



Effects of dietary ESTAQUA® yeast culture supplementation on growth, immunity, intestinal microbiota and disease-resistance against *Vibrio harveyi* in hybrid grouper (*♀Epinephelus fuscoguttatus* × *♂E. lanceolatus*)

Qi Wang^{a,b,c,d,1}, Stephen Ayiku^{a,b,c,1}, Hongyu Liu^{a,b,c,*}, Beiping Tan^{a,b,c}, Xiaohui Dong^{a,b,c}, Shuyan Chi^{a,b,c}, Qihui Yang^{a,b,c}, Shuang Zhang^{a,b,c}, Wenhao Zhou^e

^a Laboratory of Aquatic Animal Nutrition and Feed, Fisheries College, Guangdong Ocean University, Zhanjiang 524025, PR China

^b Aquatic Animals Precision Nutrition and High Efficiency Feed Engineering Research Centre of Guangdong Province, Zhanjiang, Guangdong, PR China

^c Key Laboratory of Aquatic, Livestock and Poultry Feed Science and Technology in South China, Ministry of Agriculture, Zhanjiang 524025, PR China

^d Bio-Form Biotechnology (Guangdong) Co., Ltd, PR China

^e Beijing Enhalar International Tech Co., Ltd, Beijing 100081, PR China

ARTICLE INFO

Keywords:

Yeast culture
Growth performance
Serum immunity
Intestinal microbiota
Disease resistance

ABSTRACT

This study investigated the effects of dietary supplementation of ESTAQUA® yeast culture (YC) on the growth, immunity, intestinal microbiota, and disease-resistance against *Vibrio harveyi* in hybrid grouper (*♀Epinephelus fuscoguttatus* × *♂E. lanceolatus*) at 0% (C0), 2% (N1), and 4% (N2). After 8 weeks of the feeding trial, the results indicated that the survival rate (SR), weight gain rate (WGR), and specific growth rate (SGR) of hybrid grouper were significantly higher in the N1 (2%) and N2 (4%) group than those in the control group ($P < 0.05$). Serum superoxide dismutase (SOD), catalase (CAT), total protein (TP), alkaline phosphatase (AKP), acid phosphatase (ACP), and immunoglobulin (IgM) activities were significantly higher in the N2 (4%) group ($P < 0.05$). Contrary to the control, aspartate aminotransferase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA), and glucose (GLU) were notably decreased in the N2 (4%) group ($P < 0.05$). The activity of intestinal digestive enzymes was significantly higher in the experimental groups than that in the control group ($P < 0.05$). Furthermore, at the phylum level, the relative abundance of *Actinobacteria* and *Bacteroidetes* was remarkably higher in the N2 (4%) group than that in the control group ($P < 0.05$). The relative abundance of *Blautia* and *Lactobacillus* was notably higher in the N2 (4%) group at the genus level ($P < 0.05$). Hybrid grouper in the N1 (2%) and N2 (4%) groups effectively improved intestinal villus height and width ($P < 0.05$). Lastly, the protection against *V. harveyi* challenge in hybrid grouper was enhanced in the YC treatment group, while N2 (4%) experienced the highest cumulative SR of 80%. In conclusion, the N2 (4%) treatment group is suggested as the best to improve the growth and immunity of hybrid grouper.

1. Introduction

Due to high market demand, grouper farming has been well-established in China (He et al., 2017). Unfortunately, intensive grouper farming may lead to the outbreak of diseases, which causes substantial economic losses to the grouper farming industry (Amaya et al., 2007; Jin et al., 2018). Using antibiotics is a common method to prevent and control fish infectious diseases. Nevertheless, the application of antibiotics could increase the growth and bioaccumulation of

resistant pathogens (Teuber, 2001; Yuan et al., 2019), which eventually leads to the transfer of resistant bacteria and the related factors from fish to humans (Leung et al., 2020). Therefore, people pay more attention to the use of natural immunostimulants and probiotics (such as yeast) as substitutes for traditional disease-control therapies has gained attention (Yuan et al., 2019). Probiotics, as a non-digestible feed ingredient, may influence the immunity of the host by regulating the gastrointestinal flora. It facilitates host health mainly through enzymatic digestion, host immunity enhancement, and the promotion of growth factors (Gupta

* Correspondence to: Laboratory of Aquatic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang, Guangdong 524088, PR China.

E-mail address: liuhyu@gdou.edu.cn (H. Liu).

¹ Co-first author: These authors contributed equally to the study.

<https://doi.org/10.1016/j.aqrep.2021.100922>

Received 28 June 2021; Received in revised form 19 October 2021; Accepted 22 October 2021

Available online 29 October 2021

2352-5134/© 2021 The Authors.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

et al., 2019).

The use of yeast products to improve fish growth and immune response has been a research hotspot in recent years. Yeast products can not only reduce the number of harmful bacteria in the intestinal tract by identifying, adsorbing, and eliminating the harmful bacteria, but also promote the reproduction of beneficial bacteria; moreover, it can and regulate the balance of micro-ecological zones in the animal's digestive tract, thus improving the intestinal tract's function, maintaining the intestinal tract's integrity, and improving the immunity of the animal organism (Zhou et al., 2009; Deng et al., 2013). Yeast culture (YC) is a complex yeast fermentation product composed of yeast and its growth medium. Currently, the high protein content solid fermentation culture method is generally used in the research of yeast protein feed. No non-protein nitrogen was added to the YC fermentation substrate in this study and the protein content was 50%. YC contains not only high levels of amino acids, protein content, and minerals, but also immunomodulatory factors (such as mannan oligosaccharides, β -glucan, and nucleotides) (Pongpet et al., 2016). The structural components of mannan-oligosaccharide (MOS) and β -glucan in the yeast cell wall help to promote immunity. β -glucan in YC can effectively lower cholesterol (CHO) and activate macrophages to release various immunoglobulins, such as cytokines and interleukin (Jayachandran et al., 2018). When fed with YC (12%) diet, the grass carp (*Ctenopharyngodon idella*) showed notably affected the metabolic capacity of intestinal microflora, along with good growth performance (Liu et al., 2018). MOS can block the recognition and adhesion mechanism of potential pathogens to host tissue surface molecules (competitive attachment sites), thereby improving the integrity and performance of the intestinal epithelial barrier (Gainza and Romero, 2020). Previous researchers have proved that MOS can enhance fish growth and regulate intestinal microbiota and gut morphology (Dimitroglou et al., 2010; Yuan et al., 2019). Moreover, it has shown that the addition of YC exerts a positive impact on the digestibility, feed intake, growth rate, and feed efficiency of aquatic animals (Essa et al., 2011; Berto et al., 2016). The study showed that dietary addition of 1% yeast hydrolysate or beer yeast to shrimp could improve the innate immunity and promote the growth of shrimp, as well as strengthen the resistance of shrimp to ammonia nitrogen stress (Jin et al., 2018). The addition of YC to feed helps to regulate the intestinal bacterial community of grass carp, thereby increasing growth performance and immune response (Liu et al., 2018).

Hybrid grouper is a carnivorous marine fish, which is a cross-breed between the giant grouper (*Epinephelus lanceolatus*) and brown-marbled grouper (*Epinephelus fuscoguttatus*) (Wang et al., 2020; Yin et al., 2020). Due to the high feed conversion ratio, fast growth rate, high palatability, and good market value, it has been widely cultured in many Asian countries, including China (Ye et al., 2019). A study has validated that mannan oligosaccharides could improve immunity response, growth, and resistance to bacterial disease (Reyes-Becerril et al., 2011). However, it remains elusive whether the dietary addition of YC can improve grouper's growth performance, immune response, and intestinal health. Against this backdrop, the present study aimed to evaluate the effects of dietary supplementation of YC on the growth performance, immune response, intestinal morphology, intestinal microbiota, and disease-resistance against *V. harveyi* attacks in hybrid grouper.

2. Materials and methods

2.1. Diet preparation

The components and nutrient contents of the basal diet are shown in Table 1. In the present study, there were three iso-nitrogenous and iso-lipidic experimental diets with increasing YC levels at 0%, 2%, and 4%, designated as C0 (0%), N1 (2%), and N2 (4%), respectively. The YC (fermentation substrate free of non-protein nitrogen; 50% protein content) was provided by Enhalar Biotechnology Company (Beijing, China). Wheat gluten, casein, and fishmeal (60% crude protein) were used as

Table 1

Ingredients and proximate composition of the experimental diets (DM %).

Ingredients	C0 (0%)	N1 (2%)	N2 (4%)
Fish meal ^a	34.00	32.50	31.00
Yeast culture ^d	0.00	2.00	4.00
Casein ^a	25.00	25.00	25.00
Wheat gluten ^a	6.00	6.00	6.00
Starch ^a	18.00	18.00	18.00
Fish oil ^a	8.00	8.00	8.00
Phospholipid ^a	1.00	1.00	1.00
Vitamin mixture ^b	0.50	0.50	0.50
Mineral mixture ^c	2.50	2.50	2.50
Choline chloride ^a	0.50	0.50	0.50
Antioxidant ethoxyquin ^a	0.03	0.03	0.03
Calcium Dihydrogen Phosphate ^a	1.00	1.00	1.00
Vitamin complex ^b	0.05	0.05	0.05
Attractant ^e	0.10	0.10	0.10
Yttrium oxide Y ₂ O ₃ ^a	0.1	0.10	0.10
Microcrystalline cellulose MCC ^a	3.22	2.72	2.22
Total	100	100	100
Nutrient levels			
Dry matter	92.60	92.17	92.27
Crude protein	50.57	50.91	50.61
Crude lipid	11.25	11.13	11.11
Ash	10.89	10.23	10.32

^a Ingredients purchased from Zhanjiang HaiBao Feed Factory, Zhanjiang, Guangdong, China.

^b Vitamin mixture (g/kg mixture): vitamin B1, 17.00 g; vitamin B2, 16.67 g; vitamin B6, 33.33 g; vitamin B12, 0.07 g; vitamin K, 3.33 g; vitamin E, 66.00 g; retinyl acetate, 6.67 g; VD, 33.33 g; nicotinic acid, 67.33 g; D-calcium pantothenate, 40.67 g; biotin, 16.67 g; folic acid, 4.17 g; inositol, 102.04 g; cellulose, 592.72 g (Obtained from Zhanjiang Yuehai Feed Co. Ltd., Guangdong, China). All ingredients were diluted with corn starch to 1 kg.

^c Mineral mixture (mg/g mixture): CaCO₃, 350 g; NaH₂PO₄·H₂O, 200 g; KH₂PO₄, 200 g; NaCl, 12 g; MgSO₄·7H₂O, 10; FeSO₄·7H₂O, 2 g; MnSO₄·7H₂O, 2 g; AlCl₃·6H₂O, 1 g; CuCl₂·2H₂O, 1 g; KF, 1 g; NaMoO₄·2H₂O, 0.5 g; NaSeO₃, 0.4 g; CoCl₂·6H₂O, 0.1 g; KI, 0.1 g; zeolite powder, 219.9 g. All ingredients were diluted with corn starch to 1 kg. (Obtained from Zhanjiang Yuehai Feed Co. Ltd., Guangdong, and China).

^d The yeast culture was provided by Enhalar Biotechnology Company (Beijing, China).

^e Attractant composition: taurine: glycine: betaine = 1:3:3.

protein sources, fish oil and phospholipid oil were used as lipid sources. After crushing all the coarse ingredients, they were sieved using a 60-mesh sieve, and then gradually stirred and mixed in a V-mixer type machine. Next, fish oil and phospholipid oil were added to the mixture, followed by further mixing by hand. After that, choline chloride-contained water was mixed into the mixture (nearly 30% of the net weight of the ingredient), which was then adjusted to a dough shape using a Hobart-type mixer. Next, it was processed into 2.5 mm particles with a diameter of 2.5 mm by a twin-screw extruder (F-26, South China University of Technology), dried (room temperature, 3–4 days) until the moisture was reduced to about 10%, and then refrigerated (−20 °C) until use.

2.2. Fish and feeding regime

Hybrid groupers were purchased from a local fish farm hatchery (Zhanjiang, China) and transferred to the Marine Biological Research Base of Guangdong Ocean University. They were kept in fiberglass tanks for two weeks and fed with a commercial diet to adapt to the experimental conditions. After that, all fish fasted for 24 h. A total of 270 hybrid groupers (17.56 g ± 0.02) were randomly placed into 9 fiberglass tanks (250 L), 30 fish per tank. They were fed twice a day (9:00 and 17:00) with a feed intake of 5–8% of their average body weight in three replicates to apparent satiation for 8 weeks. About 60% of the water was refreshed every day to maintain water quality. During the feeding trial, the water temperature was 28.0 ± 2.0 °C and the dissolved oxygen (DO) was within 6–8 mg/L.

2.3. Sample collection and calculations

At the end of the 8-week feeding trial, all fish fasted for 24 h before sampling. The total number of fish from each tank was recorded and they were weighed collectively. Two fish were randomly selected from each tank and stored at -20°C and their dry matter, crude protein, ash, and lipid were calculated. Six fish were randomly selected from each tank and their blood was taken using a 1 mL syringe. The blood samples were stored at 4°C for 12 h, followed by 3500 rpm centrifugation at 4°C for 10 min. The supernatant was collected and stored at -80°C for subsequent enzyme activity analysis. The midgut (0.8–1.2 cm) of three fish from each tank was taken under aseptic conditions and preserved in 10% paraformaldehyde for histological analysis. Moreover, three fish were randomly selected from each group and their midguts were taken, which were then carefully loaded into the 1.5 mL Eppendorf tube and immediately stored in liquid nitrogen for microflora analysis. The growth performance formulas were as follows:

Weight gain rate(WGR,%) = 100

$$\times \frac{\text{final body weight(g)} - \text{initial body weight(g)}}{\text{initial body weight(g)}}$$

$$\text{Feed conversion ratio(FCR)} = \frac{\text{feed intake(g)}}{\text{final body weight(g)} - \text{initial body weight(g)}}$$

Specific growth rate(SGR,%) = 100

$$\times \frac{[\ln(\text{final weight(g)}) - \ln(\text{initial weight(g)})]}{\text{days of experiment}}$$

$$\text{Protein efficiency ratio(PER)} = 100 \times \frac{\text{fish weight gain(g)}}{\text{crude protein intake(g)}}$$

$$\text{Survival rate(SR, \%)} = 100 \times \frac{\text{final fish number}}{\text{initial fish number}}$$

$$\text{Condition factor(CF, \%)} = 100 \times \frac{\text{body weight(g)}}{\text{body length(cm)}^3}$$

2.4. Analysis of diets and whole-body composition

The dry matter, crude protein, lipid, and ash of diet and whole fish were determined according to the methods of Lee (1995). The samples were dried in an oven at 105°C to a constant weight to determine the dry matter. The crude lipid content was determined using the Soxhlet extraction method (YSXT-06, XiYang instrument co., Ltd., Shanghai, China), and the crude protein content was determined using the Kjeldahl method (Kjeltac 8400, FOSS Technology and Trade Co., Ltd., Beijing, China). Moreover, the crude ash content was determined by burning in a muffle furnace (SX2-2.5-10, Qiuzuo Instrument and Equipment Co., Ltd., Shanghai, China) at 550°C for 6 h.

2.5. Serum biochemical parameters and intestinal enzyme activity analysis

Fish serum was collected. Total protein (TP, A045-2-2), malondialdehyde (MDA, A003-1-2), aspartate aminotransferase (AST, C010-2-1), alanine aminotransferase (ALT, C009-2-1), lysozyme (LZM, A050-1-1), alkaline phosphatase (ALP, A059-2-2), immunoglobulin M (IgM, H109), acid phosphatase (ACP, A060-2-2), catalase (CAT, A007-1-1), superoxide dismutase (SOD, A001-3-2), glucose (GLU, A154-1-1), triglyceride (TG, A110-1-1), and cholesterol (CHO, A111-1-1) were detected using commercial kits (Nanjing Jiancheng Biological Engineering Institute, China) as per the kit instructions.

Intestinal tissue homogenates were centrifuged and the supernatant was taken for the determination of digestive enzyme activities in the intestine using commercial kits (Jiancheng Biological Engineering

Institute) according to the manufacturer's instructions. Digestive enzymes to be measured are amylase (C016-1-1), lipase (A054-2-1), and trypsin (A080-2-1).

2.6. Intestinal microbiota analysis

As described previously (Suo et al., 2017), microbiota analysis was performed by identification of V3-V4 region of 16S rRNA gene using high throughput sequencing. In this study, the procedure was slightly altered. Total DNA was performed from the enteric bacterial communities using a soil DNA kit. Sample DNA concentration was quantified using a NanoDrop ND-2000 spectrophotometer (Thermo Science, Wilmington, DE, USA). The corresponding region of the primer was 16S V3-V4 (341F and 806R). The primer sequence of 341F was 5'-CCTACGGGNGGCWGCAG-3', and the primer sequence of 806R was 3'-GGACTACHVGGGTATCTAAT-5'. The system of PCR was 30 μL , and high-throughput sequencing was performed through Illumina HiSeq 2500 sequencing. Following this, 2% agarose gel was used to separate the amplicons. Next, they were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) as per the manufacturer's protocol. The library was constructed using NEB Next®Ultra library DNA Library Prep Kit for Illumina construction kit, and then sequenced by Illumina HiSeq 2500 sequencing system. The data were filtered by noise sequence, and the chimera sequence was detected using the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html) (Quast et al., 2013). OTU clustering was performed with 97% sequence similarity as the threshold. The UPARSE software (UPARSE v7.0.1001, <http://drive5.com/uparse/>) (Edgar, 2013) was used for sequence analysis. Next, the rarefaction curve was plotted and the alpha-diversity metrics were calculated (Simpson, Shannon, ACE, and Chao). The endemic species between different groups are shown on the Venn diagram. The PCoA of unweighted UniFrac distance was drawn in WGCNA under R version 2.15.3 and the differences among multiple samples were compared.

2.7. Histological analysis of fish intestine

Following the method of Bullerwell et al. (2016), intestinal morphological indexes were detected to determine the YC diet's effect on fish intestinal morphology (Bullerwell et al., 2016). The fixed tissues were dehydrated with gradient alcohol, cleared in toluene, transparented in xylene, and embedded in paraffin. Finally, the paraffin-embedded tissues were sectioned with a thickness of 5 μm . Intestinal tissues were stained with hematoxylin-eosin (H&E), and intestinal images were collected using a microscope (BX51 type, serial number: 9K18395; Olympus, Tokyo, Japan). A total of 12 measurements were obtained for each tissue. The data of intestinal villus height (VH), villus width (VW), and muscle thickness (MT) under 100x magnification were obtained through electronic measurement using Image-Pro Plus 6.3 software (Media Cybernetics, Inc., Rockville, USA).

2.8. Bacteria challenge test

Vibrio harveyi for challenge test was obtained from the Provincial Key Laboratory of Pathogenic Biology and Epidemiology for Aquatic Animal, Guangdong Ocean University, China. They were initially isolated from a diseased hybrid grouper and stored in 40% glycerol at -80°C . Upon challenge test, the frozen stock culture was revived. Bacteria were cultured in Luria-Bertani (LB) medium in a flat-bottomed flask (250 mL) by shaking at 180 rpm in an incubator (37°C , 20 h). Afterward, the cells were centrifuged (8000 rpm, 4°C , 10 min), with the supernatant discarded, followed by 2 washes with sterile phosphate-buffered saline (PBS). Next, they were serially diluted with PBS to attain 3.58×10^5 CFU/mL (LD 50). After the 8-week feeding trial, 12 fish were randomly selected from each replicate tank for the challenge test, and each fish was injected with 200 μL of bacterial cell suspension at LD50

(3.58×10^5 CFU/mL). In addition, 12 fish were randomly selected from each group and injected with the same dose of saline, which served as the blank control group. During challenge test, fish was not offered feed. Following the bacteria injection for 12 h, blood was sampled from two fish, which was then centrifuged (3500 rpm/min, 4 °C, 10 min), separated, and stored (-80 °C) for later analysis of ALP, ACP, LZM, and IgM activities. Fish were kept under observation. The mortality rate was recorded up to 168 h.

2.9. Statistical analysis

Data analysis was performed using SPSS23.0 (SPSS Inc., Chicago, IL, USA). The results are presented as means $\pm$ standard error of mean (SEM). All data were analyzed using a one-way analysis of variance followed by Duncan's multiple range test to determine significant differences between treatment groups. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Growth, feed utilization, and survival rate of hybrid grouper

The effects of different YC protein diets on the growth and feed utilization of hybrid grouper were illustrated in Table 2. The results showed that YC-treated fish exhibited notably improved growth performance than the control fish. FBW, WGR, and SGR were the best in the N2 (4%) groups ($P < 0.05$). FCR was notably less in the N1 (2%) and N2 (4%) groups than that in the control group, with the lowest in the N2 (4%) group ($P < 0.05$). The dietary supplementation of YC significantly improved the SR of hybrid grouper ($P < 0.05$). The excellent SR was recorded in the N1 (2%) and N2 (4%) groups. PER and CF were remarkably increased in hybrid grouper fed with N1 (2%) and N2 (4%) as compared to those in the control hybrid fish ($P < 0.05$).

3.2. Whole-body composition of fish

The YC protein effects on fish carcass analysis were illustrated in Table 3. A significant improvement in dry matter, crude protein, and ash content in fish fed with YC treatment diets were observed ($P < 0.05$), with the N2 (4%) group showing the highest significant improvement. Crude lipid content was found notably increased in the N1 (2%) group ($P < 0.05$).

3.3. Serum biochemical parameters and intestinal digestive enzyme activity

The effect of dietary supplementation of YC on serum biochemical parameters of hybrid grouper is presented in Table 4. It was observed that serum GLU was decreased in the YC group compared to the control group, with the N2 (4%) group showing the lowest serum GLU ($P < 0.05$). Dietary addition of YC increased hybrid grouper serum TP contents ($P < 0.05$), with the N2 (4%) group showing the significantly high TP content ($P < 0.05$). In our study, when hybrid grouper was fed with YC, no significant difference was found among other groups except for the significant increase of TG content in the N1 group. There was no significant difference in CHO content among groups ($P > 0.05$). The

Table 2
Effects of yeast culture supplementation on hybrid grouper growth performance.

Treatment	IBW (g/fish)	FBW (g/fish)	WGR (%)	SGR (%)	FCR	SR (%)	PER (%)	CF (g/cm ³)
C0 (0%)	17.86 $\pm$ 0.00 ^a	78.91 $\pm$ 0.68 ^a	343.49 $\pm$ 3.59 ^a	2.65 $\pm$ 0.01 ^a	1.03 $\pm$ 0.02 ^b	92.33 $\pm$ 1.17 ^a	1.54 $\pm$ 0.04 ^a	2.77 $\pm$ 0.05 ^a
N1 (2%)	17.85 $\pm$ 0.01 ^a	87.15 $\pm$ 0.43 ^b	387.13 $\pm$ 2.16 ^b	2.83 $\pm$ 0.01 ^b	0.86 $\pm$ 0.00 ^a	100.00 $\pm$ 0.00 ^b	1.85 $\pm$ 0.03 ^b	3.12 $\pm$ 0.00 ^c
N2 (4%)	17.86 $\pm$ 0.00 ^a	89.54 $\pm$ 0.50 ^b	401.39 $\pm$ 2.90 ^b	2.87 $\pm$ 0.01 ^b	0.84 $\pm$ 0.00 ^a	100.00 $\pm$ 0.00 ^b	1.87 $\pm$ 0.01 ^b	3.01 $\pm$ 0.01 ^b

The data are presented as mean $\pm$ SEM. Means in the same column with different superscripts indicate significant difference ($P < 0.05$). IBW, initial body weight; FBW, final body weight; WGR: weight gain rate; SGR, specific growth rate; FCR, feed conversion ratio; SR, survival rate; PER: protein efficiency ratio; CF, condition factor.

Table 3

Effects of dietary yeast culture supplementation on whole body composition of hybrid grouper.

Treatment	Dry matter (%)	Crude protein (%)	Lipid (%)	Ash (%)
C0 (0%)	28.32 $\pm$ 0.40 ^a	17.19 $\pm$ 0.09 ^a	6.10 $\pm$ 0.01 ^a	4.28 $\pm$ 0.00 ^a
N1 (2%)	31.02 $\pm$ 0.76 ^b	19.04 $\pm$ 0.12 ^b	6.93 $\pm$ 0.01 ^b	4.52 $\pm$ 0.012 ^b
N2 (4%)	32.92 $\pm$ 0.21 ^c	19.92 $\pm$ 0.08 ^c	6.50 $\pm$ 0.12 ^{ab}	4.73 $\pm$ 0.05 ^c

The data are presented as mean $\pm$ SEM. Means in the same column with different superscripts indicate significant difference ($P < 0.05$).

activities of serum AST and ALT in fish fed with N2 (4%) diet were lower than that of the control ($P < 0.05$).

The results of intestinal digestive enzyme activities are presented in Table 5. Intestinal trypsin, lipase, and amylase activities showed the same trends, which was significantly higher in the N1 (2%) and N2 (4%) groups than those in the control group ($P < 0.05$). However, there was no significant difference between the N1 (2%) and N2 (4%) groups ($P > 0.05$).

3.4. Serum antioxidant capacity

The results of antioxidant parameters in the serum are presented in Fig. 1. The serum SOD activity in the N2 (4%) group was notably improved after the challenge ($P < 0.05$). The increased SOD activity in the fish N2 (4%) group was dramatically higher as compared to that in the control group ($P < 0.05$). Fish in the N1 (2%) and N2 (4%) groups had dramatically increased CAT activity after challenge ($P < 0.05$), with the greatest in the N2 (4%) group. Serum MDA contents were decreased in the YC treatment groups, and it was significantly lower in the N2 (4%) group before and after the challenge test (compared to the control group) ($P < 0.05$).

3.5. Serum innate immune indexes

The activities of serum ACP, AKP, LZM, and IgM of hybrid grouper before and after the challenge test are illustrated in Fig. 2. Serum ACP activity was significantly higher after the challenge, which was remarkably higher in the N2 (4%) group relative to that in the control group ($P < 0.05$). After the challenge, AKP serum activities of the N1 (2%) and N2 (4%) groups were dramatically enhanced ($P < 0.05$), with the highest improvement observed in the N2 (4%) group. Serum LZM activity was increased in the N1 (2%) and N2 (4%) groups after the challenge, which was significantly higher in the N1 (2%) group than that in the control group ($P < 0.05$). Serum IgM activity was dramatically increased in the N1 (2%) and N2 (4%) groups after the challenge, as compared to the control group ($P < 0.05$).

3.6. Microbiota analysis in hybrid grouper intestine

3.6.1. Intestinal microbiota richness and species diversity

The rarefaction curve analysis of the samples is shown in Fig. 3A. It can be seen from the figure that rarefaction curves tended to be flat with the continuous increase in sequencing amount. The sequencing results could reflect the structure and composition of the intestinal microbial community of the grouper. As shown in Fig. 3B, the box plot position

Table 4

Effects of different yeast culture supplementation on hybrid grouper serum biochemical activity.

Treatment	TP (g/L)	GLU (mmol/L)	TG (mmol/L)	CHO (mmol/L)	ALT (U/L)	AST (U/L)
C0 (0%)	44.21 ± 0.94 ^a	4.21 ± 0.17 ^b	0.64 ± 0.01 ^a	5.40 ± 0.06 ^a	4.42 ± 0.09 ^c	25.18 ± 0.47 ^c
N1 (2%)	51.72 ± 0.81 ^b	3.85 ± 0.04 ^{ab}	0.75 ± 0.02 ^b	5.64 ± 0.13 ^a	3.93 ± 0.06 ^{ab}	21.64 ± 0.41 ^b
N2 (4%)	52.92 ± 0.88 ^b	3.58 ± 0.02 ^a	0.66 ± 0.01 ^a	5.53 ± 0.09 ^a	3.70 ± 0.02 ^a	18.25 ± 0.67 ^a

The data are presented as mean ± SEM. Means in the same column with different superscripts indicate significant difference ($P < 0.05$). TP, total protein; GLU, glucose; TG, triglyceride; CHO, cholesterol; ALT, alanine transaminase; AST, aspartate aminotransferase.

Table 5

Effects of dietary yeast culture supplementation on the intestinal digestive enzyme activities of hybrid grouper.

Treatment	Trypsin (U/mgProt)	Lipase (mU/mgProt)	Amylase (mU/mgProt)
C0(0%)	1352.58 ± 113.49 ^a	444.14 ± 20.29 ^a	318.81 ± 12.71 ^a
N1(2%)	1914.67 ± 149.13 ^b	722.32 ± 63.32 ^b	404.73 ± 39.47 ^b
N2(4%)	1894.54 ± 150.75 ^b	673.21 ± 21.37 ^b	433.94 ± 9.47 ^b

The data are presented as mean ± SEM. Means in the same column with different superscripts indicate significant difference ($P < 0.05$).

risers sharply over a range, indicating that a large number of species were found in the community. When the boxplot tended to be smooth, the species would not significantly increase as the sample size increased, indicating that the sampling was sufficient and data analysis could be carried out. Operation taxonomic units (OTUs) and Alpha diversity results in hybrid grouper intestinal microbiota are illustrated in Table 6. OTUs in groupers fed with N2 (4%) diet were dramatically higher than that in groupers fed with C0 (0%) and N1 (2%) diets ($P < 0.05$). All the OTUs made up with the mean abundance of more than one were selected for Venn diagram analysis (Fig. 4). As shown in Venn diagram analysis, a total of 540 OTUs were identified across the three treatments. The N2 (4%) group had the highest number of unique OTUs (1031), followed by C0 (0%) (771) and N1 (2%) (584). Alpha indices were represented by ACE, Chao, Shannon, and Simpson. Results showed that the N2 (4%)

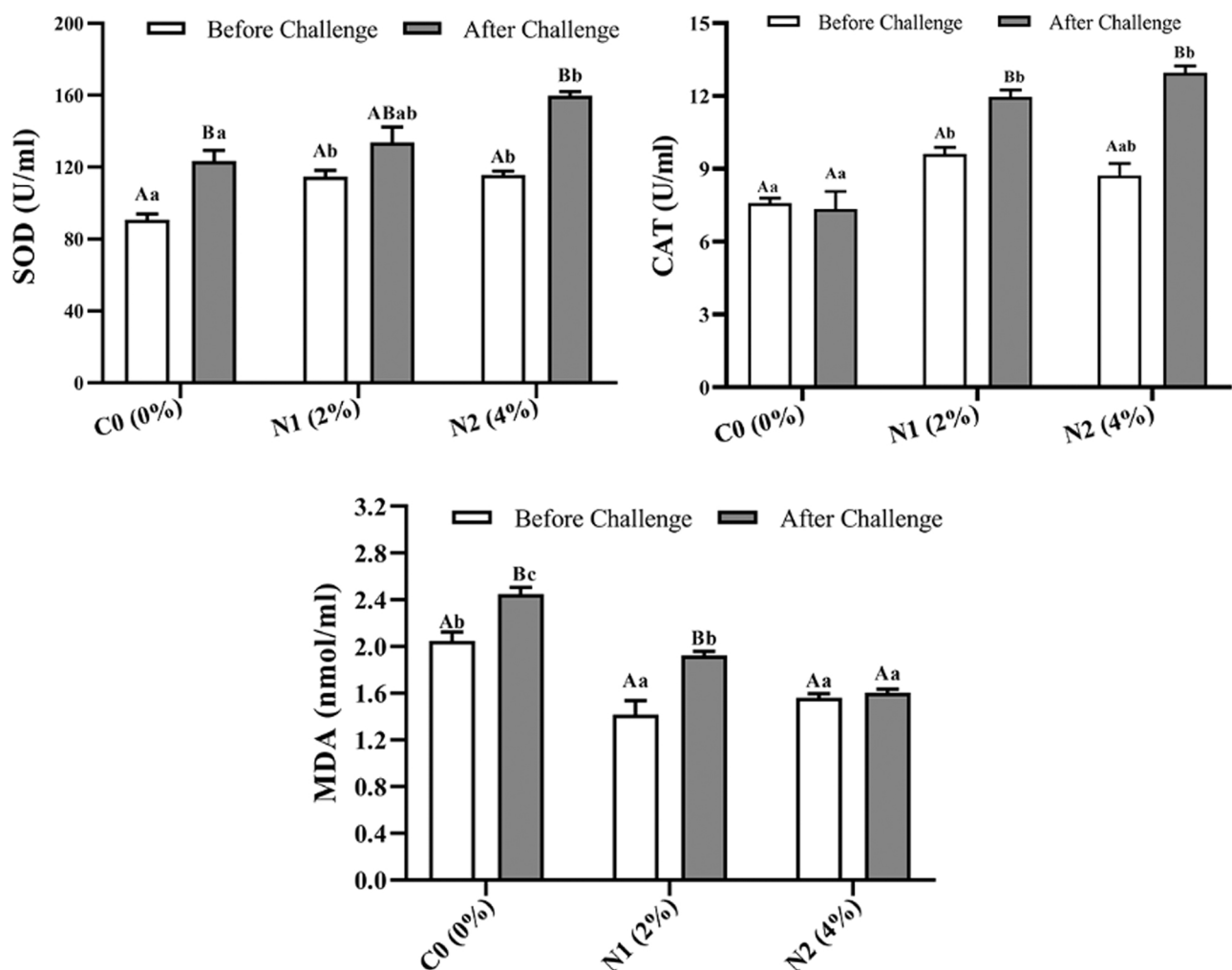


Fig. 1. Yeast culture diets effects on serum antioxidant capacity of hybrid grouper before challenge and after challenge with *V. harveyi*. The data were shown in Mean ± SEM. Different capital letters indicate significant differences before and after the challenge within the same treatment group ($p < 0.05$), and different lowercase letters indicate significant differences before and after the challenge between groups ($p < 0.05$). SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde.

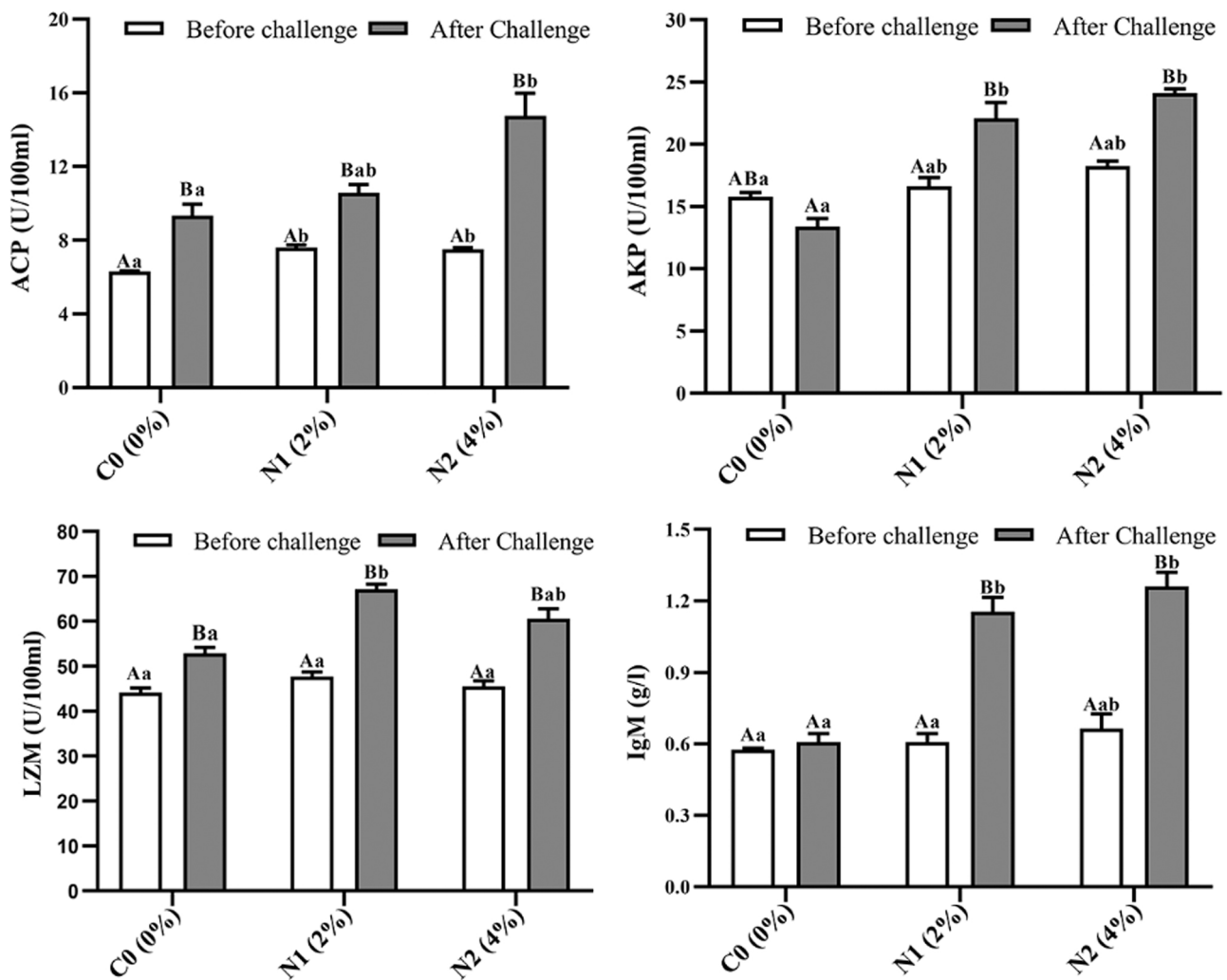


Fig. 2. Yeast culture diets effects on serum innate immune response of hybrid grouper before challenge and after challenge with *V. harveyi*. The data were shown in Mean $\pm$ SEM. Different capital letters indicate significant differences before and after the challenge within the same treatment group ($p < 0.05$), and different lowercase letters indicate significant differences before and after the challenge between groups ($p < 0.05$). ACP, acid phosphatase; AKP, alkaline phosphatase; LZM, lysozyme; IgM, immunoglobulin M.

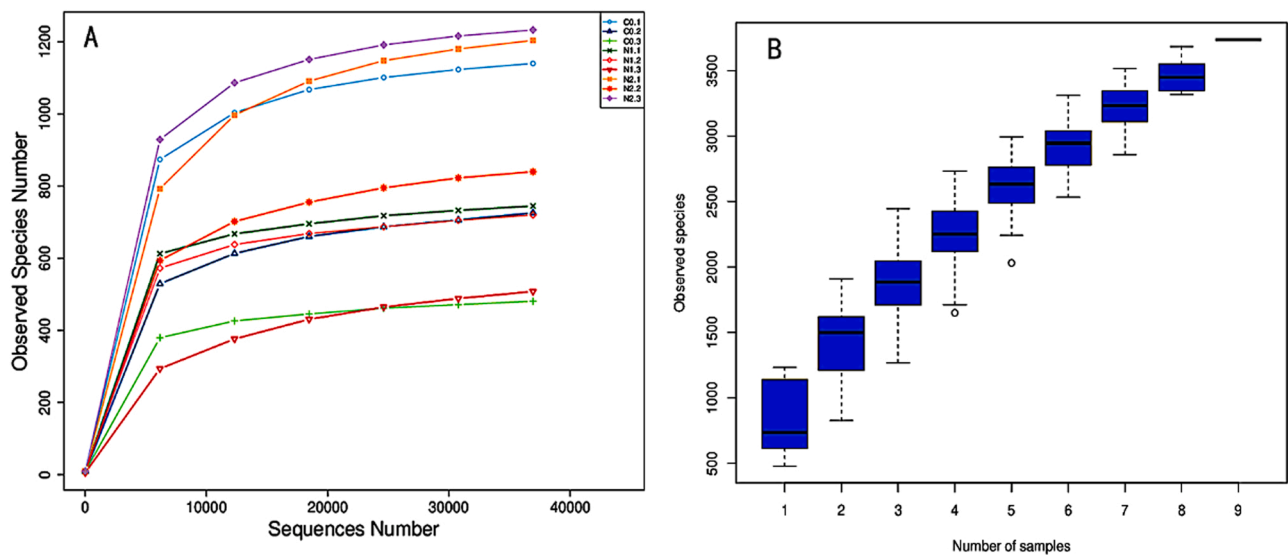


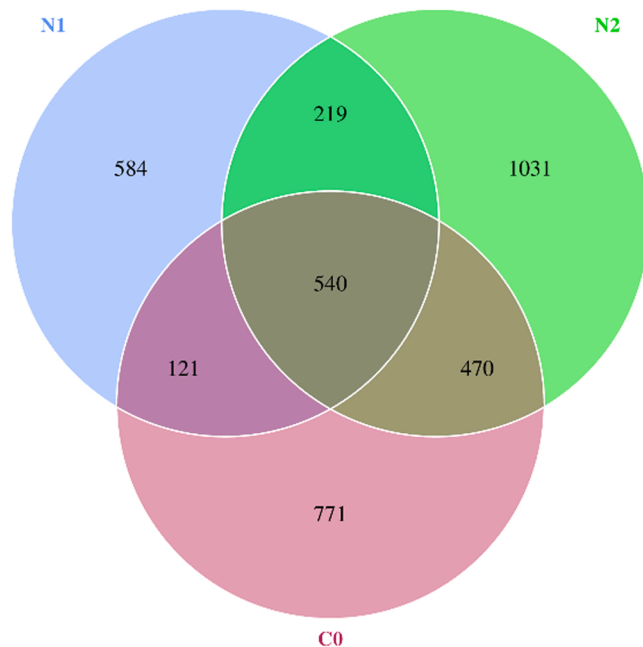
Fig. 3. A: Rarefaction curve analysis of intestinal samples in hybrid grouper from different treatment groups. B: The species accumulation boxplot. The horizontal axis represents the number of samples, and the vertical axis represents the number of species found.

Table 6

Richness and diversity indexes of intestinal bacterial in hybrid grouper fed experimental diets.

Treatment	Raw reads	Clean reads	OTU	Chao	Ace	Shannon	Simpson
C0 (0%)	76,860	69,616	770.33 ± 44.33 ^a	1136.28 ± 66.88 ^b	753.78 ± 34.88 ^a	6.91 ± 0.07 ^a	0.98 ± 0.00 ^a
N1 (2%)	85,025	80,121	797.00 ± 2.00 ^a	798.12 ± 2.87 ^a	796.00 ± 3.20 ^a	7.32 ± 0.13 ^a	0.98 ± 0.01 ^a
N2 (4%)	84,743	80,144	1292.00 ± 3.00 ^b	1258.61 ± 12.29 ^b	1258.43 ± 11.79 ^b	7.14 ± 0.05 ^a	0.97 ± 0.00 ^a

The data are presented as mean ± SEM. Means in the same column with different superscripts indicate significant difference ($P < 0.05$).

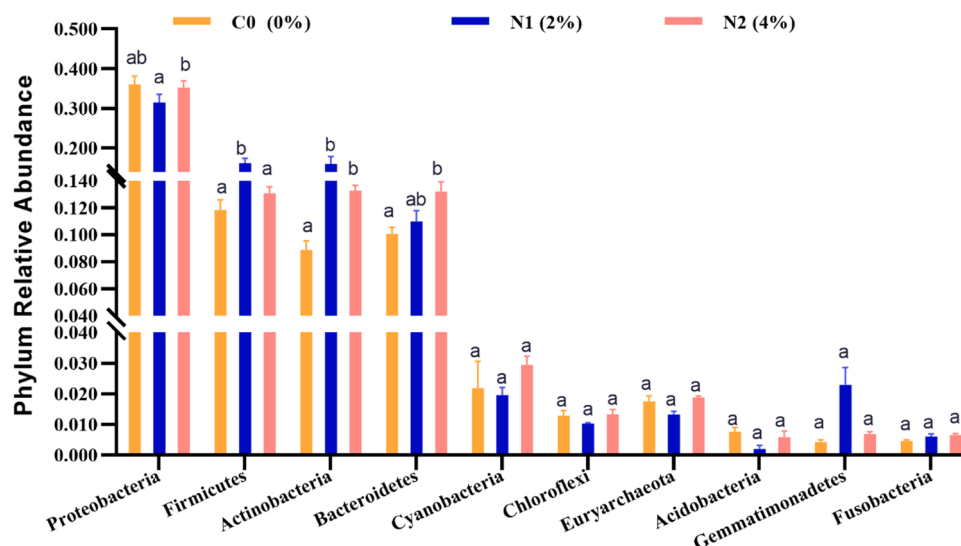
**Fig. 4.** Venn diagram of OTUs comparison.

group had the highest Chao index, but there was no significant difference as compared to the control group ($P > 0.05$). The ACE index was notably higher in the N2 (4%) group than that in the C0 (0%) and N1 (2%) groups ($P < 0.05$). The bacterial diversity indexed by Shannon was numerically increased in the N1 (2%) and N2 (4%) groups, but there was no significant difference among groups ($P > 0.05$). According to Simpson's results, no difference was found between the treated and untreated

groups ($P > 0.05$).

3.6.2. Composition characteristics of intestinal microbiota

The ten bacteria with the highest relative abundance at the phylum and genus level were shown in Fig. 5 and Fig. 6, respectively. Four dominant intestinal bacteria at the phylum level were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* (Fig. 5). The relative abundance of *Proteobacteria* was noticeably higher in the N2 (4%) group than that in the N1 (2%) group ($P < 0.05$), but not significantly different from the C0 (0%) group ($P > 0.05$). The abundance of *Bacteroidetes* in the N2 (4%) group was notably higher than that in the control group ($P < 0.05$). The relative abundance of *Actinobacteria* in hybrid grouper in the N1 (2%) and N2 (4%) groups were significantly higher than that in the control group, with no significant difference between the N1 (2%) and N2 (4%) groups ($P > 0.05$). The N1 (2%) and N2 (4%) groups had a higher relative abundance of *Firmicutes* than the C0 (0%) group, and it was significantly higher in the N1 (2%) group than that in the N2 (4%) group. At the genus level, the main intestinal bacteria were *Sphingomonas*, *Blautia*, *Lactobacillus*, and *Stenotrophomonas* in all the treatment groups (Fig. 6). The relative abundance of *Sphingomonas* was high in the N2 (4%) group, but there was no significant difference between the three groups ($P > 0.05$). *Blautia* abundance was significantly higher in the N2 (4%) group than that in the other two groups ($P < 0.05$). The hybrid grouper intestinal *Lactobacillus* were abundant in YC-fed grouper than untreated grouper ($P < 0.05$). The abundance of *Stenotrophomonas* was significantly higher in the N1 (2%) group than that in the control group, but not significantly different than the N2 (4%) group. Based on Principle Coordinate Analysis (PCoA) (Fig. 7) of unweighted UniFrac distances from all samples (microbiota composition clustered by feed-stuff type), there were distinct microbial community structure in the C0 (0%), N1(2%), and N2 (4%) groups, which indicated that the addition of YC may influence the composition of the intestinal microbial community.

**Fig. 5.** Relative abundance of intestinal bacterial of hybrid grouper at the phylum level. Upright bars denote the mean ± SEM. ($P < 0.05$). Different superscript letters indicate significant differences between groups ($P < 0.05$).

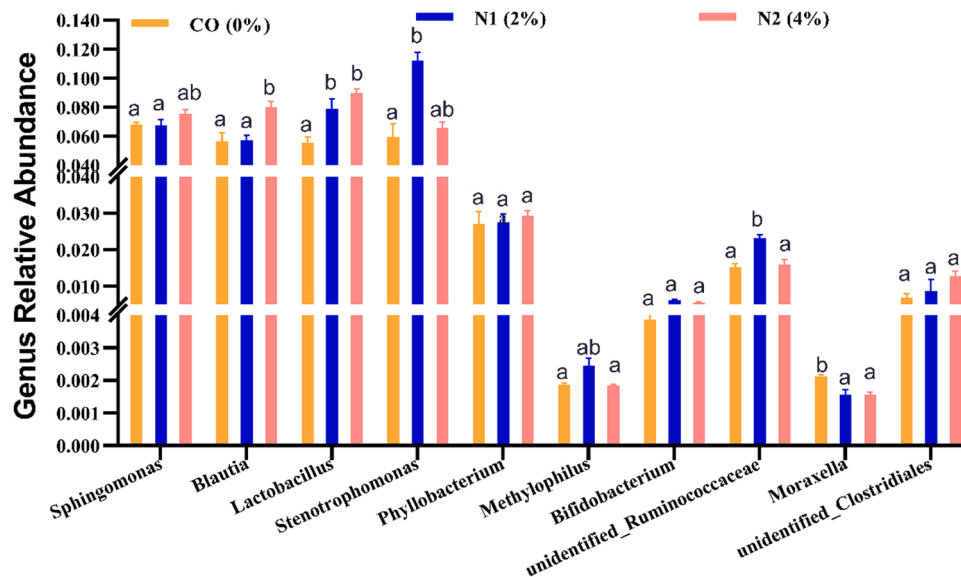


Fig. 6. Relative abundance of intestinal bacterial of hybrid grouper at the genus level. Upright bars denote the mean $\pm$ SEM. ($P < 0.05$). Different superscript letters indicate significant differences between groups ($P < 0.05$).

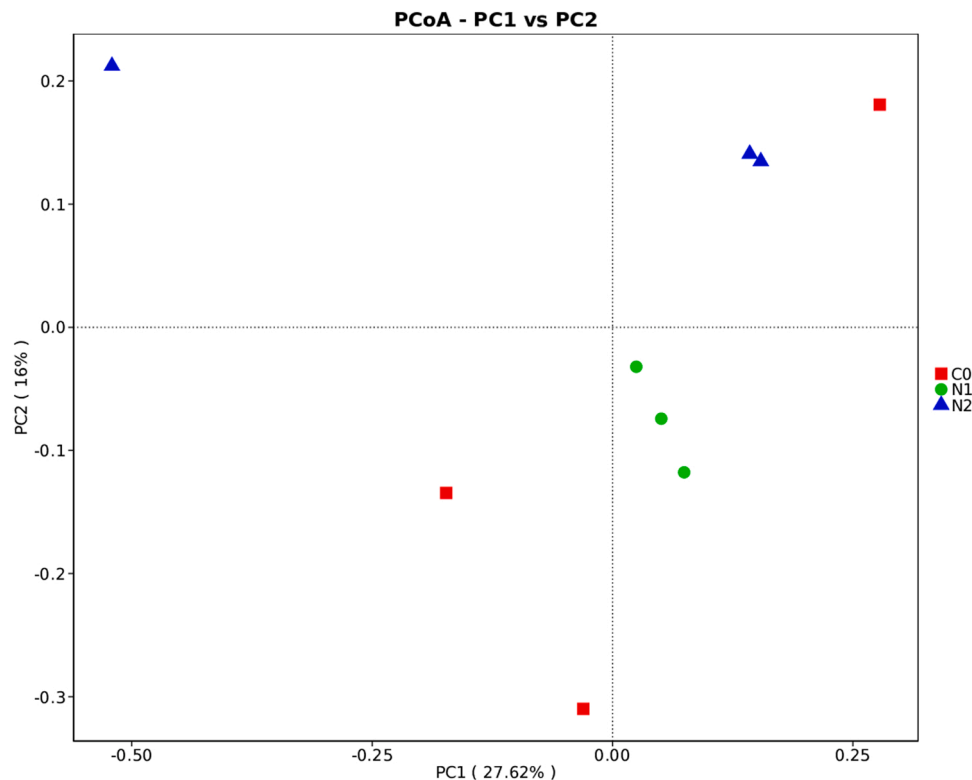


Fig. 7. Principal coordinate analysis (PCoA) of intestinal microbial community of grouper.

3.6.3. Bacterial challenge test

The cumulative fish SR after challenge with hybrid grouper for 168 h is illustrated in Fig. 8. No mortality of grouper was observed in the saline injection group. At the end of 168 h of challenge, the cumulative SR of hybrid grouper with dietary supplementation of YC was notably higher than that of the control. The highest SR of fish was recorded in the N2 group (80%), and the least registered number was found in fish fed with the control diet (20%).

3.7. Intestinal morphometric measurement

The measurement of intestinal morphometric parameters is represented in Fig. 9. The intestinal mucosal fold separation from the intestinal wall was seen in control groupers, whereas fusion of mucosal folds was observed in groupers fed with N1 (2%) and N2 (4%) diets (Fig. 9A). YC supplementation resulted in a significant increase in VH and VW ($P < 0.05$). VW and VH were significantly higher in the hybrid group fed

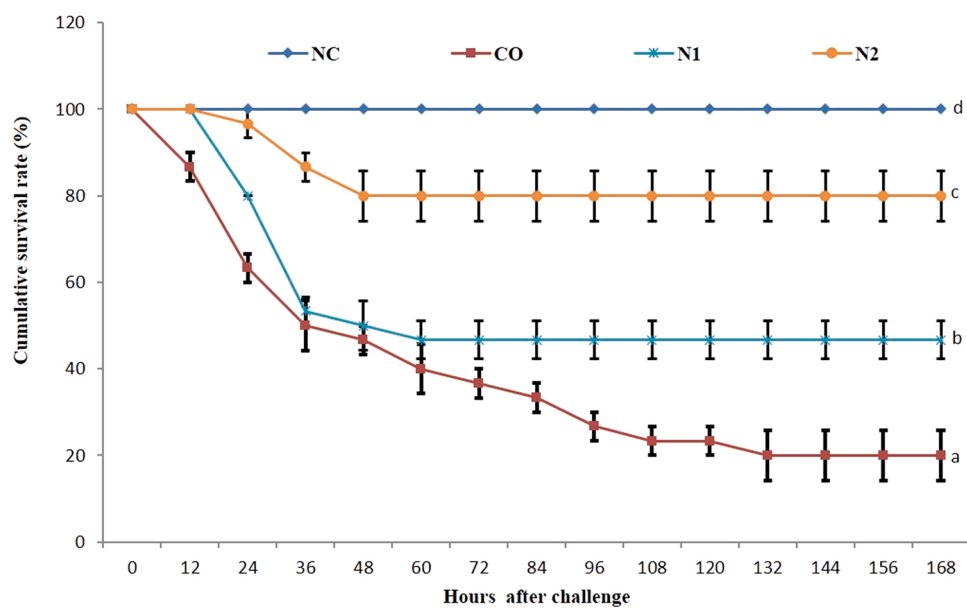


Fig. 8. Cumulative survival rate (%) of hybrid grouper after challenge with *V. harveyi*. For each treatment, values with different letter suffix differ significantly ($P < 0.05$).

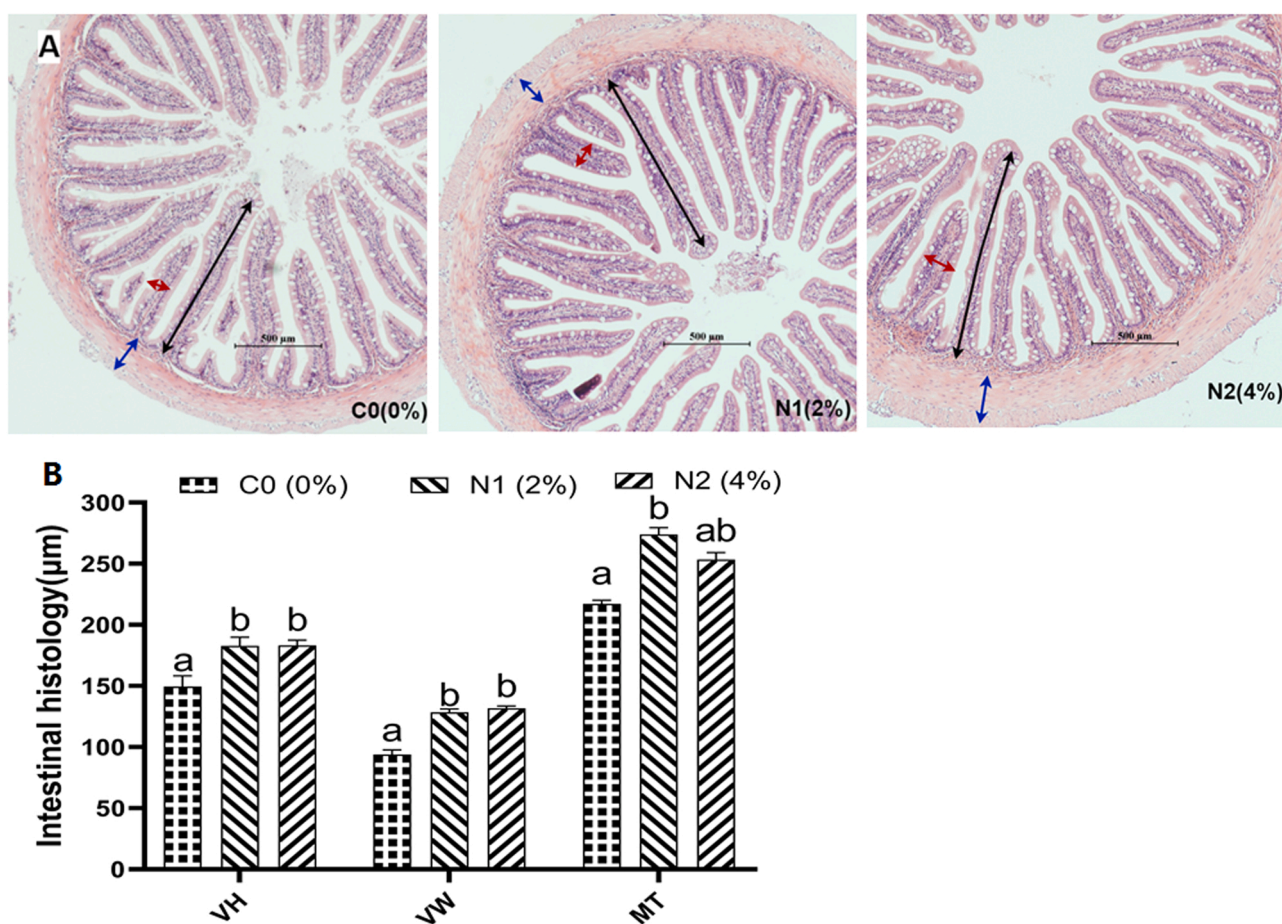


Fig. 9. Effects of supplementation of yeast culture on villus height, villus width and muscle thickness of hybrid grouper. (A) Photomicrographs. Black arrow: villus height; Red arrow: villus width; Blue arrow: muscle thickness. (B) Morphological measurements. Values are presented mean $\pm$ SEM. Different letters indicate significant differences ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with YC N2 (4%) showed than those in the control group, while no improvement was found between the N1 (2%) and N2 (4%) groups ($P < 0.05$). Among all the experimental groups, only the N1 (2%) group showed significant MT changes, as compared to the control group ($P < 0.05$) (Fig. 9B).

4. Discussion

Recently, it has been of interest to improve the growth and immune response of fish using yeast products. The evaluation of several yeast products has shown a positive influence on the growth performance and immune response of aquatic animals (Dimitroglou et al., 2010; Zhao et al., 2011; Sado et al., 2014). A previous study has proved that juveniles' leopard grouper fed with yeast diet shows improved growth and immune response (Reyes-Becerril et al., 2011). In the present study, the benefits of dietary supplementation of different levels of YC (fermentation substrate free of non-protein nitrogen; 50% protein content) in hybrid grouper were evaluated from the aspects of growth performance, intestinal morphology, digestive enzymes, intestinal microbiota, immune response, and disease resistance.

After 8 weeks of feeding trials with dietary supplementation of YC, the SR of hybrid grouper was enhanced, which may be due to the diet's nutritional fulfillment. The dietary inclusion of YC could help to increase the growth performance and feed utilization of hybrid grouper in the current study. However, FBW, WGR, and SGR were notably improved, and FCR was decreased in the dietary N1 (2%) and N2 (4%) groups, which indicated that YC could enhance the growth performance and feed utilization of hybrid grouper. This beneficial influence may be due to the bioactive compounds secreted by YC, including nucleotides, proteins, amino acids, fatty acids, vitamins, and polysaccharides. Nucleotides are especially important, which can serve as precursors of DNA and RNA in rapidly growing cells (such as intestinal tissue) in the diet via the salvage pathway, thereby sparing the organism from the cost of de novo synthesis. Moreover, the increased digestive enzyme activities are an important factor in promoting growth and feed utilization (Zhang et al., 2018). In the present study, fish in the N1 (2%) and N2 (4%) groups showed high digestive enzyme activities and improved feed utilization. Consistently, previous studies have demonstrated a similar increase in digestive enzyme and feed utilization in Seabass (*Dicentrarchus labrax*) (Tovar et al., 2002). Furthermore, intestinal microorganisms are closely involved in the absorption, digestion, and metabolism of several nutrients in aquatic animals. Specifically, the abundance of intestinal *Bacteroides* in fish in the N2 (4%) group was increased. *Bacteroides* decompose polysaccharides into simple sugars, which can be easily absorbed into the host to synthesize proteins and play mutualistic roles in fish intestine (Xia et al., 2014). Therefore, the enhanced feed utilization and growth performances in this study may be related to the effect of dietary YC on the intestinal microbiota and digestive enzymes. Based on the changes in the intestinal microbiota and digestive enzymes of hybrid grouper, it's indicated that YC could increase exogenous bacterial enzyme activities, which may lead to the increased growth performance of hybrid grouper.

Grouper whole-body composition analysis is a good indicator for studying the physiological condition of hybrid grouper. The change of feed composition will affect the nutritional composition of the whole body of fish. In this study, the YC diet had a positive influence on the protein, lipid, and ash content in the whole body of fish, which may be due to the addition of YC. YC promotes the breakdown, synthesis, digestion, and absorption of diet nutrition in the gastrointestinal tract, improving the utilization of nutrition and resulting in increased deposition of nutrition. Changes in protein and lipid content may be related to the synthesis of nutrition components and the rate of deposition, which in turn affects the changes in ash content. Similar results were observed in tilapia fed with yeast diets (Abdel-Tawwab et al., 2008), which further indicated that yeast could enhance the physiology of aquatic animals. From all above, it was plausible to state that dietary

supplementation of YC could increase intracellular enzyme production, and then promote the nutrient composition and growth performance of hybrid grouper.

Serum analysis plays an essential role in the nutrition, physiology, and health of aquatic organisms, which can indirectly reflect the health degree of aquatic animals (Hoseinifar et al., 2011). GLU can be used as a stress indicator of fish, with high blood GLU often indicative of a high level of stress (Berto et al., 2016). In the present study, dietary addition of YC decreased the serum GLU content, which may be due to the presence of β -glucan (an immunostimulant) in the YC diet. In animals, β -glucan could lower serum GLU by activating the PI3K/AKT pathway (Ma et al., 2015). β -glucans stimulated several PI3K/AKT receptors (such as Dectin-1, complement receptor 3, and toll-like receptors) to induce specific signal pathways as a sign of serum GLU reduction (Huth et al., 2000). When β -glucan was administered to fish diet, GLU content was not elevated, which invariably shows that the dietary application of β -glucan has no stress effect on fish (Misra et al., 2006). Serum TG and CHO, as components of blood lipids, are important indicators of lipid levels in fish, which reflects the metabolism, and increases energy storage of lipids in fish. High contents of TG and CHO can cause damage to the hepatopancreas of fish (Agrahar et al., 2007). In our study, when hybrid grouper was fed with YC, no significant difference was found among the other groups except for the significant TG increase in the N1 group. There was no significant difference in CHO among groups. Previous research has demonstrated that the application of yeast products contributes to decreasing both CHO and TG content in aquatic animals (Bell et al., 1999), which contradicts our results. It may be due to the non-significant effect of YC on lipid anabolism in hybrid grouper. However, the specific mechanism needs to be further investigated.

Oxidative stress, as a frequent stress response in fish, leads to a series of destructive processes (such as membrane damage and accumulation of lipid peroxidation products). As an endogenous antioxidant enzyme, SOD can improve the level of immune response and enhance the body's resistance (Reyes-Cerpa, 2018). SOD scavenges superoxide anions to produce H_2O_2 , which constitutes the first line of defense against antioxidant enzymes (Li et al., 2015). CAT, as an antioxidant enzyme, can further convert SOD-produced H_2O_2 into water and oxygen in aquatic animals' immune systems (Wang et al., 2017). MDA, the primary marker of lipid peroxidation, reflects the extent of lipid oxidation (Xu et al., 2015). Fish serum CAT and SOD activities were significantly high, while serum MDA content was significantly lower in the N2 (4%) group than those in the control group. Conforming with this study, the addition of YC to USSURI catfish (*Pseudobagrus ussuriensis*) feed could improve the antioxidant capacity, as characterized by the enhanced serum CAT and SOD activity, and decreased MDA content (Bu et al., 2019). In addition, it has been shown that feeding grass carp (*Ctenopharyngodon idella*) with yeast hydrolysate can enhance the activity of CAT and SOD in the fish (Yuan et al., 2019). Yeast cells contain vitamins (Ghazanar et al., 2015), which may enhance the immune system and promote the growth of aquatic animals (Zhang et al., 2019). Vitamin B6 compounds inhibit lipid peroxidation by scavenging oxygen free radicals (Kannan and Jain, 2004). AST and ALT are soluble enzymes present in the cytoplasm of liver cells, and their elevated activity in serum reflects liver damage (Du et al., 2017). As shown by the results of this study, YC could effectively reduce serum ALT and AST activity, which corresponds to the results of grass carp fed with YC (Liu et al., 2018). A series of active metabolism in the liver may account for this result. According to this study, dietary addition of YC can not only protect the liver, but also improve the antioxidant, and immune system of hybrid grouper.

Improving the innate immune system of fish plays a significant role in protecting them against disease infections (Lu et al., 2019). Innate immune parameters (such as LZM, AKP, ACP, TP, and IgM) were used to monitor the non-specific immunity of fish. TP, as a crucial component of the innate immune system, helps to protect fish from potentially invasive organisms (Soltanzadeh et al., 2016). In our study, serum TP activity was remarkably increased in the N2 (4%) group, which was

consistent with the report that notably enhanced serum TP modulated immune activities in gibel carp (*Carassius auratus gibelio cas III*) (Zhang et al., 2018). ACP and AKP, as important lysosomal enzymes in fish, play a crucial role in the immune response through hydrolysis and digestion of invaded pathogen (He et al., 2017). In fact, elevated ACP and AKP activities in the fish are indicative of strong immunity. In many cases, ACP and AKP are considered to be powerful markers for macrophage activation of the immune system (Jin et al., 2018). In agreement with our study, dietary supplementation of MOS in hybrid grouper stimulates AKP activity in fish (Ren et al., 2020). LZM, a useful innate immune enzyme, can lyse and digest infectious bacteria. Numerous studies related to aquatic animals have shown that yeast products exert positive effects on innate immune responses (Abu-Elala et al., 2013; Jin et al., 2018; Bu et al., 2019). In Ussuri catfish (*Pseudobagrus ussuriensis*), dietary YC supplemented at 10 g/kg improved plasma ACP, AKP, and serum LZM (Bu et al., 2019), which indicated improved innate immune response. Tukmechi and Bandboni (2014) reported that dietary supplementation of two combined yeast products in rainbow trout (*Oncorhynchus mykiss*) could improve the innate immune response (Tukmechi and Bandboni, 2014). Moreover, IgM, a useful immunoglobulin marker, has been used in evaluating fish immune response (Ortuno et al., 2002). In our results, the notably elevated serum IgM activity in the N2 (4%) group also indicated that dietary supplementation of 4% YC could enhance the non-specific immune response in hybrid grouper. Similarly, dietary addition of yeast may help to enhance the non-specific immune response in Atlantic salmon (*Salmo salar*) (Reyes-Cerpa et al., 2018) and gilthead seabream (*Sparus auratus*) (Ortuno et al., 2002). This beneficial effect could attribute to the immunostimulant property exhibited by the YC diet. Functional components of yeast (such as β -glucan and mannan-oligosaccharide) have been identified to stimulate fish immunity (Jayachandran et al., 2018). β -glucan can bind to receptor molecules on the surface of tissue phagocytes. This increases the phagocytic activity of killing and digesting bacteria (Abu-Elala et al., 2013). Dietary supplementation of β -glucan improves the innate immune response in Atlantic salmon (Vetvicka et al., 2013). Our results demonstrated a significant increase in serum ACP, AKP, and IgM activity in fish in the N2 treated group after the challenge, which suggested that dietary supplementation of 4% YC could improve the non-specific immune defense capacity of the hybrid grouper.

It is essential to understand the relationship between the host and its intestinal bacteria for the successful and efficient production of cultured fish. The intestinal microbiota composes of pathogenic and beneficial bacteria that influence fish growth and physiology. In our study, the alpha diversity indexes (OTU and ACE) were significantly higher in the N2 (4%) group than those in the control group. Consistently, dietary addition of yeast products has been shown to increase the gut microbial diversity of red drum (*Sciaenops ocellatus*) and fathead minnow (*Pimephales promelas*) (Burr et al., 2008; Lochmann et al., 2011). Dominant bacteria detected in all treatment groups at the phylum level were *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*, which is consistent with the results of existing studies (Liu et al., 2020). *Proteobacteria*, the largest phylum bacteria in the gut of marine fish, have been involved in metabolic activities (such as immune regulation, and nitrogen and carbon fixation) (Vikram et al., 2016). The rumen microbes in *Firmicutes* could degrade the fiber content of the ration (Backhed et al., 2004). *Actinobacteria* and *Bacteroidetes* not only promote the absorption of nutrients by the host but also inhibit the colonization of other harmful bacteria in the gut (Binda et al., 2018; Yang et al., 2020). In this experiment, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* were found higher in the YC treatment group than those in the control group. Similar results have been reported in grass carp (Liu et al., 2018) and mice (Geurts et al., 2014). In this study, beneficial bacteria in the YC treatment group showed remarkably higher relative abundance than those in the control group at the genus level. *Sphingomonas* was high in the N2 (4%) group, with no significant difference among the three groups ($P > 0.05$). *Sphingomonas*, the beneficial bacteria, was used as a

probiotic to inhibit the virulence of *Vibrio anguillarum* in *Labeo rohita* (Liu et al., 2020). From all above, the YC diet can promote the growth of *Sphingomonas* bacteria, thus producing antimicrobial substances to inhibit pathogens infection in the hybrid grouper. *Blautia*, a Gram-positive bacterium, produces metabolites such as acetic acid and lactic acid, and its increased level is associated with host intestinal health (Liu et al., 2008). In addition, *Lactobacillus* was identified significantly higher in fish in the YC supplemented groups, especially in the N2 (4%) treatment group. As a probiotic, *Lactobacillus* produces sticky substances in the intestine that protects the host from pathogenic infection (Reunanen et al., 2012; He et al., 2017). Furthermore, *Lactobacillus* could produce exogenous enzymes, which could increase the digestion and growth performance of fish (Ziaei-Nejad et al., 2006; Akter et al., 2019). Our results showed that dietary addition of YC can promote the growth of beneficial microorganisms in the gut of hybrid grouper, which provides a safeguard for the feed utilization, growth performance, and immunity protection of the fish. In general, the result of the intestinal microbiota composition of hybrid grouper demonstrated that significant change was associated with the dietary protein source, which may influence the digestion and immune responses. A higher level of YC protein diet had a positive influence on the digestion and immune response of hybrid grouper.

The outbreak of infectious diseases is a fundamental problem affecting the fish aquaculture industry (Flores-Miranda et al., 2011). *Vibrio* is the pathogen of the outbreak of grouper infection, and its high mortality rate has caused severe economic losses to farmers in intensive farming (Fan et al., 2019). It is a valuable method to evaluate fish's resistance to diseases by feeding fish with specific feeds and challenging them with bacterial pathogens. In this study, fish fed with dietary YC indicated significant defense against *V. harveyi* infection, particularly in the N2 (4%) group. These outcomes confirm with those who fed YC to shrimps at a 1% diet (Burgents et al., 2004). The results of Burgents et al. (2004) also show that dietary administration of YC could protect shrimp against a decline in resistance to bacterial disease (Burgents et al., 2004), which indicates that YC could enhance shrimp immune response to pathogenic infection. In another study, no mortality after 9 weeks in hybrid striped bass (*Morone chrysops* $\times$ *M. saxatilis*) fed with a 4% brewer yeast-strengthened diet was found (Li and Gatlin, 2003).

The intestinal tract's morphology and structure are crucial for the maintenance of normal intestinal function. The villus length and muscle thickness reflect the function of the intestinal wall to a certain extent (Al-Fataftah and Abdelqader, 2014; Fang et al., 2019). YC diet notably increased villus height and width of hybrid grouper, thus promoting the growth. In related work, dietary yeast hydrolysates in juvenile Jian carp increased villus height and enhanced fish growth (Yuan et al., 2019). Besides, the intestinal villus length of *L. vannamei* was increased due to the dietary addition of MOS (Zhang et al., 2012), which indicated that MOS in feed provided nutrients and promoted the growth of intestinal villi. In this study, the growth of intestinal villi surface area may be attributed to MOS components in YC.

5. Conclusion

In conclusion, this study indicated that YC products in a hybrid grouper diet could significantly improve the growth performance and serum immune responses against *V. harveyi* attacks. Furthermore, it positively influences intestinal bacterial composition and morphology. In summary, dietary supplementation of N2 (4%) YC is recommended for hybrid grouper (*Epinephelus fuscoguttatus* $\times$ *E. lanceolatus*).

Funding

This work was supported by the National Key R&D Program of China (2019YFD0900200), National Science Foundation of China (No. 31772864), Guangdong province enterprise sci-tech commissioner (GDKTP2020017800), and Natural Science Foundation of Guangdong

Province (2018A030313154 & 2020A1515011129).

Author contributions

All authors have actively contributed to the study. The author's contributions are as follows: H.Y. Liu designed the study; Qi Wang and S. Ayiku conducted the study and analyzed the data; X.H. Dong participated in the interpretation of the results; Qi Wang and S. Ayiku wrote the manuscript; Q.H. Yang, S.Y. Chi, S. Zhang and W.H. Zhou purchased the reagent supplies; H.Y. Liu revised the manuscript. All authors read and approved the final manuscript.

Ethics statement

The animal protocol was approved by the ethics review board of the Guangdong Ocean University. All procedures were performed by the standards of the National Institutes of Health guide for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978) and relevant Chinese policies.

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.

Acknowledgments

We would like to thank the Beijing Enhalar International Tech Co., Ltd (Zhanjiang, China) for kindly providing yeast in this experiment.

References

- Abdel-Tawwab, M., Abdel-Rahman, A.M., Ismael, N.E.M., 2008. Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. *Aquaculture* 280 (1–4), 185–189. <https://doi.org/10.1016/j.aquaculture.2008.03.055>.
- Abu-Elala, N., Marzouk, M., Moustafa, M., 2013. Use of different *Saccharomyces cerevisiae* biotic forms as immune-modulator and growth promoter for *Oreochromis niloticus* challenged with some fish pathogens. *Int. J. Vet. Sci. Med.* 1 (1), 21–29. <https://doi.org/10.1016/j.ijvsm.2013.05.001>.
- Agrahar, S., Pandey, K.C., Gopal, K., 2007. Biochemical alteration induced by monochrotophos in the blood plasma of fish, *channa punctatus* (Bloch). *Pestic. Biochem. Phys.* 88 (3), 268–272. <https://doi.org/10.1016/j.pestbp.2007.01.001>.
- Akter, M.N., Hashim, R., Sutriana, A., Azizah, M.N.S., Asaduzzaman, M., 2019. Effect of lactobacillus acidophilus supplementation on growth performances, digestive enzyme activities and gut histomorphology of striped catfish (*Pangasianodon hypophthalmus*), 1878 juveniles. *Aquac. Res.* 50 (3), 786–797. <https://doi.org/10.1111/are.13938>.
- Al-Fataftah, A.R., Abdelqader, A., 2014. Effects of dietary bacillus subtilis on heat-stressed broilers performance, intestinal morphology and microflora composition. *Anim. Feed Sci. Technol.* 198, 279–285. <https://doi.org/10.1016/j.anifeedsci.2014.10.012>.
- Amaya, E.A., Davis, D.A., Rouse, D.B., 2007. Replacement of fish meal in practical diets for the pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. *Aquaculture* 262 (2–4), 393–401. <https://doi.org/10.1016/j.aquaculture.2006.11.015>.
- Backhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Gordon, J.I., 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA* 101 (44), 15718–15723. <https://doi.org/10.1073/pnas.0407076101>.
- Bell, S., Goldman, V.M., Bistrian, B.R., Arnold, A.H., Ostroff, G., Forse, R.A., 1999. Effect of β -glucan from oats and yeast on serum lipids. *Crit. Rev. Food Sci. Nutr.* 39 (2), 189–202. <https://doi.org/10.1080/10408399908500493>.
- Berto, R.D., Pereira, G.D., Mourino, J.L.P., Martins, M.L., Fracalossi, D.M., 2016. Yeast extract on growth, nutrient utilization and haemato-immunological responses of Nile tilapia. *Aquac. Res.* 47 (8), 2650–2660. <https://doi.org/10.1111/are.12715>.
- Binda, C., Lopetuso, L.R., Rizzatti, G., Gibiino, G., Cennamo, V., Gasbarrini, A., 2018. Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Dig. Liver Dis.* 50 (5), 421–428. <https://doi.org/10.1016/j.dld.2018.02.012>.
- Bu, X.Y., Lian, X.Q., Wang, Y., Luo, C.Z., Tao, S.Q., Liao, Y.L., Yang, Y.H., 2019. Dietary yeast culture modulates immune response related to TLR2-MYD88-NK- κ B signaling pathway, antioxidant capability and disease resistance against *Aeromonas hydrophila* for Ussuri catfish (*Pseudobagrus ussuriensis*). *Fish Shellfish Immunol.* 84, 711–718. <https://doi.org/10.1016/j.fsi.2018.10.049>.
- Bullerwell, C.N., Collins, S.A., Lall, S.P., Anderson, D.M., 2016. Growth performance, proximate and histological analysis of rainbow trout fed diets containing Camelina sativa seeds, meal (high-oil and solvent-extracted) and oil. *Aquaculture* 452, 342–350. <https://doi.org/10.1016/j.aquaculture.2015.11.008>.
- Burgerts, J.E., Burnett, K.G., Burnett, L.E., 2004. Disease resistance of Pacific white shrimp, *Litopenaeus vannamei*, following the dietary administration of a yeast culture food supplement. *Aquaculture* 231 (1–4), 1–8. <https://doi.org/10.1016/j.aquaculture.2003.09.003>.
- Burr, G., Hume, M., Ricke, S., Nisbet, D., Gatlin III, D., 2008. A preliminary in vitro assessment of GroBiotic (R)-A, brewer's yeast and fructooligosaccharide as prebiotics for the red drum *Sciaenops ocellatus*. *J. Environ. Sci. Health B* 43 (3), 253–260. <https://doi.org/10.1080/03601230701771438>.
- Deng, D., Mei, C.F., Mai, K.S., Tan, B.P., Ai, Q.H., Ma, H.M., 2013. Effects of a yeast-based additive on growth and immune responses of white shrimp, *Litopenaeus vannamei* (Boone, 1931), and aquaculture environment. *Aquac. Res.* 44 (9), 1348–1357. <https://doi.org/10.1111/j.1365-2109.2012.03139.x>.
- Dimitroglou, A., Merrifield, D.L., Spring, P., Sweetman, J., Moate, R., Davies, S.J., 2010. Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture* 300 (1–4), 182–188. <https://doi.org/10.1016/j.aquaculture.2010.01.015>.
- Du, J.L., Cao, L.P., Jia, R., Yin, G.J., 2017. Hepatoprotective and antioxidant effects of dietary glycyrrhiza polysaccharide against tcd-induced hepatic injury and rt-pcr quantification of AHR2, ARNT2, CYP1A mRNA in Jian Carp (*Cyprinus carpio* var. Jian). *J. Environ. Sci.* 51, 181–190. <https://doi.org/10.1016/j.jes.2016.06.026>.
- Edgar, R.C., 2013. Uparse: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10 (10), 996–998. <https://doi.org/10.1038/nmeth.2604>.
- Essa, M.A., Mabrouk, H.A., Mohamed, R.A., Michael, F.R., 2011. Evaluating different additive levels of yeast, *Saccharomyces cerevisiae*, on the growth and production performances of a hybrid of two populations of Egyptian African catfish, *Clarias gariepinus*. *Aquaculture* 320 (1–2), 137–141. <https://doi.org/10.1016/j.aquaculture.2011.08.015>.
- Fan, J.Q., Chen, L.M., Mai, G.Q., Zhang, H.R., Yang, J.F., Deng, D., Ma, Y.F., 2019. Dynamics of the gut microbiota in developmental stages of *Litopenaeus vannamei* reveal its association with body weight. *Sci. Rep.* 9 (1), 734. <https://doi.org/10.1038/s41598-018-37042-3>.
- Fang, H.H., Xie, J.J., Liao, S.Y., Guo, T.Y., Xie, S.W., Liu, Y.J., Niu, J., 2019. Effects of dietary inclusion of shrimp paste on growth performance, digestive enzymes activities, antioxidant and immunological status and intestinal morphology of hybrid snakehead (*Channa maculata* × *Channa argus*). *Front. Physiol.* 10, 1027. <https://doi.org/10.3389/fphys.2019.01027>.
- Flores-Miranda, M.D., Luna-Gonzalez, A., Campa-Cordova, A.I., Gonzalez-Ocampo, H.A., Fierro-Coronado, J.A., Partida-Arangure, B.O., 2011. Microbial immunostimulants reduce mortality in whiteleg shrimp (*Litopenaeus vannamei*) challenged with *Vibrio* squaloensis strains. *Aquaculture* 320 (1–2), 51–55. <https://doi.org/10.1016/j.aquaculture.2011.08.005>.
- Gainza, O., Romero, J., 2020. Effect of mannan oligosaccharides on the microbiota and productivity parameters of *Litopenaeus vannamei* shrimp under intensive cultivation in Ecuador. *Sci. Rep.* 10 (1), 2719. <https://doi.org/10.1038/s41598-020-59587-y>.
- Geurts, L., Neyrinck, A.M., Delzenne, N.M., Knauf, C., Cani, P.D., 2014. Gut microbiota controls adipose tissue expansion, gut barrier and glucose metabolism: novel insights into molecular targets and interventions using prebiotics. *Benef. Microbes* 5 (1), 3–17. <https://doi.org/10.3920/bm2012.0065C>.
- Gupta, S., Feckaninova, A., Lokesh, J., Koskova, J., Sorensen, M., Fernandes, J., Kiron, V., 2019. Lactobacillus dominate in the intestine of Atlantic salmon fed dietary probiotics. *Front. Microbiol.* 9, 1–19. <https://doi.org/10.3389/fmicb.2018.03247>.
- He, R.P., Feng, J., Tian, X.L., Dong, S.L., Wen, B., 2017. Effects of dietary supplementation of probiotics on the growth, activities of digestive and non-specific immune enzymes in hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*). *Aquac. Res.* 48 (12), 5782–5790. <https://doi.org/10.1111/are.13401>.
- Hoseinifar, S.H., Mirvaghefi, A., Merrifield, D.L., Amiri, B.M., Yelghi, S., Bastami, K.D., 2011. The study of some haematological and serum biochemical parameters of juvenile beluga (*Huso huso*) fed oligofructose. *Fish Physiol. Biochem.* 37 (1), 91–96. <https://doi.org/10.1007/s10695-010-9420-9>.
- Huth, M., Dongowski, G., Gebhardt, E., Flamme, W., 2000. Functional properties of dietary fibre enriched extrudates from barley. *J. Cereal Sci.* 32 (2), 115–128. <https://doi.org/10.1007/s10106/jcsc.2000.0330>.
- Jayachandran, M., Chen, J., Chung, S.S.M., Xu, B., 2018. A critical review on the impacts of beta-glucans on gut microbiota and human health. *J. Nutr. Biochem.* 61, 101–110. <https://doi.org/10.1016/j.jnutbio.2018.06.010>.
- Jin, M., Xiong, J., Zhou, Q.C., Yuan, Y., Wang, X.X., Sun, P., 2018. Dietary yeast hydrolysate and brewer's yeast supplementation could enhance growth performance, innate immunity capacity and ammonia nitrogen stress resistance ability of Pacific white shrimp (*Litopenaeus vannamei*). *Fish Shellfish Immunol.* 82, 121–129. <https://doi.org/10.1016/j.fsi.2018.08.020>.
- Kannan, K., Jain, S.K., 2004. Effect of vitamin B-6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H₂O₂-treated u937 monocytes. *Free Radic. Biol. Med.* 36 (4), 423–428. <https://doi.org/10.1016/j.freeradbiomed.2003.09.012>.
- Lee, M.H., 1995. Official methods of analysis of AOAC international (16th edn). *Trends Food Technol.* 6, 382. [https://doi.org/10.1016/0924-2244\(95\)90022-5](https://doi.org/10.1016/0924-2244(95)90022-5).
- Leung, K.C., Huang, Q., St-Hilaire, S., Liu, H., Zheng, X., Cheung, K.B., Zwetsloot, I.M., 2020. Fraudulent antibiotic products on the market for aquaculture use. *Prev. Vet. Med.* 181, 105052. <https://doi.org/10.1016/j.prevetmed.2020.105052>.

- Li, J.G., Xu, Y.P., Jin, L.J., Li, X.Y., 2015. Effects of a probiotic mixture (*Bacillus subtilis* YB-1 and *Bacillus cereus* YB-2) on disease resistance and non-specific immunity of sea cucumber, *Apostichopus japonicus* (*selenka*). *Aquac. Res.* 46 (12), 3008–3019. <https://doi.org/10.1111/are.12453>.
- Li, P., Gatlin M., D., 2003. Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* × *M.saxatilis*). *Aquaculture* 219 (1–4), 681–692. [https://doi.org/10.1016/S0044-8486\(02\)00653-1](https://doi.org/10.1016/S0044-8486(02)00653-1).
- Liu, C.X., Finegold, S.M., Song, Y.J., Lawson, P.A., 2008. Reclassification of clostridium coccoides, ruminococcus hansenii, ruminococcus hydrogenotrophicus, ruminococcus luti, ruminococcus productus and ruminococcus schinkii as blautia coccoides gen. Nov., comb. Nov., blautia hansenii comb. Nov., blautia hydrogenotrophica comb. Nov., blautia luti comb. Nov., blautia producta comb. Nov., blautia schinkii comb. Nov. and description of blautia wexlerae sp nov., isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 58, 1896–1902. <https://doi.org/10.1099/ijs.0.65208-0>.
- Liu, H., Li, J.T., Guo, X.W., Liang, Y.X., Wang, W.M., 2018. Yeast culture dietary supplementation modulates gut microbiota, growth and biochemical parameters of grass carp. *Microb. Biotechnol.* 11 (3), 551–565 <https://doi.org/10.1751-7915.13261>.
- Liu, X.C., Shi, H.R., He, Q., Lin, F.M., Wang, Q., Xiao, S.Q., Zhao, H.H., 2020. Effect of starvation and refeeding on growth, gut microbiota and non-specific immunity in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Fish Shellfish Immunol.* 97, 182–193. <https://doi.org/10.1016/j.fsi.2019.11.055>.
- Lochmann, R., Phillips, H., Xie, L., 2011. Effects of a dairy-yeast prebiotic and water hardness on the growth performance, mineral composition and gut microflora of fathead minnow (*Pimephales promelas*) in recirculating systems. *Aquaculture* 320 (1–2), 76–81. <https://doi.org/10.1016/j.aquaculture.2011.08.004>.
- Lu, J.T., Qi, C.L., Limbu, S.M., Han, F.L., Yang, L., Wang, X.D., Chen, L.Q., 2019. Dietary mannan oligosaccharide (MOS) improves growth performance, antioxidant capacity, non-specific immunity and intestinal histology of juvenile Chinese mitten crabs (*Eriocheir sinensis*). *Aquaculture* 510, 337–346. <https://doi.org/10.1016/j.aquaculture.2019.05.048>.
- Ma, T., Tu, Y., Zhang, N.F., Guo, J.P., Deng, K.D., Zhou, Y., Dia, Q.Y., 2015. Effects of dietary yeast beta-glucan on nutrient digestibility and serum profiles in pre-ruminant holstein calves. *J. Integr. Agric.* 14 (4), 749–757. [https://doi.org/10.1016/S2095-3119\(14\)60843-1](https://doi.org/10.1016/S2095-3119(14)60843-1).
- Misra, C.K., Das, B.K., Mukherjee, S.C., Pattnaik, P., 2006. Effect of long term administration of dietary beta-glucan on immunity, growth and survival of Labeo rohita fingerlings. *Aquaculture* 255 (1–4), 82–94. <https://doi.org/10.1016/j.aquaculture.2005.12.009>.
- Ortuno, J., Cuesta, A., Rodriguez, A., Esteban, M.A., Meseguer, J., 2002. Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L). *Vet. Immunol. Immunopathol.* 85 (1–2), 41–50. [https://doi.org/10.1016/S0165-2427\(01\)00406-8](https://doi.org/10.1016/S0165-2427(01)00406-8).
- Pongpet, J., Ponchunchoovong, S., Payoocha, K., 2016. Partial replacement of fishmeal by brewer's yeast (*Saccharomyces cerevisiae*) in the diets of thai panga (*Pangasianodon hypophthalmus* × *Pangasius bocourti*). *Aquac. Nutr.* 22 (3), 575–585. <https://doi.org/10.1111/anu.12280>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Glockner, F.O., 2013. The SILVA ribosomal rna gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41 (D1), D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- Ren, Z.L., Wang, S.F., Cai, Y., Wu, Y., Tian, L.J., Wang, S.Q., Zhou, Y.C., 2020. Effects of dietary mannan oligosaccharide supplementation on growth performance, antioxidant capacity, non-specific immunity and immune-related gene expression of juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Aquaculture* 523, 735195. <https://doi.org/10.1016/j.aquaculture.2020.735195>.
- Reunanen, J., von Ossowski, I., Hendrickx, A.P.A., Palva, A., Vos, W.M., 2012. Characterization of the spacia pilus fibers in the probiotic lactobacillus rhamnosus gg. *Appl. Environ. Microbiol.* 78 (7), 2337–2344. <https://doi.org/10.1128/aem.07047-11>.
- Reyes-Becerril, M., Tovar-Ramirez, D., Ascencio-Valle, F., Civera-Cerecedo, R., Gracia-Lopez, V., Barbosa-Solomieu, V., Esteban, M.A., 2011. Effects of dietary supplementation with probiotic live yeast *Debaryomyces hansenii* on the immune and antioxidant systems of leopard grouper *Mycteroperca rosacea* infected with *Aeromonas hydrophila*. *Aquac. Res.* 42 (11), 1676–1686. <https://doi.org/10.1111/j.1365-2109.2010.02762.x>.
- Reyes-Cerpa, S., Vallejos-Vidal, E., Jose Gonzalez-Brown, M., Morales-Reyes, J., Perez-Stuardo, D., Vargas, D., Reyes-Lopez, F.E., 2018. Effect of yeast (*Xanthophyllomyces dendrorhous*) and plant (Saint john's wort, lemon balm, and rosemary) extract based functional diets on antioxidant and immune status of Atlantic salmon (*Salmo salar*) subjected to crowding stress. *Fish Shellfish Immunol.* 74, 250–259. <https://doi.org/10.1016/j.fsi.2017.12.061>.
- Sado, R.Y., Bicudo, A.J.A., Cyrino, J.E.P., 2014. Growth and intestinal morphology of juvenile pacu *Piaractus mesopotamicus* (Holmberg 1887) fed dietary prebiotics (mannanoligosaccharides -MOS). *An. Acad. Bras. Cienc.* 86 (3), 1517–1523. <https://doi.org/10.1590/0001-3765201420130088>.
- Soltanzadeh, S., Fereidouni, A.E., Ouraji, H., Khalili, K.J., 2016. Growth performance, body composition, hematological, and serum biochemical responses of beluga (*Huso huso*) juveniles to different dietary inclusion levels of faba bean (*Vicia faba*) meal. *Aquac. Int.* 24 (1), 395–413. <https://doi.org/10.1007/s10499-015-9933-4>.
- Suo, Y.T., Li, E.C., Li, T.Y., Jia, Y.Y., Qin, J.G., Gu, Z.M., Chen, L.Q., 2017. Response of gut health and microbiota to sulfide exposure in pacific white shrimp *Litopenaeus vannamei*. *Fish Shellfish Immunol.* 63, 87–96. <https://doi.org/10.1016/j.fsi.2017.02.008>.
- Teuber, M., 2001. Veterinary use and antibiotic resistance. *Curr. Opin. Microbiol.* 4 (5), 493–499. [https://doi.org/10.1016/S1369-5274\(00\)00241-1](https://doi.org/10.1016/S1369-5274(00)00241-1).
- Tovar, D., Zambonino, J., Cahu, C., Gatosoupe, F.J., Vazquez-Juarez, R., Lesel, R., 2002. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture* 204 (1–2), 113–123. [https://doi.org/10.1016/S0044-8486\(01\)00650-0](https://doi.org/10.1016/S0044-8486(01)00650-0).
- Tukmechi, A., Bandboni, M., 2014. Effects of *Saccharomyces cerevisiae* supplementation on immune response, hematological parameters, body composition and disease resistance in rainbow trout, *Oncorhynchus mykiss* (walbaum, 1792). *J. Appl. Ichthyol.* 30 (1), 55–61. <https://doi.org/10.1111/jai.12314>.
- Vetvicka, V., Vannucci, L., Sima, P., 2013. The effects of β - glucan on fish immunity. *N. Am. J. Med. Sci.* 5 (10), 580–588. <https://doi.org/10.4103/1947-2714.120792>.
- Vikram, S., Guerrero, L.D., Makhallanyane, T.P., Le, P.T., Seely, M., Cowan, D.A., 2016. Metagenomic analysis provides insights into functional capacity in a hyperarid desert soil niche community. *Environ. Microbiol.* 18 (6), 1875–1888. <https://doi.org/10.1111/1462-2920.13088>.
- Wang, J.X., Liang, D.Z., Yang, Q.H., Tan, B.P., Dong, X.H., Chi, S.Y., Zhang, S.G., 2020. The effect of partial replacement of fish meal by soy protein concentrate on growth performance, immune responses, gut morphology and intestinal inflammation for juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Fish Shellfish Immunol.* 98, 619–631. <https://doi.org/10.1016/j.fsi.2019.10.025>.
- Wang, L.T., Ge, C.X., Wang, J.C., Dai, J., Zhang, P.J., Li, Y.H., 2017. Effects of different combinations of bacillus on immunity and antioxidant activities in common carp. *Aquac. Int.* 25 (6), 2091–2099. <https://doi.org/10.1007/s10499-017-0175-5>.
- Xia, J.H., Lin, G., Fu, G.H., Wan, Z.Y., Lee, M., Wang, L., Yue, G.H., 2014. The intestinal microbiome of fish under starvation. *Bmc Genom.* 15, 1–11. <https://doi.org/10.1186/1471-2164-15-266>.
- Xu, L., Ran, C., He, S., Zhang, J., Hu, J., Yang, Y., Zhou, Z., 2015. Effects of dietary yeast nucleotides on growth, non-specific immunity, intestine growth and intestinal microbiota of juvenile hybrid tilapia *Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂. *Anim. Nutr.* 1 (3), 244–251. <https://doi.org/10.1016/j.aninu.2015.08.006>.
- Yang, X.Y., Dong, X.H., Yang, Q.H., Wang, G.H., Zhao, X.M., Liu, H.Y., Tan, B.P., 2020. Addition of enzyme-digested hydrolyzed porcine mucosa to low-fishmeal feed improves growth, intestinal microbiota, and intestinal peptide and amino acid transporter expressions in hybrid groupers (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Aquac. Nutr.* 27 (2), 366–385. <https://doi.org/10.1111/anu.13190>.
- Ye, H.Q., Zhou, Y.Y., Su, N.N., Wang, A.L., Tan, X.H., Sun, Z.Z., Ye, C.X., 2019. Effects of replacing fish meal with rendered animal protein blend on growth performance, hepatic steatosis and immune status in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Aquaculture* 511, 734203. <https://doi.org/10.1016/j.aquaculture.2019.734203>.
- Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., Zhang, S., 2020. Preliminary study of mechanisms of intestinal inflammation induced by plant proteins in juvenile hybrid groupers (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Fish Shellfish Immunol.* 106, 341–356. <https://doi.org/10.1016/j.fsi.2020.07.026>.
- Yuan, X.Y., Jiang, G.Z., Wang, C.C., Abasubong, K.P., Zou, Q., Zhou, Y.Y., Liu, W.B., 2019. Effects of partial replacement of fish meal by yeast hydrolysate on antioxidant capability, intestinal morphology, and inflammation-related gene expression of juvenile jian carp (*Cyprinus carpio* var. Jian). *Fish Physiol. Biochem.* 45 (1), 187–197. <https://doi.org/10.1007/s10695-018-0552-7c>.
- Zhang, J., Liu, Y.J., Tian, L.X., Yang, H.J., Liang, G.Y., Xu, D.H., 2012. Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* 33 (4), 1027–1032. <https://doi.org/10.1016/j.fsi.2012.05.001>.
- Zhang, P.Y., Cao, S.P., Zou, T., Han, D., Liu, H.K., Jin, J.Y., Zhou, W.H., 2018. Effects of dietary yeast culture on growth performance, immune response and disease resistance of gibel carp (*Carassius auratus gibelio cas III*). *Fish Shellfish Immunol.* 82, 400–407. <https://doi.org/10.1016/j.fsi.2018.08.044>.
- Zhang, R.Q., Jiang, Y., Zhou, L., Chen, Y.P., Wen, C., Liu, W.B., Zhou, Y.M., 2019. Effects of dietary yeast extract supplementation on growth, body composition, non-specific immunity, and antioxidant status of Chinese Mitten Crab (*Eriocheir sinensis*). *Fish Shellfish Immunol.* 86, 1019–1025. <https://doi.org/10.1016/j.fsi.2018.12.052c>.
- Zhao, Y.C., Ma, H.M., Zhang, W.B., Ai, Q.H., Mai, K., Xu, W., Liufu, Z.G., 2011. Effects of dietary beta-glucan on the growth, immune responses and resistance of sea cucumber, *Apostichopus japonicus* against vibrio splendidus infection. *Aquaculture* 315 (3–4), 269–274. <https://doi.org/10.1016/j.aquaculture.2011.02.032>.
- Zhou, Z.G., He, S.X., Liu, Y.C., Shi, P.J., Huang, G.X., Yao, B., 2009. The effects of dietary yeast culture or short-chain Fructo-oligosaccharides on the intestinal autochthonous bacterial communities in juvenile hybrid tilapia, *Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂. *J. World Aquac. Soc.* 40 (4), 450–459 <https://doi.org/10.1111/j.1749-7345.2009.00272.x>.
- Ziaei-Nejad, S., Rezaei, M.H., Takami, G.A., Lovett, D.L., Mirvaghefi, A.R., Shakouri, M., 2006. The effect of *Bacillus* spp. Bacteria used as probiotics on digestive enzyme activity, survival and growth in the indian white shrimp *Penaeus indicus*. *Aquaculture* 252 (2–4), 516–552. <https://doi.org/10.1016/j.aquaculture.2005.07.021>.