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Effects of nuclease-treated fermentation product of *Lactobacillus rhamnosus* GCC-3 on growth, hepatic health and gut microbiota of zebrafish (*Danio rerio*) fed a high-fat diet

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ABSTRACT

Probiotics are reported to improve the nutrition, immunity, and health of fish. Nuclease can hydrolyze nucleic acids of probiotics to produce nucleotides. The present study investigated the effect of stabilized fermentation product of nuclease-treated *Lactobacillus rhamnosus* GCC-3 (GCC-3 NT) on growth, non-specific immunity, liver health, and gut microbiota of zebrafish (*Danio rerio*). Compared to the high-fat diet (HFD) group, GCC-3 NT did not affect the growth performance of zebrafish. However, GCC-3 NT treatment can significantly increase the lysozyme activity and the total antioxidant capacity of body surface mucus. In addition, dietary GCC-3 NT significantly reduced the content of hepatic triglycerides (TAG) in zebrafish while significantly increased the expression of acyl-coenzyme A oxidases 3 (*ACOX3*) and proliferator-activated receptor γ coactivator 1α (*PGC1α*) compared with the HFD group. The 16S *r*RNA gene sequencing showed that GCC-3 NT reduced the relative abundance of Actinobacteria while increased Firmicutes at the phylum level. The relative abundance of *Rhodococcus* was significantly decreased and *Lactobacillus* and *Staphylococcus* abundance were significantly increased in the GCC-3 NT group compared to the HFD group. Furthermore, PCoA analysis showed GCC-3 NT diet had a significant effect on the autochthonous microbiota compared to the HFD diet. Together, our results showed that nuclease-treated *L. rhamnosus* fermentation product can improve the immunity, liver health and gut microbiota of zebrafish, suggesting that it can be potentially used as a functional feed additive for aquaculture.

1. Introduction

Excessive fat accumulation in the liver of aquatic animals has become one of the main problems affecting the healthy development of aquaculture (Tocher, 2003; Cheng et al., 2017). Lipid deposition impairs the quality and flavor of fish muscles while wasting feed resources (Li et al., 2012; Jia et al., 2020). In addition, the immune system and health of fish can be compromised by the excessive deposition of body fat, which ultimately results in significant economic losses for the aquaculture industry (Kirpich et al., 2015; Zhang et al., 2018). Therefore, it is crucial to find safe and effective regulatory solutions to decrease the impacts of high-fat diets.

Probiotics have been used in aquafeed to improve growth performance, immunity, disease resistance, and gut microbiota of aquatic animals (Ringø et al., 2020). Among many probiotic species, *Lactobacillus* species attract more and more attention as the beneficial effect on the growth, immune system and health of fish (Doan et al., 2021). *Lactobacillus rhamnosus* strains derived from the human intestine have been used as probiotics for humans (Tuomola and Salminen, 1998; Ouwehand et al., 2000). Consistent with the results in mammals, dietary addition of *L. rhamnosus* strains can improve fish health (Zhang et al., 2016; Klopper et al., 2018; Sewaka et al., 2019). For instance, dietary *L. rhamnosus* ATCC 53103 enhanced immune parameters in rainbow trout (*Oncorhynchus mykiss*) (Nikoskelainen et al., 2003). In red sea

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bream (*Pagrus major*), *L. rhamnosus* ATCC 53103 can be used as a feed additive to increase the growth performance and enhance immunity when compared to the control group (Dawood et al., 2016). In addition, supplementation of *L. rhamnosus* ATCC 53103 improved innate immunity and reduced gut damage in Nile tilapia (*Oreochromis niloticus*) after *Aeromonas hydrophila* infection (Ngamkala et al., 2020). In our previous study, dietary *L. rhamnosus* GCC-3 fermentation product improved the gut and liver health as well as the resistance of tilapia against *Aeromonas* infection. Importantly, the liver triglycerides of fish were reduced (Zhou et al., 2022).

Nucleic acids of the probiotic bacterium are polymers made up of nucleotides and can be hydrolyzed by nuclease to produce nucleotides (Gite et al., 1992; Koval and Dohnálek, 2018). Nucleotides, as potential function feed additives in aquaculture, have been shown to enhance growth, improve gut health, modulate innate and adaptive immune responses, as well as increase stress tolerance capacity in many fish species, such as channel catfish (*Ictalurus punctatus*), rainbow trout, turbot (*Scophthalmus maximus*), stripped catfish (*Pangasius sutchi*), Nile tilapia, red sea bream, common carp (*Cyprinus carpio*), Atlantic salmon (*Salmo salar*) (Hossain et al., 2020). In our previous study, we also found that the supplementation of nucleotides improved growth and reduced hepatic steatosis in zebrafish (Ran et al., 2021). Zebrafish (*Danio rerio*) has been used as a model for fish feed, microbiota, gut immunity, and liver health (López Nadal et al., 2020). Here, we investigated the beneficial effects of nuclease-treated *L. rhamnosus* GCC-3 fermentation product in zebrafish. The results showed that nuclease-treated *L. rhamnosus* fermentation product can be used as a feed supplement to improve fish health.

2. Materials and methods

2.1. Bacteria culture and nuclease treatment

Lactobacillus rhamnosus GCC-3 with the preserved number China General Microbiological Culture Collection Center (CGMCC) No. 21821 was cultured in lactic acid bacteria culture (MRS) medium (Oxoid, Basingstoke, UK) at 37 ℃ overnight. The stabilized fermentation product of GCC-3 was obtained by adding 3 % $CaCO_3$ (1 M) after 48 h of shake flask fermentation according to the previously described method (Zhou et al., 2022). Then, the stabilized fermentation product was mixed with fine bran at 1:1 and dried at room temperature. The concentration of GCC-3 in the dried fermentation product was 9.6×10^8 CFU/g.

On the one hand, part of GCC-3 was directly mixed with fine bran in a ratio of 1:1, dried at room temperature and added to the feed as GCC-3 treatment. On the other hand, the fermentation product was treated with 3 % nuclease (Nanning Pangbode Bioengineering, Guangxi, China) (Xie et al., 2022). After mixing, they were incubated at 30 ℃ for 48 h, and the nucleotide concentration was measured at 24 h, which was 36.388 mg/g (24.948 mg/g without nuclease). Drying at room temperature, they were added to the feed as GCC-3 nuclease treatment group (namely GCC-3 NT).

2.2. Experimental diets and animal feeding

The experimental formula for a high-fat diet for zebrafish was designed according to Zhang et al. (2019) (The feed formula was shown in Table 1). Based on the high-fat diet, 10 % GCC-3 % and 10 % nuclease-treated GCC-3 fermentation products were added to replace rice husk meal respectively. Dietary crude protein, crude fat, ash, and moisture are shown in Table 1. In feed preparation, the amount of each raw material is first accurately calculated and weighed, and then expanded step by step according to the order of mixing small raw materials and then mixing large raw materials. All the mixed raw materials were mixed with a proper amount of water to make feed, which was dried in a constant temperature oven at 90 ℃ for 90 min.

The animals used in the experiment were 2-month-old zebrafish (TU strain), which were cultured in the zebrafish circulation system and the breeding conditions referred to China Zebrafish Resource Center (CZRC). The fish were fed 6 % of their body weight every day (09:00 and 16:00) for 3 weeks. A total of four kinds of diets were fed in this experiment, and 4 biological replicates were set up in each group. There were 20 fish in each tank. All zebrafish were cultured in a recirculating system with a 12 h/12 light/dark cycle. The water temperature varied from 28.0 ℃ to 28.5 ℃, pH 7.0–7.5, and the dissolved oxygen was higher than 6 mg/L.

Table 1
Ingredients and proximate composition of diets for zebrafish.

Ingredient (g / kg diet)	Control	HFD	GCC-3	GCC-3NT
Fish meal	450.00	450.00	450.00	450.00
Flour	250.00	200.00	200.00	200.00
Soybean meal	180.00	196.00	196.00	196.00
Soybean oil	12.00	100.00	100.00	100.00
Choline chloride	2.00	2.00	2.00	2.00
Monocalcium phosphate	20.00	20.00	20.00	20.00
VC phosphate	1.00	1.00	1.00	1.00
Bentonite	61.00	7.00	7.00	7.00
Mixture	10.00	10.00	10.00	10.00
Rice husk meal	10.00	10.00	0	0
Vitamin premix[a]	2.00	2.00	2.00	2.00
Mineral premix[b]	2.00	2.00	2.00	2.00
GCC-3	0	0	100.00	0
Nuclease-treated GCC-3	0	0	0	100.00
Total	1000.00	1000.00	1000.00	1000.00
Crude protein (%, WW)	43.25	43.86	43.42	43.15
Crude fat (%, WW)	7.76	18.23	18.14	18.06
Crude ash	13.81	13.83	14.07	14.11
Moisture (%)	2.41	2.47	2.09	2.37
Crude protein (%, DW)	44.32	44.98	44.35	44.20
Crude fat (%, DW)	7.95	18.70	18.53	18.50

Note: WW and DW represent the wet weight and dry weight, respectively.
[a] Containing the following (g/kg vitamin premix): thiamine, 0.438; riboflavin, 0.632; pyridoxine·HCl, 0.908; *d*-pantothenic acid, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B-12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; retinyl acetate, 0.677; cholecalciferol, 0.116; *dl*-α-tocopherol-acetate, 12.632.
[b] Containing the following (g/kg mineral premix): $CoCl_2{\cdot}6H_2O$, 0.074; $CuSO_4{\cdot}5H_2O$, 2.5; $FeSO_4{\cdot}7H_2O$, 73.2; NaCl, 40.0; $MgSO_4{\cdot}7H_2O$, 284.0; $MnSO_4{\cdot}H_2O$, 6.50; KI, 0.68; Na_2SeO_3, 0.10; $ZnSO_4{\cdot}7H_2O$, 131.93; Cellulose, 501.09.

All fish were anesthetized with tricaine methanesulfonate (MS222) before sampling. At the end of the feeding trial, fish were weighed and the results were got with the following calculations. Weight gain (WG, %) = [100 % × (final body weight (g) - initial body weight (g))/initial body weight]; feed conversion ratio (FCR) = food intake (g)/weight gain of fish (g); survival rate (%) = (number of fish at the end of the experiment/number of fish at the start of the experiment) × 100 %. The liver was collected at 24 h after the last feeding for analysis of triglycerides content and gene expression. Body surface mucus was collected at 24 h after the last feeding for analysis of total antioxidant capacity and lysozyme activity. The gut content was collected at 4 ~ 6 h under aseptic conditions after the last feeding. The gut content samples from 6 fish were pooled as a replicate to analyze the gut microbiota of zebrafish.

2.3. Detection of triglycerides (TAG) content

TAG in the liver of zebrafish was detected using a TAG kit (Beyotime Biotechnology, Shanghai, China). This determination method referred to the manufacturer's instructions and previously described (Zhang et al., 2019).

2.4. Real-time quantitative PCR (RT-qPCR)

The RNA of zebrafish gut and liver tissue was extracted following the procedures from our previously published paper (Zhang et al., 2019).

RT-*q*PCR analysis was performed on a continuous fluorescence detector 480 system (LichtCycler® 480 Real-Time PCR System, Roche) using SYBR Green Supermix (Tiangen, China). All primers in the study were listed in Table 2. Data were analyzed by $2^{-\Delta\Delta CT}$ method using *RPS11* as the reference.

2.5. Detection of total antioxidant capacity and lysozyme activity

Under aseptic conditions, body surface mucus from 5 fish was pooled as a replicate with tweezers and then put into 100 μl phosphate-buffered saline (PBS). Total antioxidant capacity (T-AOC) and lysozyme activity in body surface mucus were determined using the T-AOC assay kit (Cominbio, Suzhou, China) and the lysozyme activity assay kit (Beyotime Biotechnology, Shanghai, China), respectively.

2.6. 16S rRNA-based analyses of gut microbiota

Gut content samples of zebrafish were collected from each treatment group 4–6 h post the last feeding. Gut content from 6 fish in each tank was pooled as a replicate. The composition of gut microbiota was analyzed as the previously published protocol (Zhang et al., 2019). In brief, the V3-V4 regions of the 16 S *r*RNA genes were amplified with the primers 338 F (ACTCCTACGGGAGGCAGCAG), 806R (GGACTACHVGGGTWTCTAAT). After the construction of the libraries, the paired-end 250-nucleotide reads were obtained using the Illumina Miseq platform. Using the DADA2 plugin, raw sequences were trimmed, quality filtered, denoised, merged, chimera, and dereplicated (DeSantis et al., 2006).

2.7. Statistical analysis

All results in this paper from four independent experiments were expressed as the mean ± standard error (SEMs). GraphPad Prism version 8.0 software was used to analyze data. The differences between the two groups were examined by the Student's t-test. The significant difference was set at $^{*}P < 0.05$.

3. Results

3.1. Effects of L. rhamnosus fermentation product on the growth performance and feed utilization of zebrafish

After 3-week feeding, the effects of nuclease-treated and untreated *L. rhamnosus* fermentation products on the growth performance of zebrafish were evaluated, and the result was presented in Fig. 1. Compared with the HFD group, the weight gain showed an increasing trend in the GCC-3 group (Fig. 1B, $P = 0.06$). Accordingly, the feed conversion ratio showed a decreasing tendency in the GCC-3 group compared with the HFD group (Fig. 1C, $P = 0.06$). In terms of survival rate, there was no difference in the four groups (Fig. 1D).

3.2. Effects of L. rhamnosus fermentation product on the non-specific immunity of zebrafish

The effects of nuclease-treated and untreated *L. rhamnosus* fermentation product on lysozyme activity and total antioxidant capacity of zebrafish were evaluated (Fig. 2). Compared with the control group, the results showed that the high-fat diet can increase the lysozyme activity (Fig. 2A, $P < 0.01$), and compared with the HFD group, GCC-3 NT treatment can further increase the lysozyme activity of body surface mucus (Fig. 2A, $P < 0.01$). Furthermore, total antioxidant capacity was increased in both GCC-3 and GCC-3 NT groups compared with HFD group (Fig. 2B, $P < 0.05$ and $P < 0.05$, respectively).

3.3. Effects of L. rhamnosus fermentation product on the liver health of zebrafish

As can be seen in Fig. 3A and B, the TAG content of zebrafish liver was significantly reduced in the GCC-3 and GCC-3 NT groups compared with the HFD group ($P < 0.05$). In addition, the expression of genes involved in lipid metabolism was detected in the liver. In comparison to the HFD group, the expression of lipid synthesis related genes including fatty acid synthase (*FAS*) and peroxisome proliferator-activated receptor gamma (*PPARγ*) had a reduced trend in the GCC-3 group (Fig. 3C, D, $P = 0.08$ and $P = 0.07$, respectively). The expression of lipolysis related genes including acyl-coenzyme A oxidases 3 (*ACOX3*) and proliferator-activated receptor γ coactivator 1α (*PGC1α*) was not affected by GCC-3 (Fig. 3E, F, $P > 0.05$), while the expression of *ACOX3* and *PGC1α* was significantly up-regulated in the GCC-3 NT group compared with the HFD group (Fig. 3E, F, $P < 0.05$).

3.4. Effects of L. rhamnosus fermentation product on the gut microbiota of zebrafish

There are 401 OTU shared by the four groups (Fig. 4A). The alpha-diversity analysis showed that the addition of GCC-3 and GCC-3 NT had no significant effect on alpha-diversity compared to the HFD group (Table 3).

As shown in Fig. 4B and C, the addition of GCC-3 and GCC-3 NT significantly affected the composition of the gut microbiota of zebrafish compared to the HFD group. At the phylum level, GCC-3 and GCC-3 NT supplementation significantly reduced the relative abundance of Actinobacteria (Fig. 4B). In contrast, Firmicutes abundance was significantly increased in GCC-3 and GCC-3 NT groups (Fig. 4B). At the genus level, the relative abundance of *Rhodococcus* was significantly decreased versus the HFD group (Fig. 4C). Dietary GCC-3 and GCC-3 NT significantly increased *Lactobacillus* and *Staphylococcus* abundance compared to the HFD group (Fig. 4C). Moreover, PCoA analysis showed substantial differences among the HFD, GCC-3, and GCC-3 NT groups, indicating that GCC-3 and GCC-3 NT diets had significant effects on the autochthonous microbiota compared to the HFD control (Fig. 4D).

4. Discussion

In the present work, dietary supplementation of *L. rhamnosus* GCC-3 fermentation product did not affect the growth performance but enhanced the non-specific immunity of zebrafish. In addition, the GCC-3 diet reduced the liver TAG and improved the gut microbiota of zebrafish. Consistent with these results, Zhou et al. (2022) showed that the addition of *L. rhamnosus* fermentation product can improve the gut and liver health as well as improve the gut microbiota of tilapia. Collectively, these studies suggest that the fermentation product of *L. rhamnosus*

Table 2
Primer sequences for *q*RT-PCR analysis.

Gene Name	Forward (5' to 3')	Reverse (5' to 3')
FAS	GGAGCAGGCTGCCTCTGTGC	TTGCGGCCTGTCCCACTCCT
PPARγ	CCTGTCCGGGAAGACCAGCG	GTGCTCGTGGAGCGGCATGT
ACOX3	TGGAAGGACATGATGCGCTTT	AGGCTGCCGGGCAAAAA
PGC1α	CCCCTTTGCCCTGACCTGCCTGAG	GAAGGACAGCTCTGATCACTGGCATTGG
RPS11	ACAGAAATGCCCCTTCACTG	GCCTCTTCTCAAAACGGTTG

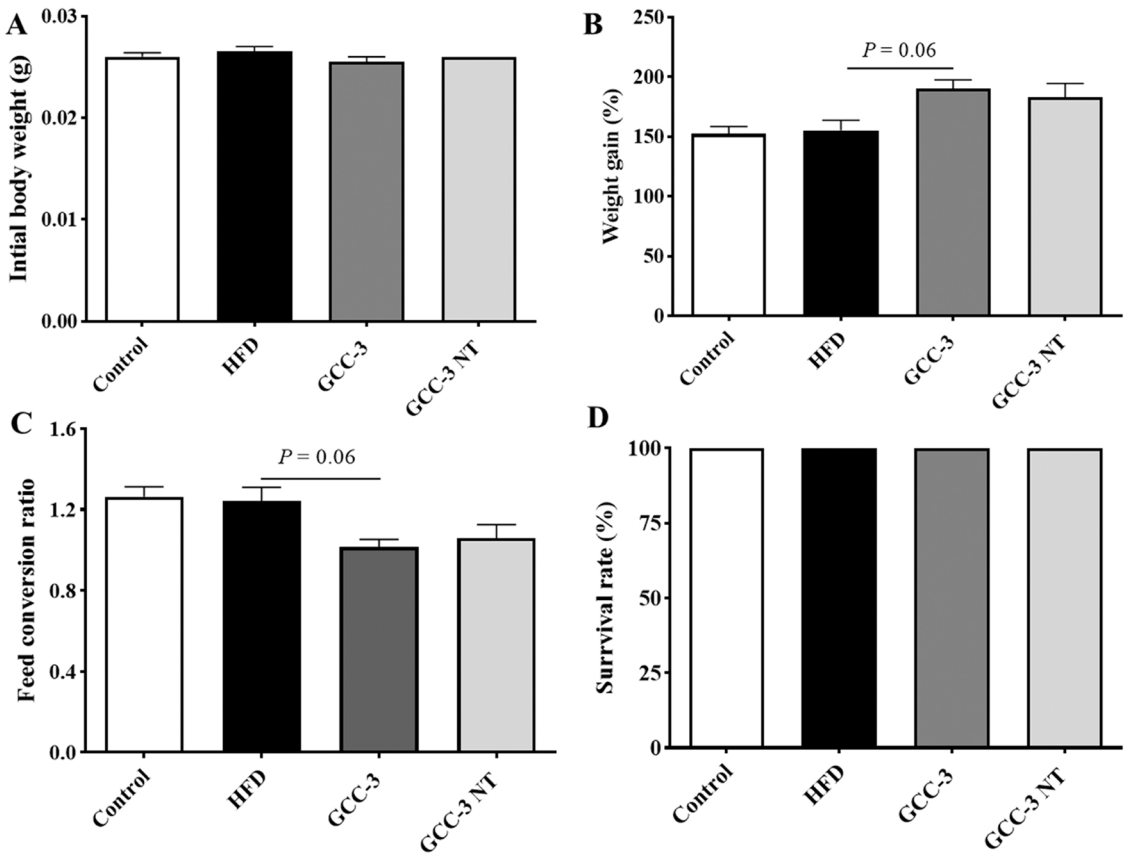

Fig. 1. Effects of fermentation products of *Lactobacillus rhamnosus* treated with nuclease (GCC-3 NT) and untreated (GCC-3) on the growth performance of zebrafish. (A) Initial body weight (g), (B) Weight gain (%), (C) Feed conversion ratio, (D) Survival rate (%). Data represent the means ± SEM of each treatment (n = 4).

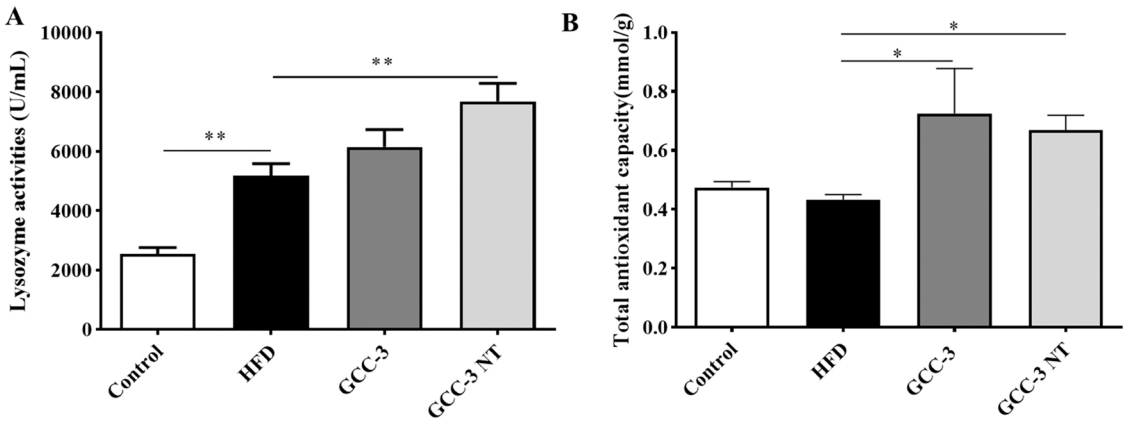

Fig. 2. Effects of fermentation products of *Lactobacillus rhamnosus* treated with nuclease (GCC-3 NT) and untreated (GCC-3) on lysozyme activity and total antioxidant capacity in body surface mucus of zebrafish. (A) Zebrafish surface mucus lysozyme activity (n = 4). (B) Total antioxidant capacity of body surface mucus (n = 4). Data represent the means ± SEM. *, $P < 0.05$; **, $P < 0.01$.

could be used as a functional additive to improve fish health. In this study, nuclease was used to treat the fermentation product of *L. rhamnosus* to further improve the function of the fermentation product. Notably, the concentration of nucleotides in the fermentation product of *L. rhamnosus* was elevated by 45.85 % after the treatment of nuclease compared to the untreated *L. rhamnosus* GCC-3. A lot of studies showed that dietary nucleotides have a growth-promotion effect in fish (Peng et al., 2013; Xu et al., 2015; Hossain et al., 2017; Ran et al., 2021). However, the addition of GCC-3 NT didn't influence the growth of zebrafish in the present work. This is consistent with previous reports on other fish species, such as channel catfish (Welker et al., 2011), Nile tilapia (Barros et al., 2013), hybrid tilapia (*Oreochromis niloticus* ♀ ×*Oreochromis aureus* ♂) (Shiau et al., 2015) and juvenile turbot (Fuchs et al., 2015), in which the inclusion of nucleotides did not affect the growth of fish. The differences in the effects of nucleotides on fish growth may be attributed to fish species, fish age, the addition level, and duration of administration (Hossain et al., 2020).

Lysozyme is an important innate defense parameter, which exists in the mucus, lymphoid tissue, serum, and other body fluids of most fish and plays a key role in resisting pathogens (Magnadottir et al., 2005; Zhuo et al., 2021). The antioxidant capacity of the body's defense system can be evaluated by the total antioxidant capacity, which can represent

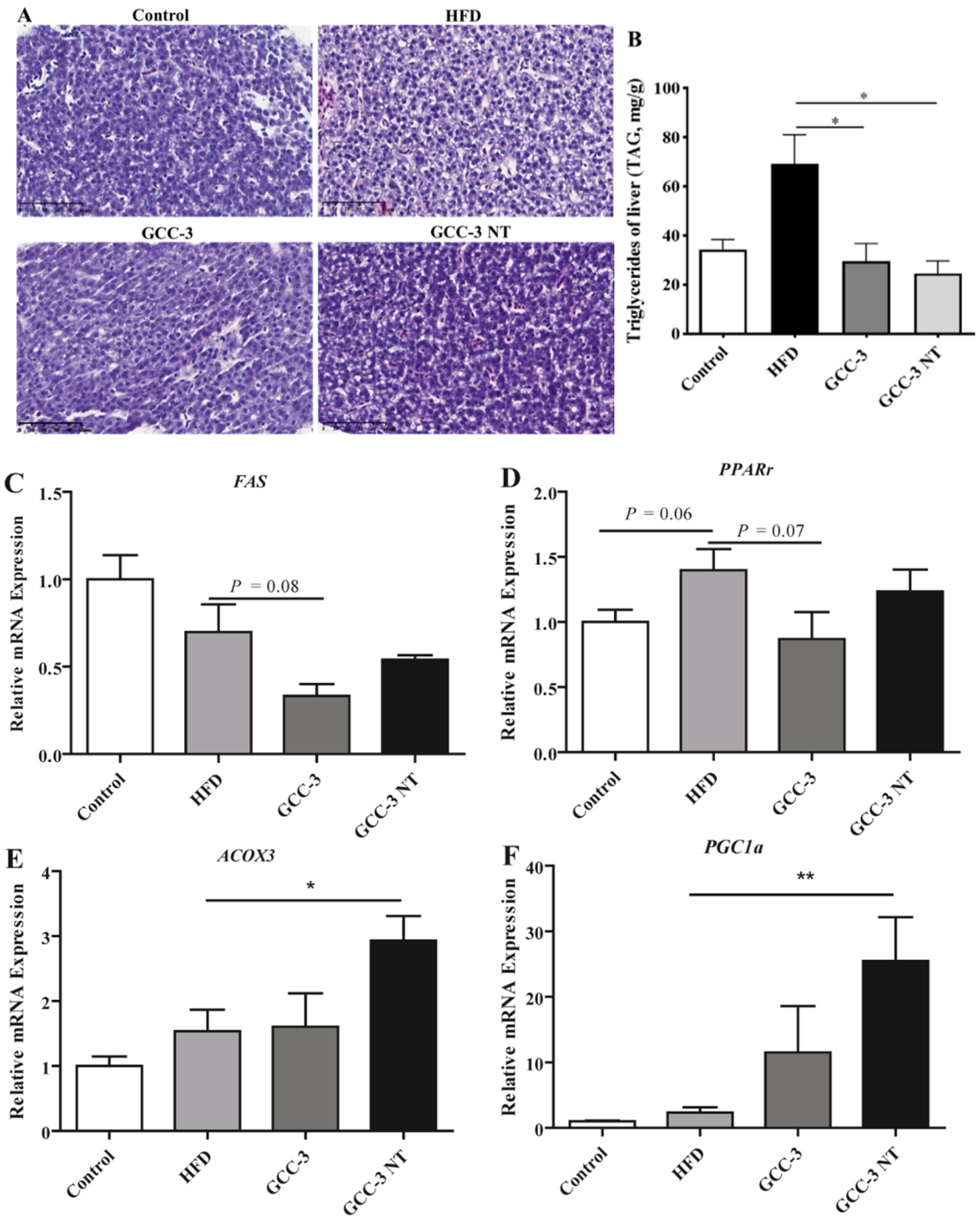

Fig. 3. Effects of fermentation products of *Lactobacillus rhamnosus* treated with nuclease (GCC-3 NT) and untreated (GCC-3) on the expression of lipid metabolism in the liver of zebrafish. (A) Detection of zebrafish liver morphology by HE staining (n = 4). (B) Triglycerides (TAG) (n = 4). (C-F) Expression of lipid metabolism related genes (n = 4). Data represent the means ± SEM. *, $P < 0.05$; **, $P < 0.01$.

and reflect the compensatory ability of the antioxidant enzyme system and non-enzymatic system of the body to external stimuli and the status of free radical metabolism of the body (Deng et al., 2013; Mohammadi et al., 2020). In the present study, dietary supplementation of GCC-3 NT significantly increased the lysozyme activity and total antioxidant capacity in the body surface mucus of zebrafish. Similarly, dietary nucleotides can enhance the non-specific immune responses by increasing the lysozyme activity in common carp (Sakai et al., 2010), red drum (*Sciaenops ocellatus*) (Cheng et al., 2011), and olive flounder (*Paralichthys olivaceus*) (Song et al., 2012). Tie et al. (2021) demonstrated that the inclusion of nucleotides increased the antioxidant capacity of grass carp (*Ctenopharyngodon idella*) infected with *Flavobacterium columnare*. The antioxidant capacity-enhancing effects of nucleotides have also been reported in other fish species such as hybrid tilapia (Xu et al., 2015), juvenile red sea bream (Hossain and Koshio, 2017), yellow catfish (*Pelteobagrus fulvidraco*) (Zhao et al., 2015) and Nile tilapia (Reda et al., 2018). Collectively, these results showed that dietary supplementation of GCC-3 NT strengthened the non-specific immunity of zebrafish and nucleotides may play a key role in the fermentation product of GCC-3 NT.

High-fat feed leads to excessive lipid accumulation, which results in an increase in oxidative stress and injury in the liver, affecting the health

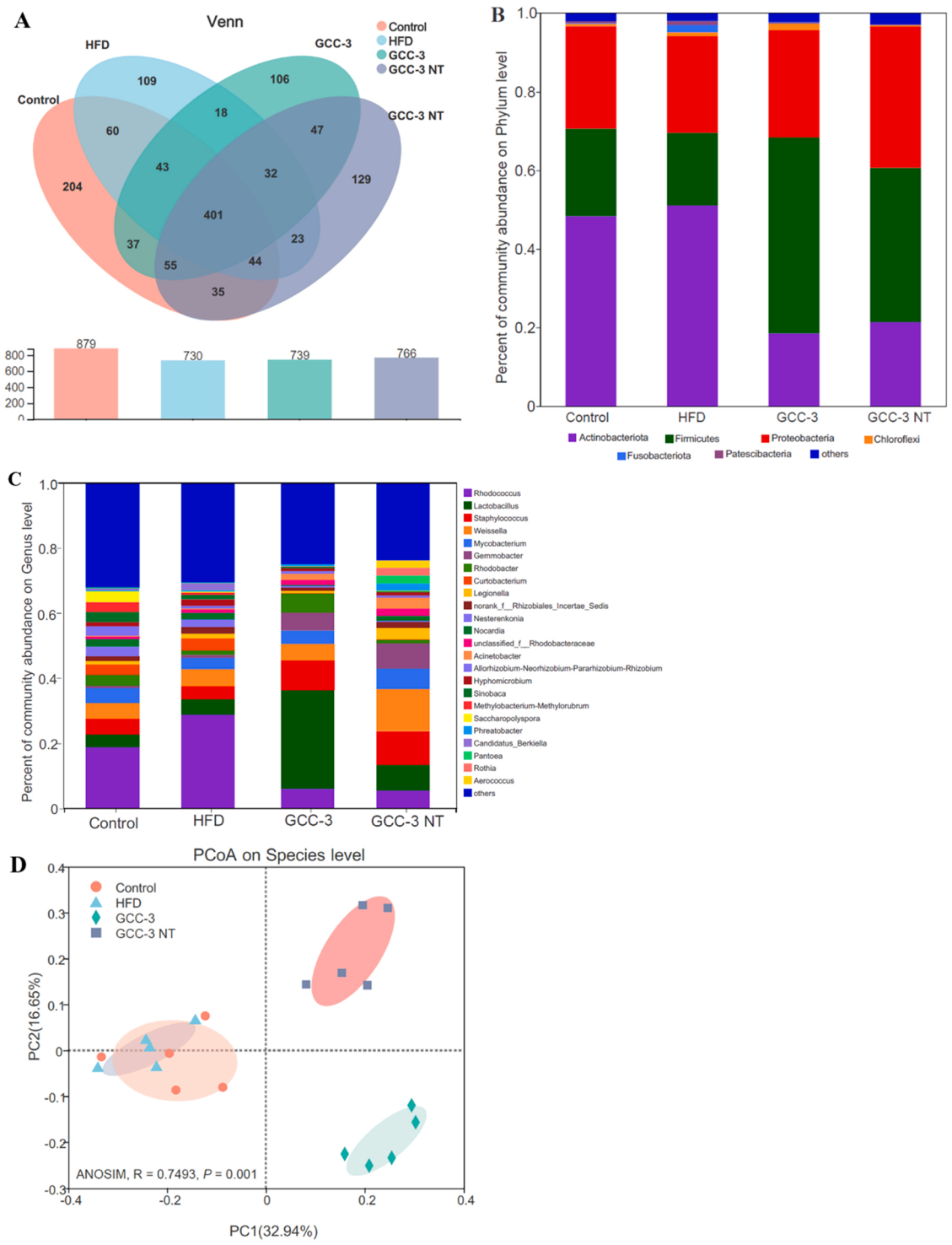

Fig. 4. Effects of fermentation products of *Lactobacillus rhamnosus* treated with nuclease on the gut microbiota of zebrafish. (A) OUT analysis, relative abundance at the phylum (B) and the genus level (C), (D) Principal coordinates analysis (PCoA) of the gut microbiota (n = 5).

Table 3
Effects of *Lactobacillus rhamnosus* fermentation product on the diversity index of zebrafish gut microbiota.

Parameters	Control	HFD	GCC-3	GCC-3 NT
Shannon	1.17 ± 0.13	1.19 ± 0.16	1.18 ± 0.11	1.12 ± 0.21
Simpson	0.37 ± 0.05	0.39 ± 0.08	0.39 ± 0.06	0.41 ± 0.10
ACE	14.08 ± 7.16	10.30 ± 8.09	12.33 ± 9.97	18.41 ± 2.28
Chao	16.33 ± 2.16	15 ± 1.41	17.33 ± 3.44	17.58 ± 1.91

of fish (Zhou et al., 2022). Dietary nucleotides were reported to benefit liver function and reduce hepatic lipid deposition in mammals (Novak et al., 1994; Pérez et al., 2004; Cai et al., 2016). However, there are few studies about dietary nucleotides regulating hepatic lipid metabolism and deposition in fish. Interestingly, a recent study found nucleotides reduced the content of TAG in the liver of zebrafish (Ran et al., 2021). Consistent with this report, the current study found that the addition of GCC-3 NT significantly reduced the TAG content in the liver of zebrafish. Ran et al. (2021) found that dietary nucleotides decreased the

expression of genes involved in lipid synthesis while increased the expression of genes involved in fatty acid oxidation in zebrafish. In line with this result, in this study, the expression of lipolysis related genes in the GCC-3 NT group was also up-regulated compared with the HFD group. These findings suggest nucleotides in the fermentation product of GCC-3 NT contributed to the hepatic lipid-lowering effect in zebrafish.

The fish gastrointestinal tract is colonized by complex gutmicrobiota (Wong and Rawls, 2012). Previous studies have shown that gut microbiota has a close connection with the growth, nutritional status, and immune system of the host fish and is easily changed by the environment, diet, stress, and development of fish (Sullam et al., 2012; Xiong et al., 2019). In this study, feeding fish with GCC-3 NT significantly changed the autochthonous microbiota, with a lower relative abundance of Actinobacteria and higher Firmicutes abundance at the phylum level compared to the HFD group. The phylum Firmicutes contains Lactic acid bacteria, *Enterococcus*, *Clostridium butyricum*, and *Bacillus*, most of which are probiotics to benefit fish health by improving the growth, immunity, and disease resistance (Wang et al., 2019). In contrast, few genera in Actinobacteria were reported to be probiotics in aquaculture (Wang et al., 2019). These results suggested that the inclusion of GCC-3 NT improved the gut microbiota of zebrafish. The effect of dietary nucleotides on gut microbiota of fish has been reported in previous studies. For example, Guo et al. (2017) found that dietary nucleotides significantly changed the gut microbiota, with a dominant phylum Proteobacteria in the control group and Fusobacteria in the nucleotides group, which reduced the energy expenditure to improve the growth of zebrafish. Mehdinejad et al. (2018) demonstrated that the supplementation of nucleotides contributed to the intestinal colonization of *Pedicoccus acidilactici* in goldfish (*Carassius auratus*). Therefore, we speculate that nucleotides play an important role in the fermentation product of GCC-3 NT in benefiting the gut microbiota of zebrafish, which needs more investigation.

5. Conclusions

Collectively, the present results indicate that dietary GCC-3 NT fermentation product did not affect the growth performance of zebrafish. However, the inclusion of GCC-3 NT enhanced the non-specific immunity of zebrafish. In addition, GCC-3 NT reduced lipid deposition by up-regulating the expression of lipogenesis genes in the liver. Moreover, the supplementation of GCC-3 NT improved gut microbiota versus the HFD group. Our findings demonstrate that nuclease-treated GCC-3 fermentation product could be considered a functional feed additive that improves fish health.

Ethical approval

This article does not contain any study of human participants by any author. All applicable international, national, and institutional guidelines for animal care and use are adhered to. All experiments and animal care procedures were approved by the Animal Care Committee of Feed Institute, Chinese Academy of Agricultural Sciences (Guarantee No.2021- AF-FRI-CAAS-001).

CRediT authorship contribution statement

The authors declare that they have no conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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