

Dietary yeast culture facilitates the growth, immune response, digestive enzyme activity, intestinal microbiota and disease resistance against *Vibrio harveyi* of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)

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Abstract

The present study aimed to investigate the effect of yeast culture (YC) supplementation at different levels (0%, 2% and 4%) on the growth, immune response, digestive enzyme activity, intestinal morphology and microbiota of hybrid grouper. After 56 days feeding trial, the results showed that the weight gain rate, specific growth rate, survival rate and protein efficiency rate of hybrid grouper were significantly improved, and the feed conversion ratio of hybrid grouper in the 4% group ($p < 0.05$) was significantly reduced. The serum total protein, acid phosphatase, alkaline phosphatase and lysozyme of hybrid grouper in the 4% group were significantly higher than those in the control group, while the alanine transaminase, aspartate transaminase and malondialdehyde were significantly lower than those in the control group ($p < 0.05$). YC treatment significantly enhanced the trypsin, lipase and amylase activities ($p < 0.05$). *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* constituted the dominant phylum in grouper intestine. The relative abundance of *Sphingomonas*, *Stenotrophomonas* and *Phyllobacterium* in the YC groups was higher than that in the control group. The length and width of intestinal villi of grouper fed with YC diets were improved ($p < 0.05$). The protection against *V. harveyi* challenge in hybrid grouper was enhanced after YC treatment group, and the hybrid groupers fed with 4% YC diets experienced the highest cumulative survival rate of 67%. All these results suggested that the supplementation of 4% YC diets improved the growth performance, digestive enzyme activity, intestinal morphology, microbiota and disease resistance against *V. harveyi* of hybrid grouper.

KEYWORDS

antioxidant capacity, growth performance, hybrid grouper, intestinal morphology, serum immunity, yeast culture

1 | INTRODUCTION

Due to environmental pollution and overexploitation of marine fishery resources, the world fishery resources are diminishing in recent years. As a result, the demand for aquatic products worldwide can only be satisfied through aquaculture (Pauly et al., 2002). Currently, intensive aquaculture remains the development direction of culture mode in the future. However, high-density intensive aquaculture tends to cause disease outbreaks, leading to the death of cultured animals (Nayak, 2010). In this context, promoting fish growth and enhancing the disease resistance have received wide concerns. It is essential for the sustainable development of the grouper industry to develop low content additives to improve disease resistance and immunity of grouper. As a new type of dietary supplements, some probiotics can replace antibiotics in aquaculture and thereby improve the health and growth of fish, which have attracted significant attention in recent years (Huang et al., 2015).

The use of yeast products to improve the growth and immune response of fish has become 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 harmful bacteria, but also promote the reproduction of beneficial bacteria and regulate the balance of micro-ecological zones in the digestive tract of animals, thus improving the functions and maintaining the integrity of the intestinal tract, as well as improving the immunity of the animal organism (Deng et al., 2013; Zhou et al., 2009). More encouragingly, the potential benefits of yeast products for aquaculture are increasing. Numerous scholars have revealed the positive effects of different yeast products on the growth performance and immune response of aquatic animals (Abass et al., 2018; Berto et al., 2016). Yeast culture (YC) is a compound yeast fermentation product composed of yeast and its growth medium, which is an ideal feed additive (Bu et al., 2019). Yeast fermentation products are rich in protein, amino acids, vitamins and trace elements, which are ideal protein sources for fish, shrimp and livestock feed. Currently, the main method used is high protein solid fermentation culture, that is adding non-protein nitrogen to the fermentation substrate to increase the protein content of the yeast fermentation products. In our study, non-protein nitrogen was added to the YC fermentation substrate, which contained 55% crude protein. YC contains protein, minerals, amino acids and immunomodulatory components such as mannan-oligosaccharides (MOS), β -glucan and nucleotides (Seo et al., 2010). The β -glucan ingredient in YC can activate macrophages and release numerous immunoglobulins, such as interleukins and cytokines, which help aquatic animals to defend against a variety of pathogens (Jayachandran et al., 2018). β -glucan has been demonstrated to exert a potent immunostimulatory effect on much freshwater and marine fish (Sealey et al., 2008; Vetvicka et al., 2013). Miest et al. (2016) have reported that the supplementation of β -glucan in the diet induces an immunomodulatory response, which can effectively increase the survival rate in turbot (*Scophthalmus maximus*) culture (Miest et al., 2016). Emerging *in vitro* studies have also unveiled that β -glucan activates a range of immune cells in mammals mainly through Toll-like

receptors (TLRs), complement receptors (CR3) and Dectin-1 receptors (Zhang et al., 2018). The addition of MOS to the diet can effectively improve the growth performance and intestinal histomorphology of fish (Dimitroglou et al., 2010; Ye et al., 2011). MOS also enhances immunity by modulating the intestinal microbiota (Ren et al., 2020; Torrecillas et al., 2011). Dietary 10^7 CFU/kg *Saccharomyces cerevisiae* can be colonized in the intestinal tract to improve the growth rate and feed efficiency of grouper, thus enhancing the resistance to streptococcus and iridovirus (Chiu et al., 2010). Besides, Ayiku et al. (2020) have shown that the addition of 2% YC in the diet can improve the growth performance, disease resistance and immune response of *Litopenaeus vannamei* (Ayiku et al., 2020b).

Hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) is a kind of marine carnivorous fish widely cultivated in southern China due to its delicious flavour and commercial importance (Ayiku et al., 2020b; Yin et al., 2018, 2020). Hybrid grouper bears the advantages of rapid growth, strong disease resistance and high nutritional value, but requires high content of fish meal in the feed because of its eating habits (Chen et al., 2020; Yang et al., 2019). Systematic studies have demonstrated the effect of YC on *Litopenaeus vannamei* (Ayiku et al., 2020a, 2020b), grass carp (*Ctenopharyngodon idella*) (Liu et al., 2018), gibel carp (*Carassius auratus gibelio* CAS III) (Zhang et al., 2018) and Ussuri catfish (*Pseudobagrus ussuriensis*) (Bu et al., 2019). However, research on YC in hybrid grouper is relatively lacking. Previous studies have shown the beneficial of YC on the growth performance, serum biochemical parameters and antioxidant capacity of hybrid grouper (Wang et al., 2019). Based on the existing studies, the present study investigated the effect of YC with non-protein nitrogen added to the fermentation substrate on the growth performance, immune response, intestinal flora and resistance to *Vibrio harveyi* infection in hybrid groupers, hoping to confer a scientific basis for the research on the healthy culture of pearl grouper and the development of environment-friendly and energy-saving feed.

2 | MATERIALS AND METHODS

2.1 | Diet preparation

The ingredients of the nutrients of the experimental diets are shown in Table 1. Fish meal, casein and wheat gluten were used as protein sources. Three iso-nitrogenous (crude protein about 50%) and iso-lipidic (crude lipid about 11%) experimental diets containing fish oil and phospholipid were prepared. YC (55% protein, fermentation substrate with non-protein nitrogen) was provided by Enhalar Biotechnology Company. Firstly, brewer's yeast was seeded into YEPD liquid medium to obtain a seed culture, and then, the seed culture was seeded into molasses medium for aerobic fermentation to obtain a liquid fermentation broth. The liquid fermentation broth was mixed with the solid medium seeded with *Aspergillus niger*, and the fermentation product was dried to produce a yeast culture. The YC used in the present study was the same as that used by Ayiku

TABLE 1 Ingredients and proximate composition of the experimental diets (DM %)

Ingredients	Treatment ^f		
	0%	2%	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 ^b	0.50	0.50	0.50
Antioxidants ^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	0.1	0.1
MCC ^a	3.22	2.72	2.22
Total	100	100	100
Nutrient levels			
Dry Matter	92.60	92.03	92.27
Crude Protein	50.57	50.36	50.72
Crude Lipid	11.25	11.34	11.50
Ash	10.89	10.17	10.18

^aIngredients purchased from Zhanjiang HaiBao Feed Factory, Zhanjiang, Guangdong, China. MCC: Microcrystalline cellulose.

^bVitamin 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. All ingredients were diluted with corn starch to 1 kg.

^cMineral mixture (mg g⁻¹ mixture): CaCO₃, 350 g; NaH₂PO₄·H₂O, 200 g; KH₂PO₄, 200 g; NaCl, 12 g; MgSO₄·7H₂O, 10 g; 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, China).

^dThe yeast products were purchased from Enhalar Biotechnology Company (Beijing, China).

^eAttractant composition: taurine: glycine: betaine = 1:3:3.

^fDiets were supplemented with YC at 0%, 2% and 4% respectively.

et al. (2020b). The nutrient composition was given with reference to the literature of Ayiku et al. (2020b). The three diets were supplemented with 0%, 2% and 4% YC respectively. All feed materials were crushed and passed through a 60-mesh sieve and then evenly mixed with fish oil, phospholipid and water in a certain proportion. Thereafter, the mixture was processed into 2.5 mm particles by a

twin-screw extruder (South China University of Technology, F-26). Finally, the feed materials were air-dried to a moisture content of 10% and stored at -20°C for use.

2.2 | Feeding trial

The hybrid groupers used in this study were obtained from a commercial hatchery (Zhanjiang, China). Prior to the feeding trial, the hybrid groupers were acclimatized to the experimental conditions and fed with a commercial feed (crude protein: 50%, crude lipid: 11%) for 2 weeks. All fish was subjected to a 24 h fasting before the experiment. There were 3 experimental groups with 3 replicates in each group. Briefly, 270 health hybrid groupers of similar size (initial weight: 17.56 ± 0.02 g) were randomly distributed into 9 fibreglass tanks (500 L), with 30 hybrid groupers in each tank. The groupers underwent satiated feeding twice a day (9:00 and 17:00) and 60% water changes daily for 8 weeks. The culture temperature was maintained at 28.0 ± 2.0°C, and the dissolved oxygen (DO) was within the range of 6–8 mg l⁻¹. Also, the culture water was used after precipitation, filtration, chlorine dioxide disinfection and aeration.

2.3 | Sample collection and calculation formula

All the fish were sampled after 24 h fasting at the end of the culture experiment. The specific growth rate (SGR), weight gain rate (WGR), survival rate (SR) and feed coefficient rate (FCR) were calculated. Two fish were randomly selected from each tank and stored at -20°C for whole-body composition analysis. The blood samples were collected from 6 groupers in each tank via tail vein using a 1 ml sterile syringe and transferred into the 1.5 ml Eppendorf tube. 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 was taken from 3 groupers in each group under aseptic condition, transferred into the 1.5 ml Eppendorf tube and immediately put into liquid nitrogen for microflora analysis. Furthermore, the midguts of another 3 groupers were collected and stored in 10% paraformaldehyde for histological analysis. Finally, 2 groupers were randomly elected from each tank and their midguts were stored in liquid nitrogen for enzyme activity analysis. The corresponding calculation formulas were as follows:

Weight gain rate (WGR, %) = 100 × (final body weight-initial body weight)/(initial body weight).

Specific growth rate (SGR, %/d) = 100 × [ln (final weight)-ln (initial weight)]/days.

Feed conversion ratio (FCR) = diet intake/wet weight gain.

Survival rate (SR, %) = 100 × (final fish number)/(initial fish number).

Protein efficiency ratio (PER) = fish weight gain/fish protein intake.

Condition factor (CF, %) = 100 × body weight/body length³.

2.4 | Analysis of diets and fish body composition

The dry matter, crude protein, lipid and ash of diet and whole fish were determined according to the methods of AOAC (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, and the crude protein content was determined using the Kjeldahl method. Moreover, the crude ash content was determined by burning in a muffle furnace at 550°C for 6 h.

2.5 | Serum and intestinal enzyme activity analysis

The supernatant of the blood samples was placed on ice for enzyme activity analysis. The levels of serum glucose (Glu), triglyceride (TG), cholesterol (CHO), catalase (CAT), superoxide dismutase (SOD), acid phosphatase (ACP), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (AKP), lysozyme (LZM), total protein (TP), malondialdehyde (MDA) and immunoglobulin M (IgM) were tested using the commercial kits (Nanjing Jiancheng Bioengineering Institute.).

The intestinal tissue sample (0.5 g) was diluted with 0.9% NaCl solution at the ratio of 1:9 (w/v). The sample was then ground on ice and centrifuged at 4°C for 10 min. Afterwards, the supernatant was transferred into an Eppendorf tube for testing. Intestinal digestive enzymes (trypsin, lipase and amylase) were determined in line with the instructions of commercial kits (Nanjing Jiancheng Biological Engineering Institute.).

2.6 | Intestinal histological analysis

In the present study, the intestinal morphological indicators were examined using the method of Ayiku et al. (2020) to determine the effect of YC on the intestinal morphology of groupers. 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. After haematoxylin-eosin staining, intestinal images were captured under a microscope (Olympus, BX51, serial number: 9K18395.). The height, width and muscle thickness of intestinal villi were measured using the Image-Pro Plus 6.3 software. A total of 12 measurements were obtained for each tissue.

2.7 | Intestinal microflora analysis

2.7.1 | DNA extraction and PCR amplification

The samples of intestinal contents were treated using the E.Z.N.A. Soil DNA kit, and the intestinal contents were extracted following

the instructions of the kit. The concentration and purity of extracted DNA were quantified using the NanoDropND-2000 spectrophotometer, and the extracted DNA was used as a template. The V3-V4 sequences of 16S rRNA gene in each sample DNA were amplified by universal primers 314F (341F: 5'-CCTACGGGNGGCWGCAG-3') and 806R (806R: 3'-GGACTACHVGGGTATCTAAT-5'). PCR reaction system (30 µl) was as follows: 15 µl Phusion Master Mix (2×), 1.5 µl upstream and downstream primers (2 µmol/L), 10 µl template DNA and 2 µl ddH₂O. The reaction procedures were as follows: pre-denaturation at 98°C for 1 min; 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s and final extension at 72°C for 5 min. The PCR products were electrophoretic with 2% agarose gel. The sequence bands between 400 and 500 bp were selected, and the gel was cut. In addition, the GeneJEF (Thermo Scientific) Gel Extraction kit was used to recover and purify the target bands.

2.7.2 | Library construction and sequencing

Libraries were constructed using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs) library building kits for library construction and then sequenced by Illumina HiSeq 2500 sequencing system.

2.7.3 | Sequencing data analysis

The removal of low-quality Reads was conducted by using Cutadapt (V1.9.1, <http://cutadapt.readthedocs.io/en/stable/>) (Asshauer et al., 2015). The sample data were separated from the Reads obtained by barcode. The reads obtained from the original data after the preliminary quality control of barcode and primer sequences need to be processed to remove the chimera sequence. The read sequence was compared with the species annotation database to detect the chimera sequence (<https://github.com/torognes/vsearch/>) (Martin,), and finally, the chimera sequence was removed to obtain the final valid data (Clean Reads). All the Clean Reads of all samples were clustered using the Uparse software (Uparse v7.0.1, <http://www.drive5.com/uparse/>) (Hass et al., 2011), and the sequence was clustered into Operational Taxonomic Units (OTUs), with 97% consistency (Identity) by default. Moreover, the representative sequence of OTUs was selected. The sequence with the highest frequency in OTUs was selected as the representative sequence of OTUs according to the principle of its algorithm. The sequence of OTUs was annotated, and the Mothur method was used to analyse the species annotation with SILVA132's (<http://www.arb-silva.de/>) (Edgar, 2013) and SSUrRNA database (Wang et al., 2007). QIIME software (Version 1.9.1) was used to calculate Chao1, Shannon, Simpson and ace indexes. The dilution curve was drawn using the R software (Version 2.15.3). The differences of microflora structure in different samples were analysed and compared by using unweighted UniFrac distance algorithm and principal coordinate analysis (PCoA).

TABLE 2 Effects of yeast culture supplementation on hybrid grouper growth performance

Treatment	IBW(g/fish)	FBW(g/fish)	WGR(g/fish)	SGR(g/fish)	FCR	SR(%)	PER(%)	CF(%)
0%	17.86 ± 0.00	78.91 ± 0.68 ^b	341.90 ± 3.59 ^b	2.65 ± 0.01 ^b	1.03 ± 0.02 ^a	92.33 ± 1.17 ^b	1.54 ± 0.04 ^b	2.77 ± 0.05 ^b
2%	17.87 ± 0.01	81.53 ± 0.76 ^{ab}	356.29 ± 4.17 ^{ab}	2.71 ± 0.02 ^{ab}	0.94 ± 0.01 ^b	94.22 ± 0.89 ^b	1.73 ± 0.22 ^a	2.87 ± 0.02 ^{ab}
4%	17.85 ± 0.04	82.32 ± 0.66 ^a	361.00 ± 3.21 ^a	2.73 ± 0.01 ^a	0.92 ± 0.05 ^b	100 ± 0.00 ^a	1.72 ± 0.04 ^a	3.10 ± 0.07 ^a

Note: The data are presented as mean ± SEM. Means in the same column with different superscripts indicate significant difference ($p < 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively.

Abbreviations: CF, Condition factor; FBW, Final body weight; FCR, Feed conversion ratio; IBW, Initial body weight; PER, Protein efficiency ratio; SGR, Specific growth rate; SR, Survival rate; WGR, Weight gain rate.

2.8 | Experimental challenge test

For the challenge test of hybrid groupers with *Vibrio harveyi*, the frozen *Vibrio harveyi* (Provincial Key Laboratory of Pathogenic Biology and Epidemiology for Aquatic Animal, Guangdong Ocean University,) was placed in a 250 ml flask containing Luria-Bertani (LB) culture medium and cultured in an incubator at 37°C and 180 r/min for 20 h. The supernatant was discarded after centrifugation at 4°C and 8000 r/min for 10 min. Subsequently, the cells were washed twice with aseptic phosphate buffer solution (PBS) and continuously diluted to 3.58×10^5 CFU/ml (LD50) with PBS. After 8 weeks of feeding trial, 12 groupers were randomly selected from each replicate group for the challenge test, with the saline injection group as the control. Each grouper was injected with 200 µl bacterial suspension and fed during the challenge period. Two fish were randomly selected 12 h after bacterial injection, and the blood samples were collected. The sample was centrifuged for 10 min at 4°C and 3500 r/min. The supernatant was collected and stored at -80°C for the determination of ACP, alkaline phosphatase (ALP), LZM and IgM. The survival rate of groupers in each experimental group was recorded 168 h after the challenge test. The formula for cumulative survival rate was as follows:

Cumulative survival rate (%)

$$= 100 \times \frac{\text{Total survival rate in each treatment after challenge}}{\text{Total number of grouper challenged for same treatment}}$$

2.9 | Statistical analysis

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

3 | RESULTS

3.1 | Growth performance

The effect of YC supplementation on the growth performance of hybrid grouper is shown in Table 2. The WGR and SGR of hybrid groupers in the 4% group were significantly higher than those of hybrid groupers in the 0% group ($p < 0.05$), but there was no significant difference between the 2% and 4% groups ($p > 0.05$). The survival rate of hybrid groupers in the 4% group was significantly higher than that of hybrid groupers in the 0% and 2% groups ($p < 0.05$). The FCR of hybrid groupers in the 2% and 4% groups was significantly lower than that of hybrid groupers in the 0% group, but there was no significant difference between the 2% and 4% groups ($p > 0.05$). The PER and CF of hybrid groupers in the 4% group were significantly higher than those of hybrid groupers in the 0% group ($p < 0.05$). However, there was no significant difference between the 2% and 4% groups ($p > 0.05$).

3.2 | Whole-body compositions

The effects of YC at different levels on the whole-body composition of hybrid grouper are shown in Table 3. Compared with those of the controls, the dry matter, crude protein and crude lipid of hybrid groupers fed with YC were significantly enhanced with the increase in YC level ($p < 0.05$). The dry matter, crude protein and crude lipid of hybrid groupers in the 4% group were significantly higher ($p < 0.05$) than those in the 0% group. Also, the ash content of hybrid groupers in the 0% and 4% groups was significantly higher than that of hybrid groupers in the 2% group ($p < 0.05$).

3.3 | Serum biochemical parameters, antioxidant enzymes and immune enzyme activities

As shown in Table 4, the serum TP levels of hybrid groupers in the 4% group were significantly higher than those in the 0% group ($p < 0.05$), but no significant difference was observed between the 4% group and 2% group ($p > 0.05$). The serum ALT and AST levels of hybrid groupers in the 4% group were significantly lower than those in the 0% and 2% groups ($p < 0.05$). There were no significant difference in serum GLU, TG and CHO levels between the control group and experimental groups ($p > 0.05$).

The activities of SOD, MDA and CAT in the serum of hybrid groupers before and after the challenge test are shown in Figure 1. There was no significant difference in SOD among the treatment groups before the challenge test ($p > 0.05$). The serum SOD and MDA concentrations were significantly increased after the challenge compared with those before the challenge ($p < 0.05$). The serum SOD was raised with the increase in YC addition level, but there was no significant difference among all groups ($p > 0.05$). There was no significant difference in serum CAT between the control and experimental groups before and after the challenge. In addition, the content of serum MDA

in the 2% and 4% groups was significantly lower than that in the 0% group before and after the challenge ($p < 0.05$).

The activities of ACP, AKP, LZM and IgM in the serum of hybrid groupers before and after the challenge are shown in Figure 2. The activity of serum ACP was significantly increased after the challenge compared with that before the challenge ($p < 0.05$). The ACP activity of hybrid groupers in the 4% group was significantly higher than that in the control group ($p < 0.05$). After the challenge, the serum AKP activity in the 2% and 4% groups was significantly elevated compared with that in the 0% group ($p < 0.05$). The LZM activity in the 4% group was significantly higher than that in the 0% group after the challenge ($p < 0.05$). The IgM activity in the 2% group before the challenge was significantly higher than that in the 0% group ($p < 0.05$). However, there was no significant difference among the treatment groups after the challenge ($p > 0.05$).

3.4 | Intestinal digestive enzyme activity

As shown in Table 5, no significant differences were found in the trends of intestinal trypsin, lipase and amylase activities among all groups. Trypsin, lipase and amylase in the YC treatment groups were significantly higher than those in the control group ($p < 0.05$), with the 4% group showing the highest levels.

3.5 | Analysis of intestinal microflora

3.5.1 | Intestinal microbiota richness and species diversity

Figure 3a shows the sample rarefaction curve. It could be seen that the corresponding curve of each group of samples tended to be smooth with the increase in the sequence number, suggesting

TABLE 3 Effects of dietary yeast culture on whole-body conventional components of hybrid grouper (% dry weight)

Treatment	Dry matter	Crude protein	Lipid	Ash
0%	28.32 ± 0.40 ^b	17.19 ± 0.09 ^b	6.10 ± 0.01 ^b	4.28 ± 0.00 ^a
2%	28.98 ± 0.41 ^b	17.64 ± 0.05 ^a	6.19 ± 0.10 ^b	4.04 ± 0.03 ^b
4%	29.67 ± 0.20 ^a	17.89 ± 0.04 ^a	6.45 ± 0.21 ^a	4.37 ± 0.04 ^a

Note: The data are presented as mean ± SEM. Means in the same column with different superscripts indicate significant difference ($p < 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively.

TABLE 4 Effects of dietary yeast culture supplementation on serum biochemical indices of grouper.

Treatment	TP (mmol/L)	GLU (mmol/L)	TG (mmol/L)	CHO (mmol/L)	ALT (U/L)	AST (U/L)
0%	44.21 ± 0.94 ^b	4.21 ± 0.17	0.64 ± 0.01	5.40 ± 0.06	4.42 ± 0.09 ^a	25.18 ± 0.47 ^a
2%	45.94 ± 0.50 ^{ab}	4.29 ± 0.06	0.67 ± 0.01	5.52 ± 0.14	4.64 ± 0.06 ^a	25.96 ± 1.00 ^a
4%	47.89 ± 0.36 ^a	4.31 ± 0.15	0.65 ± 0.00	5.41 ± 0.05	4.13 ± 0.03 ^b	23.96 ± 0.38 ^b

Note: The data are presented as mean ± SEM. Means in the same column with different superscripts indicate significant difference ($p < 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively.

Abbreviations: ALT, Alanine transaminase; AST, Aspartate aminotransferase; CHO, Cholesterol; GLU, Glucose; TG, Triglyceride; TP, Total protein.

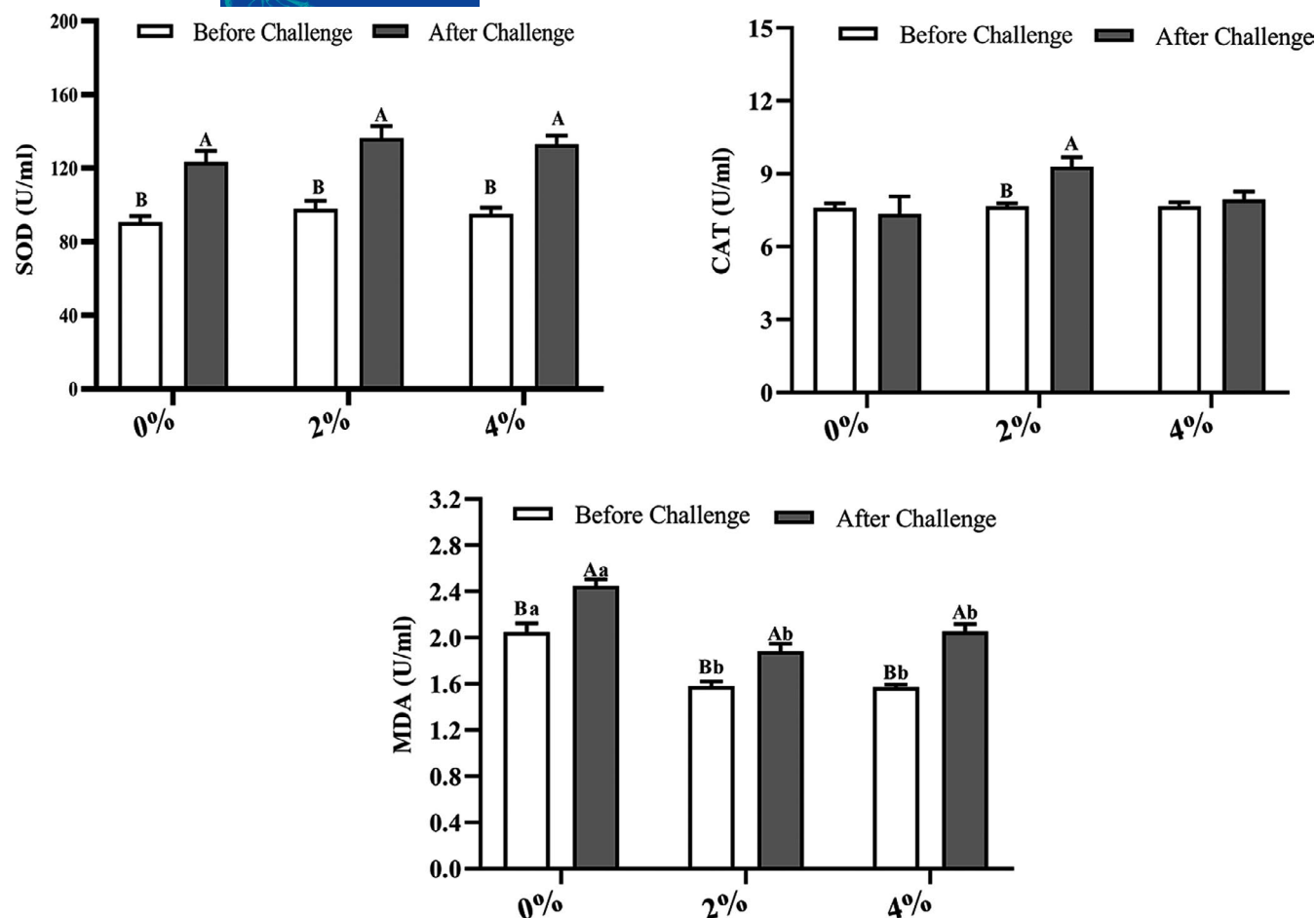


FIGURE 1 Yeast culture diet effects on serum antioxidant enzyme activity of hybrid grouper before challenge and after challenge with *Vibrio harveyi*. Upright bars denote the mean $\pm$ SEM. Different capital letters indicate a comparison before and after challenge in the same group, and different lowercase letters indicate a comparison before and after challenge between different groups. The same letter indicates no significant differences among groups ($p > 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively. SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde

that the amount of sequencing data was gradually becoming reasonable, with more data yielding only a small number of new species. The existing data can reflect the characteristics of microbial diversity in the samples. A species accumulation box plot chart was drawn to analyse the relationship between species diversity and sample size. As shown in Figure 3b, the box plot position rises sharply over a range, indicating the existence of large number of species was found in the community. When the box plot tended to be smooth, the species would not significantly increase with the increase in sample size, indicating that the sampling was sufficient and data analysis could be carried out. The OTUs and alpha diversity index of intestinal microflora operation taxon of hybrid groupers are shown in Table 6. The OTUs and Alpha diversity index (Chao, Ace, Shannon, Simpson) of different samples were calculated. The results showed that the OTUs and alpha diversity indexes had no significant difference between groups ($p > 0.05$). The Venn diagram (Figure 3c) was obtained after 97% consistency of OTUs clustering. Venn diagram analysis showed that there were 426 OTUs shared among the three groups. The mean OUTs of the 0%, 2% and 4% groups were 962, 340 and 594 respectively. The

0% and 4% groups shared the most OTUs at 390. In contrast, the 0% and 2% groups shared 124 OTUs.

3.5.2 | Taxonomic composition of intestinal microorganisms

The 4 kinds of intestinal bacteria with the most abundant relative abundance in each group were *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* at the phylum level (Figure 4). *Proteobacteria* was the dominant phylum of all samples, with the 2% group (69.83%) showing the highest abundance, followed by the 4% group (60.64%) and 0% group (29.40%). *Firmicutes* (0% group, 22.35%; 2% group, 8.51%; 4% group, 16.47%), *Actinobacteria* (0 group %, 14.16%; 2% group, 4.81%; 4% group, 5.81%) and *Bacteroides* (0% group, 11.20%; 2% group, 5.29%; 4% group, 4.83%) came next. The intestinal microbial composition of the 3 groups at the genus level is shown in Figure 5. The 4 genera with the highest relative abundance in each group were as follows: group 0%: *Sphingomonas* (4.99%), *Stenotrophomonas* (3.20%), *Phyllobacterium*

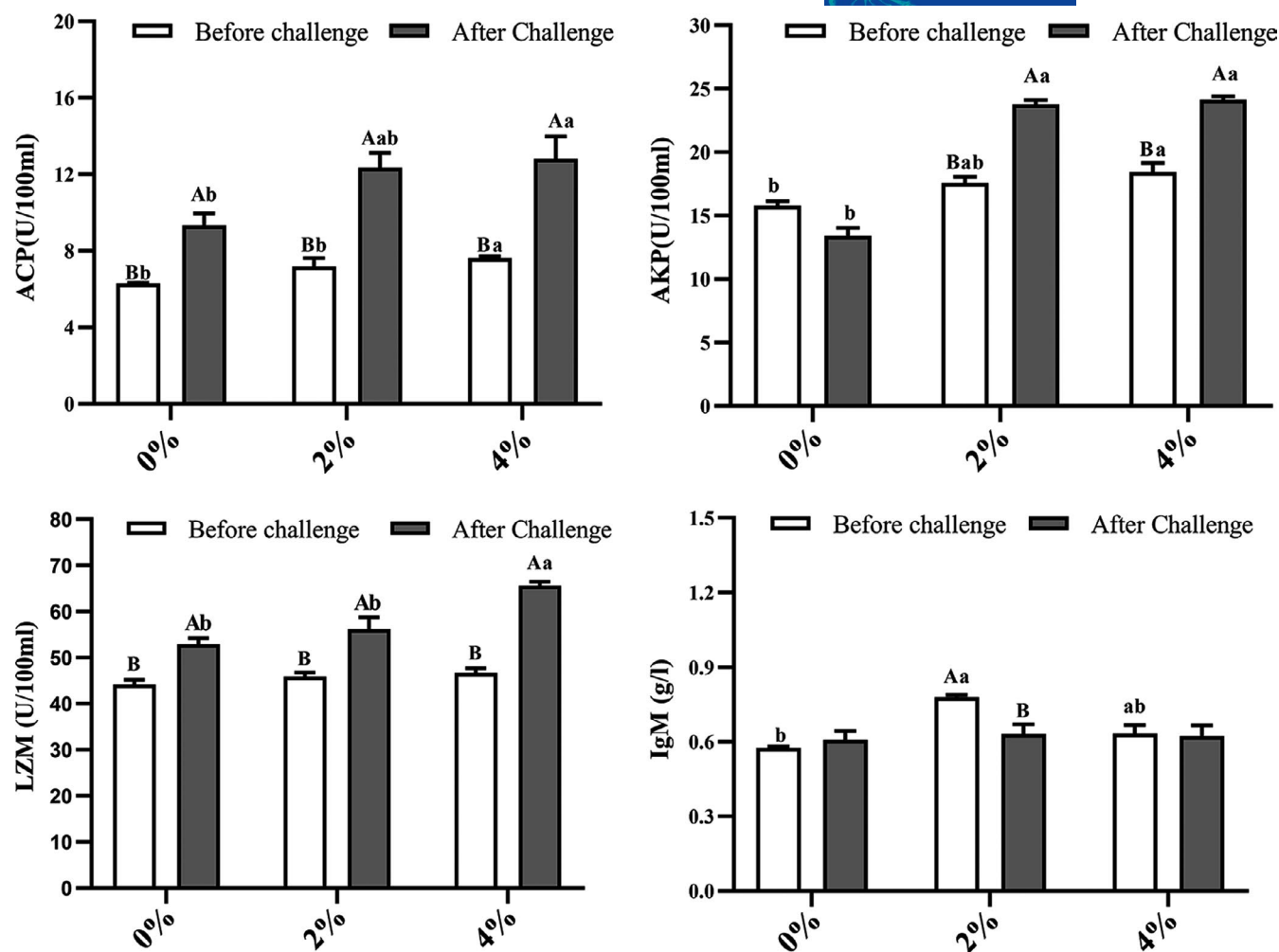


FIGURE 2 Yeast culture diet effects on serum immune enzyme activity of hybrid grouper before challenge and after challenge with *Vibrio harveyi*. Upright bars denote the mean $\pm$ SEM. Different capital letters indicate a comparison before and after challenge in the same group, and different lowercase letters indicate a comparison before and after challenge between different groups. The same letter indicates no significant differences among groups ($p > 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively. ACP, acid phosphatase; AKP, alkaline phosphatase; LZM, lysozyme; IgM, immunoglobulin M

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)
0%	1352.58 $\pm$ 113.49 ^b	444.14 $\pm$ 20.29 ^c	318.81 ^c $\pm$ 12.71 ^c
2%	2497.57 $\pm$ 146.34 ^a	631.08 $\pm$ 59.67 ^b	442.93 ^c $\pm$ 16.40 ^b
4%	2768.15 $\pm$ 217.92 ^a	862.79 $\pm$ 68.52 ^a	537.17 $\pm$ 20.47 ^a

Note: The data are presented as mean $\pm$ SEM. Means in the same column with different superscripts indicate significant difference ($p < 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively.

(4.61%) and *unidentified_Ruminococcaceae* (4.73%); group 2%: *Sphingomonas* (4.31%), *Perluclidibaca* (13.71%), *Stenotrophomonas* (16.84%) and *Phyllobacterium* (11.33%); group 4%: *Sphingomonas* (27.40%), *Stenotrophomonas* (6.57%), *Phyllobacterium* (14.29%) and *Faecalibacterium* (3.21%).

3.6 | Multi-sample comparative analysis

The principal coordinate analysis (PCoA) based on Weighted Unifrac at the OTU level is shown in Figure 6a. The YC group samples were separated from the control group samples, indicating the differences in the structural composition of intestinal microorganisms between the two groups. In contrast, the distance between the 2% and 4% groups was closer, indicating a high degree of community similarity between the two sample groups. Besides, all the groupers were assigned to two major clusters according to the UPGMA method (Figure 6b). One of the clusters consisted of the members of the 2% and 4% groups, and the other cluster contained the members of the 0% group, which was consistent with the results of PCoA. It was indicated that the YC group samples had similar microbiota composition. *Proteobacteria* and *Firmicutes* were the two bacteria with the highest relative abundance in each group. Thus, the addition of YC to feed can influence the structure of the intestinal flora of hybrid grouper.

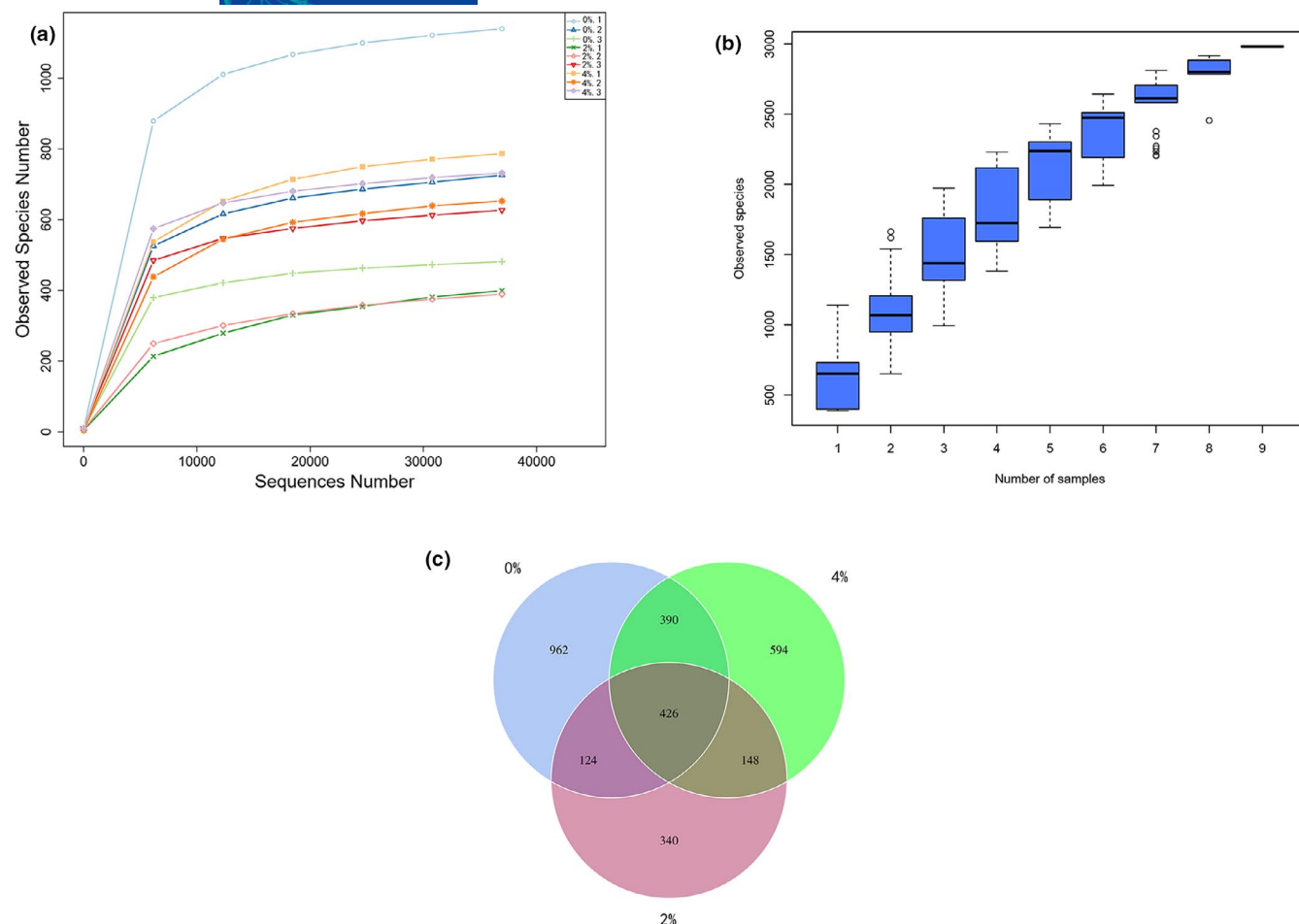


FIGURE 3 (a) Analysis of rarefaction curves of hybrid grouper gut samples. (b) The species accumulation box plot. The horizontal axis represents the number of samples, and the vertical axis represents the number of identified OTUs. (c) Venn diagram of OTUs comparison. Diets were supplemented with YC at 0%, 2% and 4% respectively

TABLE 6 Richness and diversity indexes of intestinal bacterial in hybrid grouper fed experimental diets

Index	Raw reads	Clean reads	OTUS	Chao	Ace	Shannon	Simpson
0%	73232	69616	814.67 ± 207.44	772.54 ± 268.52	650.56 ± 138.26	7.54 ± 0.70	0.98 ± 0.00
2%	81539	75850	542.67 ± 76.88	589.26 ± 93.86	587.24 ± 107.90	5.88 ± 1.75	0.88 ± 0.05
4%	86092	80143	777.33 ± 45.06	834.66 ± 21.64	815.21 ± 22.59	6.40 ± 0.89	0.76 ± 0.15

Note: The data are presented as mean ± SEM. Means in the same column with different superscripts indicate significant difference ($p < 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively.

3.7 | Intestinal histological analysis

The intestinal tissue section is shown in Figure 7a. It could be seen that the intestinal mucosal folds of groupers in the 0% group were disorderly arranged and separated from the intestinal wall. The groupers in the 2% group had slight separation, and the groupers in the group 4% had no separation, with the intestinal epithelium neatly arranged. The groupers in the 4% group had thicker muscles and wider villi compared with the groupers in the other two groups. The intestinal morphology of hybrid

groupers was measured (Figure 7b). The villus height of groupers in the 4% group was significantly higher than that of groupers in the 0% group ($p < 0.05$). There was no significant difference in villus height between the 2% and 4% groups ($p > 0.05$). The villus width of groupers in the 4% group was significantly higher than that of groupers in the 0% and 2% groups. The muscle thickness was increased with the increase in YC level, with the 4% group showing the highest muscle thickness. Besides, there was no significant difference in muscle thickness among these groups ($p > 0.05$).

FIGURE 4 Histograms of intestinal bacterial community abundance at phylum level between three groups of hybrid grouper. Diets were supplemented with YC at 0%, 2% and 4% respectively

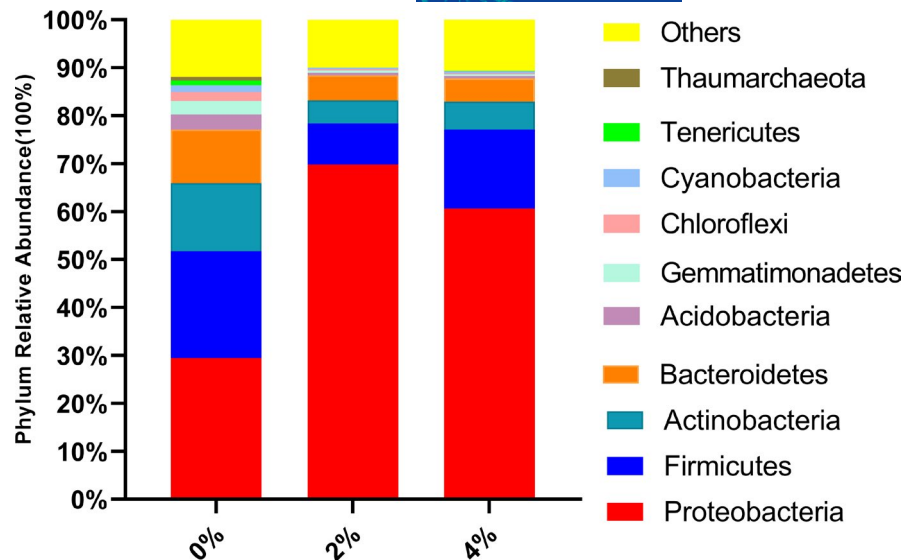


FIGURE 5 Histograms of intestinal bacterial community abundance at genus level between three groups of hybrid grouper. Diets were supplemented with YC at 0%, 2% and 4% respectively

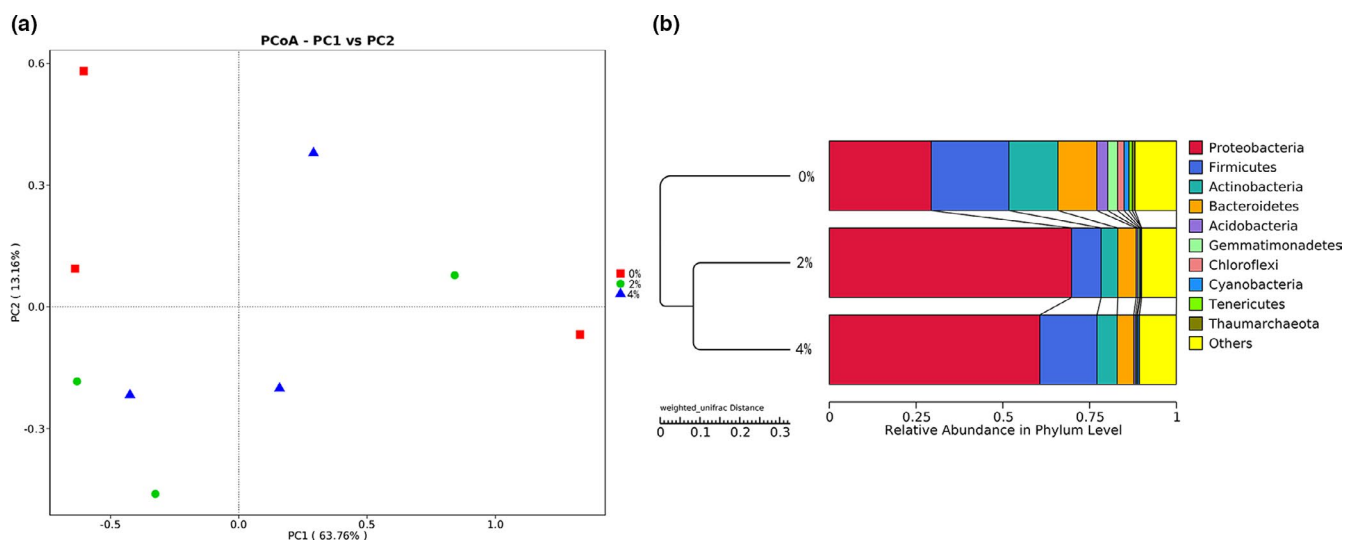
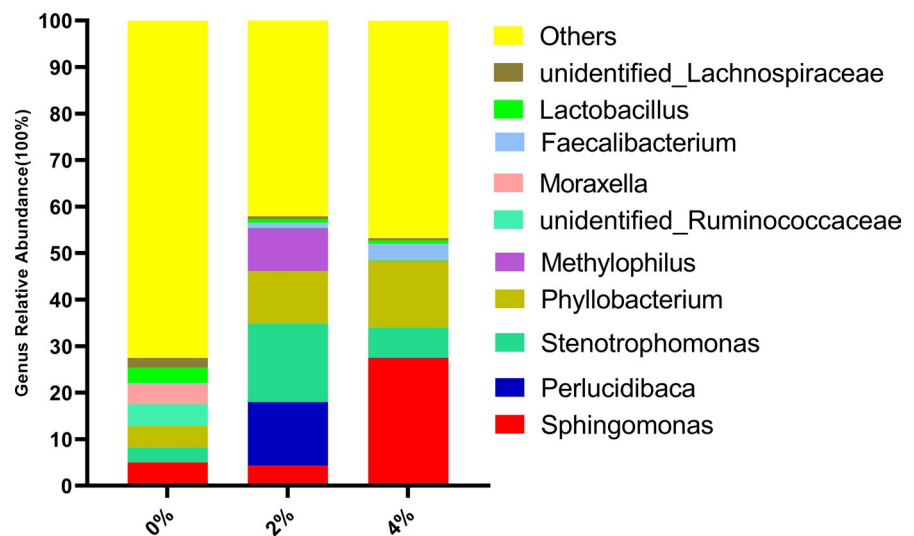


FIGURE 6 (a) Principal coordinate analysis based on Weighted Unifrac at OTU level. (b) Bacteria UPGMA clustering analysis. Diets were supplemented with YC at 0%, 2% and 4% respectively

(a)

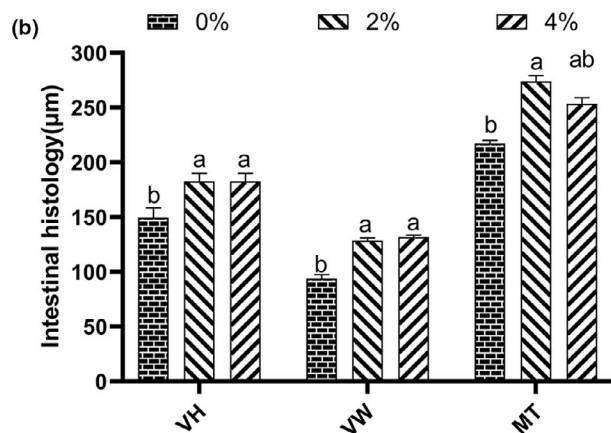
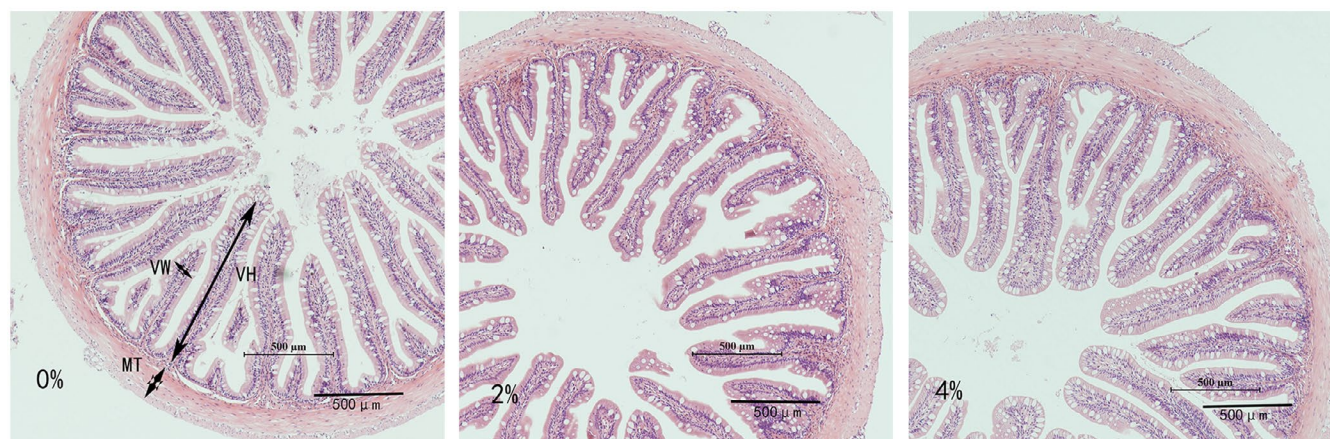


FIGURE 7 Effects of dietary yeast culture supplementation on intestinal tissue section (a) and morphological measurements (b). Upright bars denote the mean $\pm$ SEM. In Figure 8a, the VH, VW and MT double-headed arrows indicate intestinal villus height, villus width and muscle thickness respectively. Diets were supplemented with YC at 0%, 2% and 4% respectively

3.8 | Challenge test

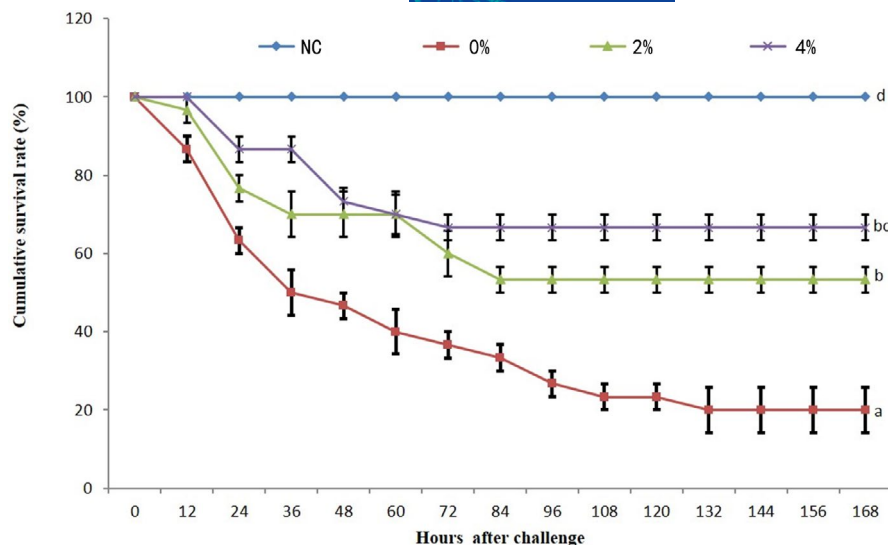
The cumulative survival rate of groupers in each group was counted after 168 h of challenge (Figure 8). The mortality of groupers was not observed in the saline injection group. The cumulative survival rate of groupers in the 2% and 4% groups was significantly higher ($p < 0.05$) than that in the C0 (0%) group. The L2 (4%) group had the highest cumulative survival rate (67%), followed by the 2% group (55%) and 0% group (20%).

4 | DISCUSSION

YC is a product with complex composition, which contains the nutrients within the yeast cells and the yeast cell metabolites produced by fermentation (Liu et al., 2018). A series of studies have demonstrated that yeast extracts, yeast hydrolysates or YC can promote the health and growth of animals (Ayiku et al., 2020a; Berto et al., 2016; Jin et al., 2018). The present also provided supporting evidence that the supplementation of YC in the diets improved the growth performance and feed efficiency of hybrid groupers. Yeast

products have been shown to improve the growth performance of the fish concerned (Berto et al., 2016; Liu et al., 2018; Yuan et al., 2019). For instance, Chiu et al. (2010) have found that the addition of *Saccharomyces cerevisiae* to the diet can significantly improve the weight gain and feed efficiency of groupers (Chiu et al., 2010). The addition of yeast extracts in the diet can contribute to the feed intake and specific growth rate of Nile tilapia (*Oreochromis niloticus*) (Berto et al., 2016). Consistently, this study showed that YC addition exerted positive effects on the feed efficiency of hybrid groupers. Therefore, it was suggested that improving feed utilization could be one of the important ways for YC to promote the growth of hybrid groupers. Accumulating studies have shown that the addition of 2% YC to the feed can significantly reduce the FCR of *Litopenaeus vannamei* (Ayiku et al., 2020b), and moreover, the addition of 1% and 2% brewer's yeast feed notably improves the feed efficiency of bass (*Morone chrysops* $\times$ *Morone saxatilis*) (Li et al., 2004), which may be due to the contribution of yeast cells and extracellular metabolites (proteins, amino acids, vitamins, nutrient metabolites and enzymes) in YC. These substances can enhance the activity of digestive enzymes and facilitate the absorption and metabolism of nutrients, thus promoting growth (Ayiku et al., 2020a; Gao et al., 2008).

FIGURE 8 Cumulative survival rate of hybrid grouper fed yeast culture after 168 h of *Vibrio harveyi* infections. For each treatment, values with different letter suffix differ significantly ($p < 0.05$). Diets were supplemented with YC at 0%, 2% and 4% respectively



Intestinal digestive enzyme activity reflects the digestive capacity of fish. In this study, the intestinal digestive enzymes showed an increasing trend after YC treatment, which was consistent with the results of growth performance. Similar studies have been reported on Nile tilapia (*Oreochromis niloticus*) (Goda et al., 2012) and sea bass (*Dicentrarchus labrax*) (Tovar et al., 2002). It has also been demonstrated that the addition of 1% yeast hydrolysate and brewer's yeast to the shrimp diet significantly up-regulates mTOR-related gene expression, thereby promoting protein synthesis (Jin et al., 2018). However, the addition of *Saccharomyces cerevisiae* fermentation products to the feed has no significant effect on the growth performance of Nile tilapia cultured in cages (He et al., 2009; Zhang et al., 2018). The discrepancies in fish growth performance can be attributed to the differences in culture conditions, fish species and diet nutrient composition. In the present study, the rich nutrients of YC may promote the growth of hybrid grouper, but its exact mechanism of action remained to be further investigated.

Diet provides nutrients for fish, and the type of diet consumed by fish impacts the body composition. The nutritional value of fish mainly depends on the protein and lipid content of the muscle. The present study indicated that the level of YC added to the diet significantly affected the body composition of hybrid groupers. The supplementation of YC in the diet significantly increased the dry matter, crude protein, crude lipid and ash content of hybrid groupers, with the hybrid groupers fed with 4% YC showing the highest levels. Similarly, the supplementation of 2% YC in the diet improves the crude protein, crude lipid, dry matter and ash content of *Litopenaeus vannamei* (Ayiku et al., 2020a, 2020b). Abass et al. (2018) have also shown that the addition of 7% brewer's yeast to the diet significantly increases the crude protein and crude lipid in juvenile Nile tilapia (Abass et al., 2018). The supplementation of YC in the diet can enhance the activity of intracellular enzymes that contribute to growth performance and nutrient accumulation. For the present study, the addition of 4% YC to the diet effectively promoted the protein and lipid deposition in groupers.

Blood biochemical indicators can indirectly reflect the fish's metabolic condition and health status (Xie et al., 2021). As a kind

of beneficial probiotics, the regulatory effect of YC on the animal body can be reflected by biochemical blood indexes. Aung (2019) and Hassaan et al. (2018) have found that the addition of YC to the diet improves the blood biochemical parameters of animals (Aung et al., 2019; Hassaan et al., 2018). The serum TP content reflects the nutritional metabolism of the organism and is closely related to the immune system of the fish organism (Hossain et al., 2017; Tan et al., 2017). In the present study, we found that supplementation of YC in the diet affected the serum TP content. Similarly, the addition of yeast cell walls (β -glucan and mannan-oligosaccharides) to the diet can increase the total concentrations of protein and globulin in Nile tilapia (Abu-Elala et al., 2018), suggesting that YC may be an immune activator in hybrid groupers. As part of blood lipids, serum TG and CHO are essential indicators that reflect the lipid level of the fish body, indicating the metabolism and increase in lipids and energy storage in fish (Agrahar et al., 2007; Kumar et al., 2018; Xie et al., 2021). Blood GLU can be used as an indicator of stress in fish, with a high level of GLU indicating a high state of stress (Hossain et al., 2016). In the present study, the relevant results of GLU, TG and CHO levels confirmed that the addition of YC had no adverse effect on groupers. ALT and AST are essential indicators of liver health, both of which exist in the cytoplasm of hepatocytes. When hepatocytes are damaged, hepatocyte membrane permeability is increased, inducing the release of ALT and AST into the bloodstream and thus causing significant serum enzyme activity changes (Hassaan et al., 2018). The present study demonstrated that the addition of YC to the diet decreased the activities of AST and ALT, indicating that YC exerted a protective effect on the liver against free radical damage, which was consistent with existing studies' findings (Ayiku et al., 2020a, 2020b).

The oxidation reaction in the animal body produces harmful energy and reactive oxygen radicals, and excessive free radicals can damage the phospholipid bilayer of cell membranes (Wang, Zuo, et al., 2016a, 2016b, 2016c.). SOD and CAT are essential components of the endogenous antioxidant defence system, reflecting the body's antioxidant strength. SOD converts harmful metabolites

into hydrogen peroxide, while CAT decomposes hydrogen peroxide into oxygen and water to protect cells (Campa-Codova et al., 2002; Tavares-Sanchez et al., 2004). MDA is a primary marker of lipid peroxidation that reflects the extent of lipid oxidation (Xu et al., 2015). In the present study, YC supplementation in the diet decreased the serum MDA content, while the SOD content was increased with the increase in YC level after the challenge. The addition of YC had no significant effect on the CAT content. Similar findings have been reported on grass carp (*Ctenopharyngodon idella*) (Liu et al., 2018) and catfish (*Pseudobagrus ussuriensis*) (Bu et al., 2019). β -glucan in yeast products can reduce lipid peroxidation and protect the body from oxidative damage (Sener et al., 2005). In this study, the addition of 4% YC to the diet benefited the antioxidant capacity of hybrid groupers.

In the present study, ACP, AKP, LZM and IgM served as indicators of innate immune parameters in hybrid groupers. Innate immunity is the first line of defence against pathogen invasion in the host. LZM, a hydrolytic enzyme important for innate immunity, is commonly used to indicate the stress response and disease resistance of aquatic organisms (Saurabh et al., 2008). IgM, the main immunoglobulin in the serum of fish, can be used as a marker of immune status (Bag et al., 2009). ACP, a macrophage lysosomal marker enzyme, plays an essential role in the intracellular digestion of phagocytic antigens (Song et al., 2006). AKP is also an crucial component of the natural immune system that can dephosphorylate pro-inflammatory compounds (Lalles., 2019). In the present study, there was an upward trend in innate immune parameters after the challenge. Zhang et al. (2018) have shown that the addition of 4 g of YC per 100 g of diet can improve the disease resistance and immunity of silver crucian carp (*Carassius auratus gibelio cas III*) partly through the TLR2 pathway (Zhang et al., 2018). MOS and β -glucan are the main functional components of the yeast cell wall (Abu-Elala et al., 2018). MOS can combine with bacteria and viruses to effectively alleviate the harm of bacteria, thus enhancing humoral and cellular immunity (Ren et al., 2020). Dietary supplementation of 0.2%–0.3% MOS significantly enhances the intestinal ACP, AKP and LZM activities of Chinese mitten crab (*Eriocheir sinensis*) (Zhang et al., 2019). As an immune response matrix, β -glucan activates the immune-related signal pathway, prompts immune cells to release downstream signal molecules and then improves the body's immune responses (Lee et al., 2014). β -glucan has been proven to enhance non-specific immunity in turbot (*Scophthalmus maximus*) (Miest et al., 2016), rainbow trout (*Oncorhynchus mykiss*) (Chettri et al., 2013) and Atlantic salmon (*Salmo salar* L.) (Engstad & Robertsen, 1993). Still, the mechanism of dietary YC supplementation in the innate immune system of groupers needs further exploration.

As an essential part of the intestinal mucosal barrier, intestinal microorganism forms a complex intestinal microbial system with intestinal epithelial cells, immune cells etc. Many microorganisms colonized in the intestinal tract can transform the nutrients in the diet into forms that can be used by the host, which contributes to the maintenance of the intestinal morphology, digestion and immunity of animals (He et al., 2021; Hooper & Gordon, 2001). In

the current study, however, YC addition had no remarkable effect on OTUS and alpha diversity. The dominant phylum in healthy animals are *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroides* (Wang, Zuo, et al., 2016). In the present study, all the groups of hybrid groupers were dominated by *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroides* at the phylum level. These bacteria formed the core flora in the grouper intestine, which was consistent with the results of existing studies (Yang et al., 2019; Ye et al., 2020). The relative abundance of *Proteobacteria* in the YC treatment groups showed an increasing trend, while the relative abundance of *Firmicutes*, *Actinobacteria* and *Bacteroides* showed a decreasing trend. Consistently, Zhao et al. (2015) have revealed that the addition of non-protein nitrogen (nitrate) has no significant effect on ruminal alpha diversity and that *Proteobacteria* abundance is increased with nitrate addition (Zhao et al., 2015), which is consistent with the results of our study. The non-protein nitrogen in the YC fermentation substrate is converted to protein by the yeast, and furthermore, the YC contains a variety of metabolites. The present study may be the result of a combination of the two. At the genus level, the genera *Sphingomonas*, *Perlucidibaca*, *Stenotrophomonas* and *Phyllobacterium* made up the core microbial genera. YC supplementation in the diet had a positive effect on the relative abundance of *Sphingomonas*. Some studies have shown that healthy Atlantic salmon have a prosperous abundance of *Sphingomonas* in their gut microbes (Wang et al., 2017). Also, the carotenoids produced by *Sphingomonas* bear antioxidant properties and can scavenge free radicals produced in the body (Basta et al., 2004). *Stenotrophomonas* inhibits pathogenic microorganisms and acts as the dominant bacterium in the gut of healthy animals (Destoumieux-Garzon et al., 2016). In our study, the abundance of *Stenotrophomonas* and *Phyllobacterium* was elevated after YC treatment, which is consistent with the findings of Liao et al. (2019). Our results also showed the presence of *Faecalibacterium* in the YC treatment groups. *Faecalibacterium* produces a short-chain fatty acid (SCFA) called butyric acid, which is the main nutrient for cell regeneration and repair of intestinal epithelial tissue cells (Miquel et al., 2014). *Faecalibacterium* has been identified as an important butyric acid-producing bacterium in the caecum of chickens (Duncan et al., 2002).

The animal intestine plays a vital role in nutrient absorption and metabolism. The increase in villi height and width expands the absorption area of the intestine, which contributes to the digestion and absorption of nutrients (Dimitroglou et al., 2010). Furthermore, the muscle thickness of the intestine determines the efficiency of absorption (Geda et al., 2012). In the present study, it was found that YC supplementation improved villi height, villi width and muscle thickness. The same findings have also been reported on *Litopenaeus vannamei* (Ayiku et al., 2020a, 2020b). Dimitroglou et al. (2008) have demonstrated that the supplementation of MOS in the diet can significantly increase the length of the intestinal villi of rainbow trout and affect the intestinal structure by increasing the density of microvilli. (Dimitroglou et al., 2008). Whether the changes in intestinal morphology of hybrid groupers in this study were also caused by MOS supplementation in the YC needs to be further verified.

Juvenile grouper is susceptible to bacterial infection. *Vibrio harveyi* is the most common pathogen causing bacterial diseases in groupers (Li et al., 2019). At present, there is a lack of effective methods to treat *Vibrio* disease in groupers. Through the challenge test, the survival rate of aquatic animals can evaluate the immunity and disease resistance of the body. The survival rate of groupers fed with YC was significantly higher than that of control groupers at the end of the challenge test. It has been previously reported that the addition of YC to the diet can significantly improve the survival rate of *Litopenaeus vannamei* (Jin et al., 2018; Tendencia et al., 2004). Both β -glucan and MOS, the primary nutrients of YC, activate the immune system and induce immune responses (Bu et al., 2019; Vetvicka et al., 2013). This study showed that the addition of 4% YC to the diet effectively improved the resistance of hybrid groupers to *Vibrio harveyi* infection.

5 | CONCLUSION

The present study demonstrates that the addition of YC to the diet improves the growth performance, immune response, digestive enzyme activity, intestinal microbiota and disease resistance against *Vibrio harveyi* of hybrid groupers. In summary, YC is an ideal diet additive, and 4% YC is recommended in the diet of hybrid groupers.

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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 analysed 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.

ETHICAL 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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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