

Partially replacing dietary fish meal by *Saccharomyces cerevisiae* culture improve growth performance, immunity, disease resistance, composition and function of intestinal microbiota in channel catfish (*Ictalurus punctatus*)

Qiang Hao^{a,b,1}, Rui Xia^{a,1}, Qingshuang Zhang^a, Yadong Xie^a, Chao Ran^c, Yalin Yang^c,
Wenhao Zhou^d, Fuwei Chu^e, Xiaomei Zhang^e, Yu Wang^e, Zhen Zhang^{c,**}, Zhigang Zhou^{a,f,*}

^a Sino-Norway Joint Lab on Fish Gut Microbiota, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

^b Norway-Sino Joint Lab on Fish Gut Microbiota, Institute of Biology, Norwegian University of Science and Technology, Trondheim, 7491, Norway

^c Key Laboratory for Feed Biotechnology of the Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

^d Beijing Enhonor International Tech Co., Ltd., Beijing, 100081, China

^e Shandong Dayang Agriculture and Animal Husbandry Science and Technology Development Co., Ltd., Shandong, 277319, China

^f Institute of Animal Husbandry and Veterinary, Jiangxi Academy of Agricultural Science, Nanchang, 330200, China

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ABSTRACT

The aim of the present study was to investigate the partial replacement of fish meal by *Saccharomyces cerevisiae* culture on growth performance, immunity, composition and function of intestinal microbiota and disease resistance in channel catfish (*Ictalurus punctatus*). Two equal nitrogen and energy diets were prepared including a basal diet (containing 10% fish meal, Control) and an experimental diet (replacing 20% of the fish meal of the basal diet with yeast culture, RFM). Channel catfish were fed with the diets for 12 weeks. The results showed that weight gain and condition factor were significantly increased, and FCR was significantly decreased in RFM group ($P < 0.05$). The gene expression of intestinal *HIF1α* was significantly increased in RFM group ($P < 0.05$), while the expressions of *NF-κB* in the intestine and liver were significantly decreased ($P < 0.05$). The relative abundance of Firmicutes tended to increase, and the *Turicibacter* had an upward trend ($0.05 < P < 0.2$). In addition, the survival rate of channel catfish was significantly increased in RFM group after challenged with *Aeromonas veronii* Hm091 and *Aeromonas hydrophila* NJ-1 ($P < 0.05$). Compared with intestinal microbiota of channel catfish of control group, intestinal microbiota of channel catfish of RFM group significantly increased the expression of *HIF1α*, and decreased the expression of *IL-1β* and *TNF-α* ($P < 0.05$) in germ-free zebrafish. Intestinal microbiota induced by RFM diet also significantly increased disease resistance to *Aeromonas veronii* Hm091 and *Aeromonas hydrophila* NJ-1. In conclusion, replacement of fish meal by the yeast culture improved the growth, immunity and disease resistance of channel catfish, and intestinal microbiota of channel catfish induced by the yeast culture played a critical role in these effects.

1. Introduction

As an important protein source in feed, fish meal has the characteristics of high protein content, good palatability, less antinutritional factors, balanced composition of essential amino acids, high content of calcium, phosphorus, iodine and selenium, and many kinds of vitamins [1]. However, the rapid increase of fish meal demand and the decline of

marine fishery resources have led to the shortage of fish meal resources, the rising price of fish meal and the reduction of profits in the feed industry. It is an important way for the sustainable development of aquaculture to reduce the use of high price protein sources and use appropriate protein sources instead of fish meal [2]. Plant protein sources (such as soybean meal, miscellaneous meal, etc.) are important alternatives, but there are also problems such as insufficient resources,

* Corresponding author.

** Corresponding author.

E-mail addresses: zhangzhen@caas.cn (Z. Zhang), zhouzhigang03@caas.cn (Z. Zhou).

¹ Equal contributors.

nutritional imbalance, and anti-nutritional factors [3]. Thus, there is an emerging need for suitable and sustainable novel feed ingredients for aquaculture. In the past ten years, the research on single cell protein and its application in aquatic feed as a substitute for fish meal have been widely concerned.

Single cell protein refers to the cells of yeast, bacteria, actinomycetes, molds, algae and higher fungi, which are cultivated on a large scale and used as the protein source of human food and animal feed [4–6]. Yeast products are widely used in feed as functional additives for aquatic animals. However, many researchers have gradually begun to pay attention to the possibility of yeast source products as feed raw materials. Yeast is a kind of protein feed with high nutritional value, which contains relatively high protein content (45–55%) and can be produced in large quantities at low cost [7,8]. By adding a certain proportion of yeast products to replace fish meal in feed, the cost of feed raw materials can be reduced and the shortage of feed protein raw materials can be alleviated. At the same time, the yeast products are rich in organic acid, oligosaccharide, flavoring substance and aromatic substance [9], which can enhance the digestion and absorption of aquatic feed, improve growth and metabolism of fish, and improve the survival rate of fish [10–12].

Channel catfish (*Ictalurus punctatus*) is the most important freshwater aquaculture species in the United States, accounting for more than 60% of the total fish production in the United States [13,14]. In 1984, China introduced channel catfish from the United States. Since then, the scale of cultivation has gradually expanded. Due to its strong adaptability, fast growth rate and delicious meat, the scale of aquaculture has been increased year by year [15–17]. In 2020, the national aquaculture output of channel catfish reached to 308,488 tonnes, which has become one of the excellent aquaculture species in China (China fishery statistical yearbook, 2021). The yeast culture has high content of crude protein, which can be used as an alternative protein source. However, there were fewer reports on application of fish meal replacing by yeast culture in channel catfish. Therefore, in order to provide theoretical basis for the development of low fish meal artificial feed for channel catfish, yeast culture was used to replace fish meal in this study to evaluate its effects on the growth performance, immune response, intestinal microbiota, and disease-resistance against *Aeromonas veronii* Hm091 and *Aeromonas hydrophila* NJ-1 in channel catfish.

2. Materials and methods

2.1. Bacteria, yeast product and diet preparation

Saccharomyces cerevisiae Sa-10 was preserved in China General Microbiological Culture Collection Center (CGMCC no. 6120). *A. veronii* Hm091 was preserved in China General Microbiological Culture Collection Center with its preservation number CGMCC no.22536. *A. hydrophila* NJ-1 was a gift from Dr. Yongjie Liu from Nanjing Agricultural University (Nanjing, China). *Saccharomyces cerevisiae* culture (ESTAQUA®) was provided by Enhalar Biotechnology Company (Beijing, China), and was fermented based on the patent CN107937292A together with technique guidance of Sino-Norway Joint Lab on Fish Gut Microbiota. In brief, when obtaining yeast culture powder, *Saccharomyces cerevisiae* Sa-10 was aerobically fermented in a molasses-based medium (150 g of cane molasses, 10 g of ammonium sulfate, 1.6 g of yeast extract, 1.5 g of magnesium sulfate, 0.08 g of anhydrous calcium chloride, 1.5 g of potassium dihydrogen phosphate, 0.3 g of defoamer in 1 L of sterile water, pH 6.2). The grains were added to the content to form a slurry and undergo anaerobic fermentation to enhance cell wall destruction. Finally, the fermented product was enriched with nucleotides and acetic acid, and this product contained 48.56% more nucleotides and 90.19% more acetic acid than it obtained using the original production method (SI Table 1).

The main source of protein in the basal diet were soybean meal, rapeseed meal and fish meal, and the main source of lipid was soybean

Table 1

Feed formulation and chemical composition of diets for channel catfish.

Ingredient	g/kg (air dry basis)	
	Control	RFM
Rice bran	100.00	100.00
Flour	150.00	150.00
Soybean meal	250.00	250.00
Rapeseed meal	150.00	154.00
Fish meal	100.00	80.00
Chicken meal	100.00	100.00
DDGS	50.00	50.00
SCC Sa-10 ^a	0.00	20.00
Bentonite	15.00	10.00
Lys-HCl	2.00	2.00
Methionine	0.50	0.50
Choline Chloride (50%)	2.00	2.00
Ca(H ₂ PO ₄) ₂	20.00	20.00
Soybean oil	40.00	41.00
VC phosphate	0.50	0.50
Premix ^b	20.00	20.00
Total	1000	1000
Chemical composition (g/kg)		
Crude protein	380.4	376.7
Crude fat	112.9	116.1
Crude ash	102.2	98.6
Moisture	54.3	66.7

^a Provided by Enhalar Biotechnology Company, Beijing, China, its composition was as follows: Crude protein, 67.73%; Crude fat, 3.18%.

^b Containing vitamin premix and mineral premix. (1) Vitamin premix containing the following (g/kg): 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. (2) Mineral premix containing the following (g/kg): CoCl₂·6H₂O, 0.074; CuSO₄·5H₂O, 2.5; FeSO₄·7H₂O, 73.2; NaCl, 40.0; MgSO₄·7H₂O, 284.0; MnSO₄·H₂O, 6.50; KI, 0.68; Na₂SeO₃, 0.10; ZnSO₄·7H₂O, 131.93; Cellulose, 501.09.

oil. According to previous research results [18,19], in this study the experimental diet was prepared by 20 g/kg *Saccharomyces cerevisiae* culture (SCC) to replace the same amount of fish meal in the basic diet. The feed formulation and approximate analysis were shown in Table 1.

2.2. Fish husbandry and feeding

All experiments and animal care procedures were approved by Institute of Feed Research of the Chinese Academy of Agricultural Sciences by the China Council for Animal Care (Assurance No. 2019-AF-FRI-CAAS-001). Channel catfish was purchased from a fish farm in Hebei, China, and transported to the International Agricultural High-tech Industrial Park of the Chinese Academy of Agricultural Sciences, Langfang, Hebei, China. The channel catfish was domesticated in circulating water for a week before the experiment.

After 24 h of starvation, channel catfish with similar body weight (initial body weight 24.49 ± 0.11 g) were randomly divided into 2 groups. 6 replication tanks were assigned to each treatment, and each tank containing 12 fish. During the trial period, channel catfish were fed 3 times a day (7:00, 12:00 and 18:00). The fish were fed with basic feed and experimental feed for 12 weeks. Initially, they were fed daily at 1.5% of their initial body weight for 2 weeks. From the third week, the feeding amount increased to 2.5% of their initial body weight for 6 weeks. Starting from the ninth week, the feeding amount increased to 4% of their initial body weight for another 3 weeks, and from the twelfth week, the feeding amount increased to 5% of their initial body weight for 1 week. The capacity of the feeding tank was 90 L, the water inflow was 120 L/h, the water temperature was 25.5 °C, the dissolved oxygen (DO) was ≥ 6 mg/L, the ammonia nitrogen content was < 0.2 mg/L, and the nitrite concentration was < 0.06 mg/L.

2.3. Measurement of growth performance and feed utilization efficiency

At the end of the experiment, fish were fasted for 24 h, and then weighed after being anaesthetized with 3-aminobenzoic acid ester methanesulfonate. The weight gain (WG, %), feed coefficient (FCR), survival rate (%), condition factor were calculated according to the method [20].

2.4. Histological observation of intestine and liver

At the end of the experiment, 1 fish was randomly selected from each of the 6 replication tanks for liver and hindgut histological analysis after 24 h fasting ($n = 6$). The samples were dehydrated in 4% paraformaldehyde, and then embedded in paraffin. The sections were stained with hematoxylin and eosin (HE), sealed with neutral gum, and observed with microscope (Leica DMIL-LED, Germany). The K-Viewer image analysis software was used for the image analysis.

2.5. Relative quantification expression of immune genes

Relative quantification analysis was used to evaluate the effect of feeding RFM diet on the immunity of channel catfish. Total RNA was extracted from liver and intestine of channel catfish using the TRIzol Reagent extraction method ($n = 6$). According to the instructions of the reverse transcription kit (TianGen, Beijing, China), 2 μ g RNA was used as a template to reverse transcribe into cDNA, and then the cDNA was used as a template for Quantitative real-time PCR reaction (TianGen, Beijing, China). The primers are shown in Table 2.

2.6. Western blot analysis of immune protein expression

The intestine and liver of channel catfish were treated with Hank's Balanced Salt (14065056, Gibco™, Grand Island, N.Y.) containing protease/phosphatase inhibitor cocktail (5872, Cell Signaling Technology). The equivalent proteins were loaded in 12% SDS-PAGE. After electrophoresis, the proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA) for immunoblotting. The membrane incubated with antibody GAPDH (1:3000, SAB2701826, Sigma-Aldrich), NF- κ B-p65 (1:1000, LS-C31758, LifeSpan BioSciences, Inc.) at 4 °C overnight. Secondary peroxidase conjugated antibodies were used (anti-mouse: 1:5000, A21010, Abbkine, Wuhan, China; or anti-rabbit: 1:1000, CW0103, CWBIO, CoWin Biotech Co. Ltd., Beijing, China). The blots were developed by chemiluminescence using an ECL kit (WBKLS0500, Merck). The optical density of the bands was determined by the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.7. Analysis of the quantity and structure of intestinal microbiota

At the end of the 12-week feeding experiment, the intestinal contents of channel catfish were collected and mixed into a duplicate at 4 h after the last feeding. The 16s rRNA gene sequencing technique was used to analyze the intestinal microbiota of channel catfish. The 16s V3–V4 region was amplified by using the primers 338F_806R-F (5'-ACTCCTACGGGAGG-CAGCAG-3') and 338F_806R-R (5'-GGACTACHVGGGTWTCTAAT-3'). The

16s rRNA gene sequencing was performed on the Illumina platform in Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China).

The total number of bacteria was quantified by *q*-PCR, using Universal bacteria primers (forward 5'-ACTCCTACGGGAGGAGCAGT-3', reverse 5'-ATTACCGCGGCTGCTGGC-3') [21]. DNA was extracted from each sample by using a bacterial genomic DNA extraction kit (Tiangen, Beijing, China). According to the principle of homologous recombination, the bacterial 16S rDNA was cloned into the pLB vector (Tiangen, Beijing, China) as the target gene to form a recombinant plasmid. For each standard plasmid, the copy number was calculated based on the length of the PCR product and the average quality of a DNA base pair. The results were expressed as the number of Log10 copies of bacterial 16S rDNA per milligram of intestinal contents.

2.8. Bacterial disease resistance

At the end of the 12-week feeding experiment, the channel catfish was subjected to a bacterial challenge experiment. The pathogenic *A. veronii* Hm091 and *A. hydrophila* NJ-1 were obtained from the Sino-Norway Joint Lab on Fish Gut Microbiota. Channel catfish were injected intraperitoneally with *A. veronii* Hm091 and *A. hydrophila* NJ-1. The dose for the challenge was Hm091 of 1.3×10^7 CFU per fish and NJ-1 of 8.5×10^7 CFU per fish [22]. The cumulative survival rate of groups were observed for 48 h after bacteria challenge and fish were not fed at the challenge period.

2.9. Functions of intestinal microbiota tested by germ-free zebrafish

In order to evaluate roles of the intestinal microbiota of channel catfish induced by RFM diet on immune response. Germ-free (GF) zebrafish were prepared following established protocols as described previously [23]. Intestinal contents of channel catfish were collected and resuspended in PBS. 30 GF zebrafish and 30 ml of gnotobiotic zebrafish medium (GZM) were included in each tank. Then 300 μ L suspension (10^6 CFU/ml) was added to each tank of GF zebrafish at 4 dpf. One group of GF zebrafish was not treated, and the other two groups were transplanted with intestinal microbiota of control or RFM-treated fish. 9 replicates were assigned to each treatment. After 3 days of colonization, some the larvae were sampled to evaluate the expression of immune regulatory genes, and the primers are shown in Table 2. The other larvae were challenged with mixture of *A. veronii* Hm091 and *A. hydrophila* NJ-1. The dose of Hm091 and NJ-1 were 1.5×10^7 CFU/ml in this study. The zebrafish mortality was observed for 20 h after infection.

2.10. Statistical analysis

Graph Prism 8 software (GraphPad Software Inc. San Diego, CA, USA) was used to analyze the experimental data. The results were presented as mean with standard error mean (mean $\pm$ SEM). Student's *t*-test was used to make the difference between the two groups of data. Difference was considered as significant when $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***), P between 0.05 and 0.2 was considered there was a trend of difference between groups.

Table 2

The primer sequences of immune gene of channel catfish for *q*PCR quantitative analysis.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
18sRNA	GAGAAACGGCTACCACATCC	GATACGCTCATTCCGATTACAG
HIF1 α	CCAGCAGTCCAGCAGATTACTTC	GCAGCAGGTGGAGAGGAGAGG
NF- κ B	GACGAGGTGGACGAGGGCTAC	CTGAGGCGGAAGGCATTGATGAG
IL-1 β	GGCTGTGTGTTGGGAATCT	TGATAAACCAACCGGGACA
TNF- α	AAGGAGAGTTGCCTTTACCG	ATTGCCCTGGGTCTTATGG
IL-10	TCACGTCATGAACGAGATCC	CCTCTTGCAATTCACCATATCC

3. Results

3.1. Growth performance and survival of channel catfish

After feeding experimental diets to channel catfish for 12 weeks, the results showed that fish of RFM group exhibited notably improved growth performance than the control fish. The weight gain (Fig. 1A and B, $P < 0.05$) and the condition factor (Fig. 1D, $P < 0.05$) in the RFM group were significantly higher than the control group, and the FCR in the RFM group was significantly lower than the control group (Fig. 1C, $P < 0.05$). There was not significantly different in the survival rate (Fig. 1E, $P > 0.05$) between RFM groups and control group.

3.2. Effect of RFM diet on the liver health in channel catfish

Histological changes of liver were observed, the result showed that RFM diet reduced microvesicular fat lesions and infiltration of inflammatory cell (Fig. 2). Moreover, compared with the control group, the expression of pro-inflammatory mediator NF- κ B significantly reduced in the RFM group both at the transcriptional and protein level ($P < 0.05$, Fig. 3A and B).

3.3. Effect of RFM diet on the intestinal health in channel catfish

As shown in Fig. 4, the intestinal structural integrity of channel catfish was improved and infiltration of inflammatory cells was reduced in RFM group compared with the control group. Meanwhile, compared with the control group, the intestinal expression of *HIF1 α* was significantly increased ($P < 0.05$, Fig. 5A), and the intestinal expression of NF- κ B was significantly reduced in RFM diet ($P < 0.001$, Fig. 5B).

3.4. RFM diet protected channel catfish against bacterial infection

To investigate the disease resistance of the channel catfish in control group and RFM group, channel catfish were injected intraperitoneally with *A. veronii* Hm091 and *A. hydrophila* NJ-1. At 28 h post challenge, the mortality rate of the control group was 100%, while the mortality rate of the RFM group was 66.7% ($P < 0.05$, Fig. 6). The results showed that RFM diet significantly increased the resistance of channel catfish against Hm091 and NJ-1 compared with control group.

3.5. Effect of RFM diet on intestinal microbiota of channel catfish

Compared with the control group, the total number of bacteria in the

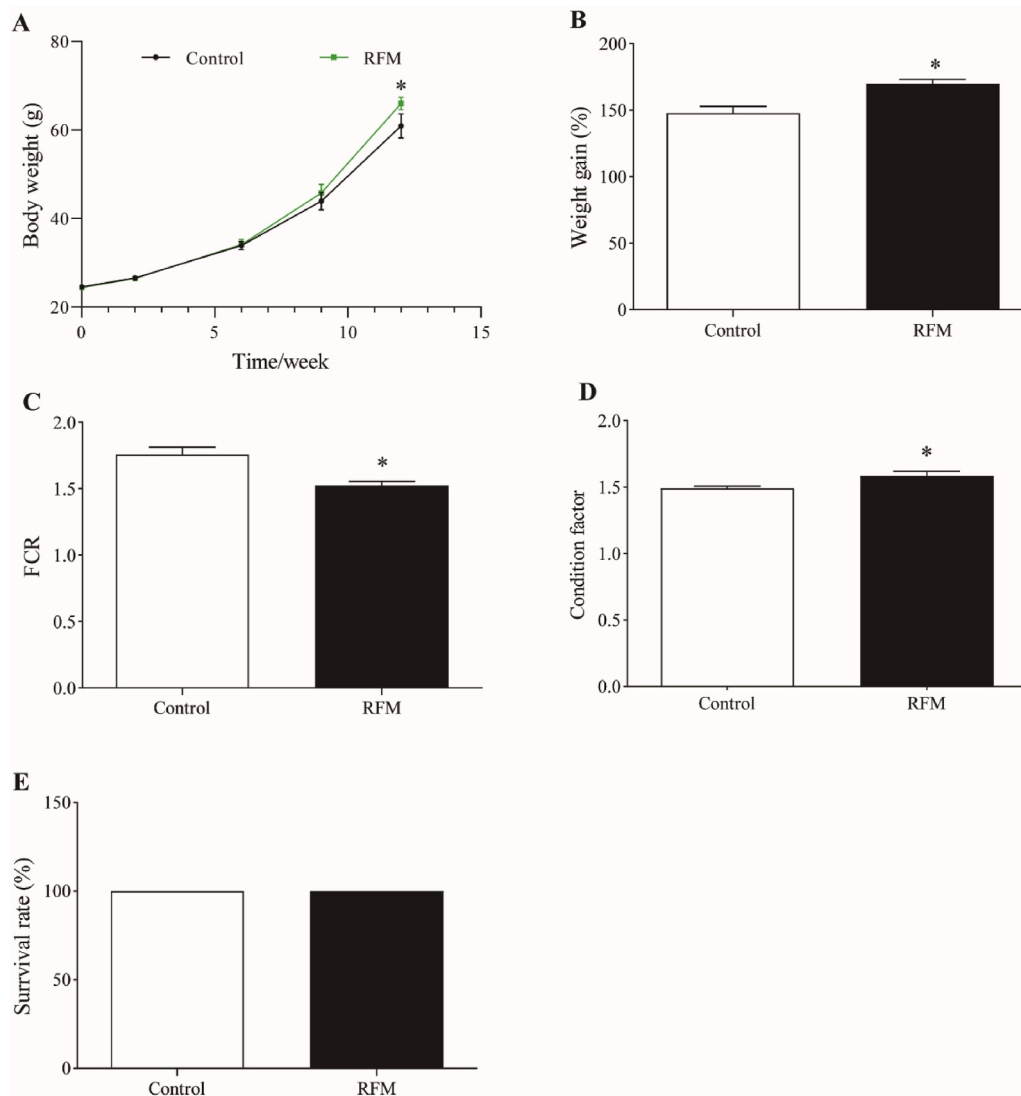


Fig. 1. Effects of RFM diet on the body weight, weight gain, FCR, survival rate and condition factor of channel catfish. (A) Body weight, (B) Weight gain (%), (C) FCR, (D) Condition factor, (E) Survival rate. Data are presented as the means $\pm$ SEM ($n = 6$). Differences are considered significant at $P < 0.05$ (*).

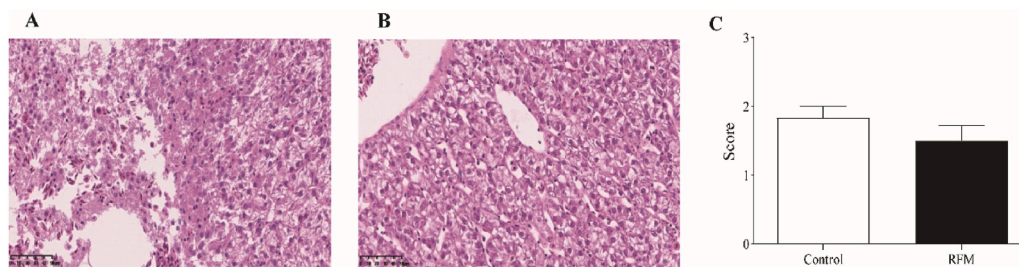


Fig. 2. Effects of RFM diet on the histology (H&E staining) of liver of channel catfish. (A) Control, (B) RFM. (C) Score of liver inflammation. Data are presented as the means $\pm$ SEM ($n = 6$).

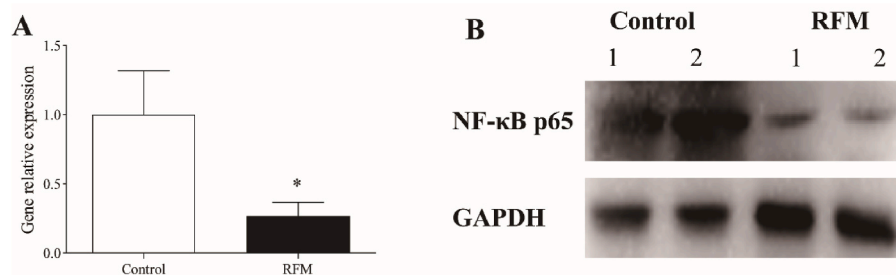


Fig. 3. Effect of RFM diet on the expression of NF- κ B gene and protein in liver of channel catfish. (A) qPCR showing the gene expression of NF- κ B in the liver, (B) Western blot showing the protein expression of NF- κ B in the liver. Data are presented as the means $\pm$ SEM ($n = 6$). Differences are considered significant at $P < 0.05$ (*).

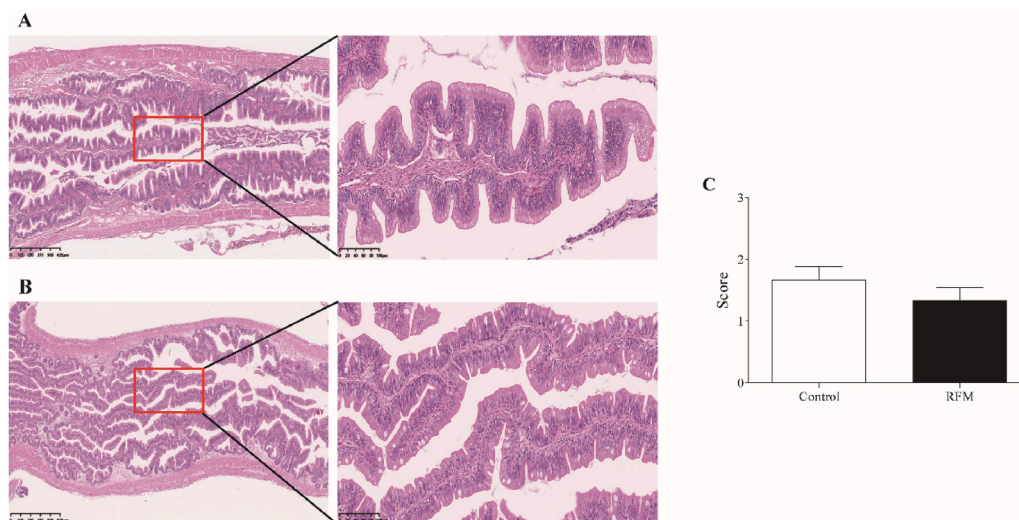


Fig. 4. Effects of RFM diet on the histology (H&E staining) of intestine of channel catfish. (A) Control, (B) RFM. (C) Score of intestinal histopathology. Data represent the means $\pm$ SEM ($n = 6$).

RFM group significantly increased (Fig. 7A). The 16s rRNA gene sequencing technique was used to analyze the intestinal microbiota of channel catfish. The results showed that Firmicutes, Proteobacteria and Bacteroidetes were the dominant phyla. Compared with control group, the relative abundance of Firmicutes had an upward trend (Fig. 7B; Table 3). The dominant genera were *Romboutsia*, *Turicibacter* and *Brvi-bacillus*, and the *Turicibacter* had an upward trend in RFM treatment ($0.05 < P < 0.2$, Fig. 7C; Table 4). The results of PcoA analysis showed that the structure of intestinal microbiota was altered by the RFM diet (Fig. 7D).

3.6. Effects of intestinal microbiota of channel catfish induced by RFM diet on the expression of genes associated with host health in the GF zebrafish model

Compared with GF zebrafish colonized microbiota from the control group, the expression of *HIF1 α* in GF zebrafish colonized microbiota from RFM group was significantly increased ($P < 0.01$, Fig. 8A), while the expression of NF- κ B was reduced ($P > 0.05$, Fig. 8B). Meanwhile, we found that the treatment group transplanted from RFM diet significantly decreased the expression of pro-inflammatory genes (*IL-1 β* , *TNF- α*) ($P < 0.01$, Fig. 8C and D) and increased the expression of anti-inflammatory genes (*IL-10*) ($P > 0.05$, Fig. 8E).

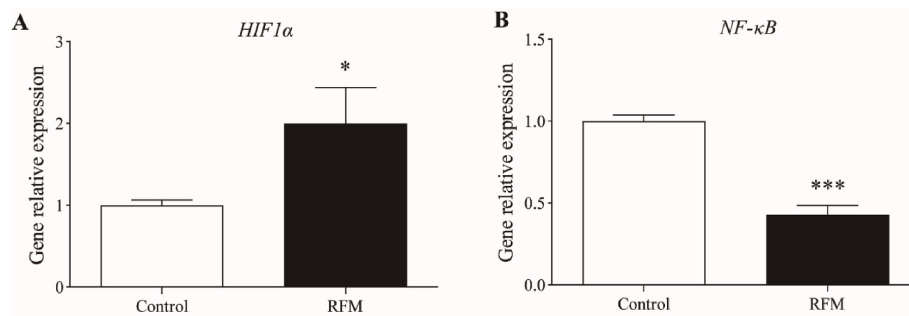


Fig. 5. Effect of RFM diet on the intestinal expression of *HIF1α* and *NF-κB* in channel catfish. (A) *HIF1α*, (B) *NF-κB*. Data are presented as the means $\pm$ SEM (n = 6). Differences are considered significant at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

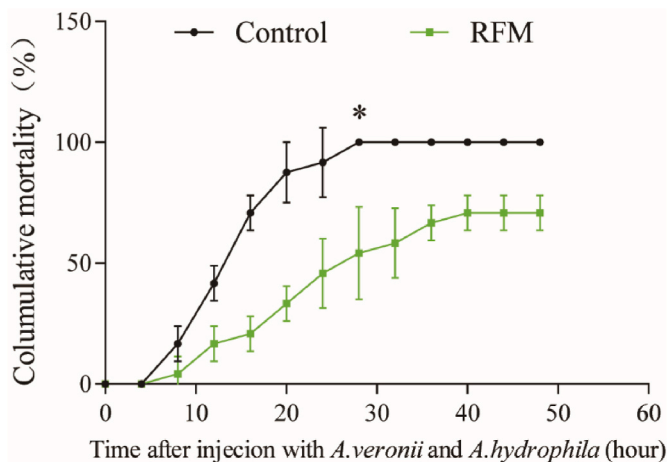


Fig. 6. Protection ability of RFM diet in channel catfish. Data are presented as the means $\pm$ SEM (n = 3). Differences are considered significant at $P < 0.05$ (*).

3.7. Effects of intestinal microbiota of channel catfish induced by RFM diet on the resistance of GF zebrafish against *Aeromonas*

To further investigate the effects of intestinal microbiota of channel catfish fed the RFM diet or the control diet, GF zebrafish colonized with either intestinal microbiota of channel catfish were challenged with mixture of *A. veronii* Hm091 and *A. hydrophila* NJ-1. Mortality was recorded in every 2 h. The results showed that compared with the control group, the treatment group colonized with intestinal microbiota of channel catfish of RFM diet significantly decreased the mortality rate against the *A. veronii* Hm091 and *A. hydrophila* NJ-1 ($P < 0.001$, Fig. 9).

4. Discussions

In recent years, the replacement of fish meal with single cell protein has become a research hotspot of animal nutritionists, and yeast is the most used single cell protein in aquatic feed. Many studies have shown that it is feasible to use feed yeast instead of fish meal in aquatic animals, but it is related to many factors, such as culture species, fish feeding habits and so on. Indexes such as growth rate and feed utilization rate are usually used as the criteria for evaluating the feeding effect [24].

The present study showed that partially replacing fish meal by yeast culture could significantly improve the growth status and feed conversion utilization rate of channel catfish, which was more suitable for the growth of channel catfish. In previous studies, when using yeast culture instead of fish meal to feed white shrimp (*Litopenaeus vannamei*), FBW and WGR increased, FCR and HSI decreased [18]. The body length, body weight and survival time of tilapia (*Oreochromis mossambicus*) larvae

were greatly improved when the larvae were fed with single cell protein diet [25]. This may be due to feed yeast can produce or release various digestive enzymes after cell lysis in the digestive tract of aquatic animals. These enzymes can effectively promote the absorption of nutrients in the digestive tract of animals, so as to improve the weight gain and reduce the feed coefficient [26,27].

Most of the previous researches on fish meal replacement in aquaculture have focused on the effects on the growth performance of fish, but gut health cannot be ignored either. The gut of animals plays a major role in nutrition digestion and absorption and in providing immune barriers against disease infection [28]. It is well known that morphological and histological assessment of the gut is crucial, which may be affected by dietary nutrient absorption, and also shows immune blockade against pathogen infection. Previous studies have shown that mannan oligosaccharides, as a component of yeast culture, can improve the intestinal tract of white shrimp and European lobster (*Homarus gammarus* L.), which is consistent with our results [29,30]. In this study, compared with the control group, RFM diet improved the intestinal structural integrity and reduced inflammatory cell infiltration. Intestinal tract is a highly hypoxic tissue. *HIF1α* contributes to maintaining hypoxic environment, intestinal barrier, nutrient absorption and immune regulation [31]. Studies have shown that *HIF1α* can upregulate claudins and mucins to enhance the integrity of the epithelial barrier, thereby promoting microbial colonization. And it can also downregulate *NF-κB* signaling and the production of anti-inflammatory cytokines, thereby reducing intestinal inflammation [32]. *NF-κB* is a first responder to harmful cellular stimuli and plays an important role in many biological processes including innate and adaptive immunity [33]. In this study, compared with the control group, the expression of *HIF1α* in the intestine of RFM group was significantly increased, and the expression of *NF-κB* in the intestine of RFM group was significantly decreased, which indicated that the positive effect of *Saccharomyces cerevisiae* culture on the expression of *HIF1α* and *NF-κB* may contribute to its impact on intestinal health.

Intestinal microbiota plays a key role in nutrition, immunity and resistance against invasive pathogens [34]. Generally speaking, the most common phyla in intestinal microbiota of fish are Firmicutes and Proteobacteria [35], which is also consistent with our research. Our study showed that the abundance of Firmicutes in the RFM group was higher than the control group. Among them, *Turicibacter* is one of the most genus of Firmicutes, and its abundance is also higher than the RFM group. The majority of bacteria in the phylum Firmicutes play an important role in the fermentation of undigested carbohydrates and amino acids into short-chain fatty acids [36]. As described earlier, *Turicibacter* could produce short-chain fatty acids, and it was positively correlated with the content of butyric acid [37], which is a functional fatty acid that inhibits enteritis and repairs the intestine, and *Turicibacter* deficiency could lead to intestinal diseases in animals [38]. Combined with the results of intestinal microbiota transplant in the GF zebrafish model, we also found that the treatment group colonized with intestinal

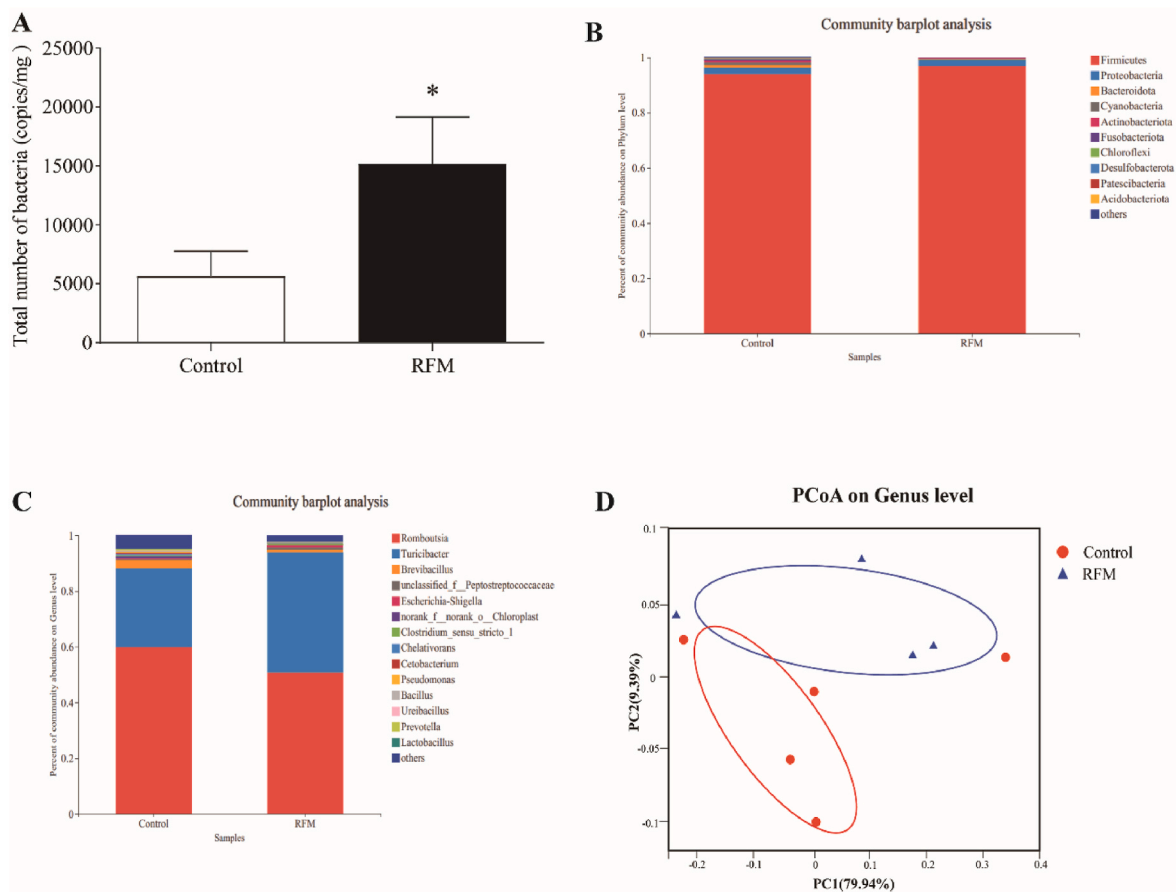


Fig. 7. Effects of RFM diet on the intestinal microbiota of channel catfish. (A) The total number of bacteria in channel catfish fed with control and RFM diet. The relative phylum (B) and genus (C) abundance of the microbiota. PCoA of the intestinal microbiota (D) of the intestinal microbiota (n = 4).

Table 3

Intestinal microbiota at main phylum level of channel catfish fed with control diet and RFM diet.

Bacteria Phylum (%)	Control	RFM	P Value
Firmicutes	93.97 ± 4.63	96.42 ± 6.49	0.24
Proteobacteria	2.51 ± 2.10	2.55 ± 4.62	0.76
Bacteroidetes	0.82 ± 0.60	0.40 ± 0.77	0.90
Cyanobacteria	0.92 ± 1.79	0.10 ± 0.17	0.84
Actinobacteria	0.64 ± 0.46	0.31 ± 0.54	0.76
Fusobacteria	0.56 ± 0.58	0.01 ± 0.01	0.98
Chloroflexi	0.16 ± 0.14	0.03 ± 0.06	0.98
Desulfobacterota	0.07 ± 0.10	0.02 ± 0.04	0.94
Acidobacteria	0.06 ± 0.06	0.02 ± 0.04	0.90
Patescibacteria	0.07 ± 0.07	0.01 ± 0.01	0.97

Table 4

Intestinal microbiota at main genus level of channel catfish fed with control diet and RFM diet.

Bacteria Genus (%)	Control	RFM	P Value
<i>Romboutsia</i>	59.83 ± 3.91	51.52 ± 12.93	0.90
<i>Turicibacter</i>	28.47 ± 11.05	41.88 ± 21.41	0.16
<i>Brevibacillus</i>	2.96 ± 4.05	0.91 ± 1.67	0.94
<i>Escherichia-Shigella</i>	0.21 ± 0.17	1.03 ± 2.04	0.90
<i>unclassified_f_Peptostreptococcaceae</i>	0.33 ± 0.62	0.77 ± 0.52	0.33
<i>norank_f_norank_o_Chloroplast</i>	0.90 ± 1.77	0.10 ± 0.17	0.72
<i>Chelativorans</i>	0.48 ± 0.62	0.26 ± 0.47	0.94
<i>Clostridium_sensu_stricto_1</i>	0.21 ± 0.20	0.48 ± 0.43	0.24
<i>Cetobacterium</i>	0.56 ± 0.58	0.01 ± 0.01	0.98
<i>Pseudomonas</i>	0.31 ± 0.26	0.16 ± 0.27	0.94

microbiota of channel catfish of RFM diet had effect to improve the immune system and disease resistance. Based on the above evidence, we speculate that the RFM diet can improve intestinal health which mainly mediated by intestinal microbiota.

The outbreak of infectious diseases is the bottleneck of the development of aquaculture. *Aeromonas* can cause motile aeromonad septicemia, which can lead to a large number of deaths of intensive high-density cultured freshwater fish and cause serious economic losses to farmers [22,39]. In aquatic animals, the survival rate after bacterial attack is usually used to determine the immune response to disease [40]. The non-specific immune system of channel catfish plays an important role in protecting it from bacterial infection. In this study, the survival rate of channel catfish fed with RFM was increased after challenged with a mixture of *A. veronii* Hm091 and *A. hydrophila* NJ-1, which was consistent with the results of shrimp fed with 2% yeast culture [18]. The increased resistance to *Vibrio harveyi* indicated that yeast culture could stimulate and prolong the immune response of rockfish to pathogenic infection. A large number of these chemicals, such as β -Glucan, chitin and mannan oligosaccharide showed immune stimulation after pathogen attack [41,42], so as to improve survival rate. Therefore, the effect of yeast culture on bacterial resistance indicated that feeding yeast culture could effectively improve the resistance of channel catfish to *Aeromonas* infection.

5. Conclusion

In conclusion, yeast culture can be used as a substitute for fish meal in aquafeed of channel catfish. In this study, the results showed that partially replacing dietary fish meal by *Saccharomyces cerevisiae* culture

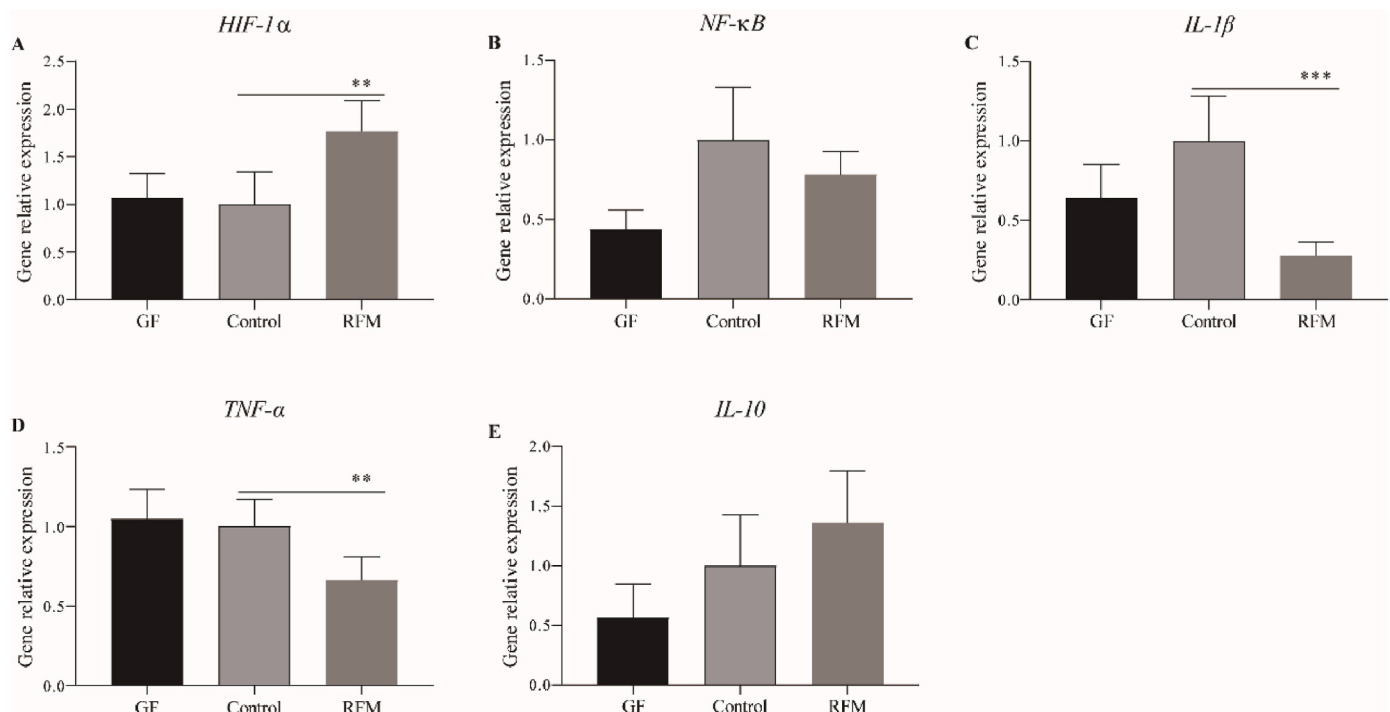


Fig. 8. The effect of RFM induced intestinal microbiota of channel catfish on genes associated with host health in the GF zebrafish. (A) *HIF1α*, (B) *NF-κB*, (C) *IL-1β*, (D) *TNF-α*, (E) *IL-10*. Data are presented as the means $\pm$ SEM (n = 6). Differences are considered significant at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

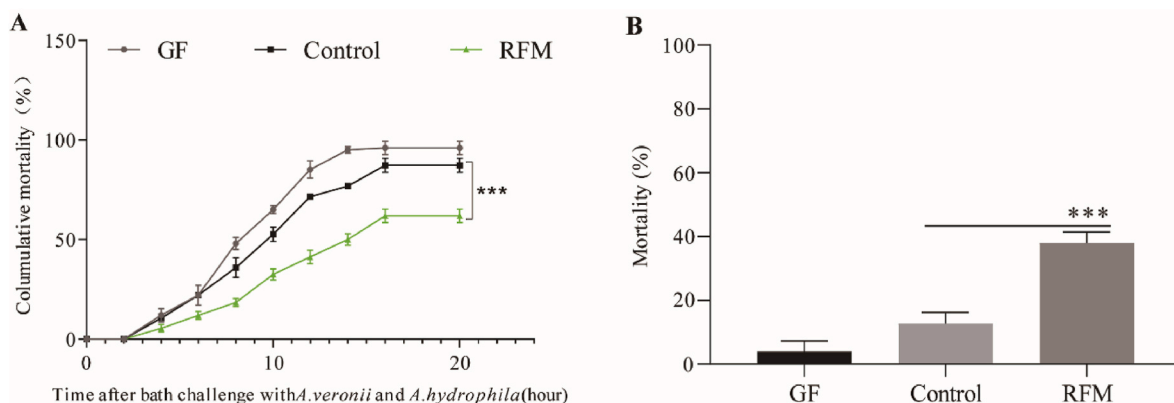


Fig. 9. Effects of RFM induced intestinal microbiota of channel catfish on cumulative mortality in the GF zebrafish against pathogenic *Aeromonas*. Data are presented as the means $\pm$ SEM (n = 3). Differences are considered significant at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

improve the growth performance and feed utilization rate of channel catfish, effectively improve the immune function of channel catfish, maintain intestinal health and improve the ability of channel catfish to resist *Aeromonas* infection, and further showed the intestinal microbiota induced by the *Saccharomyces cerevisiae* culture played a critical role in probiotic effects of *Saccharomyces cerevisiae* by using the germ-free zebrafish model.

CRediT authorship contribution statement

Qiang Hao: Formal analysis, Writing – original draft, Writing – review & editing, conducted the experiments and biochemical analyses, analyzed the data, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Rui Xia:** Formal analysis, Writing – original draft, Writing – review & editing, conducted the experiments and biochemical analyses, analyzed the data, All authors participated in writing draft, reviewing

and editing the manuscript. All authors read and approved the final manuscript. **Qingshuang Zhang:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Yadong Xie:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Chao Ran:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Yalin Yang:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Wenhao Zhou:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology, All authors participated in writing

draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Fuwei Chu:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Xiao-mei Zhang:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Yu Wang:** Methodology, Writing – original draft, Writing – review & editing, developed the methodology. All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Zhen Zhang:** Resources, Formal analysis, Writing – original draft, Writing – review & editing, designed study and provided the laboratory resources, analyzed the data, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript. **Zhigang Zhou:** Resources, Writing – original draft, Writing – review & editing, designed study and provided the laboratory resources, All authors participated in writing draft, reviewing and editing the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

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

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Appendix A. Supplementary data

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