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Effects of dietary yeast culture on shrimp growth, immune response, intestinal health and disease resistance against *Vibrio harveyi*

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ABSTRACT

The current study was conducted to evaluate the effects of different levels of yeast culture (YC) supplementation at 0% (YC 0%), 1% (YC 1%), and 2% (YC 2%) on growth, feed conversion ratio, body composition, intestinal morphology, microflora, immune response, and resistance to *Vibrio harveyi* infection in *Litopenaeus vannamei*. After 8-weeks feeding trial, the results showed significant improvement ($p < .05$) in the final weight, weight gain rate, specific growth rate, survival rate and low feed conversion ratio in YC groups than the control. Serum total protein, superoxide dismutase, catalase, alkaline phosphatase, acid phosphatase, lysozyme, and phenol oxidase in shrimps fed diet YC (2%) were significantly higher ($p < .05$), whereas significantly decreased trend in serum cholesterol, triglyceride, aspartate aminotransferase, and alanine aminotransferase ($p < .05$) were observed in YC (2%) diet. Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes were the core phylum bacteria found in the shrimp intestines. At the genus level, opportunistic pathogenic bacteria, *Vibrio* was significantly decreased ($p < .05$) while beneficial bacteria *Pseudoalteromonas* was increased in YC (2%) group. Intestinal villus height and width in shrimps fed YC diets were significantly improved than the control diet ($p < .05$). YC groups challenged test significantly showed ($p < .05$) improved shrimps immune response against *V. harveyi* infections with YC (2%) recording the highest percentage survival rate (70%). The present study demonstrated that supplementing YC (2%) can improve growth, intestinal microbiota, intestinal morphology, and immune response against *V. harveyi* infections in *L. vannamei*.

1. Introduction

Litopenaeus vannamei (Pacific white shrimp) is a worldwide cultured animal among the crustacean species because of its rapid growth, tender flesh, and high nutritional value [1]. For the past years, disease occurrences have led to substantial economic loss in the shrimp aquaculture industry [1–3]. Preventing pathogenic microorganisms using antibiotics have been widely associated with problems such as development and bioaccumulation of antibiotic resistant pathogens [4,5]. As an alternative to traditional disease-control, the use of probiotics, prebiotics, and natural immunostimulants has been of growing interest [4]. Non-digestible feed ingredient, prebiotics has the potentials of affecting host immunity by exclusively modulating metabolic activities in the gut, therefore, stimulating the host intestinal balance [6]. Due to abundant vitamins, proteins, pigments nucleotides, β-glucan content of single-cell proteins such as microalgae, yeast, and bacteria, they are often used as feed additives in aquaculture [7].

Single cell protein of some yeast, for example, *Saccharomyces cerevisiae* and *Candida* species have been used as probiotics [8,9]. The potential probiotics effects of *S. cerevisiae* on several aquatic species are enhanced immunity, growth, and protection against pathogen infections [10–12]. Yeast culture (YC) is a complex fermented product, which contains yeast cell and its metabolic products. YC possesses high protein, lipid, and B-vitamins [13], and as a source of prebiotics, it contains β-glucan, chitin, nucleic acid, and oligosaccharides. Mannan oligosaccharides (MOS) and β-glucan structural components of yeast cell walls have the capacity of enhancing immunity. The B-glucan component in yeast culture activates the macrophage and releases various immunoglobulins, interleukin and cytokines to defend the fish against various pathogens [14]. Fish, when fed yeast culture diet, increase the abundance of *Actinobacteria* in the intestinal microbiota [15]. *Actinobacteria* maintain intestinal permeability, down regulate

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inflammation by IL-4 and IL-I3, and induces regulatory T-cells [16]. The complex carbohydrate (MOS) content of yeast in the intestine inhibit pathogen colonization by blocking pattern recognition molecules and serves as binding site of potential pathogen instead of directly colonizing the gut walls [17]. Proven effects have shown that (MOS) can improve fish growth performance, gut morphology, modulating the intestinal microbiota [11,18], and elevating immunity and stress-resistance ability [19,20]. Reports have shown that yeast β-1,3-glucan has immunostimulatory capacity in shellfish against viral or bacterial infections [21]. A further report on β-glucan fed to *Penaeus monodon* postlarvae enhanced vibriosis resistance in shrimp [21]. Studies have demonstrated positive effects of YC on feed intake and nutrient digestibility, which in turn improved feed efficiency and growth of aquatic animals [22,23]. Dietary 1% yeast hydrolysate or brewer's yeast (Guangdong Hinabiotech Co., Ltd and Tech-bank Aquatic Feed Co., Ltd respectively) supplementation in diet improved growth performance, immune response, and ammonia nitrogen resistance of Pacific white shrimp [1]. Zhang et al. [24],reported that dietary supplementation of YC (4 g/100 g diet) (Enhalor Biotechnology Company (Beijing, China)) resulted in better growth performance, enhanced immune response, and improved the protection against *Aeromonas hydrophila* in gibel carp (*Carassius auratus gibelio*). A range of studies have shown that dietary YC supplementation improved growth, immune response, and diseases resistance in aquatic animal, however, information regarding YC effect in *L. vannamei* is limited.

Therefore, this research was conducted to evaluate the effect of YC supplementing fish meal (FM) concentration at 1% and 2% in *L. vannamei* diets by assessing growth performance, serum immune response, intestinal microbiota, intestinal morphology, and resistance against *V. harveyi.* The findings from this study might provide useful information on the formulation of immune response and disease resistant diets for *L. vannamei* production.

2. Materials and methods

2.1. Ingredients and experimental diet preparation

The ingredients and the nutrient profile of the basal diet are displayed in Table 1. Three experimental diets were formulated by supplementing YC at 0%, 1%, 2% designated as YC (0%), YC (1%) and YC (2%) respectively. The YC derived from *S. cerevisiae* was provided by Enhalor Biotechnology Company (Beijing, China) and contains 50.0% crude protein, 0.29% crude lipid, 4.0% moisture, and 9.3% ash on dry matter basis. The basal diet contains fishmeal (60% crude protein), soybean meal, peanut meal and shrimp shell powder meal as a protein source and fish oil, soybean oil, and phospholipid oil as lipid sources. Prior to diet preparation, all the coarse ingredients were milled into powder using a hammer mill machine, sieved through 80 mm mesh before mixing thoroughly with minerals and vitamins in a V-mixer machine. Phospholipid oil, soybean oil, and fish oil were added to mixtures and mixed by hand. Water containing Choline Chloride was blended in the mixture (approximately 25% of the total ingredient weight) to dough form which was divided into 1 kg and 3 kg. They were separately pelleted using meat grinder machines containing different discs with holes sizes of 1.0 mm and 1.5 mm to make spaghetti pellet. Pellets were oven-dried at 90 °C for 15 min, followed by air drying using electrical fan until moisture content reduced to about 10%. Pellets were broken into pieces, sieved into a proper length of 2.00 mm and 3.0 mm. After packaging pellets into seal bags, they were then stored at −20 °C until feeding.

2.2. Shrimp and rearing conditions

Juveniles of L. *vannamei* with the same genetic background, free from pathogens were obtained from Zhanjiang Allied Pacific Aquaculture Limited to Marine Biological Research Base of Guangdong Ocean University where the experiment was conducted. Shrimps were conditioned in indoor rearing fiberglass tanks for four weeks and fed with a commercial diet. Having starved shrimps for 12 h, those with an average weight of 0.36 g ± 0.002 were selected for the experiment. A group of 40 healthy shrimps was stocked into each fiberglass tank containing 200 L of sieved seawater. They were offered diet four times daily (7.00, 11.00, 17.00 and 21.00) at 5–8% of their average body weight in four replicates. Before first feeding, 70% of water in each tank was replaced with new seawater every day. Feed intake, mortality, and water quality parameters were monitored. The rearing temperature was 28.0 ± 2.0 °C whiles dissolved oxygen (DO) was within 6–8 mgl^{-1}.

Table 1
Ingredients and proximate composition of the experimental diets (%).

Ingredients	CO (0%)	YC (1%)	YC (2%)
Fish meal[A]	20.00	19.26	18.51
Yeast culture	0.00	1.00	2.00
Soybean meal[A]	I8.00	18.00	18.00
Peanut meal[A]	18.00	18.00	18.00
Shrimp shell powder[A]	7.00	7.00	7.00
High-gluten flour[A]	20.00	20.00	20.00
Fish oil[A]	1.80	1.80	1.80
Soybean oil[A]	1.80	1.80	1.80
Phospholipid oil[A]	1.80	1.80	1.80
Vitamin mixture[B]	0.20	0.20	0.20
Mineral element mixture[C]	0.50	0.50	0.50
Attractant[A]	0.10	0.10	0.10
Antioxidants ethoxyquin[A]	0.03	0.03	0.03
Choline chloride 99%[A]	0.50	0.50	0.50
92% monobasic calcium phosphate[A]	1.00	1.00	1.00
Vc polyphosphate 35%[E]	0.09	0.09	0.09
Antimony trioxide[D]	0.50	0.50	0.50
CMCC[D]	8.86	8.32	8.02
Total	100.00	100.00	100.00
Nutrient levels			
Dry matter	89.16	89.06	89.67
Crude protein	42.20	42.19	42.21
Crude lipid	8.01	7.95	7.90
Ash	8.94	8.89	9.05

A. Ingredients purchased from Zhanjiang HaiBao Feed Factory, Zhanjiang, Guangdong, China.
B. Vitamin mixture supplied the following per kg of the diet: vitamin A, 22,500 IU; vitamin D3, 6,000 IU; vitamin E, 200 mg; vitamin K3, 40 mg; vitamin B1, 30 mg; vitamin B2, 45 mg; vitamin B6, 35 mg; vitamin B12, 0.25 mg; calcium pantothenate, 150 mg; niacin, 225 mg; folic acid, 12.5 mg; biotin, 0.5 mg; inositol, 500 mg (Obtained from Zhanjiang Yuehai Feed Co. Ltd., Guangdong, China).
C. Mineral element mixture provided the following per kilogram of diet: Fe, 60 mg; Zn, 24 mg; Mn, 16 mg; Cu, 1.4 mg; Co, 0.2 mg; Se, 0.1 mg; I, 0.2 mg (Obtained from Zhanjiang Yuehai Feed Co. Ltd., Guangdong, China).
D. Obtained from Shanghai Macklin Biochemical Co. Ltd., 1288 Guangdong Rd., Shanghai, China.
E. Obtained from Shantou Xilong Chemical Factory, Guangdong, China.

2.3. Sample collection

After feeding trials for 8 weeks, all shrimps were starved feed for 24 h before sampling. The total numbers, as well as body weight of shrimps from each tank, were recorded. Per tank, two shrimps were randomly picked, stored at −20 °C for carcass analysis. Hemolymph samples were drawn from the first abdominal segment of ten shrimps per tank with the aid of 1 mL sterile syringe into 1.5 Eppendorf tubes. The tubes were kept at 4 °C overnight before centrifuging at 3500 rpm/min for 10 min at 4 °C, supernatants were separated and stored at −80 °C for enzymatic activities analysis. Mid-guts (0.8–1.2 cm) were aseptically removed from three shrimps in each tank and fixed into 10% paraformaldehyde for histological analysis. Furthermore, another three mid-guts per tank were randomly sampled gently loaded into empty 1.5 Eppendorf tubes and immediately stored in liquid nitrogen for

microbiota analysis.

2.4. Growth performance analysis

Shrimp were weighed from each tank at the initial stage and 8 weeks after feeding trial. The total amount of diet fed to each of the experimental groups and the control group was calculated after 8 weeks of the feeding trials. The final weight (FW), weight gain rate (WGR), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated using the following formula;

$$\mathrm{SR}\,(\%) = 100 \times \frac{\textit{Shrimp final number}}{\textit{Shrimp initial number}};\ \mathrm{FCR} = \frac{\text{Total dry feed intake}}{\text{Total wet weight gain by shrimp (g)}};$$

$$\mathrm{SGR}\,(\%) = 100 \times \frac{In\,[\textit{Final body weight}\,(g)] - \ln\,[\textit{Initial body weight}\,(g)]}{\text{number of culture days}};$$

$$\mathrm{WGR}\,(\%) = 100 \times \frac{[\textit{Final body weight}\,(g) - \textit{Initial body weight}\,(g)]}{\textit{Initial body weight}\,(g)}$$

2.5. Chemical analysis of feed and whole body composition

Proximate compositions of formulated feed and shrimp carcass samples were examined using a standard method [25]. Dry matter was assessed by oven-drying samples at 105 °C until a constant weight was obtained. Crude protein was established following the Kjeldahl method which involves using boric acid to trap NH_3 and was calculated as N x 6.25. The quantity of ash was tested by burning 2 g of samples in a muffle furnace at 550 °C for 4 h. Crude lipid was examined by the ether-extraction method using Soxtect System HT.

2.6. Serum immune analysis

The serum collected from shrimp were used to analyze parameters such as Glucose (Glu), Triglyceride (TG), Cholesterol (CHO), Catalase (CAT), Superoxide dismutase (SOD), Acid phosphatase (ACP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Lysozyme (LZM), and Phenoxidase (PO). These parameters were determined using commercial kits (Nanjing Jiancheng Biological Engineering Institute, China) following the company's protocol. A unit of SOD activity was described as the quantity of enzyme required to yield 50% inhibition of the nitroblue tetrazolium reduction (NBT) rate measured at 550 nm using ELISA microplate reader and is expressed as unit/millilitre (U/ml). A unit of CAT activity was defined as the amount of enzyme needed to decrease absorbance of 0.01/min at 405 nm in a microplate reader and is expressed as unit/millilitre (U/ml). AST and ALT activities were determined following the calorimetric method of Reitman and Frankel's [26] decided by standard curve acquired by contrasting assay between experimental method and Carmen's unit (1 Carmen's unit = 0.482 IU/L, 25 °C) at an absorbance peak of 510 nm. ACP and AKP activities of the serum were determined by spectrophotometer at 520 nm. A unit of AKP activity was defined as the volume of an enzyme that reacted with the matrix to produced 1 mg phenol in 30 min at 37 °C. A unit of ACP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg phenol in 30 min at 37 °C. A unit of LZM activity was defined as the sum of the enzyme required to cause decrease absorbance rate of 0.001 min^{-1} mL^{-1}. The PO activity was well-defined as the amount of enzyme required to produce an increase in absorbance of 0.001 min^{-1}. TP was quantified as described by Ref. [27] using bovine serum albumin as a standard.

2.7. Histological analysis of shrimp intestine

In order to determine the effect of the YC diet on shrimp intestinal morphology, we examined intestinal morphological indexes as described by Ref. [28]. Briefly, after the fixation process, intestinal segments retrieved from Bouin's liquid were dehydrated with graded concentrations of alcohol, cleared in toluene, equilibrated in xylene, and inserted in paraffin to form wax blocks. About 5 μm of wax blocks with tissue segments were sliced by using a rotary microtome and then stained with hematoxylin-eosin (H&E). Intestinal images were captured by using a microscope (Olympus, model BX51, Serial number: 9K18395, Tokyo, Japan). Electronic measurements were taken using the software, Image-Pro Plus 6.3 (Media Cybernetics, Inc., Rockville, USA) to obtain data on intestinal villus height (VH), villus width (VW) and muscle thickness (MT) at 100× magnifications. From each tissue, 12 measurements were taken.

2.8. Microbiota analysis

Using high-throughput sequencing to identify the V3–V4 region of 16S rRNA gene, microbiota analysis was performed according to Ref. [29] with slight changes. Bacterial genomic DNA was extracted from the stool sample using Soil DNA Kit by following the manufacturer's instructions. Sample DNA concentration was quantified using NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The 16S ribosomal V3–V4 hypervariable region of bacteria gene was amplified by polymerase chain reaction (PCR) (95 °C for 2 min, followed by 27 cycles at 98 °C for 10 s, 62 °C for 30 s, and 68 °C for 30 s with final elongation at 68 °C for 10 min) using universal bacteria primers (341F: 5′-CCTACGGGNGGCWGCAG-3'; 806R: 3′-GGACTACHVGGGTATCTAAT-5′). PCR reactions were carried out in triplicate 25 μL mixture containing 2.5 μL of TransStart Buffer, 2 μL of dNTPs, 1 μL of each primer, and 20 ng of template DNA. Through Illumina Hiseq 2500 sequencing, high-throughput sequencing was performed. Amplicons were then separated by 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) by following the manufacturer's protocol before being quantified with QuantiFluor-ST (Promega, U.S.). Purified amplicons were pooled in equimolar and paired-end sequenced (2×250) on an Illumina platform according to the standard protocols.

Quality filtering on the raw reads was performed by filtering to obtain the high-quality clean reads according to the Cutadapt. The reads were compared with the reference database (Silva database, https://www.arb-silva.de/) using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) [30] to detect chimera sequences, and then the chimera sequences were removed and finally, clean reads were obtained. Sequence analysis was performed by Uparse software (Uparse v7.0.1001,http://drive5.com/uparse/) [31] where ≥ 97% similarities were assigned to the same OTUs. Alpha diversity was applied in analyzing the complexity of species diversity for samples through 4 indices, including Chao1, Shannon, Simpson, and ACE. Unique species among groups were shown by Venn analysis.

2.9. Challenge test

Bacteria *Vibrio harveyi* (HY99) was acquired from Guangdong provincial key laboratory of pathogenic biology, Fisheries College, Guangdong Ocean University. They were grown in Luria-Bertani (LB) media in 250 ml flat bottom flask by shaking in an incubator at 37 °C in 180 rmp for 20 h. The cells were centrifuged at 8,000 rpm for 10 min at 4 °C and supernatant discarded. The cells were washed twice with sterile phosphate-buffered saline (PBS). Using serial dilutions method, graded concentrations (10^6, 10^7, 10^8 and 10^9 CFU/ml) of *V. harveyi* was used to conduct preliminary bacteria challenge experiment. 0.1 ml of each concentration of *V. harveyi* was intramuscularly injected into the

third abdominal segment of ten shrimps (average weight 9.7 ± 0.1 g) in two replicates each in tanks containing 50 L of seawater. Mortalities were observed for 168 h, and calculation of best median lethal dose 50 (LD50) was determined (10^8 CFU/ml).

At the end of the feeding trial, 40 shrimps from each treatment were randomly redistributed into four tanks for bacteria resistance test. Another ten shrimps per each experimental group were randomly selected and intramuscularly injected with PBS which was indicated as the negative control (NC). The other four replicate tanks were challenged with 0.1 ml of *V. harveyi* cell suspensions (10^8 CFU/ml) by intramuscular injection. During the challenge trials, shrimps were still fed with their normal experimental diets. From ten shrimps per each treatment, hemolymph was sampled after 24 h of bacteria injection and centrifuged at 3500 rpm/min at 4 °C for 10 min, sera were separated and stored at −80 °C for subsequent analysis of ACP, ALP, PO, and LZM activities. The PBS and the other three bacteria challenge groups per treatment were kept under monitoring, and mortality was recorded up to 168 h. Cumulative mortalities were calculated using the formula shown below.

$$\text{Cumulative mortality, } \% = 100 \times \frac{\textit{Total mortality in each treatment after challenge}}{\textit{Total number of shrimp challenged for same treatment}}$$

2.10. Statistical analysis

The results in this experiment were presented as Mean ± Standard Error (SE). Statistical significant differences were established by using One-Way Analysis of Variance (ANOVA) at 5% level of probability and differences between means were compared using Tukey Range Test. Statistical analysis was carried out in SPSS for Windows, version 22 (SPSS Inc.) Chicago, IL, USA.

3. Results

3.1. Shrimps growth performance, feed conversion ratio and survival rates

The growth performance, feed conversion ratio, and survival rates of *L. vannamei* juveniles fed experimental diets are shown in Table 2. Diet supplemented with YC significantly showed improved growth performance and feed conversion ratio than the control group (YC (1%)) ($p < .05$). No significant differences ($p > .05$) had occurred between YC (1%) and YC (2%) supplemented diets in terms of FW, WGR, SGR, and FCR. YC supplemented groups improved survival rate during the experimental trial period, however, no significant differences ($p > .05$) have been found among all the experimental groups.

3.2. Proximate whole body composition of L. vannamei

Proximate whole body compositions of *L. vannamei* fed experimental diets are presented in Table 3. There was a significant improvement in the dry matter ($p < .05$) of the YC (2%) group but not Y (1%) when compared to the YC (0%) group. Crude protein and ash were significantly high ($p < .05$) in YC (2%) group, followed by YC (1%) compared to the YC (0%) group. In addition, crude lipid was significantly higher ($p < .05$) in YC (2%) group than the YC (1%) and YC (0%) treatment groups.

Table 3
Effects of YC on *L. vannamei* proximate whole body composition.

Treatment	YC (0%)	YC (1%)	YC (2%)
Dry Matter (%)	21.45 ± 0.48^{a}	22.60 ± 0.35^{ab}	23.68 ± 0.25^{b}
Crude Protein (%)	15.12 ± 0.07^{a}	15.65 ± 0.02^{b}	16.00 ± 0.10^{c}
Crude Lipid (%)	2.26 ± 0.15^{a}	2.26 ± 0.01^{a}	2.76 ± 0.10^{b}
Ash (%)	2.55 ± 0.02^{a}	2.82 ± 0.04^{b}	2.89 ± 0.01^{b}

The values are presented as the mean ± SE. The values in the same row with different superscripts are significantly different ($p < .05$) (n = 4).

Table 4
Effects of YC on serum biochemical indexes in *L. vannamei*.

Treatment	YC (0%)	YC (1%)	YC (2%)
TP(mmol/L)	22.43 ± 0.77^{a}	23.15 ± 0.62^{ab}	25.14 ± 0.41^{b}
GLU(mmol/L)	2.53 ± 0.03^{b}	2.46 ± 0.06^{b}	2.07 ± 0.44^{a}
TG(mmol/L)	0.78 ± 0.01^{ab}	0.81 ± 0.00^{b}	0.75 ± 0.01^{a}
CHO(mmol/L)	1.24 ± 0.02^{a}	1.29 ± 0.01^{a}	1.23 ± 0.00^{a}
CAT(U/ml)	3.33 ± 0.06^{a}	3.65 ± 0.03^{b}	3.91 ± 0.07^{c}
SOD(U/ml)	938.76 ± 35.0^{a}	1148.99 ± 24.88^{b}	1210.66 ± 27.13^{b}
AST(U/L)	3.24 ± 0.06^{ab}	3.37 ± 0.01^{b}	3.09 ± 0.03^{a}
ALT(U/L)	5.21 ± 0.02^{b}	5.10 ± 0.06^{b}	4.83 ± 0.05^{a}

The values are presented as the mean ± SE. The values in the same row with different superscripts are significantly different ($p < .05$) (n = 4).

3.3. Evaluation of serum biochemical indexes

Serum biochemical and antioxidant activities are presented in Table 4. Significantly improved ($p < .05$) TP (but not higher than YC (1%)) was detected in YC (2%) treatment in comparison to the YC (0%). There was no significant difference ($p > .05$) between the GLU of YC (0%) and YC (1%), however, GLU of YC (2%) decreased significantly ($p < .05$). TG concentration in serum was significantly low ($p < .05$) in YC (2%) (but not lower than YC (0%)) supplemented group. CHO showed no significant difference ($p > .05$) among all the treatment groups when compared to the untreated YC (0%) group. All YC treated groups (YC (1%) and YC (2%)) showed a significant rising tendency ($p < .05$) of SOD and CAT activities with the highest been observed in YC (2%) fed group. The activities of serum ALT and AST in shrimp fed YC (1%) and YC (2%) diet were clearly lower than the YC (0%) diet ($p < .05$).

3.4. Serum innate immune responses

Serum innate immune response in shrimps before and after 24 h *V. harveyi* challenge test is presented in Fig. 1. Before the challenge test, the result showed significant improvement ($p < .05$) in serum ACP and LZM activity with shrimps fed YC (1%) and YC (2%) supplemented diet

Table 2
Effects of YC on growth performance and feed conversion ratio in *L. vannamei*.

Treatment	YC (0%)	YC (1%)	YC (2%)
IW(g shrimp^{-1})	0.36 ± 0.001	0.36 ± 0.002	0.36 ± 0.000
FW(g shrimp^{-1})	9.79 ± 0.08^{a}	10.66 ± 0.06^{b}	10.54 ± 0.05^{b}
WGR%	2617.31 ± 43.05^{a}	2824.37 ± 49.94^{b}	2789.75 ± 32.32^{b}
SGR%day^{-1}	6.17 ± 1.35^{a}	6.36 ± 1.29^{b}	6.34 ± 1.24^{b}
FCR	1.35 ± 0.02^{b}	1.29 ± 0.02^{ab}	1.24 ± 0.01^{a}
SR%	85.00 ± 85.00^{a}	91.25 ± 91.25^{a}	87.50 ± 87.50^{a}

Values are presented as means ± SE. The values in the same row with different superscripts are significantly different ($p < .05$) (n = 4).

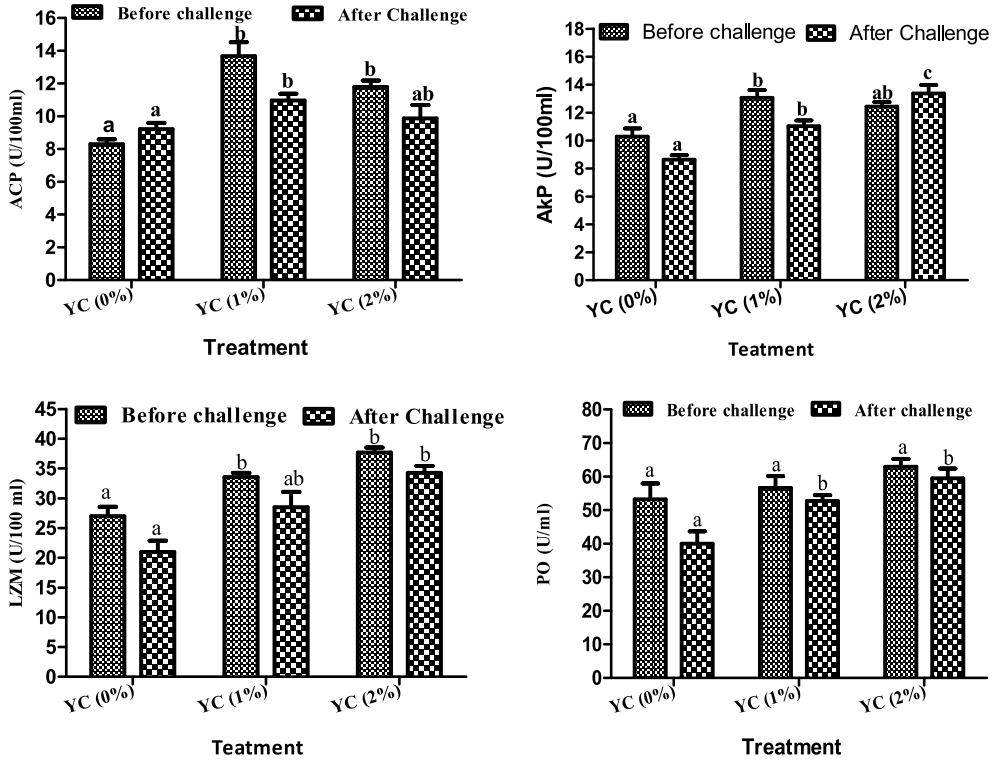

Fig. 1. Effects of YC on serum AKP ACP LZM and PO activity in *L. vannamei* before the challenge, and after 24 h post-challenge with *V. harveyi*. Upright bars denote the mean ± SE. Bars labeled with different letters denote significant difference ($p < .05$) (n = 4) among the treatment groups.

when compared to the YC (0%) diet. After 24 h bacteria challenge, serum AKP, ACP (but not YC (2%)), PO and LZM (but not YC (1%)) were also increased ($p < .05$) in YC (1%) and YC (2%) treated groups (Fig. 1) than the YC (0%) group.

3.5. Microbiota analysis in shrimp gut

3.5.1. Intestinal microbiota richness and species diversity

Operation taxonomic units (OTUs) and Alpha diversity data in *L. vannmei* intestinal microbiota are presented in Table 5. OTUs in shrimp fed YC (1%) and YC (2%) diet were significantly ($p < .05$) higher than the YC (0%). Venn diagram analysis showed that a total of 224 OTUs were shared among all the treatments. The individual OTUs were abundant in YC (2%) (535) group, followed by YC (1%) (177) with YC (0%) (64) having the least (Fig. 2). YC (1%) and YC (2%) treatment groups significantly improved ($p < .05$) chao1 and ACE estimator indices than the YC (0%) group. In addition, the Shannon and Simpson estimator index ranged between 5.08 to 5.82 and 0.86 to 0.91 respectively.

Table 5
Richness and diversity indexes of intestinal bacterial in *L. vannamei* fed experimental diets.

Treatment	YC (0%)	YC (1%)	YC (2%)
Raw Read	85597.39	92351.69	96510.46
Clean Read	80134.65	90462.33	94354.54
Tags	70600.16	89633.33	91688.00
OTU	342.35 ± 22.05^{a}	425.77 ± 19.67^{a}	632.91 ± 26.24^{b}
Chao	382.34 ± 9.67^{a}	463.78 ± 21.07^{b}	666.96 ± 15.97^{c}
ACE	424.96 ± 9.48^{a}	483.85 ± 8.34^{b}	728.97 ± 13.51^{c}
Shannon	5.08 ± 0.03^{a}	5.46 ± 0.02^{b}	5.82 ± 0.05^{c}
Simpson	0.86 ± 0.07^{a}	0.88 ± 0.00^{a}	0.91 ± 0.01^{b}

The data are presented as Mean ± SE. Means in the same row with different superscripts indicate significant difference ($p < .05$) (n = 4).

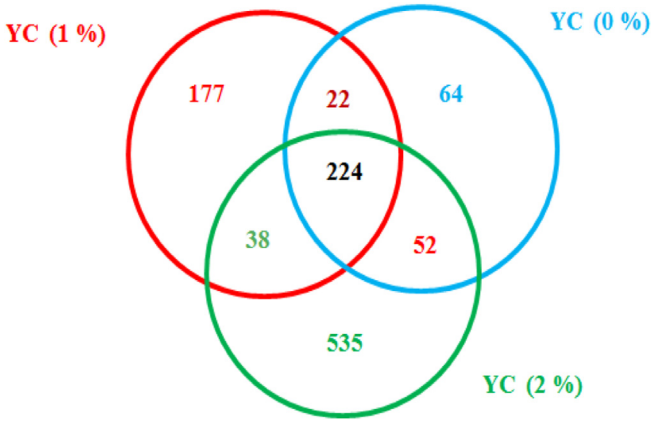

Fig. 2. Venn diagram indicating OTUs shared among *L. vannamei* fed with YC diets.

3.5.2. Gut microbiota composition of L. vannamei fed with YC supplemented diets

The top ten most abundant intestinal bacteria at phylum and genus levels are displayed in Figs. 3 and 4. The four dominant intestinal bacteria at the phylum level were Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes (Fig. 3). Proteobacteria was significantly decreased ($p < .05$) in YC (2%) fed groups while Bacteroidetes and Actinobacteria increased. Although Firmicutes was abundant in YC (1%) and YC (2%) fed group, no significant increase had occurred in any of the experimental groups. At the genus level (Fig. 4), *Vibrio*, *Motilinas, Pseudoalteromonas,* and *Candidatus bacilloplasma* were abundant in all the experimental groups. However, *Vibrio* was significantly decreased ($p < .05$) while *Pseudoalteromonas* was abundant in the YC (2%) groups than in YC (0%) group ($p < .05$).

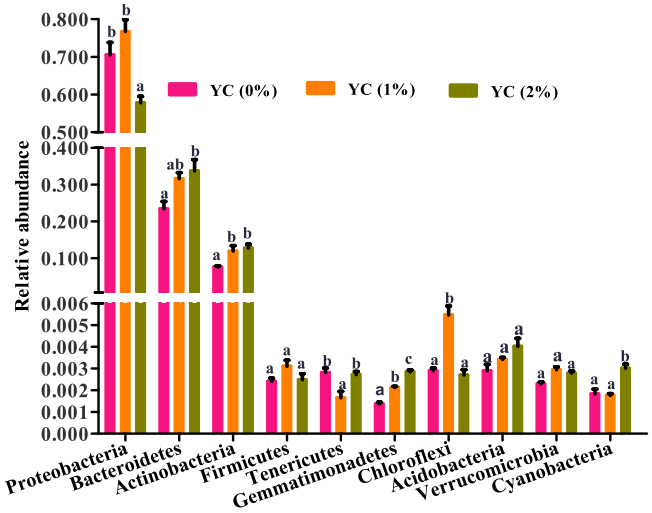

Fig. 3. Effects of YC on intestinal bacterial structure and composition in shrimps at the phylum level of the taxonomy. Upright bars denote the mean ± SE. ($p < .05$) (n = 4). Bars labeled with different letters denote significant differences ($p < .05$) among treatment groups.

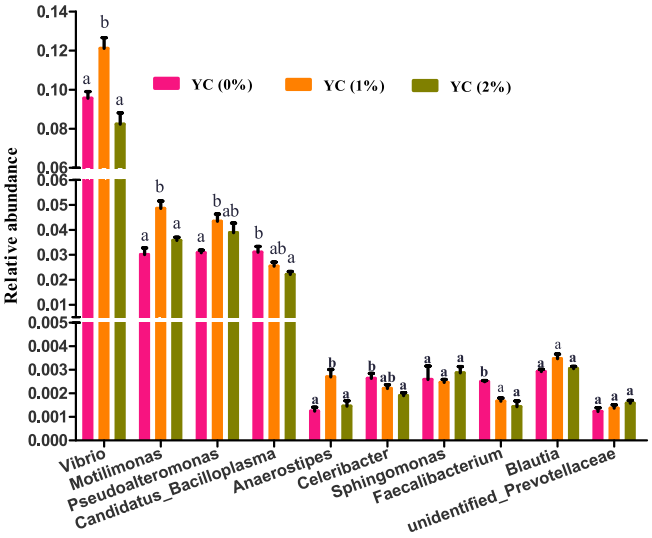

Fig. 4. Effects of YC on the relative abundance of intestinal bacteria in *L. vannamei* at the genus level. Upright bars denote the mean ± SE. ($p < .05$) (n = 4). Bars labeled with different letters denote significant differences ($p < .05$) among treatment groups.

3.6. Histological analysis of shrimp intestine

Mid-intestine photomicrographs and morphological measurements of *L. vannamei* fed with experimental diets are displayed in Fig. 5. No morphological distortions of villus attachment to their intestinal muscle wall were detected in shrimps fed the YC (1%) and YC (2%) diets (Fig. 5A). Taller and wider villi were found in shrimp fed with YC (1%) and YC (2%) diets than the YC (0%) (Fig. 5B). VH was not significantly high ($p > .05$) in YC (1%) compared to YC (2%) whiles VW was significantly high ($p < .05$) in YC (2%) compared to YC (1%) group.

3.7. Challenge test

At the end of 168 h challenge test, no mortality of *L. vannamei* was observed in the PBS-injected group. However, *L. vannamei* post-challenge with *V. harveyi* in the experimental group's started dying before 12 h (Fig. 6). YC (1%) and YC (2%) diet fed groups showed low cumulative mortality rates than the YC (0%) diet (Fig. 6) with the least recorded in YC (2%) fed group. The mean cumulative mortality rates in YC (0%), YC (1%), and YC (2%) groups were 80%, 50.0%, and 30.0%, respectively.

4. Discussion

Yeast culture is a fermented yeast product which contains yeast cell wall and its metabolic fermentation products [32]. A range of studies has established that baker's yeast (*S. cerevisiae*), YC or yeast extract has the capacity of improving growth and animal health [33–35]. The present study demonstrated improved significant FBW, WGR, and SGR, and decreased FCR among shrimps fed YC (1%) and YC (2%) diets than the YC (0%). In agreement with the current study, Jin et al. [1], reported that supplementation of 1% yeast products enhanced growth and feed conversion ratio in *L. vannamei*. The addition of 1%–3% yeast products in the diet of shrimp showed an improvement in the final body weight and specific growth rate [1,36]. Similar growth performance and feed conversion ratio were reported in *L. vannamei* fed with yeast-based additive [33]. Both 1% and 2% dietary supplementation of yeast nucleotides or baker's yeast improved growth and feed efficiency of *Babylonia areolata* [34]. In contrast, hybrid tilapia fed YC diets under cage culture system did not significantly improve growth and feed conversion ratio [37]. The discrepancies in fish growth and feed conversion ratio might be due to the difference in culture systems, fish species and nutrient composition in the diets. The improved growth performance observed in the present study could be attributed to the valuable nutrients in YC such as protein, carbohydrate, vitamins, and nucleic acids, which might have played major nutritional roles in improving shrimp growth. A different reason may be that YC triggered digestive enzyme activities which could influence the nutrient digestibility in shrimp [38–40]. Shrimps fed YC (1%) and YC (2%) groups had a high survival rate without any significant differences being observed when compared with YC (0%) group. Hybrid striped bass fed both (1% and 2%) supplemented brewer's yeast or the prebiotic Grobiotick AE diet was reported to have improved survival rate [41].

Analysis of whole-body composition is a good marker of shrimp physiology and change in feed composition can adversely affect nutrient content in shrimp whole body. In this experiment, positive influence of the YC diet on protein, lipid, and ash content in shrimp's whole body has been observed. Also, Abdel-Tawwab et al. [42], observed similar results in tilapia fed with yeast diets, indicating yeast could enhance the physiology of aquatic animals. From the above results, it could be said that dietary YC supplementation increase intracellular enzyme production which promotes the growth and nutrient composition of Pacific whiteleg shrimps.

Serum analysis provides information on the wellbeing of aquatic animals as it has an essential role to play on the physiological, nutritional, and pathological status of aquatic organisms [43]. TP is a crucial component of the innate immune system, protecting shrimp from potentially invasive organisms [44–46]. In our study, dietary YC, particularly 2% significantly improved serum TP. Zhang et al. [47], reported a significant enhancement in serum TP in grass carp which modulated immune activities. Yeast nucleotide supplemented diet enhanced serum TP in shrimp thus may serve as an immunostimulant for shrimp [35]. TG and CHO are part of the shrimp's body fat, which activities in serum have been used in determining lipid metabolism [48]. YC (2%) diets displayed a decreased trend of serum TG and CHO which contradict the findings reported by Ref. [1]. Normally, an increase in serum glucose is an indicator of stress in aquatic animals [49]. YC (1%) and YC (2%) groups in the present study demonstrated low glucose content than the YC (0%) group; this unveiled that feeding Pacific whiteleg shrimp with YC (1%) and YC (2%) may not expose the shrimps to stress. Metabolic activities in animals produce several Reactive Oxygen Species (ROS) which may lead to oxidative stress. SOD have been used as a molecular marker for assessing oxidative stress condition in shrimps, owing to its

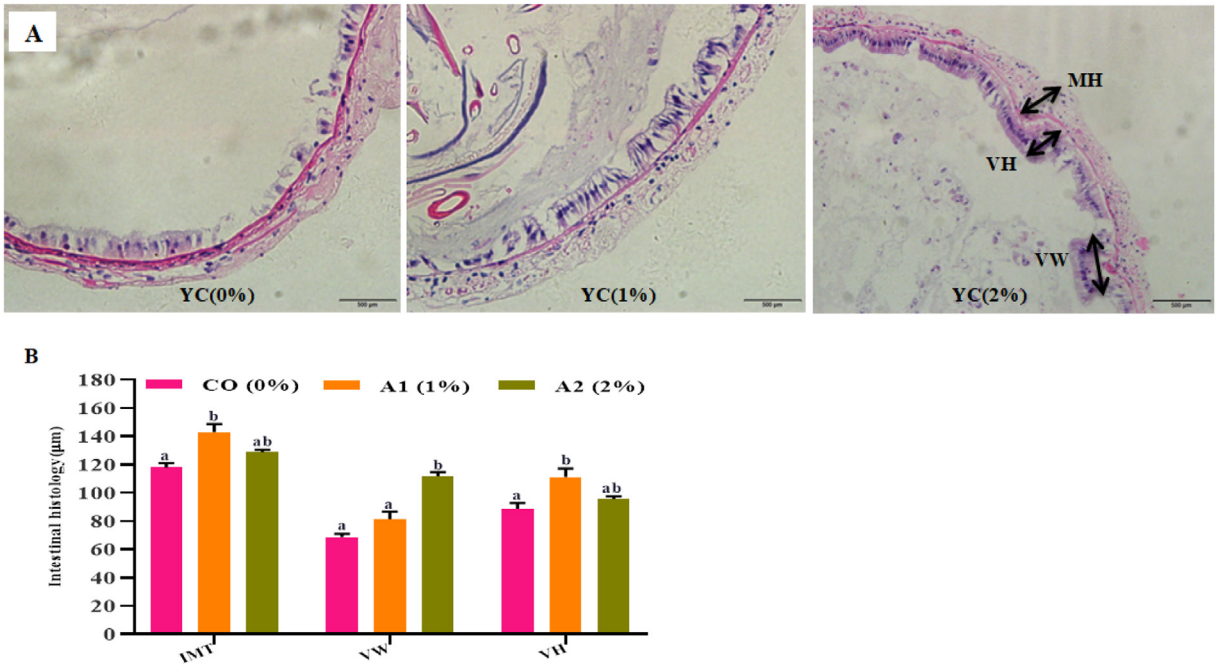

Fig. 5. Photomicrographs (A) and Morphological measurements (B) of the mid-intestine of *L. vannamei* fed YC diets. Upright bars denote the mean ± SE (n = 4). In figure (5A), arrows MT, VH, and VW represent intestinal muscle thickness, villus height, and villus width respectively. Upright bars labeled with different letters denote significant differences ($p < .05$) among groups.

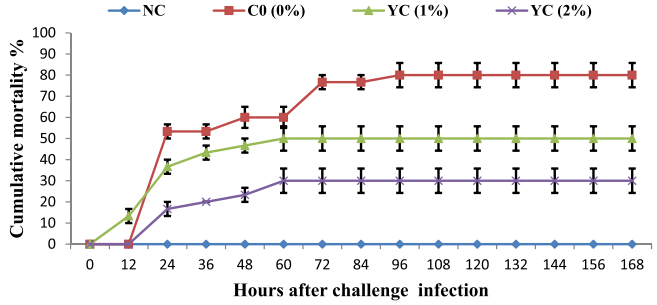

Fig. 6. Cumulative mortality of *L. vannamei* fed YC after 168 h of *V. harveyi* infections. Every plotted value denote mean ± SE of ten shrimp per three replicates (n = 3).

ability to convert superoxide anions to hydrogen peroxide and oxygen which form the first line of antioxidant enzymatic defense [50]. CAT being an antioxidant enzyme has the ability to catalyze the conversion of hydrogen peroxide molecules into water and oxygen during immune responses in aquatic animals [51]. Shrimp serum SOD and CAT activities were highly elevated in treated groups compared to the untreated group in this study. Similar to our study, improvement in SOD and CAT activities have been reported in shrimps fed with yeast, *Rhodosporidium paludamentum* [52]. Furthermore, Yuan et al. [11],reported enhanced SOD and CAT in yeast hydrolysates diets fed to grass carp (*Cyprinus carpio*). Yeast cell contains vitamins [53] which may have beneficial effects on improving the antioxidant immune system of aquatic animals [36]. In cell culture studies, B-complex vitamins reduce oxidative stress caused by H_2O_2 in monocyte cells [54]. AST and ALT are soluble enzymes found in the cytoplasm of liver cells, and their high activities in serum reflect liver damage [55]. It was observed that YC had no adverse effect on shrimp which could be seen in the serum ALT and AST levels. This result corresponds to the YC diet fed in grass carp [56], which could be that active metabolism had occurred in the liver. The present study demonstrated that the YC could enhance the antioxidant immune system in the shrimps as well as protect liver tissues.

Actually, like other crustaceans, Pacific whiteleg shrimp lack an adaptive immune system [57] to resist infections, thus depends on the innate immune system to override pathogenic attack [58]. PO, LZM, ACP, and AKP are key immune enzymes that form part of the innate immune responses in shrimps. ACP is a key lysosomal enzyme that plays a critical role in immunity and has been used as a marker of macrophage activation in animal models [59,60] while AKP hydrolyzes phosphate conjugates extracellular enzyme in many organic compounds (carbohydrates, lipids, and proteins) [60,61]. LZM plays defensive roles against several types of bacterial infections through cleaving N-acetylmuramic acid and N-acetylglucosamine bonds in the cell walls of bacteria, thus damage the cell wall and kills bacteria [62]. PO induces antimicrobial substance into the serum to enhance phagocytosis in the shrimp hemocytes. Various studies on aquatic animals have demonstrated that yeast products have positive effects on innate immune responses. In Ussuri catfish (*Pseudobagrus ussuriensis*), dietary YC supplement at 10% improved plasma ACP, AKP, and serum LZM indicating improved innate immune response [63]. Tukmechi and Bandboni [64] reported that supplementation of two combined yeast products in rainbow trout (*Oncorhynchus mykiss*) diets enhanced innate immune responses. With Pacific whiteleg shrimp, improved innate immune response was reported when proPO gene was expressed in shrimps fed with yeast hydrolysates [1]. In the present work, shrimps fed diets containing YC (1%) and YC (2%) modulated ACP, AKP, LZM, and PO activities before and after shrimps were challenged with *V. harveyi*. YC (1%) and YC (2%) diets improving innate immune responses in shrimps may be due to the presence of β-glucan [65]. Yeast cell β-glucan contains binding receptors that wave on the cell surface to phagocyte pathogens. The binding effects increase the activities of phagocyte cells in overriding, destroying and digesting bacteria [66]. β-glucan extracted from yeast cell wall in feed, enhanced innate immune response of Atlantic salmon challenge with several pathogens [67].

Shrimp intestine is an important organ for nutrient breakdown and absorption, contributing to protection against pathogenic infections, thus plays crucial roles in growth and also improve the immunity of the host [68]. Microbiota diversity and richness shape metabolism and protection activities in shrimps to outweigh pathogenic infections. Irrespective of the diets used in this study, the most abundant intestinal

bacterial communities in *L. vannamei* at the phyla level were *Proteobacteria*, *Bacteroidetes, Actinobacteria*, and *Firmicutes*. Earlier findings by Ref. [56] on grass carp reported *Fusobacteria*, *Bacteroidetes*, *Firmicutes,* and *Proteobacteria* as the most abundant phyla intestinal bacterial populations in grass carp fed with YC for 10 weeks. The discrepancies of *Fusobacteria* dominating grass carp intestinal bacterial might be due to differences in fish species. *Proteobacteria* is the most predominant intestinal bacterial currently been found in *L.vannamei* intestine [69]. The richness variation of *Actinobacteria, Bacteroidetes*, and *Firmicutes* in the intestine of *L. vannamei* intestine could be influenced by the culture environment and available nutrient composition in the diet [69]. The relative abundances of these three major intestinal bacteria in this work suggested how well shrimps were adapted to the culture environment and YC diets. These bacteria, *Bacteroidetes*, *Firmicutes,* and *Actinobacteria*, have been well known to play a major role in the physiology of the host health through metabolic activities [70] such as fermentation of non-digestible carbohydrates which in turn produces short chain fatty acids (SCFAs) [71]. Butyrate is a key product from *Firmicutes* which stimulate the health of the intestinal mucosa [72]. The phylum *Actinobacteria* is a gram-positive bacteria having probiotic effect of producing phenol oxidase which secretes antibacterial, antioxidant, and mucin, contributing to intestinal health of the host [73,74]. *Actinobacteria* have been increased in YC (1%) and YC (2%) diet groups than the YC (0%), signifying that YC could improve immune responses in shrimps. *Vibrio* is a common pathogenic bacterium in *L.vannamei* [75,76]. *Pseudoalteromonas* produce several anti-microbial compounds [77] which are involved in the production of marine bioactive and hydrolytic enzymes through antifouling, antibacterial, and anti-biofilm activities in marine animal and its environment [78]. Recognizing its anti-infective roles and probiotic characteristics, strains of these bacteria have been used as probiotics to reduce pathogenic infection of *Vibrio* species in shrimp aquaculture. In the treated groups, YC (2%) group remarkably reduced colonization of *Vibrio* species in *L. vannamei* intestine. However, the YC (1%) group increased the abundance of *Vibrio* species which warrants further investigations.

As an important tissue in digestion processes, intestinal morphology and structures are essential for nutrient assimilation and the maintenance of normal intestinal functions [79–81]. The functioning of the intestinal wall depends on villi length as it helps in better nutrient absorption and also improve growth performances [79,82]. Currently, YC diets fed to *L. vannamei* significantly enhanced villus height and villus width which translated into improved growth. According to Yuan et al. [4], yeast hydrolysates fed to juvenile Jian carp increased villus height and enhanced growth performance in the fish. Furthermore, dietary MOS increased intestinal villus length in *L. vannamei* [19]; this indicates that dietary MOS provided nutrient which enlarged the intestinal villus structures. The growth in intestinal villus surface area in the present study may be attributed to MOS which is a component in YC.

Infectious disease outbreaks have become a core problem and the most preventive factor in shrimp production [83]. *Vibrio* species are well identified as causative agents for infectious disease outbreak in shrimp culture, causing serious economic losses to farmers under intensive culture through mortalities [84–86]. Feeding shrimps with diet and later challenging them with bacteria pathogens have been a useful method in assessing shrimps resistance to disease infections. In the present study, shrimp fed YC diets showed significant defense against *V. harveyi* infection, particularly YC (2%) group. These results are consistent with that of [87] who fed YC to shrimps at 1% diet. Their results also showed improved resistance of shrimps against *V. harveyi* as compared to the control. Increased resistance of shrimps against *V. harveyi* suggests YC could stimulate and extend the immune response of shrimp against pathogenic infections.

In summary, the present study indicates that YC product in *L. vannamei* diet can significantly improve growth performance, and serum immune responses against *V. harveyi* attack, as well as positively influence intestinal bacterial composition and morphology, thus YC can be used as a feed supplement in *L. vannamei* diet.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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