



Effects of dietary *Saccharomyces cerevisiae* on growth, intestinal and liver health, intestinal microbiota and disease resistance of channel catfish (*Ictalurus punctatus*)

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ABSTRACT

Probiotics are an eco-friendly alternative to antibiotics and play an important role in ensuring the sustainability of aquaculture. The aim of the present study was to investigate the effects of *Saccharomyces cerevisiae* on the growth, intestinal and liver health, intestinal microbiota, and disease resistance of channel catfish (*Ictalurus punctatus*). Channel catfish were randomly allotted into two treatments, control group (basal diet) and SCC group (basal diet with 2% *Saccharomyces cerevisiae* culture), and fed for 12 weeks. The results showed that the weight gain of SCC group was significantly increased ($P < 0.05$) and FCR was significantly decreased ($P < 0.05$) when compared with the control group. SCC diet significantly improved the intestinal structural integrity of channel catfish and increased the number of intestinal goblet cell. The expression of intestinal gene *HIF1α* was increased in SCC group, 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, while Proteobacteria had a tendency to decrease ($0.20 > P > 0.05$). In addition, the survival rate of channel catfish was significantly increased in SCC group after being challenged with mixture of *A. veronii* HM091 and *A. hydrophila* NJ-1. In conclusion, the supplementation of *Saccharomyces cerevisiae* culture improved the growth, intestinal and liver health and disease resistance of channel catfish.

1. Introduction

China has the world's largest aquaculture industry, accounting for more than 60% of global production. In 2018, the national fish production has reached about 179 million tons (FAO. The State of World Fisheries and Aquaculture 2020). However, at the same time, disease problems have become increasingly prominent under the intensive and industrialized farming systems (Gui, Tang, Li, Liu, and De Silva, 2018), and bacterial diseases accounted for the highest proportion, leading to the overuse of antibiotics. Improper use of antibiotics will destroy intestinal microbiota in the aquaculture environment, and threaten food safety and human health (Chen et al., 2020). People pay more attentions to restrict the uses of antibiotics in aquaculture, and probiotics may be

one of the most promising approaches (Castex, Daniels, and Chim, 2014). Probiotics have many positive impacts, such as activating the host's immunity, inhibiting the growth of pathogenic microorganisms, balancing the host's intestinal microbiota and improving the host's metabolism (Hoseinifar, Sun, Wang, and Zhou, 2018; Subuntith Nimrat, Suksawat, Boonthai, and Vuthiphandchai, 2012).

Nowadays, yeast has been widely used as probiotics in aquaculture. Yeast products could reduce the number of harmful bacteria in the intestine of fish, and promote the reproduction of beneficial bacteria. In addition, yeast products could improve intestinal function and maintain intestinal integrity, thereby improving growth performance and immunity of fish (Islam, Rohani, and Shahjahan, 2021; Jahan, Islam, Rohani, Hossain, and Shahjahan, 2021; Rohani et al., 2021). Yeast culture is a

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complex yeast fermentation product, including yeast, its metabolites, and culture medium (Barnes, Durben, Reeves, and Sanders, 2006; Sanderson and Jolly, 1994). The first generation of yeast culture is DVAQUA® of Diamond V Mills Inc., IA. It is yeast culture of *Saccharomyces cerevisiae*, and contains "unknown growth factors", which could adjust the microecological balance of the intestinal tract of aquatic animals, so as to improve the production performance of animals (Barnes et al., 2006; Sanderson and Jolly, 1994). The experiments of hybrid tilapia (*Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂) showed that DVAQUA® could affected the autochthonous gut bacteria community and improved non-specific immunity (He et al., 2009, 2011a; Zhou et al., 2009, 2011). The second generation of yeast culture is Saccharoculture of Veterinary Pharmaceuticals, Korea. It not only contains yeast culture of *Saccharomyces cerevisiae*, but also has *Bacillus amyloliquefaciens*, with a live bacterial content of more than 4 billion/g (He et al., 2011b). Saccharoculture is also rich in a large number of β-Glucan, amino acids, antimicrobial peptide and other metabolites. The experiments of crucian carp (*Carassius auratus*) and juvenile common carp (*Cyprinus carpio*) both showed that Saccharoculture could improve weight gain, special growth rate and survival rate after *A. hydrophila* infection of fish (He et al., 2011b; Huang et al., 2015).

Studies have shown that nucleotides and short-chain fatty acids have a very positive significance for improving disease resistance of the host. Nucleotides, as essential substances for cell synthesis, have physiological functions such as enhancing growth, immune responses, and disease resistance in various fish species (Guo et al., 2017, 2019; Ran et al., 2021; Xu et al., 2015). Short-chain fatty acids could significantly promote the weight gain and daily feeding rate of fish, improve intestinal digestive enzyme activities, energy metabolism, and inhibit the reproduction of harmful bacteria (Liu et al., 2014; Wang et al., 2021; Zhang et al., 2020). Because short-chain fatty acids (especially acetic acid, Guo et al., 2017, 2019; Ran et al., 2021) and nucleotides (especially AMP, Wang et al., 2021; Zhang et al., 2020) have these prominent probiotic effects, the third generation of yeast culture was developed in China, which is characterized by high acetic acid and high AMP.

Channel catfish (*Ictalurus punctatus*) is an important freshwater aquaculture fish. Due to its wide adaptability, miscellaneous food habits, strong disease resistance, fast growth, high yield, and tender meat, the scale of aquaculture has expanded rapidly and the degree of intensification has been continuously improved. Channel catfish was used as the research subjects in this study, and we evaluated the effects of *Saccharomyces cerevisiae* culture (ESTAQUA®) on the growth, intestinal and hepatic health, intestinal microbiota and disease resistance of channel catfish.

2. Material and methods

2.1. Bacteria and culture conditions

Aeromonas veronii HM091 was preserved in China General Microbiological Culture Collection Center with its preservation number CGMCC no.22536. *Aeromonas hydrophila* NJ-1 was a gift from Dr. Yongjie Liu from Nanjing Agricultural University (Nanjing, China). *Saccharomyces cerevisiae* Sa-10 was preserved in China General Microbiological Culture Collection Center with its preservation number CGMCC no.6120. *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. The fermented product was enriched with nucleotides and acetic acid by adding the processes of liquid low-temperature anaerobic fermentation and solid-state enzymatic aerobic fermentation, 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 cell wall of *Saccharomyces cerevisiae* Sa-10 was destroyed by high temperature, which allowed its contents to exert their potential functional roles.

Table 1

Feed formulation and chemical composition of diets for channel catfish.

Ingredient	g/kg (air dry basis)	
	Control	SCC
Rice bran	100.00	100.00
Flour	150.00	150.00
Soybean meal	250.00	230.00
Rapeseed meal	150.00	144.00
Fish meal	100.00	100.00
Chicken meal	100.00	100.00
DDGS	50.00	50.00
SCC Sa-10 ^a	0.00	20.00
Bentonite	15.00	20.85
Lys-HCl ^b	2.00	2.40
Methionine ^b	0.50	0.55
Choline Chloride (50%)	2.00	2.00
Ca(H ₂ PO ₄) ₂	20.00	20.00
Soybean oil	40.00	39.70
VC phosphate	0.50	0.50
Premix ^c	20.00	20.00
Total	1000	1000
Chemical composition (g/kg)		
Crude protein	380.4	380.5
Crude fat	112.9	114.1
Crude ash	102.2	107.7
Moisture	54.3	54.8

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

^b The additive quantities of Lys-HCl and methionine in the two feeds were adjusted according to the differences between SCC and SBM (China Feed-database Information Network Centre).

^c 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): CoCl₂·6 H₂O, 0.074; CuSO₄·5 H₂O, 2.5; FeSO₄·7 H₂O, 73.2; NaCl, 40.0; MgSO₄·7 H₂O, 284.0; MnSO₄·H₂O, 6.50; KI, 0.68; Na₂SeO₃, 0.10; ZnSO₄·7 H₂O, 131.93; Cellulose, 501.09.

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. 2018-AF-FRI-CAAS-001). The experiment was conducted in the International Agricultural High-tech Industrial Park of the Chinese Academy of Agricultural Sciences, Langfang, Hebei, China. Channel catfish was purchased from a fish farm in Hebei and domesticated in a circulating water system 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 with 6 repetitive per group at a density of 12 fish per 90 L tank. The basal diet was formulated with fish meal, soybean meal, rapeseed meal as the main protein source, and soybean oil as the main fat source. Based on previous findings, supplementation of 2% yeast culture could improve the growth performance, immunity and disease resistance of juvenile largemouth bass, hybrid grouper and shrimp (Ayiku et al., 2020; Feng et al., 2021; Wang et al., 2022), and could relieve liver damage and inflammatory response induced by CCL₄ (Tao et al., 2021), so in this study the experimental diet was prepared by 20 g/kg *Saccharomyces cerevisiae* culture (SCC, ESTAQUA®) to replace the same amount of soybean meal (SBM) in the basic diet. The feed formulation and approximate analysis were shown in Table 1.

During the trial period, channel catfish were fed to apparent satiation by hand three times (7:00, 12:00 and 18:00) daily for 12 weeks. The fish 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. 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

To evaluate the effect of SCC on performance, the fish were weighed at 2 week, 6 week, 9 week, and 12 week. Weight gain (WG, %) = $100\% \times [(final\ body\ weight\ (g) - initial\ body\ weight\ (g))/initial\ body\ weight\ (g)]$, feed conversion ratio (FCR) = food intake (g)/weight gain of fish (g), survival rate (SR, %) = (number of fish at the end of the experiment/number of fish at the start of the experiment) $\times 100\%$, and condition factor = $100 \times body\ weight\ (g)/[body\ length]^3$.

2.4. Histological observation of intestine and liver

After 24 h fasting, the channel catfish was anesthetized with 3-aminophenolic acid ester methanesulfonate. 1 fish was randomly selected from each of the 6 replication tanks for liver and hindgut histological analysis (n = 6). The liver and hindgut of channel catfish were collected, 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 images were analyzed with K-Viewer image analysis software.

2.5. Relative quantification expressions of intestine and liver health related genes

The liver and hindgut tissues of channel catfish were taken for RNA extraction (n = 6), and the total RNA was extracted using the TRIzol Reagent extraction method (Invitrogen, Carlsbad, CA, USA). 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 the cDNA was used as a template for Quantitative real-time PCR reaction (TianGen, Beijing, China). The primers were shown in Table 2.

2.6. Western blot analysis of NF- κ B-p65

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 same amounts of total protein were loaded in 12% SDS-PAGE for electrophoresis, and then transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). After the PVDF membrane was blocked non-specific binding with TBST containing 5% skimmed milk powder, and incubated with primary antibodies, i.e., antibodies against GAPDH (1:3000, SAB2701826, Sigma-Aldrich), NF- κ B-p65 (1:1000, LS-C31758, LifeSpan BioSciences, Inc.). 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) and the ChemiDoc XRS+ System (BIO-

RAD). The optical density of the bands was determined by the ImageJ software (National Institutes of Health, Bethesda, MD, USA). Table 3.

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 at 4–6 h after the last feeding. 12 fish from each group were randomly selected for intestinal contents sampling, and intestinal content samples from 3 fish were mixed into a replicate sample (n = 4). The 16 s rRNA gene sequencing technique was used to analyze the intestinal microbiota of channel catfish. According to the manufacturer's instructions, DNA was extracted from each sample using a bacterial genomic DNA extraction kit (Tiangen, Beijing, China).

For the DNA copy of the total bacteria, the obtained DNA was used as a template to amplify 16 S rRNA, using Universal bacteria primers (forward 5'-ACTCCTACGGGAGGCAGAGT-3', reverse 5'-ATTACCGCGGCTGCTGGC-3') with specific barcodes for each samples (Zhang et al., 2019).

2.8. Bacterial disease resistance

In order to evaluate the effect of supplementary SCC on the disease resistance of channel catfish, the fish were challenged with mixture of highly pathogenic *A. veronii* HM091 and *A. hydrophila* NJ-1. *A. veronii* HM091 and *A. hydrophila* NJ-1 were placed in a 250 ml flask containing LB culture medium and cultured in an incubator at 37 °C and 180 r/min for 20 h. The concentration of Hm091 and NJ-1 were 6.5×10^7 CFU/ml and 4.25×10^8 CFU/ml before challenge test, respectively. After 12 weeks of feeding trial, each fish was injected with pathogenic bacteria. The size of the challenge tank was 50 $\times$ 50 $\times$ 40 cm, the final challenge concentration of Hm091 was 2.1×10^5 CFU/g fish body weight, and the final challenge concentration of NJ-1 was 1.3×10^6 CFU/g fish body weight. There were two groups: group A was the control group; group B was SCC group, each treatment group had 3 replicates, and each replicate had 8 fish. The survival rates of the two groups were calculated and fish were not fed in the challenge period.

2.9. 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 $\pm$ SEM. Paired t-tests were used to make the difference between the two groups of data. Difference was considered as significant when $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***)

Table 3

Growth performance and morphological parameters of channel catfish fed experimental diets at 12 weeks.

	Control	SCC
Initial weight (g/fish)	24.57 $\pm$ 0.11	24.48 $\pm$ 0.12
Final weight (g/fish)	60.92 $\pm$ 2.68	65.09 $\pm$ 2.18 *
WG (%)	147.95 $\pm$ 10.06	165.91 $\pm$ 8.84 *
FCR	1.76 $\pm$ 0.11	1.56 $\pm$ 0.09 *
SR (%)	100 $\pm$ 0	100 $\pm$ 0
CF (g/cm3)	1.49 $\pm$ 0.05	1.54 $\pm$ 0.07

Table 2

The primer sequences of immune gene of channel catfish for qPCR quantitative analysis.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
18sRNA	GAGAAACGGCTACCATCC	GATACGCTCATTCCGATTACAG
HIF1 α	CCAGCAGTCAGCAGATTACTTCC	GCAGCAGGTGGAGAGGAGAGG
NF- κ B	GACGAGGTGGACGAGGGCTAC	CTGAGGCGGAAGGCATTGATGAG

3. Results

3.1. Growth performance and survival of channel catfish

During the experimental period, no mortality of channel catfish in each treatment was observed. At 12 weeks, the weight gain of fish in the SCC group was significantly higher than the control group ($P < 0.05$, Fig. 1A, B; Table 1), and the FCR was significantly lower than the control group ($P < 0.05$, Fig. 1C; Table 1). There was not significantly different in condition factor between SCC group and control group ($P > 0.05$, Fig. 1E; Table 1).

3.2. Effects of SCC diet on the intestinal health of channel catfish

The effects of SCC diet on histological changes of intestine were investigated. As shown in Fig. 2, the intestinal structural integrity of channel catfish, as scored by comparing intestinal structural integrity and intestinal cells, was improved in SCC group compared with the control. Intestinal epithelial cells were more organized, and goblet cells were significantly increased (Fig. 2). Moreover, the genes expression related to intestinal health was detected. Compared with the control group, the expression of the hypoxia inducible factor (*HIF1 α*) in the intestine fed with SCC diet had a rising trend ($0.2 > P > 0.05$, Fig. 3A). The gene expression of pro-inflammatory mediator *NF- κ B* was

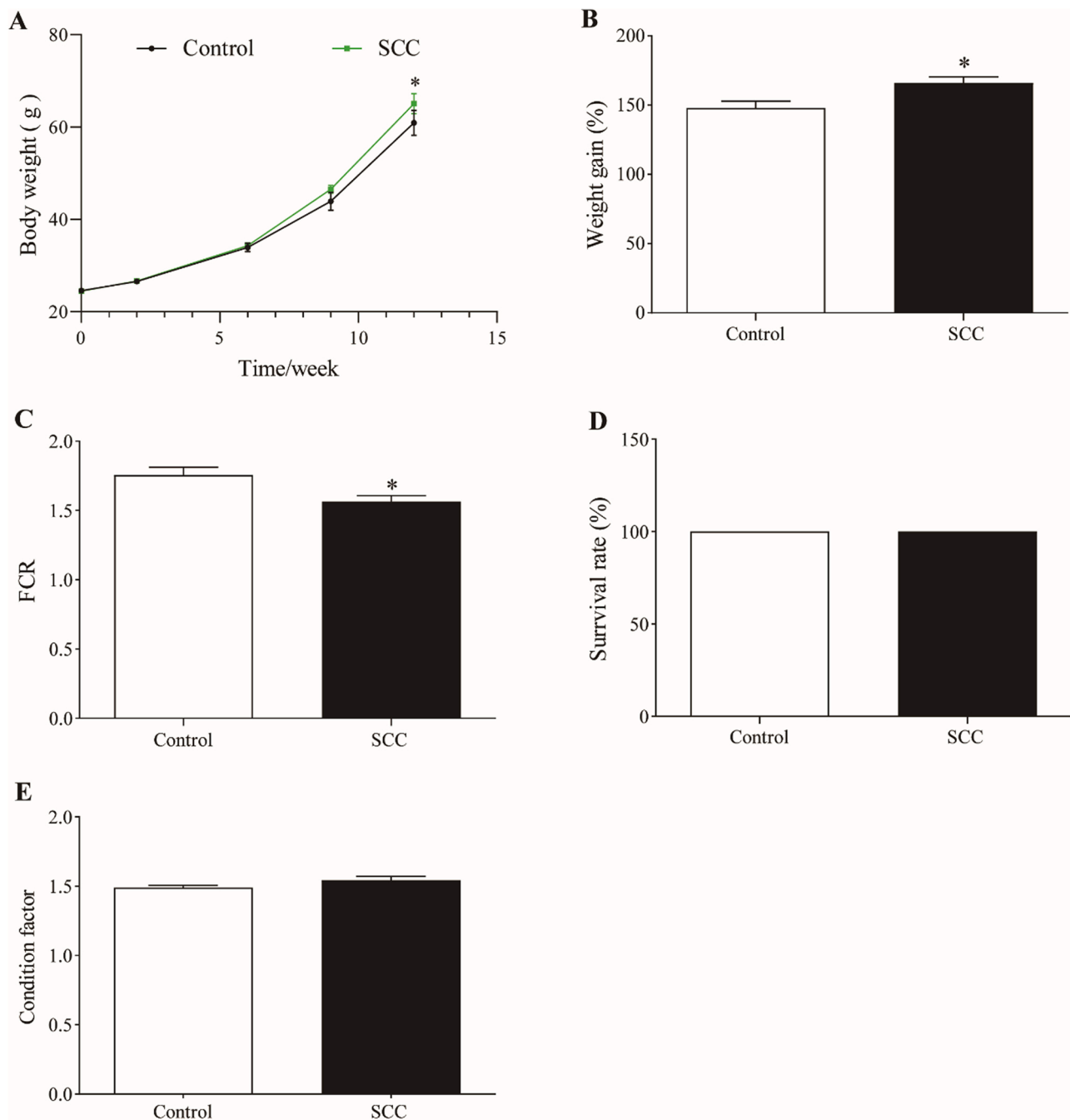


Fig. 1. Effects of SCC diet on the body weight, weight gain, FCR, survival rate and condition factor of channel catfish. (A) Body weight, (B) Weight gain (%), (C) FCR, (D) Survival rate, (E) Condition factor. Data are presented as the means ($\pm$ SEM) ($n = 6$). Differences are considered significant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

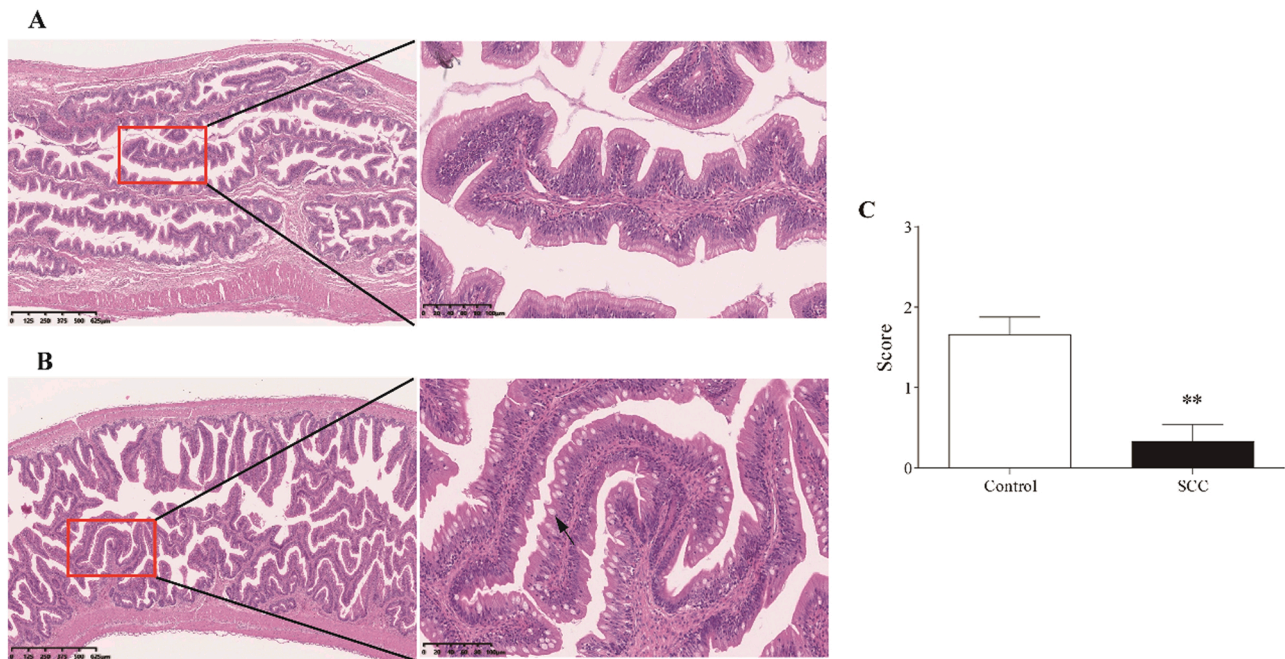


Fig. 2. Effects of SCC diet on the histology (H&E staining) of intestine of channel catfish. (A) Control, (B) SCC. (C) Score of intestinal histopathology (Evaluation parameters: abnormal lamina propria, loosening of enterocytes from basal membrane, edema and number of goblet cell). Data represent the means (± SEM) (n = 6). Differences are considered significant at $P < 0.05$ (*) and $P < 0.01$ (**).

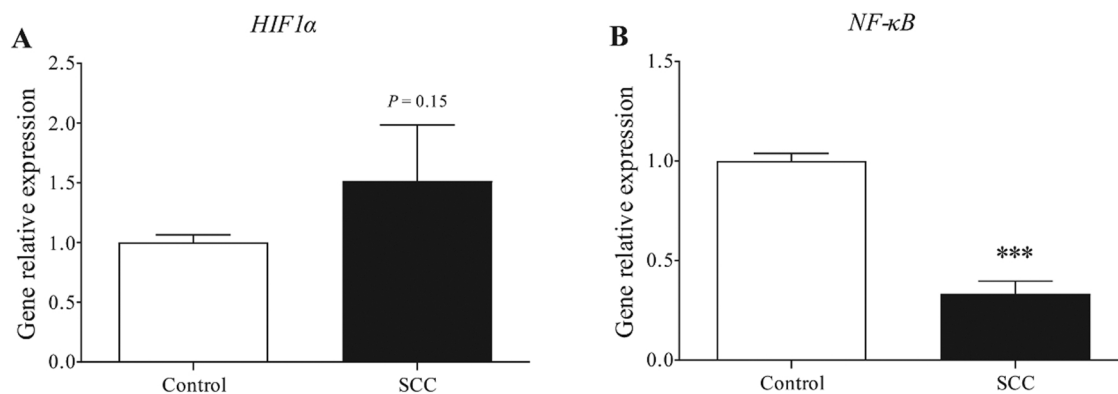


Fig. 3. Effect of SCC diet on the intestinal expression of *HIF1α* and *NF-κB* in channel catfish. (A) *HIF1α*, (B) *NF-κB*. Data are presented as the means (± SEM) (n = 6). Black arrows indicated goblet cell. Differences are considered significant at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

significantly decreased in SCC group ($P < 0.05$, Fig. 3B).

3.3. The effect of SCC diet on intestinal microbiota of channel catfish

The total amount of gut bacteria was detected after 12 weeks of feeding. As shown in Fig. 4A, there was no significant difference in the total number of bacteria between control group and SCC group. At the phylum level (Fig. 4B; Table 4), Firmicutes is dominant. Compared with the control group, the relative abundance of Firmicutes had an upward trend in SCC group ($0.2 > P > 0.05$). At the genus level (Fig. 4C; Table 5), the dominant bacteria were *Romboutsia*, *Turicibacter* and *Brvibacillus*. *Romboutsia* had a downward trend; *Turicibacter* had an upward trend ($0.2 > P > 0.05$) and *Brvibacillus* was significantly decreased ($P < 0.05$) after feeding the SCC diet. The results of PcoA analysis showed that the intestinal microbiotas of channel catfish fed the two diet was completely separated at the level of both phylum and genus (Fig. 4D, E).

3.4. Effects of SCC diet on the liver health of channel catfish

Compared with the control group, SCC diet significantly reduced infiltration of inflammatory cells and the formation of necrosis (Fig. 5). Compared with the control group, the expression of pro-inflammatory mediator *NF-κB* significantly decreased in SCC group ($P < 0.05$, Fig. 6A). Consistently, western blot also showed that SCC diet significantly decreased the expression of *NF-κB* at protein level (Fig. 6B).

3.5. SCC diet protected channel catfish against bacterial infection

The disease resistance of the channel catfish in control group and SCC group was evaluated by intraperitoneal injection of *A. veronii* Hm091 and *A. hydrophila* NJ-1. At 28 h post challenge, all fish of the control group had died, while the mortality of the SCC group at 28 h was 62.5%. At 48 h, the mortality of SCC group was 66.6%. The survival rate of channel catfish against *A. veronii* Hm091 and *A. hydrophila* NJ-1 was significantly improved in SCC group ($P < 0.05$, Fig. 7).

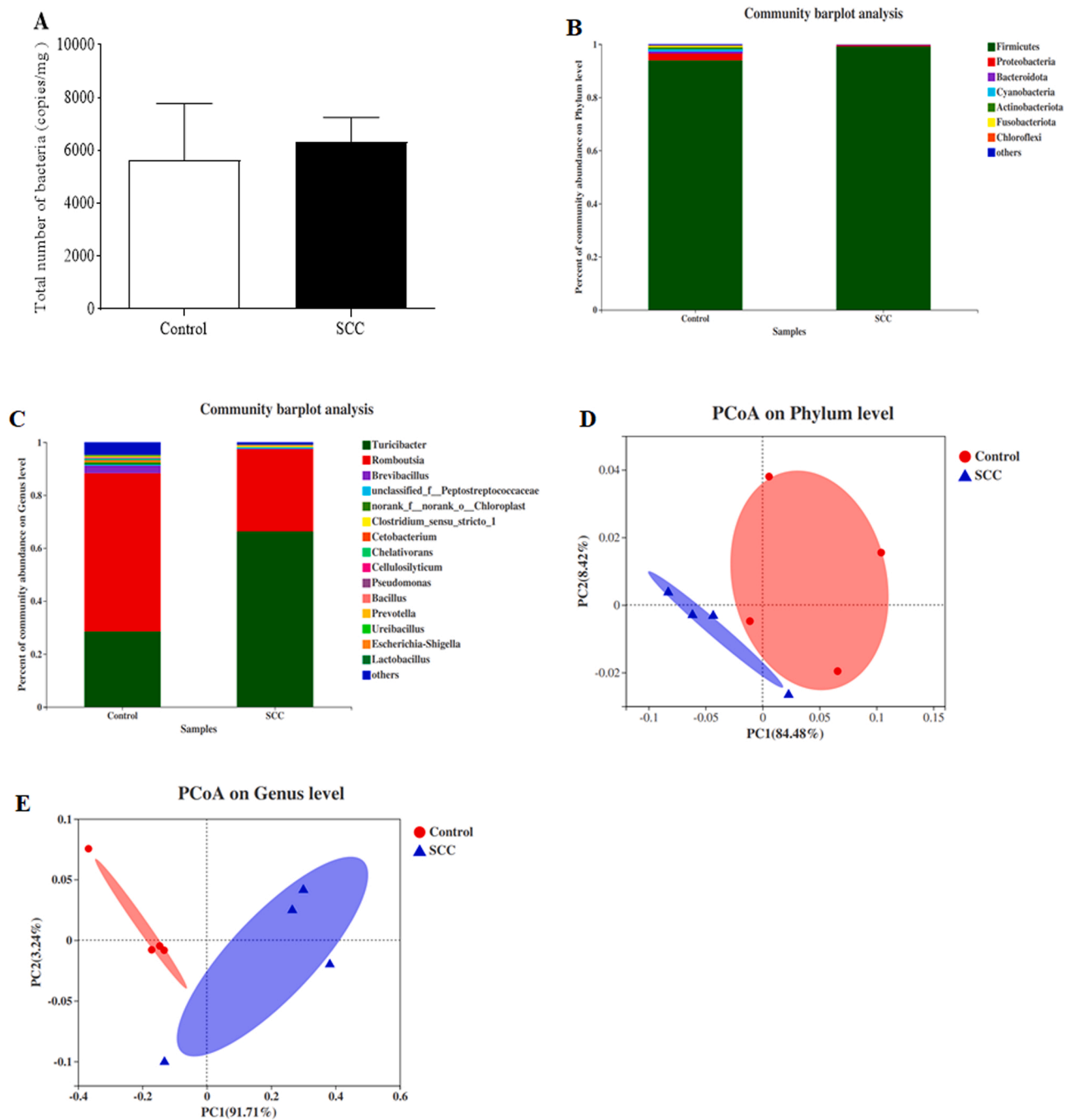


Fig. 4. Effects of SCC diet on the intestinal microbiota of channel catfish. (A) The total number of bacteria in channel catfish fed with control diet and SCC diet. The relative phylum (B) and genus (C) abundance of the microbiota of channel catfish fed with control diet and SCC diet. PCoA of the intestinal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

Table 4

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

Bacteria Phylum (%)	Control	SCC	P Value
Firmicutes	93.97 ± 4.63	99.26 ± 0.50	0.11
Proteobacteria	2.51 ± 2.10	0.23 ± 0.19	0.06
Bacteroidota	0.82 ± 0.60	0.34 ± 0.44	0.31
Cyanobacteria	0.92 ± 1.79	0.01 ± 0.01	0.31
Actinobacteriota	0.64 ± 0.50	0.05 ± 0.05	0.11
Fusobacteriota	0.56 ± 0.58	0.07 ± 0.09	0.19
Chloroflexi	0.16 ± 0.14	0.01 ± 0.01	0.06

4. Discussions

Yeast culture is a kind of microecological product which has good palatability and growth promoting effect. A large number of experiments have studied the mechanism of yeast culture improving animal production performance. It is generally believed that yeast culture can improve the gastrointestinal environment, accelerate the metabolism of microorganisms, adjust the structure of bacteria in animals (Ernesto Ceseña, Vega-Villasante, Aguirre-Guzman, Luna-Gonzalez, and Campa-Cordova, 2021; Licon-Jain et al., 2020; Nimrat et al., 2021). Studies have reported that adding appropriate amount of yeast culture to aquatic feed can significantly improve the specific growth rate and

Table 5

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

Bacteria Genus (%)	Control	SCC	P Value
<i>Turicibacter</i>	28.47 ± 11.05	67.67 ± 23.52	0.11
<i>Romboutsia</i>	59.83 ± 3.91	29.73 ± 23.67	0.31
<i>Brevibacillus</i>	2.96 ± 4.05	0.08 ± 0.10	0.03
<i>unclassified_f_Peptostreptococcaceae</i>	0.33 ± 0.62	0.65 ± 0.38	0.31
<i>norank_f_norank_o_Chloroplast</i>	0.90 ± 1.77	0.01 ± 0.01	0.56
<i>Clostridium_sensu_stricto_1</i>	0.21 ± 0.20	0.46 ± 0.14	0.19
<i>Cetobacterium</i>	0.56 ± 0.58	0.04 ± 0.04	0.19
<i>Chelativorans</i>	0.48 ± 0.62	0.01 ± 0.01	0.03
<i>Cellulosilyticum</i>	0.05 ± 0.03	0.34 ± 0.31	0.06
<i>Pseudomonas</i>	0.31 ± 0.26	0.03 ± 0.03	0.06

weight gain of white shrimp (Deng et al., 2013), Nile tilapia (*Oreochromis niloticus*) (Abdel-Tawwab, Abdel-Rahman, and Ismael, 2008; Islam et al., 2021), juvenile beluga (*Huso huso*) (Hoseinifar, Mirvaghefi, and Merrifield, 2011), rainbow trout (*Onchorhynchus mykiss*) (L. Wang et al., 2018) and rohu (*Labeo rohita*) (Jahan et al., 2021). In this experiment, similar effects were also observed. The results showed that adding an appropriate amount of yeast culture improved the growth performance and feed utilization of channel catfish. This may be attributed to the valuable nutrients of yeast culture, such as proteins, carbohydrates and nucleic acids, which play major nutritional roles in promoting fish growth (Ernesto Ceseña et al., 2021; Licón-Jain et al., 2020; S Nimrat et al., 2021).

The liver and intestines are the most important organs for absorbing nutrients and maintaining normal function (Fang et al., 2019; Vizcaino et al., 2014). Liver is an important metabolic organ that can regulate various physiochemical functions including synthesis, secretion and metabolism of xenobiotics (Tao et al., 2021). Damage to the liver could lead to these physiochemical function disorder. NF- κ B is a ubiquitous transcription factor that plays an important roles in many biological processes including innate and adaptive immunity (W. Wang et al., 2019). In this study, compared with the control group, the expression of NF- κ B in the liver of SCC group was significantly decreased, which indicated that the effect of *Saccharomyces cerevisiae* culture on the expression of NF- κ B may contribute to its impact on hepatic health. The intestinal mucosa provides a physiologic and immunologic barrier to a wide range of microorganisms and foreign substances. The overlying mucus-gel layer is the first line of defense. Formation of the mucus gel is through goblet secretion of polymeric mucin glycoprotein and act as a medium for protection, lubrication, and transport between the luminal contents and the epithelial lining. In this present study, it was observed that the supplementation of SCC could increase goblet cell number of intestines, which is consistent with the results of previous (Islam et al., 2021). Previous studies showed that mannan oligosaccharides as a component of yeast (*Saccharomyces cerevisiae*) have the capacity to increase villus length of white shrimp and rohu (Jahan et al., 2021; Yuan

et al., 2019), and the addition of yeast polysaccharides increased the number of intestinal goblet cell (Zhu et al., 2012). Therefore, it can be speculated that prebiotic increased goblet cell number, resulting in improving intestinal defense. Tissue damage is usually accompanied with up-regulation of the expression of nuclear factor NF- κ B (De Santis, Bartie, Olsen, Taggart, and Tocher, 2015). HIF1 α is a transcription factor that can regulate the expression of the tight junctions and antimicrobial peptide-related genes of the intestine (Zhang et al., 2019). In this study, the supplementation of SCC significantly up-regulated the expression of HIF-1 α and decrease the expression of NF- κ B. These results were also consistent with the results of histological changes of intestine.

Intestinal mucosa is the first barrier for pathogen invasion, and intestinal bacteria is beneficial to maintain the barrier function of intestinal mucosal system (Tlaskalová-Hogenová et al., 2004). Beneficial bacteria in intestine can regulate the balance of immune response,

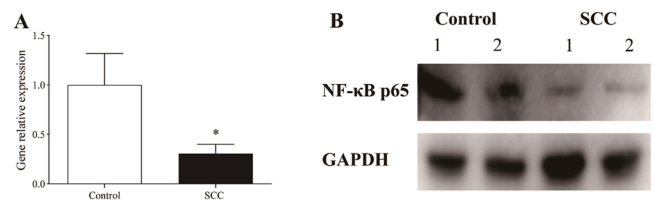


Fig. 6. Effect of SCC diet on the liver of expression of NF- κ B gene and protein in channel catfish. (A) qPCR showing the gene expression of NF- κ B in the liver, (B) Western blot showing the protein expression of NF- κ B in the liver. Data are presented as the means ($\pm$ SEM) ($n = 6$). Differences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

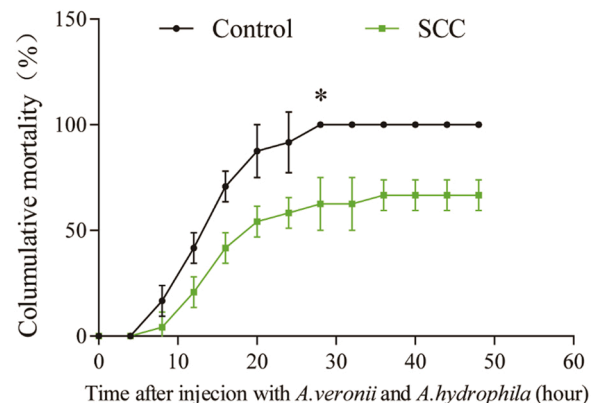


Fig. 7. Protection ability of SCC diet in channel catfish against *A. veronii* Hm091 and *A. hydrophila* NJ-1. Data are presented as the means ($\pm$ SEM) ($n = 3$). Differences are considered significant at $P < 0.05$ (*).

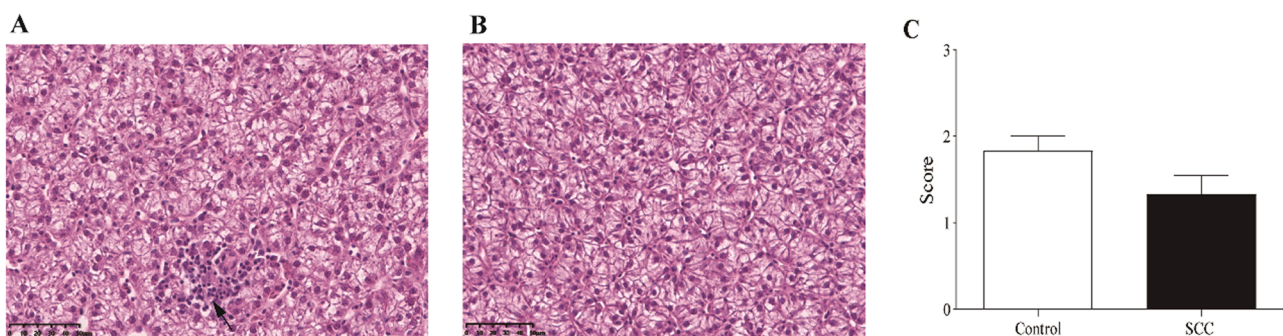


Fig. 5. Effects of SCC diet on the histology (H&E staining) of liver of channel catfish. (A) Control, (B) SCC. (C) Score of liver histopathology (Evaluation parameters: changes in the nucleus, changes in the cytoplasm, hepatocytes vacuolization). Black arrows indicated infiltration of inflammatory cell. Data are presented as the means ($\pm$ SEM) ($n = 6$).

nutrient absorption, internal environment stability and other beneficial functions of the host (Cahenzli, Köller, Wyss, Geuking, and McCoy, 2013; Hooper and Macpherson, 2010; Hooper, Midtvedt, and Gordon, 2002), but harmful bacteria can increase the permeability of the mucosal layer, thus allowing bacteria and macromolecules in food to pass through the mucosal barrier (Ma et al., 2021). In this study, the most abundant intestinal bacterial communities in channel catfish at the phyla level were Firmicutes, Proteobacteria and Bacteroidetes. Previous studies showed that Fusobacterium, Bacteroidetes, Firmicutes and Proteobacteria were the most abundant intestinal microbiota in grass carp fed with yeast culture (Yu et al., 2014). Firmicutes has been known to play a major role in the physiology of host health through metabolic activities, such as the fermentation of indigestible carbohydrates, which in turn produces short chain fatty acids (Yu et al., 2014). Butyrate is a key product of Firmicutes that promotes the health of the intestinal mucosa. The number of Firmicutes in the SCC group was higher than the control group, suggesting that the addition of SCC could improve the immune response of channel catfish.

Acute challenge experiment can directly observe the body's resistance to bacterial infection, thus reflecting the body's immune ability. *Aeromonas* is the pathogen of infectious disease outbreaks in freshwater fish. *Aeromonas* septicemia caused serious economic losses in freshwater aquaculture (Yu et al., 2014; Rathinam et al., 2021). Feeding freshwater fish with diet and then challenging them with bacteria pathogens have been a useful way to assess the resistance of fish to disease infections. Studies have reported that cell wall of baker's yeast (*Saccharomyces cerevisiae*) can improve the survival rate of apanese seabass (*Lateolabrax japonicus*) against *Aeromonas* (Yu et al., 2014). Dietary administration of Diamond V XP Yeast Culture® can protect shrimp (*Litopenaeus vannamei*) against a decline in resistance to *Vibrio* sp. 90–69B3 (Burgents, Burnett, and Burnett, 2004). Our results also showed that SCC diet enhanced the resistance of channel catfish to *A. veronii* and *A. hydrophila*. This may be attributed to the presence of β -glucan in the cell wall of yeast, and it contains specific phagocytic cells receptors that help to bind receptors molecules which will help to release signal molecules that finally stimulate the production of new WBCs, thereby contributing to the improvement of disease resistance of fish (Akhter, Wu, Memon, and Mohsin, 2015; Rohani et al., 2021).

In conclusion, diet supplemented with a certain proportion of *Saccharomyces cerevisiae* culture (ESTAQUA®) promoted the growth performance and feed utilization of channel catfish, improved the intestinal and hepatic health, modulated the structure of intestinal microbiota, and enhanced the ability of channel catfish to resist the infection of *A. veronii* and *A. hydrophila*.

CRedit authorship contribution statement

Zhigang Zhou and Zhen Zhang designed study and provided the laboratory resources; Rui Xia and Qiang Hao conducted the experiments and biochemical analyses; Yadong Xie, Qingshuang Zhang, Chao Ran, Yalin Yang, Wenhao Zhou, Fuwei Chu, Xiaomei Zhang and Yu Wang developed the methodology. Rui Xia, Qiang Hao and Zhen Zhang analyzed the data. 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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