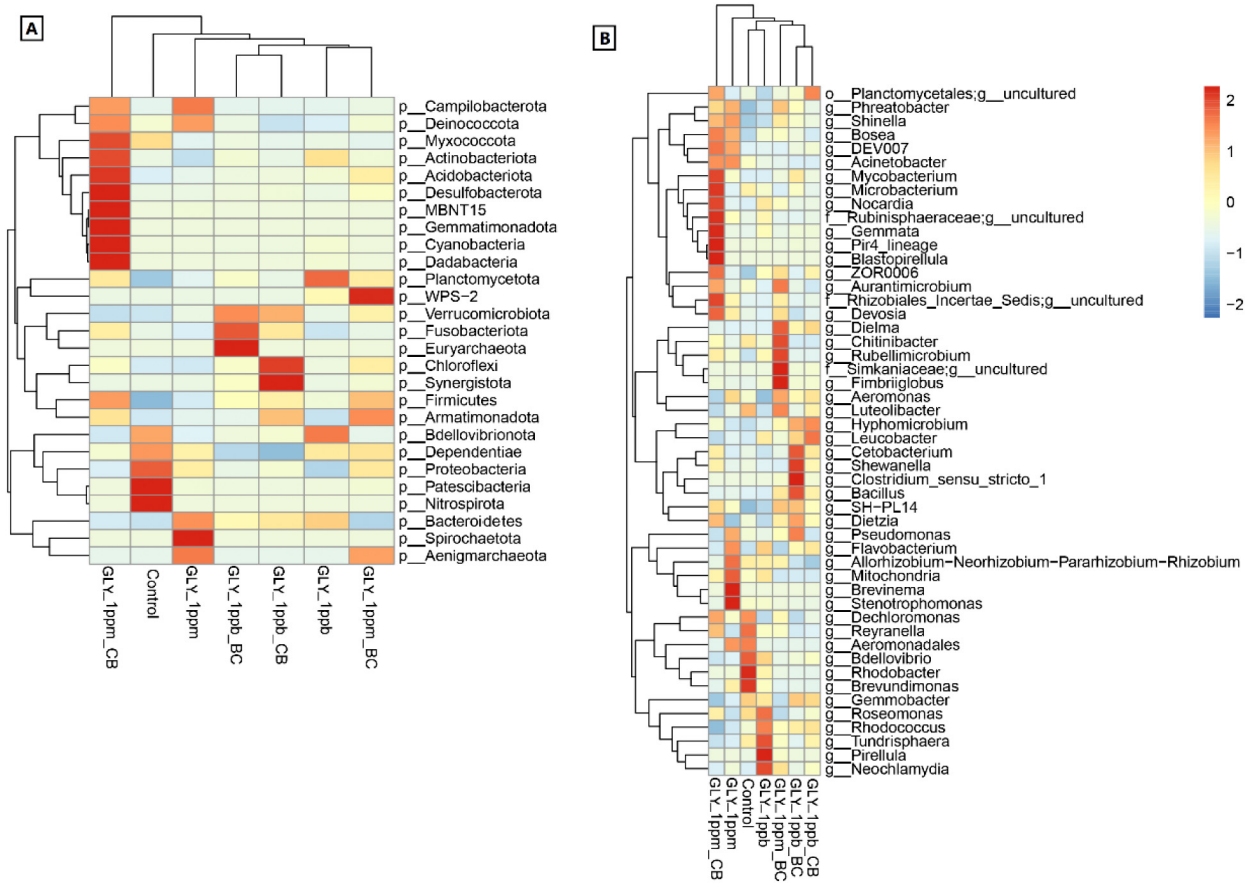


Fig. 3. Intestinal microbial community composition of crucian carp in different treatments. A. Clustering heatmap of species abundance at phylum level. B. Clustering heatmap of species abundance at genus level. Colour represents the degree of species abundance, horizontal clustering is sample information, vertical clustering is microbial species information, leftmost is microbial species clustering tree, and top is sample clustering tree. The gradual change from blue to red reflects the change of abundance from low to high.

total bile acids, TCA, cholic acid and L. lactic acid were lower in GLY 1 ppm group than those in the Control ($p < 0.01$). Other significant decreases observed in GLY 1 ppm group include acetic acid, propanoic acid, and indoleacetic acid ($p < 0.05$). In contrast, after adding probiotics, TCA, cholic acid, TCDCA, butyric acid, *L.* lactic acid and indoleacetic acid in GLY 1ppm_BC group and GLY 1ppm_CB group increased significantly compared with the GLY 1 ppm group ($p < 0.05$).

3.7. Correlations among intestinal microbiota, growth variables and differential metabolites

The correlations between gut microbiota and growth variables or differential metabolites were determined by RDA (Fig. 5). *Proteobacteria* at phylum level and *Rhodobacter*, *Gemmobacter* at genus level in the Control, were positively correlated with length, weight, and fat ratio. Comparatively, *Bacteroidetes* at phylum level and *Flavobacterium* at genus level in GLY 1 ppm group, was negatively correlated with CF, fat ratio and SGR. In contrast, *Actinobacteriotes* at phylum level and *leifsonia* at genus level in GLY 1ppm_BC group and GLY 1ppm_CB group were positively correlated with SGR (Fig. 5A). Similarly, *Bacteroidetes* at phylum level and *Aeromonas*, *Flavobacterium* at genus level in GLY 1 ppm group, was negatively correlated with these eight identified metabolites (Fig. 5B).

Correlation network analysis was performed to filter the most significant association of high confidence ($r > 0.5$ or < -0.5, $p < 0.05$). Fig. 6A showed a simple network that five phylum-level intestinal microbiota interact with these growth variables, and *Roseomonas* at genus level positively correlated with fat ratio and SGR. Fig. 6B demonstrated a complex interactive network among gut metabolites, that metabolites can be both positively and negatively affected by gut microbiota. Among them, TCA, cholic acid, TCDCA, *L.*lactic acid, and propanoic acid were significantly negatively correlated with *Bacteroidetes*. In addition, *Actinobacteriotes* at phylum level and *leifsonia* at genus level in GLY 1ppm_BC group and GLY 1ppm_CB group were significantly positively correlated with acetic acid and indoleacetic acid.

4. Discussion

The present study investigated the impacts of GLY on growth performance and the potential protective effects of BC and CB in crucian carp. Exposure to GLY caused evident bioconcentration of GLY in the intestine, gut microbiota dysbiosis, and growth retardation in crucian carp. However, the addition of BC or CB weakened these deficits by modulating intestinal composition and contributing to increase GLY-reduced gut LPS activity, bile acids and fatty acids. To our knowledge, this is the first study to provide evidence for the positive roles of probiotics in alleviating GLY-induced impairments in fish.

Increasing evidence revealed that GLY and its preparations have adverse impacts on the growth performance of aquatic organisms (Frontera et al., 2011; Annett et al., 2014; Yang et al., 2019b). For instance, GLY exposure could lead to lower weight gain of fish species by affecting food intake or conversion efficiency (Salbego et al., 2010; Giaquinto et al., 2017). In addition, negative correlation between the GLY exposure concentration and SGR was observed in crossbred red tilapia (Muhammad et al., 2021). Moderate visceral fat is beneficial for the growth and development of fish. Our present results showed that 1 mg/L GLY exposure significantly reduced final weight, MSI, SGR and visceral fat deposition, which suggest

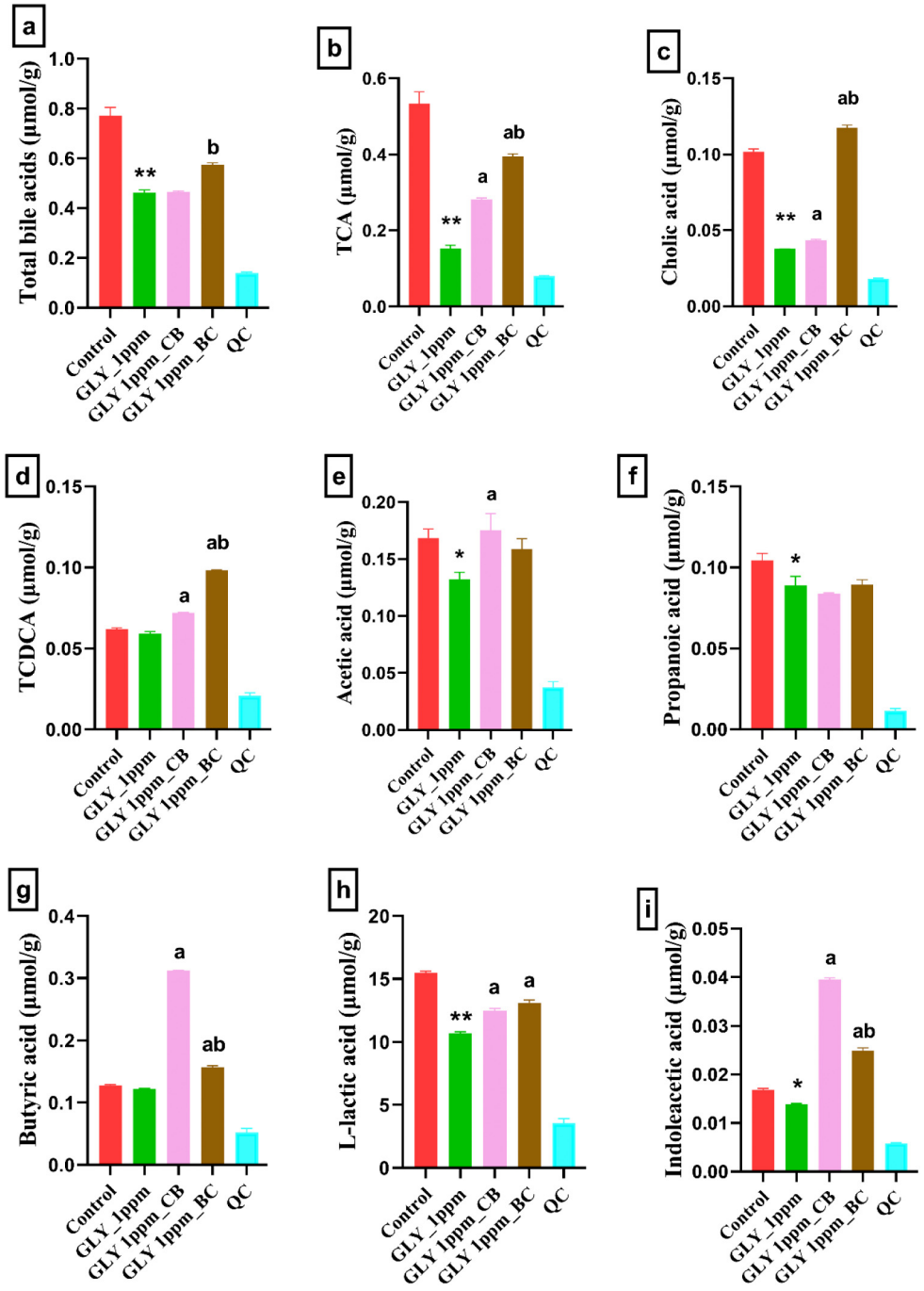


Fig. 4. Intestinal quantitative metabolites in crucian carp in different treatments. a-d: Total bile acids (The sum of all identified 20 bile acid metabolites), TCA (Taurocholic acide), Cholic_acid, TCDCA (Taurochenodeoxycholic acid). e-g: Acetic_acid, Propanoic acid, Butyric acid. h: *L.* lactic acid. i: Indoleacetic acid. $n = 3$, $*p < 0.05$, $**p < 0.01$, compare with Control; ^{a} $p < 0.05$, compare with GLY_1ppm; ^{b} $p < 0.05$, GLY 1ppm_CB compare with GLY 1ppm_BC. Quality Control (QC).

that GLY exposure indeed affects the growth of the juvenile crucian carp. More importantly, in contrast, probiotics (BC and CB) attenuated the detrimental effects of GLY, which was supported by related research that the routine feed supplemented with appropriate levels of BC could promote the WGR and SGR of juvenile gibel carp (*Carassius auratus gibelio*) (Yu et al., 2018). Another study also showed that CB could improve the growth performance (WG and SGR) of shrimp (*Litopenaeus vannamei*) (Duan et al., 2017). These results suggest that BC and CB could reduce the GLY-caused growth retardation.

The intestine of crucian carp was determined with higher bioaccumulation of GLY than the other tested organs in our present study. This is consistent with the results of grass carp (*Ctenopharyngodon idella*) from freshwater aquaculture ponds (Yan et al., 2022). This indicates that the intestine should be treated as the primary organ for GLY-induced toxicity assessment. Intestinal health is vital for normal growth of the organisms (Qi et al., 2021). Thus, in the present study, histopathological observation was firstly conducted and revealed that 1 mg/L GLY exposure caused basement membrane damage of intestinal tissues in crucian carp, and this is similar to that observed in zebrafish after exposure to GLY (Ding et al., 2021). Furthermore, the activities of digestive enzymes reflect the ability to digest, absorb and utilize nutrients, which is directly related to the growth and health status of organisms (Yang et al., 2019b). Previous study also showed that the activities of AMS and LPS in intestine of the Chinese mitten crab (*Eriocheir sinensis*) were markedly decreased after exposure to 48.945 mg/L GLY for

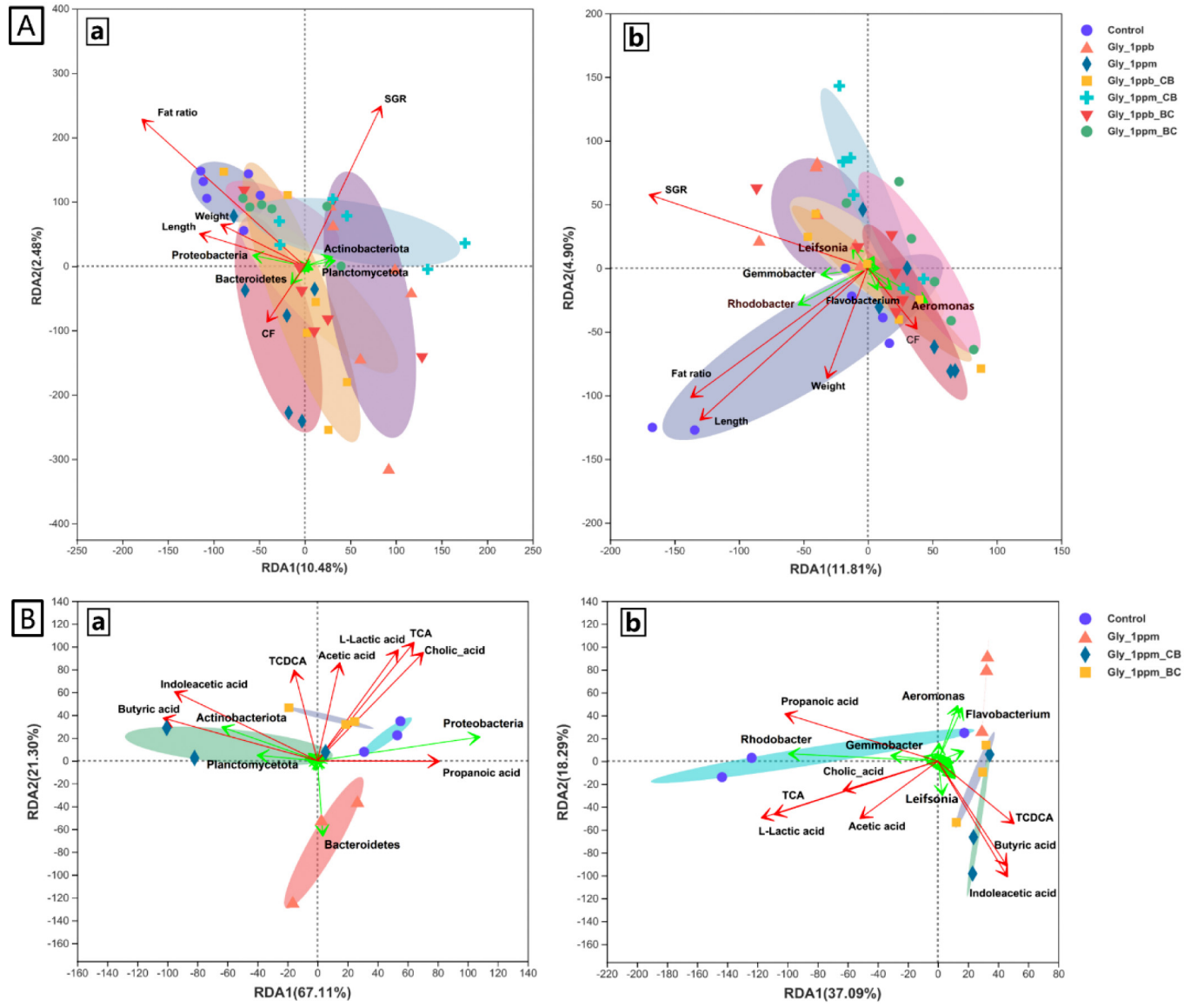


Fig. 5. Correlation among intestinal microbiota, growth variables and differential metabolites in different treatments. Growth variables include length, weight, specific growth rate (SGR), visceral fat ratio and condition factor (CF). A. Correlation between intestinal microbiota and growth variables. B. Correlation between intestinal microbiota and differential metabolites. a: RDA on phylum level, *Actinobacteriota*, *Bacteroidetes*, *Planctomycetota, Proteobacteria*. b: RDA on genus level, *Aeromonas*, *Flavobacterium*, *Gemmobacter*, *Leifsonia*, *Rhodobacter*.

7 days (Yang et al., 2019b). Subsequently, we herein determined the activity of digestive enzymes including AMS, LPS and TRY in both serum and intestine of crucian carp. Our results also showed that GLY decreased AMS, LPS and TRY activities in intestine, generating adverse impacts on the intestinal digestive function. In comparison, the presence of probiotics CB or BC almost eliminated the histopathological damage of intestine and increased the activities of AMS, LPS and TRY in gut. Considering that the probiotics like BC was applied to improve the digestive ability and increase the activities of AMS, LPS and TRY in aquaculture species (Amoah et al., 2019), our present results provided hard evidence to supporting that the addition of probiotics (CB or BC) could diminish the GLY-induced gut injury and bioaccumulation, which might contribute to the attenuated growth retardation.

Probiotics, prebiotics and synbiotics could improve the functionality of aqua feed by upgrading growth, reproduction, immunity and disease resistance in fish (Rohani et al., 2022). Microbiome analysis of our present study indicated that 1 mg/L GLY significantly increased the diversity of gut microbiota in crucian carp. Meanwhile, *Proteobacteria* abundance decreased at phylum level and *Flavobacterium* (harmful bacteria) abundance increased at genus level. This is bearing a resemblance to a recent report which revealed that 3.5 mg/L GLY increased *Fusobacteria* but decreased *Proteobacteria* in zebrafish gut (Ding et al., 2021). *Proteobacteria* was prevalent in the gut of zebrafish at phylum level, which is primarily responsible for the utilization of amino acids in the intestine and also used as a microbial signature of dysbiosis in gut microbiota (Davis et al., 2016; Shin et al., 2015). The alteration of *Bacteroidetes* and *Proteobacteria* composition in the gut of crucian carp (*Carassius auratus gibelio*) indicated a disequilibrium of the microbial community after exposure to diazinon (another commonly used organophosphate pesticide) and resulted in further negative impacts on the host health status (Tang et al., 2021). Given the association between gut microbiota and growth performance (Jahan et al., 2021), the alterations of *Bacteroidetes* at phylum level and *Flavobacterium* at genus level might be primarily responsible for the negative growth performance caused by GLY. These results clearly suggest that the GLY-induced growth retardation of crucian carp might be related to the dysbiosis of intestinal microbiota. Probiotics were shown to provide increased resistance against durable dysbiosis of gut microbiota in fish (Rohani et al., 2022; Simón et al.,

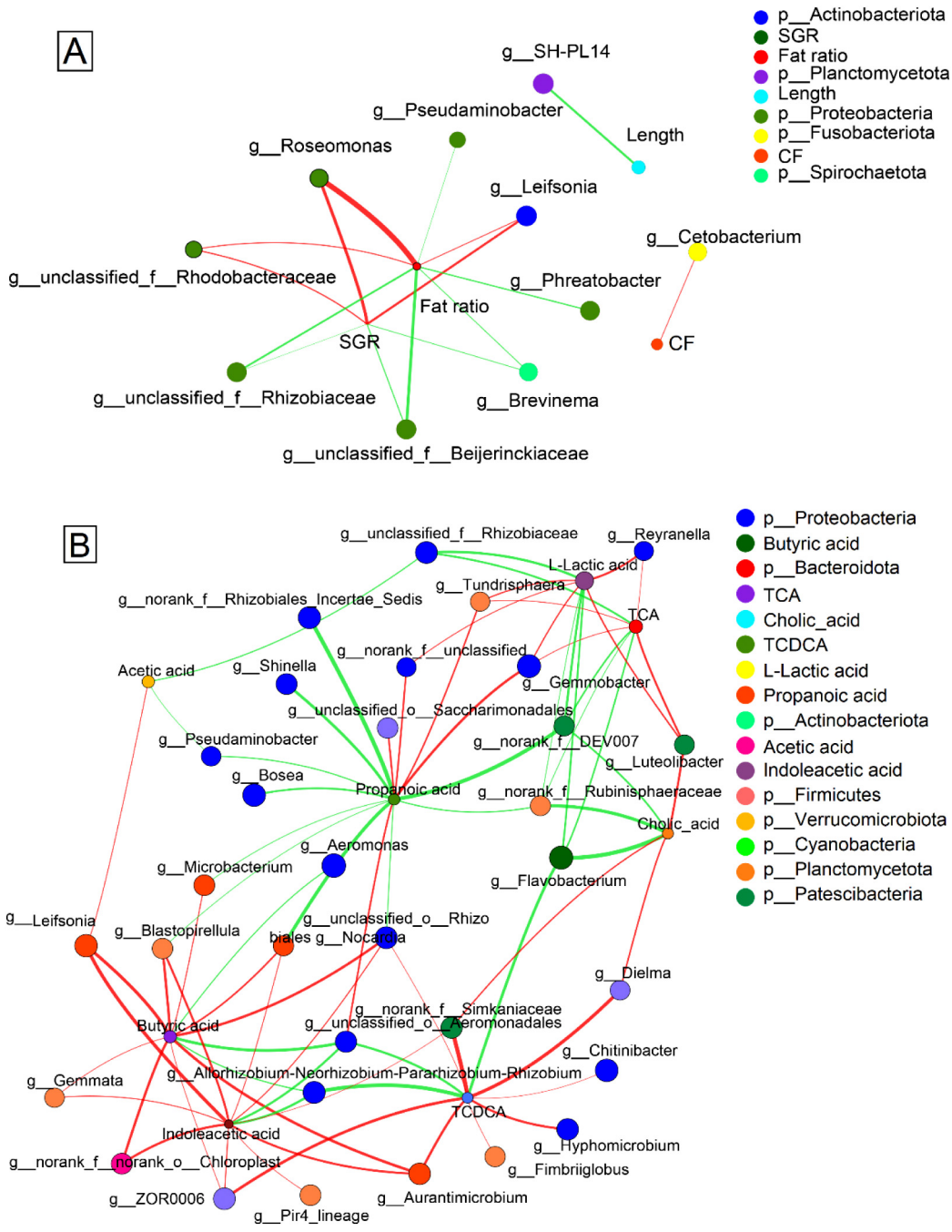


Fig. 6. Two-factor correlation network. A. Correlation network between intestinal microbiota and growth variables. B. Correlation network between intestinal microbiota and differential metabolites. Top 30 of total abundance of classification level. The thickness of the lines reflects the strength of the correlations, green lines indicate negative correlations, and red lines indicate positive correlations. $r > 0.5$ or < -0.5, $p<0.05$.

2021). BC has been one of the most commonly used probiotics in feed additives for decades (Kuebutornye et al., 2019), and CB is increasingly being used to promote the gut health of aquaculture animals (Chen et al., 2021; Duan et al., 2017). Another study showed that dietary CB increased the diversity of intestinal microbiota and altered the microbiota composition with reduced relative abundances of *Firmicutes* and *Bacteroidetes* (Liu et al., 2021b). Moreover, the CB could reduce the abundance of pathogenic bacteria *Aeromonas* in the gut microbiota of Mandarin fish (Chen et al., 2021). This is consistent with our present results which indicated by lower relative abundances of *Bacteroidetes* and *Aeromonas* in GLY_1ppm + BC/CB groups than those in GLY 1 ppm group. These results indicate that the probiotics BC or CB could improve the impeded growth performance by remodeling the GLY-induced gut microbial dysbiosis.

In gut, microbial products and metabolites also fundamentally contribute to the intestinal health, including bile acids, SCFAs, indoleacetic acid, etc. (Gao et al., 2021). Bile acids, produced from cholesterol in the liver and metabolized in the intestine for lipid metabolism, can also affect host metabolism by modulating gut microbial composition (Wahlström et al., 2016). Dietary bile acids distinctly remodeled intestinal microbiota by reducing the abundance of some potential pathogenic bacteria (Li et al., 2021). Previous study reported that GLY could disrupt the bile acid synthesis by raising endogenous retinoic acid in chick and frog embryos (Paganelli et al., 2010; Fathi et al., 2020). In our present study, the most abundant bile acid metabolites were identified as TCA, which followed by cholic acid, TCDCA and chenodeoxycholic acid. Our results demonstrate that most of the bile acids decreased with varying degrees in gut of exposed fish to

GLY. However, supplement with BC or CB relieved the GLY-induced bile acids reduction. Intestinal microbiota can also degrade carbohydrates and other nutrients to produce SCFAs with a carbon chain number of 1 to 6, which mainly include acetic acid, propionic acid, butyric acid and isobutyric acid, and account above 90 % of the total content of SCFAs (González-Bosch et al., 2021). The SCFAs were produced by gut microbiota during the fermentation of partial and nondigestible polysaccharides to prevent the translocation of bacteria and microbial toxins (Gao et al., 2021). A recent study showed that GLY had no discernable effects on bile acids and SCFAs from the colon simulating bioreactor model, which also determined with targeted metabolite analysis (Krause et al., 2020). While our present results revealed that exposure to GLY altered the profiles of bile acids and SCFAs in the gut towards unhealthy condition. More importantly, our present results also suggested that BC or CB weakened the GLY-induced disorder of SCFAs production in gut. This might be attributed to that the added probiotics could promote microbial metabolism by optimizing the microbiota structure and production of SCFAs like butyrate (Zhang et al., 2018; Stoeva et al., 2021). Furthermore, indoleacetic acid plays a protective role in guarding the inner mucus layer of gut from bacteria translocation (Gao et al., 2021). L. lactic acid is abundant in the intestine and has obvious effects on eliminating pathogenic bacteria and maintaining gut barrier function (Ren et al., 2018). However, the intestinal levels of these two metabolites were all significantly reduced by GLY but increased in BC and CB treatments. These findings above suggested that exposure to GLY could induce intestinal damage by decreasing the beneficial gut microbiota metabolites (bile acids, SCFAs, indoleacetic acid and L. lactic acid), while the addition of BC and CB could increase these metabolites, which suggests the potential protective or ameliorative effects of the added probiotics. Notably, this study confirmed that the efficacy of BC and CB in GLY-exposed crucian carp were slightly different. In terms of growth performance, visceral fat and SCFAs metabolic profiles, CB might be better to restore these GLY-induced impairments than BC, which may mainly attributed to that CB can benefit the gut health by increasing the population of lactic acid bacteria and the production of acetic acid, n-butyric acid, n-valeric acid, and SCFAs in the gut (Fan et al., 2021).

Recent researches were focused on the modulation of intestinal microbiota composition and their metabolites for maintaining or promoting host health (Chen et al., 2021; Rohani et al., 2022; Sun et al., 2021b). The ingested nutrients were converted by gut microbiota into metabolites that target either the microbiota population or host cells. Metabolites perform critical functions as transmitters between gut microbiota and host cells. Naturally, gut microbiota composition and resulting metabolites thus determine host development and health, as well as the status of certain diseases (Sittipo et al., 2019). Although Li et al. (2021) identified the disturbed metabolisms in brains, kidneys and livers of goldfish (*Carassius auratus*) following chronic exposure to GLY-based herbicide, our present study suggested that GLY-perturbed gut microbiota composition and GLY-attenuated microbial metabolites were responsible for negative growth performance in exposed crucian carp. The present data also showed that BC and CB interact positively with other commensal beneficial bacteria to regulate gut metabolic profiles. Modulation of gut microbiota by BC and CB supplementation was shown to an improved growth performance via increasing GLY-reduced gut metabolisms.

5. Conclusion

This study revealed the impacts of GLY on the growth performance of crucian carp and especially the beneficial roles of aquaculture probiotics (BC and CB) in reducing the health risk of GLY. It further indicated that GLY-induced microbiota dysbiosis, reduction of LPS activity, decreases of bile acids and fatty acids in gut contributed to the growth retardation of crucian carp. More importantly, it also verified that the probiotics (BC and CB) played positive roles in improving the intestine health which benefit to the recovery of growth retardation. Even the internal mechanisms need further investigations, this study highlights the addition of probiotics might be a noteworthy strategy for GLY pollution control in freshwater fish aquaculture, and also suggests the potential application of probiotics in reducing the health risks of GLY in aquatic environment.

CRediT authorship contribution statement

Biao Yan: Investigation, Data curation, Methodology, Formal analysis, Writing -original draft. **Jian Han:** Conceptualization, investigation, Writing-review & editing, Supervision. **Yumiao Sun:** Investigation, Methodology. **Lei Lei:** Data curation, Formal analysis. **Jing Yuan:** Data curation, Formal analysis, Validation. **Zhixian Qiao, Jun Men & Xin Wang:** Methodology, Formal analysis. **Yongyong Guo:** Investigation, Methodology, Formal analysis. **Qidong Wang:** Conceptualization, investigation, Writing-review & editing, Supervision. **Bingsheng Zhou:** Funding acquisition, Project administration.

Data availability

The authors do not have permission to share data.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This research was supported by the National Key Research and Development Program of China (No. 2018YFD0900701), the National Natural Science Foundation of China (No. 32002396), Youth Innovation Promotion Association CAS (No. 2022344) and the State Key Laboratory of Freshwater Ecology and Biotechnology (No. 2019FBZ03).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.158260.

References

Amoah, K., Huang, Q.C., Tan, B.P., Zhang, S., Chi, S.Y., Yang, Q.H., Liu, H.Y., Dong, X.H., 2019. Dietary supplementation of probiotic Bacillus coagulans ATCC 7050, improves the growth performance, intestinal morphology, microbiota, immune response, and disease confrontation of Pacific white shrimp,Litopenaeus vannamei. Fish Shellfish Immunol. 87, 796–808.

Annett, R., Habibi, H.R., Hontela, A., 2014. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. J. Appl. Toxicol. 34 (5), 458–479.

Arun, K.B., Madhavan, A., Sindhu, R., Emmanual, S., Binod, P., Pugazhendhi, A., Sirohi, R., Reshmy, R., Awasthi, M.K., Gnansounou, E., Pandey, A., 2021. Probiotics and gut microbiome - prospects and challenges in remediating heavy metal toxicity. J. Hazard. Mater. 420, 126676.

de Brito Rodrigues, L., Gonçalves Costa, G., da Silva, L.R., de Oliveira, R., Lundgren, Thá.E., Morais Leme, D., Cestari, M.M., Koppe Grisolia, C., Campos Valadares, M., de Oliveira, G.A.R., 2019. Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 842, 94–101.

Brovini, E.M., Cardoso, S.J., Quadra, G.R., Vilas-Boas, J.A., Paranaíba, J.R., Pereira, R.O., Mendonça, R.F., 2021. Glyphosate concentrations in global freshwaters: are aquatic organisms at risk? Environ. Sci. Pollut. Res. Int. 28 (43), 60635–60648.

Chen, L., Lam, J.C.W., Tang, L., Hu, C., Liu, M., Lam, P.K.S., Zhou, B., 2020. Probiotic modulation of lipid metabolism disorders caused by perfluorobutanesulfonate pollution in zebrafish. Environ. Sci. Technol. 54 (12), 7494–7503.

Chen, X., Yi, H., Liu, S., Zhang, Y., Su, Y., Liu, X., Bi, S., Lai, H., Zeng, Z., Li, G., 2021. Probiotics improve eating disorders in Mandarin fish (Siniperca chuatsi) induced by a pellet feed diet via stimulating immunity and regulating gut microbiota. Microorganisms 9 (6), 1288–1312.

Davis, D.J., Doerr, H.M., Grzelak, A.K., Busi, S.B., Jasarevic, E.A., Ericsson, C., Bryda, E.C., 2016. Lactobacillus plantarum attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish. Sci. Rep. 6, 33726.

Ding, W., Shangguan, Y., Zhu, Y., Sultan, Y., Feng, Y., Zhang, B., Liu, Y., Ma, J., Li, X., 2021. Negative impacts of microcystin-LR and glyphosate on zebrafish intestine: linked with gut microbiota and microRNAs? Environ. Pollut. 286, 117685.

Duan, Y., Zhang, Y., Dong, H., Wang, Y., Zheng, X., Zhang, J., 2017. Effect of dietary Clostridium butyricum on growth, intestine health status and resistance to ammonia stress in Pacific white shrimp Litopenaeus vannamei. Fish Shellfish Immunol. 65, 25–33.

El-Saadony, M.T., Patra, A.K., Kar, I., Tiwari, R., Dawood, M.A.O., Alagawany, M., Dhama, K., Abdel-Latif, H.M.R., 2021. The functionality of probiotics in aquaculture: an overview. Fish Shellfish Immunol. 117, 36–52.

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). J. World Aquacult. Soc. 52 (6), 1194–1208.

Faria, M., Bedrossiantz, J., Ramírez, J.R.R., Mayol, M., García, G.H., Bellot, M., Prats, E., Garcia-Reyero, N., Gómez-Canela, C., Gómez-Oliván, L.M., Raldúa, D., 2021. Glyphosate targets fish monoaminergic systems leading to oxidative stress and anxiety. Environ. Int. 146, 106253.

Fathi, M.A., Han, G., Kang, R., Shen, D., Shen, J., Li, C., 2020. Disruption of cytochrome P450 enzymes in the liver and small intestine in chicken embryos in ovo exposed to glyphosate. Environ. Sci. Pollut. Res. Int. 27 (14), 16865–16875.

Feng, P., Ye, Z., Kakade, A., Virk, A.K., Li, X., Liu, P., 2018. A review on gut remediation of selected environmental contaminants: possible roles of probiotics and gut microbiota. Nutrients 11 (1), 22–41.

Fisheries and Fisheries Administration Bureau of the Ministry of Agriculture and Rural Areas, National Aquatic Technology Promotion Station, China Society of Fisheries, 2018. China Fishery Statistical Yearbook-2018. China Agriculture Press, Beijing.

Fogliatto, S., Ferrero, A., Vidotto, F., 2020. Current and future scenarios of glyphosate use in Europe: are there alternatives? Adv. Agron. 163, 219–278.

Folmar, L.C., Sanders, H.O., Julin, A.M., 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch. Environ. Contam. Toxicol. 8 (3), 269–278.

Frontera, J.L., Vatnick, I., Chaulet, A., Rodríguez, E.M., 2011. Effects of glyphosate and polyoxyethylenamine on growth and energetic reserves in the freshwater crayfish cherax quadricarinatus (Decapoda, Parastacidae). Arch. Environ. Contam. Toxicol. 61 (4), 590–598.

Gao, B., Emami, A., Nath, S., Schnabl, B., 2021. Microbial products and metabolites contributing to alcohol-related liver disease. Mol. Nutr. Food Res. 65 (5), e2000023.

Giaquinto, P.C., de Sá, M.B., Sugihara, V.S., Gonçalves, B.B., Delício, H.C., Barki, A., 2017. Effects of glyphosate-based herbicide sub-lethal concentrations on fish feeding behavior. Bull. Environ. Contam. Toxicol. 98 (4), 460–464.

Giommi, C., Ladisa, C., Carnevali, O., Maradonna, F., Habibi, H.R., 2022. Metabolomic and transcript analysis revealed a sex-specific effect of glyphosate in zebrafish liver. Int. J. Mol. Sci. 23 (5), 2724–2740.

González-Bosch, C., Boorman, E., Zunszain, P.A., Mann, G.E., 2021. Short-chain fatty acids as modulators of redox signaling in health and disease. Redox Biol. 47, 102165.

Hai, N.V., 2015. The use of probiotics in aquaculture. J. Appl. Microbiol. 119 (4), 917–935.

Islam, S.M., Rohani, M.F., Shahjahan, M., 2021. Probiotic yeast enhances growth performance of Nile tilapia (Oreochromis niloticus) through morphological modifications of intestine. Aquac. Rep. 21, 100800.

Jahan, N., Islam, S.M., Rohani, M.F., Hossain, M.T., Shahjahan, M., 2021. Probiotic yeast enhances growth performance of rohu (Labeo rohita) through upgrading hematology, and intestinal microbiota and morphology. Aquaculture 545, 737243.

Kitada, Y., Muramatsu, K., Toju, H., Kibe, R., Benno, Y., Kurihara, S., Matsumoto, M., 2018. Bioactive polyamine production by a novel hybrid system comprising multiple indigenous gut bacterial strategies. Sci. Adv. 4 (6), eaat0062.

Krause, J.L., Haange, S.B., Schäpe, S.S., Engelmann, B., Rolle-Kampczyk, U., Fritz-Wallace, K., Wang, Z., Jehmlich, N., Türkowsky, D., Schubert, K., Pöppe, J., Bote, K., Rösler, U., Herberth, G., von Bergen, M., 2020. The glyphosate formulation roundup LB plus influences the global metabolome of pig gut microbiota in vitro. Sci. Total Environ. 745, 140932.

Kuebutornye, F.K.A., Abarike, E.D., Lu, Y., 2019. A review on the application of bacillus as probiotics in aquaculture. Fish Shellfish Immunol. 87, 820–828.

Lee, S., Lee, Y.M., Kim, K.H., Kim, H.C., Park, C.J., Park, J.W., Noh, G.E., Kim, W.J., Hwang, H.K., 2018. Effects of food availability on growth performance and immune-related gene expression of juvenile olive flounder (Paralichthys olivaceus). Fish Shellfish Immunol. 80, 348–356.

Li, Y., Wang, S., Hu, Y., Cheng, J., Cheng, X., Cheng, P., Cui, Z., 2021. Dietary bile acid supplementation reveals beneficial effects on intestinal healthy status of tongue sole (Cynoglossus semiliaevis). Fish Shellfish Immunol. 116, 52–60.

Liu, J., Dong, C., Zhai, Z., Tang, L., Wang, L., 2021a. Glyphosate-induced lipid metabolism disorder contributes to hepatotoxicity in juvenile common carp. Environ. Pollut. 269, 116186.

Liu, Y., Liu, C., An, K., Gong, X., Xia, Z., 2021b. Effect of dietary Clostridium butyricum supplementation on growth performance, intestinal barrier function, immune function, and microbiota diversity of Pekin ducks. Animals 11 (9), 2514–2531.

López Nadal, A., Ikeda-Ohtsubo, W., Sipkema, D., Peggs, D., McGurk, C., Forlenza, M., Wiegertjes, G.F., Brugman, S., 2020. Feed, microbiota, and gut immunity: using the zebrafish model to understand fish health. Front. Immunol. 11, 114–129.

Muhammad, U.A., Yasid, N.A., Daud, H.M., Shukor, M.Y., 2021. Glyphosate herbicide induces changes in the growth pattern and somatic indices of crossbred red tilapia (O. niloticus × O. mossambicus). Animals (Basel) 11 (5), 1209–1222.

Paganelli, A., Gnazzo, V., Acosta, H., López, S.L., Carrasco, A.E., 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chem. Res. Toxicol. 23, 1586–1595.

Pang, M., Luo, W., Fu, B., Yu, X., Zhou, Y., Tong, J., 2018. Transcriptomic profiles of brain provide insights into molecular mechanism of feed conversion efficiency in Crucian Carp (Carassius auratus). Int. J. Mol. Sci. 19 (3), 858–872.

Qi, M., Tan, B., Wang, J., Liao, S., Deng, Y., Ji, P., Song, T., Zha, A., Yin, Y., 2021. The microbiota-gut-brain axis: a novel nutritional therapeutic target for growth retardation. Crit. Rev. Food Sci. Nutr. 1, 1–26.

Ren, C., Dokter-Fokkens, J., Figueroa Lozano, S., Zhang, Q., de Haan, B.J., Zhang, H., Fass, M.M., de Vos, P., 2018. Lactic acid bacteria may impact intestinal barrier function by modulating goblet cells. Mol. Nutr. Food Res. 62 (6), e1700572 In this issue.

Rohani, M.F., Islam, S.M., Hossain, M.K., Ferdous, Z., Siddik, M.A., Nuruzzaman, M., Padeniya, U., Brown, C., Shahjahan, M., 2022. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: upgrading growth, reproduction, immunity and disease resistance in fish. Fish Shellfish Immunol. 120, 569–589.

Sakashita, M., Sato, M., Kondo, S., 2019. Comparative morphological examination of vertebral bodies of teleost fish using high-resolution micro-CT scans. J. Morphol. 280 (6), 778–795.

Salbego, J., Pretto, A., Gioda, C.R., de Menezes, C.C., Lazzari, R., Radünz Neto, J., Baldisserotto, B., Loro, V.L., 2010. Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in piava (Leporinus obtusidens). Arch. Environ. Contam. Toxicol. 58 (3), 740–745.

Shin, N.R., Whon, T.W., Bae, J.W., 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33, 496–503.

Simón, R., Docando, F., Nuñez-Ortiz, N., Tafalla, C., Díaz-Rosales, P., 2021. Mechanisms used by probiotics to confer pathogen resistance to teleost fish. Front. Immunol. 12, 653025.

Sittipo, P., Shim, J.W., Lee, Y.K., 2019. Microbial metabolites determine host health and the status of some diseases. Int. J. Mol. Sci. 20 (21), 5296–5322.

Solomon, K.R., Thompson, D.G., 2011. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J. Toxicol. Environ. Health B 6 (3), 289–294.

Stoeva, M.K., Garcia-So, J., Justice, N., Myers, J., Tyagi, S., Nemchek, M., McMurdie, P.J., Kolterman, O., Eid, J., 2021. Butyrate-producing human gut symbiont, Clostridium butyricum, and its role in health and disease. Gut Microbes 13 (1), 1–28.

Sula, E., Aliko, V., Barceló, D., Faggio, C., 2020. Combined effects of moderate hypoxia, pesticides and PCBs upon crucian carp fish, Carassius carassius, from a freshwater lake-in situ ecophysiological approach. Aquat. Toxicol. 228, 105644.

Sun, B., Liu, M., Tang, L., Hu, C., Huang, Z., Zhou, X., Chen, L., 2021a. Probiotic supplementation mitigates the developmental toxicity of perfluorobutanesulfonate in zebrafish larvae. Sci. Total Environ. 799, 149458.

Sun, B., Liu, M., Tang, L., Hu, C., Huang, Z., Chen, L., 2021b. Probiotics inhibit the stunted growth defect of perfluorobutanesulfonate via stress and thyroid axes in zebrafish larvae. Environ. Pollut. 290, 118013.

Tang, J., Wang, W., Jiang, Y., Chu, W., 2021. Diazinon exposure produces histological damage, oxidative stress, immune disorders and gut microbiota dysbiosis in crucian carp (Carassius auratus gibelio). Environ. Pollut. 269, 116129.

Van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., Morris Jr., J.G., 2018. Environmental and health effects of the herbicide glyphosate. Sci. Total Environ. 616–617, 255–268.

Wahlström, A., Sayin, S.I., Marschall, H.U., Bäckhed, F., 2016. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metab. 24 (1), 41–50.

Wang, A., Ran, C., Wang, Y., Zhang, Z., Ding, Q., Yang, Y., Olsen, R.E., Ringø, E., Bindelle, J., Zhou, Z., 2019. Use of probiotics in aquaculture of China-a review of the past decade. Fish Shellfish Immunol. 86, 734–755.

Wang, J.X., Cheng, Y.F., Pan, X.H., Luo, P., 2022. Tissue-specific accumulation, transformation, and depuration of fipronil in adult crucian carp (Carassius auratus). Ecotoxicol. Environ. Saf. 232, 113234.

Yan, B., Lei, L., Chen, X., Men, J., Sun, Y., Guo, Y., Yang, L., Wang, Q., Han, J., Zhou, B., 2022. Glyphosate and glufosinate-ammonium in aquaculture ponds and aquatic products: occurrence and health risk assessment. Environ. Pollut. 296, 118742.

Yang, G., Cao, H.Z., Jiang, W.H., Hu, B.J., Jian, S.Q., Wen, C.G., Kajbaf, K., Kumar, V., Tao, Z.Y., Peng, M., 2019a. Dietary supplementation of Bacillus cereus as probiotics in Pengze crucian carp (Carassius auratus var. Pengze): effects on growth performance, fillet quality, serum biochemical parameters and intestinal histology. Aquac. Res. 50 (8), 2207–2217.

Yang, X., Song, Y., Zhang, C., Pang, Y., Song, X., Wu, M., Cheng, Y., 2019b. Effects of the glyphosate-based herbicide roundup on the survival, immune response, digestive activities and gut microbiota of the chinese mitten crab,Eriocheir sinensis. Aquat. Toxicol. 214, 105243.

Yu, Y., Wang, C., Wang, A., Yang, W., Lv, F., Liu, F., Liu, B., Sun, C., 2018. Effects of various feeding patterns of Bacillus coagulans on growth performance, antioxidant response and Nrf2-Keap1 signaling pathway in juvenile gibel carp (Carassius auratus gibelio). Fish Shellfish Immunol. 73, 75–83.

Zhang, S., Xu, J., Kuang, X., Li, S., Li, X., Chen, D., Zhao, X., Feng, X., 2017. Biological impacts of glyphosate on morphology, embryo biomechanics and larval behavior in zebrafish (Danio rerio). Chemosphere 181, 270–280.

Zhang, J., Chen, X., Liu, P., Zhao, J., Sun, J., Guan, W., Johnston, L.J., Levesque, C.L., Fan, P., He, T., Zhang, G., Ma, X., 2018. Dietary Clostridium butyricum induces a phased shift in fecal microbiota structure and increases the acetic acid-producing bacteria in a weaned piglet model. J. Agric. Food Chem. 66 (20), 5157–5166.

Zhang, P., Lu, G., Sun, Y., Yan, Z., Dang, T., Liu, J., 2022. Metagenomic analysis explores the interaction of aged microplastics and roxithromycin on gut microbiota and antibiotic resistance genes of Carassius auratus. J. Hazard. Mater. 425, 127773.

Zhou, L., Limbu, S.M., Qiao, F., Du, Z.Y., Zhang, M., 2018. Influence of long-term feeding antibiotics on the gut health of zebrafish. Zebrafish 15 (4), 340–348.

Zhou, Y., Guo, W., Lei, L., Sun, Y., Li, R., Guo, Y., Yang, L., Shi, X., Han, J., Zhou, B., 2021. Bis (2-ethylhexyl)-tetrabromophthalate induces zebrafish obesity by altering the brain-gut axis and intestinal microbial composition. Environ. Pollut. 290, 118127.