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Effects of reducing dietary fishmeal with yeast supplementations on *Litopenaeus vannamei* growth, immune response and disease resistance against *Vibrio harveyi*

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ABSTRACT

The aim of this experiment was to investigate the effects of reducing dietary fishmeal (FM) with yeast culture (SYC) supplementation on growth, immune response, intestinal microbiota, intestinal morphology, and disease resistance of *Litopenaeus vannamei*. A total of 480 shrimps with an average initial body weight of 0.35 ± 0.002 g were randomly distributed into twelve tanks. Three isonitrogenous (40.00 crude protein) and isolipidic (8.00 crude lipids) diets with yeast culture supplementing fishmeal were formulated. The groups were divided into two (2) namely control group and experimental groups. The formulations of the groups were control (0 %, without yeast culture) and the experiment groups (SYC) [(1 % of yeast culture), and (2 % of yeast culture)]. Each diet was delivered in four replicate per treatment group. The results indicate significant improvement on the growth indices (specific growth rate, weight gain rate, survival rate and lower feed conversion ratio) with yeast culture treatment group after 56 days feeding trials ($P < 0.05$). Total hemolymph protein, superoxide dismutase, catalase, alkaline phosphatase, acid phosphatase, lysozyme and phenoxidase were enhanced but low aspartate aminotransferase, alanine aminotransferase, and glucose were observed in shrimp fed yeast culture diets ($P < 0.05$). The SYC groups showed insignificant differences in hemolymph cholesterol and triglyceride. Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant bacteria found in all the SYC groups. At the genus level, *Vibrio* was significantly decreased ($P < 0.05$) in 2 % yeast culture diets supplemented group whereas the beneficial bacteria *Pseudoalteromonas* was significantly enhanced. Moreover, intestinal villus length and width in shrimps fed yeast culture diets were improved ($P < 0.05$). Dietary yeast culture supplementation can improve growth, intestinal health, immune response, and resistance against *Vibrio harveyi* infections in *L. vannamei*.

1. Introduction

Global aquaculture production is increasing with continuous demand for aqua-feeds production expansion. Commercial production of aqua-feeds from 1995 to 2008 increased from 7.6 million metric tons to 29.2 million metric tons with an expectation of reaching 71.0 million metric tons in 2025 (Tacon et al., 2011). The increasing production of aqua-feeds faces challenges such as high cost and low supply of feed material component (Deng et al., 2013a, b). Fishmeal is an excellent protein source for the growth and health of shrimp; however, prices have inflated due to increase demand which had led to extensive utilization of plant protein (Bulbul et al., 2016). Diet with low fishmeal but high plant protein content frequently affect growth performance, metabolism, and health status in farmed shrimp (Ding et al., 2015) due to imbalance nutrient, high anti-nutritional factors, and fiber content that affect feed intakes, palatability, and digestibility (Cummins et al., 2013; Jannathulla et al., 2017; Taher et al., 2017). In shrimp, strategies used to develop feed supplement with low-fishmeal diet enhance immunity and disease resistance are important to curb disease outbreaks. For instance, nucleotide (90 mg kg^{-1}) supplemented in low fishmeal diet (18 %) was reported to improve immune response, gut health and disease resistance of shrimp (Guo et al., 2016).

Yeasts are single-cell protein, either alive to feed food organisms or after processing is commonly use as feed ingredients in aquaculture (Jin

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et al., 2018). Yeasts contain rich source of protein (45–55 % crude protein) and vitamin B-complex (Zhang et al., 2018). As feed supplement, yeasts have been used to compensate amino acids and vitamins in diet formulated with cereal grains (Olvera-Novoa et al., 2002). Furthermore, yeast can be easily produced from carbon- rich substrate by-products such as molasses, citrus pulp, fruit waste (*Candida utilis*) and paper industrial wastes on large scale production. It has been considered as a cheap source of dietary feed supplement (Olvera-Novoa et al., 2002). Some studies have indicated that yeast culture is capable of improving health of shrimp by influencing the microbial ecosystem in the digestive tract; this improves digestion and assimilation of nutrients which enhance immunity and growth performance (Burgents et al., 2004; Jin et al., 2018; Li et al., 2009). However, yeast strain and process method may vary in nutrient composition in the yeast product (Guo et al., 2019). Brewers' yeasts (*Saccharomyces cerevisiae*) are natural product of the brewing industry, which at its conventional product form contains 40–47 % crude protein (Guo et al., 2019). Natural fermentation helps in degradation of cell wall in the yeast and cereal grains, this releases nutrients (protein, vitamin, lipid) as well as immunostimulatory compounds (β-glucans, mannan-oligosaccharides and polyamines), which could enhance growth performance, immune response and metabolic activity in shrimps. It is confirmed that yeast culture can also reduce population of pathogenic bacteria settlement on shrimp gut, thus helps in participating in active structuring and shaping of gut wall to improve feed intakes and increases nutrient digestibility (Deng et al., 2013a, b; Marques et al., 2006; Patra and Mohamed, 2003).

Pacific white shrimp (*Litopenaeus vannamei*) remains the dominant shrimp aquaculture practice in China and also the world owing to the advantage of having high growth performance, taste of flesh and good consumer preference (Chen et al., 2019a, b, c; Hu et al., 2009). *L. vannamei* production in China reached 16.7 million tons in 2017 (Xiong et al., 2018) and many of its culture practices depend on more than 36 % crude proteins which is provided by fishmeal (Guo et al., 2016; Smith et al., 1985). The unreliable supply and increase in price of fishmeal has led to the utilization of plant protein. However, poor feed utilization, growth performance, immune stimulation, damage of the intestine structure and changes in the gut microbiota are associated with high plant protein diets (Guo et al., 2016). Previous studies observed the significance of yeast hydrolysates in improving the growth performance, antioxidant responses, the innate immunity, diseases resistance and intestinal diversity in shrimp (Jin et al., 2018; Yuan et al., 2017; Zhou et al., 2018). Much is unknown about the effects of yeast culture products derived from brewer's yeast, whether it could improve *L. vannamei* growth performance and its health. Therefore, the objective of the present study was to evaluate the effects of supplementing high quality protein yeast culture in low fishmeal diet on growth performance, hemolymph immune response, intestinal health, and disease resistance of *L. vannamei*.

2. Materials and methods

2.1. Yeast product and diet preparation

Yeast culture was produced at Enhalor Biotechnology Company in Beijing, China. Briefly, in obtaining yeast culture powder, *Saccharomyces cerevisiae* was aerobically fermented in molasses based medium. Cereal grain was added into a content to form slurry and fermented anaerobically to enhance breakage of cell wall. After drying, the mixture was milled to desirable final particle size. The nutrient composition in the yeast culture powder was listed in Table 1.

The main source of protein in the basal diet was brown fishmeal and the other two diets contained increasing concentrations of yeast culture supplement at 1 % and 2 % of the fishmeal. The three diets were referred to as Control (0 % of no yeast culture), SYC (1 % of yeast culture) and SYC (2 % of yeast culture). The diet was prepared by thoroughly blending core ingredients with minerals and vitamins. Lipid source was added to the mixtures and mixed by hand. They were then mixed with water to form dough before processing in meat mincer machine to obtain spaghetti like pellets. Pellets were dried until moisture content decreased to about 10 %. Having cut into approximately 2.00 mm length, they were packaged into seal bags and freeze dried at -20 ℃ until used. The three experimental diets formulated contained 40 % crude protein and 8 % lipid crude (Table 2).

Table 1
Nutrient content in yeast culture powder (% dry weight).

Chemical composition	Yeast culture
Crude Protein	55.00
Crude Lipid	0.53
Moisture	3.97
Essential amino acids (%)	
Histidine	1.27
Arginine	3.97
Isoleucine	2.10
Leucine	3.25
Lysine	3.29
Methionine	0.74
Threonine	1.69
Phenylalanine	2.31
Cystine	0.42
Valine	2.47
ΣEAA	21.51
Non-Essential Amino Acids	
Alanine	2.97
Aspartic acid	4.65
Glutamic acid	8.91
Glycine	2.14
Proline	1.93
Serine	1.62
Tyrosine	1.17
ΣNEAA	23.40

2.2. Rearing condition

Zhanjiang Allied Pacific Aquaculture Limited supplied juveniles of *L. vannamei* used in this experiment. The shrimps were acclimatized in in-door concrete tanks ((4.5 m (L) ×3.45 m (W) ×1.8 m (H)) at the Marine Biological Research Base of Guangdong Ocean University for 28 days and fed with commercial diet containing 45 % crude protein and 10 % crude lipids. To begin with the experiment, shrimps were weighed after 12 h of starvation, and those with approximately 0.35 g ± 0.002 wt were divided into twelve fiberglass tanks (0.3 m^3) containing 200 L sieved seawater. In all, 40 shrimps were stocked into a tank and fed four times daily in four replicates per treatment at 8–10 % of their average body weight. Feed intakes were monitored to evaluate feed conversion ratio, and one third of the seawater in each tank was replaced every 24 h with continuous oxygenation via air stones. Water quality parameters such as temperature during experimental period ranged from 26.5 ˚C to 30.5 ˚C, dissolved oxygen 6.0 mg L^{-1} to 6.5 mgL^{-1}, and pH 7.9 to 8.1.

2.3. Sample collection

At the end of the 56 days feeding trials, hemolymph samples were taken from pericardial cavity of eight shrimps into 1.5 mL Eppendorf tubes, where they were centrifuged at 4000 rpm/min for 10 min at 4 ˚C; supernatants were collected and stored at −20 ˚C then used for enzymatic analyses. Shrimps were countered, weighed and length measured from each tank to determine growth performance. The whole body of two shrimps was sampled and freeze dried at −20 ˚C for proximate chemical composition. Three shrimp was pooled, mid-guts (0.6–1.0 cm) was dissected and fixed into 10 % paraformaldehyde for histological analysis. Another three mid-guts were immediately collected, loaded into liquid nitrogen for microbiota analysis.

Table 2
Ingredients and nutritional composition in experimental diets (% dry weight).

Ingredient (%)	Control (0 %)	SYC (1 %)	SYC (2 %)
Fish meal (60 % protein)[A]	20.00	19.18	18.38
Yeast culture (55 % protein)	0.00	1.00	2.00
Peanut meal[a]	I8.00	18.00	18.00
Soybean 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	1.89	1.97
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[c]	0.10	0.10	0.10
Antioxidants ethoxyquin[c]	0.03	0.03	0.03
Choline chloride 99 %[d]	0.50	0.50	0.50
92 % monobasic calcium phosphate[d]	1.00	1.00	1.00
Vc polyphosphate 35 %[e]	0.09	0.09	0.09
Antimony trioxide[e]	0.50	0.50	0.50
CMCC[D]	8.86	8.41	8.15
Total	100.00	100.00	100.00
Nutrient levels			
Dry matter	89.16	89.12	89.21
Crude protein	40.18	40.25	40.22
Crude lipid	8.00	8.20	8.05
Ash	8.94	8.89	8.81

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

[b] Vitamin premix supplied the following per kg of the diet: vitamin A, 22,500 IU; vitamin D3, 6000 IU; vitamin E, 200 mg; vitamin K3, 40 mg; vitamin B1, 30 mg; vitamin B2, 45 mg; vitain 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 premix 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.

2.4. Bacterial challenge test

2.4.1. Bacterial preparation and preliminary test

Vibrio harveyi was used to conduct shrimp disease resistance test. This microorganism were cultured in Luria-Bertani (LB) media at 37 °C for 20 h and centrifuged at 8000 rpm/min for 10 min at 4 °C. The bacteria cells were washed twice with aseptic phosphate buffer saline (PBS) at pH of 7.2 to get rid of culture media. They were serially diluted into four different concentrations (10^6, 10^7, 10^8, and 10^9 CFU/mL) where 100 µl of *V. harveyi* was intramuscularly inoculated at third abdominal segment of the shrimp, ten shrimps in a tank with two replicate per *V. harveyi* concentration. 7 days mortalities of shrimps was observed and suitable median lethal dose 50 (LD50) was achieved with 10^8 CFU/mL concentrations.

2.4.2. Experimental challenge test

The experimental challenge test was conducted with 10 shrimp per fiberglass tanks containing 50 L of sieved seawater in five repeat per treatment group. Each shrimp in four repeat tanks per treatment was intramuscularly inoculated with *V. harveyi* containing 10^8 CFU/mL cells suspended in 100 µl PBS, whereas the negative control group was injected using PBS with the same volume as bacterial infected group. Shrimps were fed thrice with their respective experimental diets during the infection period. At the 12 h after infection, hemolymph samples were collected from different challenge groups, these were used to examine innate immune response in shrimp. Twelve hours interval mortalities were observed for 7 days. Cumulative mortalities curves were calculated using the formula presented below.

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

2.5. Chemical composition analysis

Chemical analyses of the yeast culture, diets and shrimps whole body were assessed using methods described by AOAC International (2012). Crude protein (N x 6.25) was determined by Kjeldahl method. Crude lipid was assessed by ether extraction using Soxtec system. Dry matter was evaluated by oven drying samples at 105 °C until constant weight was obtained. Ash was evaluated by inclination of 2 g samples into muffle furnace at 550 °C for 3 h. Amino acid analysis of yeast culture powder was measured after acid hydrolysis in 6 M HCl for 24 h at 110 °C separated by ion-exchange chromatography and reacted with ninhydrin post column derivatization using Hitachi L-8800 Amino Acid Analyzer.

2.6. Intestinal microbiota analysis

Total genomic DNA of microbes in shrimp mid-gut was extracted using E.Z.N.A. stool DNA Kit (OMEGA, US) following the manufacturer's protocol. Amplification and sequencing of the V3-V4 hypervariable region of the bacterial 16S DNA gene was performed using barcoded primers 515 F (GTGCCAGCMGCCGCGGTAA) 806R (GGACTACHVGGGTWTCTAAT). Polymerase chain 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. Using Illumina MiSeq platform, 16S rRNA tag-encoded high throughput sequencing were performed at Novogene Company (Beijing, China). Sequencing reads were assigned to individual sample with their unique barcode before analyzing with QIIME software package (Quantitative Insights Into Microbial Ecology) and UPARSE pipeline (Quast et al., 2013). Reads were clean and grouped into Operational Taxonomic Units (OTUs) at uniqueness threshold of 97 %. Alpha diversity was applied in analyzing complexity of species diversity for samples through 4 indices, including Chao1, Shannon, Simpson, and ACE using QIIME. The shared and unique species among groups were showed on Venn diagram.

2.7. Histological analysis of shrimp intestine

Dietary effects of yeast culture diet on shrimp intestinal morphology was determined as described by Zhang et al. (2009). Briefly, mid-guts were fixed in Bouin's solution, dehydrated with graded concentrations of alcohol, cleared in toluene, equilibrated in xylene, inserted in paraffin to form wax blocks and sectioned at 5 µm. Samples after being stained with hematoxylin–eosin was captured using microscope (Olympus, model BX51, Serial number: 9K18395, Tokyo, Japan) and electronic measurement on intestinal villus height (VH), villus width (VW) and muscle thickness (MT) at 100× magnifications were taken using software Image-Pro Plus 6.3 (Media Cybernetics, Inc., Rockville, USA). Villus height was determined by taken measurement from the tip of the villus to the villus-crypt junction. Villus width was evaluated by taking measurement at middle portion of the villus. Muscle thickness was at the base of crypt to the base of the muscular is mucosae.

2.8. Hemolymph biochemical analysis

The hemolymph 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 (Reitman and Frankel, 1957) decided by standard curve acquired by contrasting assay between experimental method and Carmen's unit (1 Carmen's unit = 0.482 IU/L, 25 ℃) at an absorbance peak of 510 nm. ACP and AKP activities of the hemolymph 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 ℃. 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 ℃. 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 Bradford (1976) using bovine hemolymph albumin as a standard.

3. Statistical analysis

SPSS for Windows, version 22 (SPSS Inc. Chicago, IL, USA) was used for statistical analysis. Data was compared by One-Way Analysis of Variance followed by Tukey Range Test where statistical significant differences were established at the $P < 0.05$ level.

4. Results

4.1. Growth performance, survival, and feed efficiency

At the end of the feeding trials, growth performance and feed utilization of shrimp increase with the SYC inclusion levels in the diets ($P < 0.05$). FBW, WGR, and SGR parameters significantly increased in shrimp fed diet SYC (1 %) and SYC (2 %) as compared to the control group ($P < 0.05$) (Table 3). PER and CK were substantially improved in SYC (2 %) supplementation groups whereas the FCR and HSI were significantly lower in SYC (2 %) group ($P < 0.05$) ($P < 0.05$). The different inclusion rates of SYC significantly increased SR than control treatments ($P < 0.05$). The SYC (2 %) treatment group had the highest SR than the SYC (1 %) group ($P > 0.05$).

4.2. Shrimp whole body composition

The whole body composition parameters of shrimp fed SYC supplementation groups were significantly affected with the exception of crude lipid content ($P > 0.05$). Dietary SYC (1 %) and SYC (2 %) groups' results were significantly higher in terms of dry matter and crude protein in the shrimp whole body, as compared to control group ($P < 0.05$). Ash content was only improved in the shrimp fed SYC (2 %) diet group ($P < 0.05$) (Table 4).

4.3. Hemolymph biochemical parameters

The effects of dietary SYC supplementation on hemolymph biochemical parameters are displayed in Table 5. Although MDA, and AST exhibited a decreasing tendency among the SYC groups, there were no significant differences in the MDA and AST between SYC (1 %) and SYC (2 %) groups ($P > 0.05$). The CHO and TG content were increased among SYC treatment groups, however no significant difference was found when compared to control group ($P > 0.05$). TP concentration in hemolymph of the shrimp was significantly increased as yeast culture inclusion increased from control (0 %) to SYC (2 %) ($P < 0.05$). The increasing SYC supplementation showed a decreasing trend of shrimp hemolymph GLU action. Lower level of GLU content was observed in SYC (2 %) compared to the other group ($P > 0.05$). Statistical differences showed that SOD was first enhanced in SYC (1 %) group and then further increased with SYC (2 %) supplementation compared to control group ($P < 0.05$), whereas CAT content in SYC groups differ with

Table 3
Growth performance, feed utilization and morphological indices of *L. vannamei* fed with different levels of yeast culture diet for 8 weeks.

Treatment	Control (0 %)	SYC (1 %)	SYC (2 %)
IBW (g/shrimp)	0.35 ± 0.002^{a}	0.35 ± 0.000^{a}	0.35 ± 0.001^{a}
FBW (g/shrimp)	10.28 ± 0.98^{a}	11.52 ± 0.14^{b}	11.73 ± 0.28^{b}
WGR (%)	2909.26 ± 24.24^{a}	3182.70 ± 40.44^{b}	3243.19 ± 81.40^{b}
SGR (%day^{-1})	6.03 ± 0.03^{a}	6.23 ± 0.03^{b}	6.26 ± 0.04^{b}
FCR	1.28 ± 0.01^{b}	1.18 ± 0.02^{a}	1.15 ± 0.16^{a}
PER (%)	233.63 ± 0.55^{a}	237.17 ± 0.14^{b}	237.92 ± 0.04^{b}
SR%	84.37 ± 2.37^{a}	94.37 ± 2.13^{b}	97.50 ± 1.44^{b}
CK (g/cm^3)	0.72 ± 0.01^{a}	0.73 ± 0.00^{ab}	0.74 ± 0.00^{b}
HSI (%)	6.63 ± 0.23^{b}	5.99 ± 0.21^{ab}	5.52 ± 0.14^{a}

The data was showed as in mean ± SE. The data in the same row with different superscripts are significantly different (Tukey, $P < 0.05$; $n = 4$).
Initial mean body weight (IBW, g/shrimp).
Final mean body weight (FBW, g/shrimp).
Weight gain (WG, %) = 100 × (final mean weight - initial mean weight)/initial mean weight.
Specific growth rate (SGR, %day^{-1}) = 100 × (LnWt − LnWi)/t.
Feed conversion rate (FCR) = feed consumed (g, dry weight)/weight gain (g, wet weight).
Survival rate (SR, %) = 100 × (final amount of shrimp)/(initial amount of shrimp).
Condition factor (CF, g/cm^3) = 100 × body weight (g)/body length (cm) ^{3}.
Hepatopancreas somatic index (HSI, %) = 100 × liver weight (g)/body weight (g).

Table 4
Proximate composition in whole body of *L. vannamei* fed with different levels of yeast culture diet for 8 weeks (% dry weight).

Treatment	Control (0 %)	SYC (1 %)	SYC (2 %)
Dry matter	24.01 ± 0.27^{a}	24.98 ± 0.40^{b}	24.45 ± 0.34^{b}
Crude protein	15.66 ± 0.16^{a}	16.63 ± 0.15^{b}	17.01 ± 0.18^{b}
Crude lipid	2.03 ± 0.12^{a}	2.00 ± 0.21^{a}	2.14 ± 10^{a}
Ash	2.33 ± 0.07^{a}	2.69 ± 0.10^{a}	3.20 ± 0.12^{b}

The data are presented as the mean ± S.E. The data in the same row with different superscripts are significantly different (Tukey, $P < 0.05$; $n = 4$).

Table 5
Hemolymph biochemical parameters of *L. vannamei* fed with different levels of yeast culture diet for 8 weeks.

Treatment	Control (0 %)	SYC (1 %)	SYC (2 %)
TP ($ugml^{-1}$)	23.50 ± 0.39^{a}	25.84 ± 0.24^{b}	27.80 ± 0.45^{c}
GLU ($mmolL^{-1}$)	2.08 ± 0.10^{b}	1.78 ± 0.08^{b}	1.49 ± 0.07^{a}
TG ($mmolL^{-1}$)	0.56 ± 0.01^{a}	0.55 ± 0.02^{a}	0.57 ± 0.00^{a}
CHO ($mmolL^{-1}$)	0.93 ± 0.09^{a}	0.99 ± 0.06^{a}	1.02 ± 0.04^{a}
MDA ($mmolL^{-1}$)	3.11 ± 0.07^{b}	2.71 ± 0.03^{a}	2.55 ± 0.10^{a}
CAT (Uml^{-1})	3.94 ± 0.09^{a}	4.38 ± 0.20^{b}	4.62 ± 0.08^{b}
SOD (Uml^{-1})	926.6 ± 18.84^{a}	988.50 ± 5.12^{b}	1065.24 ± 14.72^{c}
ALT (UL^{-1})	3.12 ± 0.15^{b}	2.54 ± 0.06^{a}	2.87 ± 0.12^{ab}
AST (UL^{-1})	4.92 ± 0.02^{b}	4.62 ± 0.05^{a}	4.51 ± 0.04^{a}

The data was showed as in mean ± SE. The value in the same row with different superscripts are significantly different (Tukey, $P < 0.05$; $n = 4$). Where; TP (Total protein), GLU (glucose), TG (Triglyceride), CHO (Cholesterol); CAT (Catalase); SOD, (Superoxide dismutase); AST (Aspartate aminotransferase); ALT (Alanine aminotransferase) and MDA (Malondialdehyde).

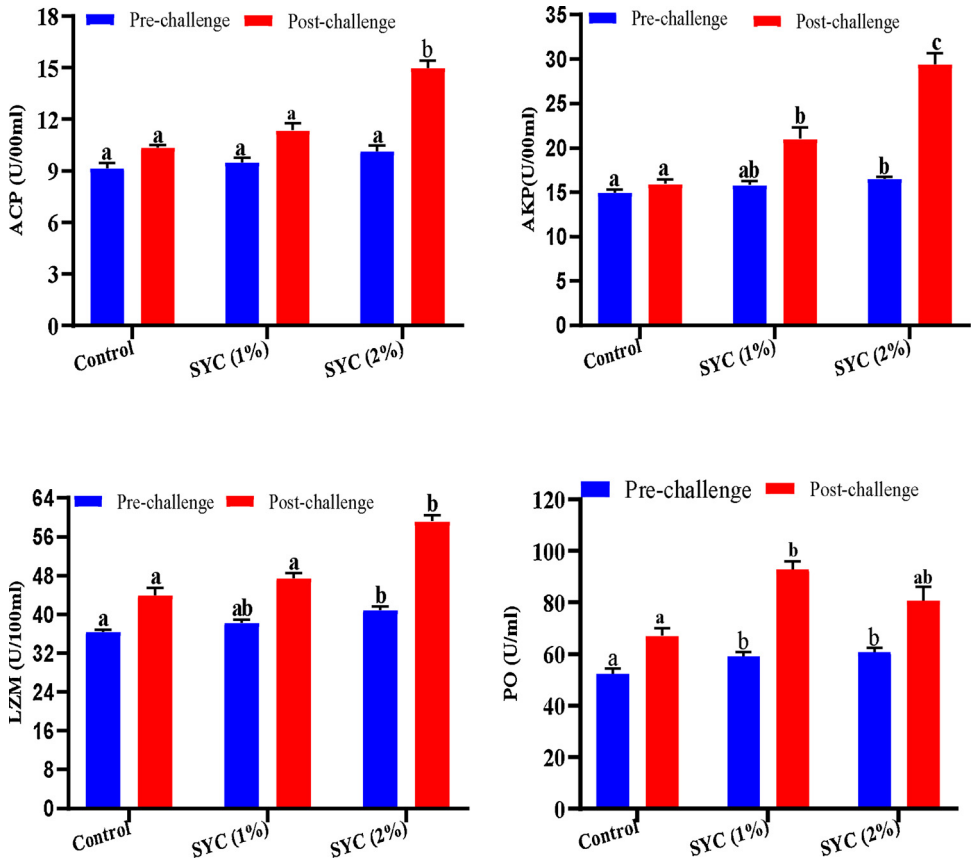

Fig. 1. Yeast culture diets effects on hemolymph innate immune activity of L.*vannamei* pre-challenge and 12 h post-challenge with V. harveyi. The data were showed as in mean ± SE (n = 4). The graphs with the same color having different superscript showed significantly different (P < 0.05).

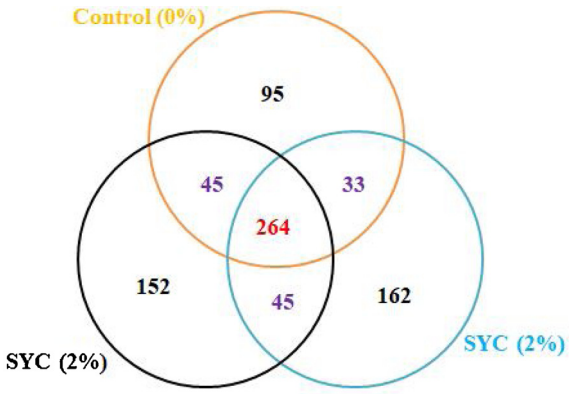

Fig. 2. Venn diagram showing the OTU distribution of L.*vannamei* intestinal microbiota fed with different diets.

different inclusion levels (P < 0.05). The highest CAT content was observed in the SYC (2 %) group without significant differences being observed between SYC (1 %) and SYC (2 %) groups (P > 0.05). ALT in hemolymph was significantly elevated in the control group compared to SYC treatments with the SYC (1 %) experiencing lowest ALT activities (P < 0.05).

4.4. Hemolymph innate immune indexes

The hemolymph innate immune activities (ACP, AKP, LZM and PO) before challenge and after 12 h challenge test are presented in Fig. 1. Before challenge, significant levels of LZM and AKP were maintained in the SYC groups (P < 0.05) while the ACP levels slightly increased in the SYC groups than the control group (P > 0.05). The PO activities were slightly enhanced in shrimp fed SYC diets before challenge (P < 0.05). After 12 h *V. harveyi* injection, the innate immune parameters (LZM, ACP, and AKP) were significantly increased in the SY0.C (2 %) group (P < 0.05). However, the PO significantly increased in the SYC (1 %) after12 hours *V. harveyi* injection (P < 0.05). Before and after challenge, the SYC groups indicated significant differences in the innate immune parameters (P < 0.05).

4.5. Microbial composition

The Chao and Ace represent the quantity of bacteria community species without respect to abundance of each species. The Shannon and Simpson indexes are influenced by species richness and species evenness which account for the bacteria community in the treatment group. Ace, Chao and Simpson indexes were significant higher in the SYC groups as compared to control group (P < 0.05). Shannon index showed a decreased trend in the SYC groups' supplementation in the intestinal bacteria of shrimp (P > 0.05). The number of unique OUT was presented in the Venn diagram (Fig. 2). The mean OUT in the control, SYC (1 %) and SYC (2 %) group were 95, 152 and 632 respectively. The intestinal bacteria richness in SYC groups was higher than the control (Table 6).

4.5.1. The intestinal bacteria composition and the abundance in shrimp

The top ten highest relatively abundant bacteria at phyla level are presented in Table 6a. The most abundant phyla bacteria observed were Proteobacteria, Bacteroidetes, and Actinobacteria in control, SYC (1 %) and SYC (2 %) groups. Proteobacteria abundance in the treatment groups was much more than the untreated group with no significance being found among the treatment groups (P > 0.05); whereas abundance of Bacteroidetes and Actinobacteria in the control group were

Table 6
Intestinal bacteria diversity indexes and estimate richness of *L. vannamei* fed with different levels of yeast culture diet for 8 weeks.

Index	Control (0 %)	SYC (1 %)	SYC (2 %)
Raw Reads	82771.33 ± 829.00^{a}	86480.25 ± 262.28^{b}	87505.00 ± 336.14^{b}
Clean Reads	80101.03 ± 39.4^{a}	80178.00 ± 20.18^{a}	80324.50 ± 33.07^{a}
Tag	80067.33 ± 8.19^{a}	80168.00 ± 10.44^{b}	80264.33 ± 28.41^{c}
OTU	302.00 ± 5.58^{a}	408.33 ± 17.6^{b}	541.25 ± 16.32^{c}
Chao	227.84 ± 9.46^{a}	368.02 ± 6.40^{b}	451.02 ± 23.03^{c}
Ace	239.54 ± 16.48^{a}	382.13 ± 4.39^{b}	456.48 ± 15.30^{c}
Shannon	0.88 ± 0.00^{b}	0.82 ± 0.01^{a}	0.83 ± 0.00^{a}
Simpson	3.89 ± 0.23^{a}	4.15 ± 0.15^{b}	5.01 ± 0.32^{c}

The data was showed as in mean ± SE. Means in the same row with different superscripts indicated significant difference (Tukey, $P < 0.05$; n = 4).

Table 6a
Relative abundance (%) of phyla intestinal bacteria of *L. vannamei* fed with different levels of yeast culture diet for 8 weeks.

Phyla	Control (0 %)	SYC (1 %)	SYC (2 %)
Proteobacteria	67.74 ± 38.20^{a}	69.12 ± 3.55^{a}	69.43 ± 1.01^{a}
Bacteroidetes	14.73 ± 2.10^{a}	14.89 ± 2.48^{a}	17.12 ± 0.05^{b}
Actinobacteria	7.67 ± 0.03^{a}	10.73 ± 0.31^{ab}	12.56 ± 1.24^{b}
Firmicutes	0.25 ± 0.01^{a}	0.26 ± 0.02^{a}	0.29 ± 0.05^{a}
Tenericutes	0.15 ± 0.02^{a}	2.17 ± 0.32^{b}	0.17 ± 0.04^{a}
Gemmatimonadetes	0.13 ± 0.00^{a}	0.13 ± 0.13^{a}	0.15 ± 0.03^{a}
Chloroflexi	0.30 ± 0.00^{a}	0.30 ± 0.00^{a}	4.69 ± 0.00^{b}
Acidobacteria	0.30 ± 0.03^{a}	1.02 ± 0.60^{b}	0.49 ± 0.03^{ab}
Verrucomicrobia	0.25 ± 0.00^{b}	0.16 ± 0.01^{a}	0.12 ± 0.00^{a}
Cyanobacteria	0.38 ± 0.02^{a}	0.36 ± 0.05^{a}	0.54 ± 0.00^{b}

The data was showed as in mean ± SE. Means in the same row with different superscripts indicated significant difference (Tukey, $P < 0.05$; n = 4).

significantly lower than that of SYC (2 %) but not in SYC (1 %) group ($P < 0.05$).

Vibrio belonging to the phylum *Proteobacteria* was the most abundant microbe observed at genus level (Table 6b). *Vibrio* in the control group was significantly higher than the SYC groups representing 9.53 %, 5.54 % and 3.10 % as in the control, SYC (1 %) and SYC (2 %) respectively ($P < 0.05$). There was a significant abundance of *Pseudoalteromonas* and *Sphingomonas* in SYC groups as compared to the control group ($P < 0.05$). The abundance of *Pseudoalteromonas* and *Sphingomonas* in the control, SYC (1 %) and SYC (2 %) account for 4.51 %, 5.90 % and 7.51 % as well as 0.16 %, 0.26 % and 0.46 % respectively.

4.6. Intestinal histological analysis

The effects of the experimental diet on *L. vannamei* intestinal morphology are presented in Fig. 3. Shrimp fed with the control diet have morphological changes. The mucosal folds separation from intestinal wall was seen in shrimps fed the control diets whereas fusion of mucosal folds was observed in shrimps fed with SYC (1 %) and SYC (2 %) diets.

Table 6b
Relative abundance (%) of genera intestinal bacteria of *L. vannamei* fed with different levels of yeast culture diet for 8 weeks.

Genera	Control (0 %)	SYC (1 %)	SYC (2 %)
Vibrio	9.53 ± 0.44^{c}	5.54 ± 0.50^{b}	3.10 ± 0.24^{a}
Motilimonas	5.37 ± 0.69^{a}	9.21 ± 0.61^{ab}	7.44 ± 0.38^{b}
Pseudoalteromonas	4.51 ± 0.24^{a}	5.90 ± 0.17^{b}	7.36 ± 0.50^{c}
Candidatus-Bacilloplasma	0.23 ± 0.02^{a}	0.23 ± 0.02^{a}	0.15 ± 0.01^{b}
Anaerostipes	0.25 ± 0.01^{a}	0.26 ± 0.01^{a}	0.32 ± 0.02^{a}
Celeribacter	0.36 ± 0.02^{a}	0.63 ± 0.04^{b}	0.30 ± 0.02^{b}
Sphingomonas	0.16 ± 0.02^{b}	0.26 ± 0.02^{b}	0.49 ± 0.05^{c}
Unidentified-Prevotellaceae	0.15 ± 0.00^{b}	0.10 ± 0.00^{a}	0.18 ± 0.01^{b}
Faecalibacterium	0.16 ± 0.00^{a}	0.17 ± 0.00^{a}	0.13 ± 0.00^{a}
Blautia	0.27 ± 0.01^{a}	0.24 ± 0.02^{a}	0.26 ± 0.01^{a}

The data was showed as in mean ± S.E. Means in the same row with different superscripts indicated significant difference (Tukey, $P < 0.05$; n = 4).

The measurement of intestinal morphometric parameters are represented in Fig. 4. There was significant increase in VH and WH with SYC supplementation ($P < 0.05$). The shrimp fed SYC (2 %) showed significant higher VH over SYC (1 %) ($P < 0.05$), whiles there were no improvement in VW observed between SYC (1 %) and SYC (2 %) treatment groups ($P > 0.05$). The results of MT displayed no significant changes in all the experimental groups ($P > 0.05$).

4.7. Pathogenic challenge test

The cumulative mortality rate of *L. vannamei* challenged with *V. harveyi* for 168 h was presented in Fig. 5. Feeding shrimp at different levels of SYC supplementation in diets showed significant improved resistance to *V. harveyi* infection ($P < 0.05$). All shrimps fed with SYC treatment indicated significant low cumulative mortality rates as compared to the control diet ($P < 0.05$). The 168 h percentage cumulative mortality rates in Control (0 %), SYC (1 %) and SYC (2 %) groups were 60 %, 40.0 %, and 16.7 %, respectively.

5. Discussion

In the present study, a yeast culture product was used to replace small amounts of fishmeal in the experimental diets of *L. vannamei*. At the end of feeding trials, growth parameters (FBW, WGR, and SGR) of the shrimp fed SYC supplementation diets were significantly increased than the control group. In addition, the growth indices slightly increased in SYC (2 %) compared to SYC (1 %) indicating SYC (2 %) improve better growth performance. Earlier studies indicated that, yeast product enhanced the growth performances and immune function in L.*vannamei* which conform to the present study (Deng et al., 2013a, b; Qiu and Davis, 2017; Yang et al., 2009). Furthermore, shrimp feed utilization performances (FCR and PER) were improved by SYC treatment groups in the current study. FCR decreased while PER increased in SYC (2 %) in the present study. The shrimps feed utilization on yeast diet was improved on the commercial scale production of shrimp (Deng et al., 2013a, b). This may be attributed to energy produced by glucanase which is involved the breakdown of glucan in the intestine by secreting more digestive enzymes to aid in protein deposition; thereby influencing the growth performance and feed utilization of shrimps (Chen et al., 2019a, b, c). Besides, the presence of manna oligosaccharides help in growth of intestinal microvilli structures which improve the absorption areas in shrimp; this enhance nutrient utilization and growth performance of shrimp (Zhang et al., 2012). Another reason maybe that SYC diets activates digestive enzyme activities which influence the nutrient digestibility in the shrimps (Ayiku et al., 2020). The survival rate of the shrimp was improved in the dietary SYC groups indicating an improvement in the wellbeing of the SYC groups. HSI of SYC (2 %) was lower compared to the control and SYC (1 %) groups in the current study. The low level of HSI may be related to the shrimps enhanced metabolism because of an improvement of protein synthesis in the shrimp body and enhanced the immunity as a result of glycogen consumption and lipid of hepatocytes (Chen et al., 2019a, b, c). Therefore, the findings of the present study indicated an improvement in the growth performance and feed utilization in the SYC groups than the control group.

The increase in ash and crude protein in a whole body composition indicate improve nutrient utilization of shrimp. Dietary SYC (2 %) group in this study was observed to improve crude protein and ash content in shrimps whereas crude lipid content was not enhanced. Similarly, Chinese mitten crab was fed with yeast diet, crude protein of the body tissue was elevated, which increased its growth performances (Zhang et al., 2019) while another study reported no significant change

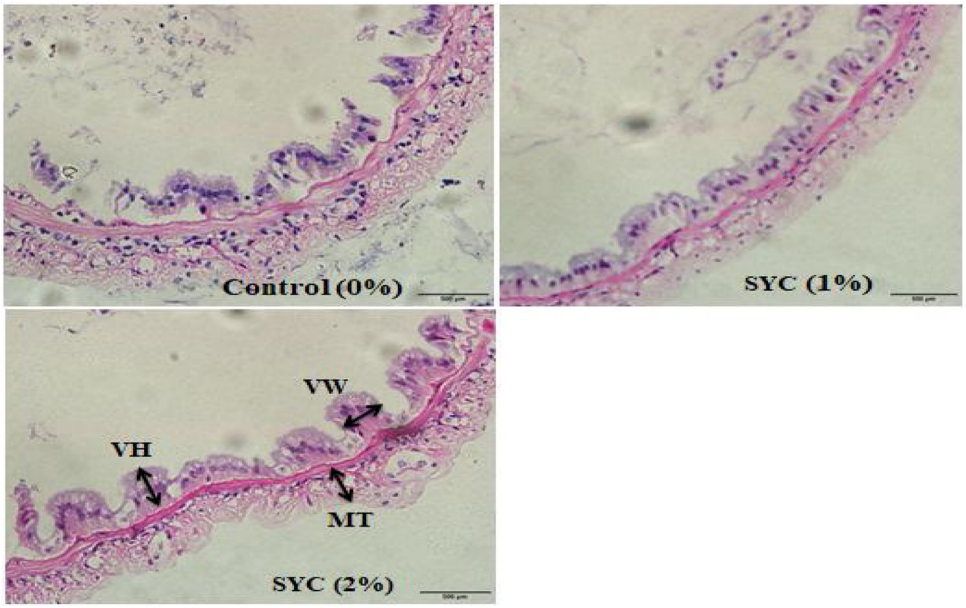

Fig. 3. Intestinal histology of L. *vannamei* fed with different levels of yeast culture diet for 8 weeks. Shrimp intestine transverse photomicrograph segments were presented as: VH - Villus Height, VW- Villus Width and MT - Muscles Thickness.

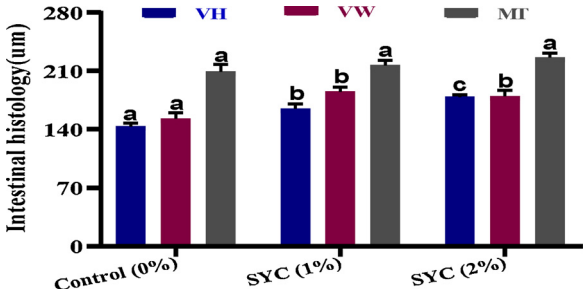

Fig. 4. Intestinal morphology measurements of L. *vannamei* fed with different levels of yeast culture diet for 8 weeks. Graph showed the mean ± SE (Tukey, $P < 0.05$; $n = 4$). The graphs with the same color having different superscript showed significant difference. Where VH − Villus Height, VW − Villus Width, and MT − Muscles Thickness.

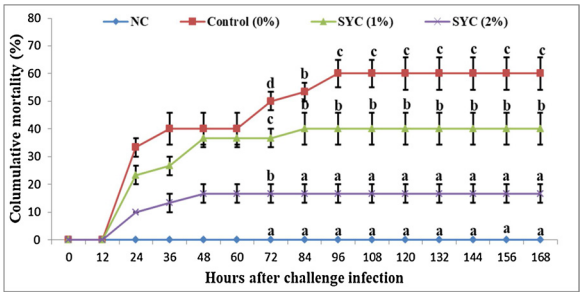

Fig. 5. Cumulative mortality of L. *vannamei* post-challenge with *V. harveyi* for 168 h. Every plotted value denoted as in mean ± SE of ten shrimp per three replicates (Tukey, $P < 0.05$; $n = 3$).

in ash, crude lipid and crude protein in the body proximate composition of shrimp (Jin et al., 2018). The present study did not focus on digestibility. However, we can speculate that the findings of the current study imply that SYC (2 %) enhanced growth performance than other groups.

Hemolymph lipids, proteins, and glucose may provide insight into metabolism. Research studies reported that, TP content is very essential for reflecting immune responses (Hossain et al., 2017; Xiong et al., 2018). The present study depicts that SYC groups significantly increased the TP content (Table 5). Similarly, β-glucan and mannan oligosaccharides improved the TP concentration and innate immune responses in the aquatic animal (Abu-Elala et al., 2018). CHO is an important constituent of lipoprotein, that helps in lipid absorption and transport in shrimps (Kumar et al., 2018). It is involved in processing steroid hormones which function in reproduction and moulting (Kumar et al., 2018). CHO supports more structural integrity to cell membranes against environmental distortion, such as thermal and salinity stress (Kumar et al., 2018; Yang et al., 2016). Additionally, dietary CHO increases the utilization efficacy of the vital pigment in shrimp (Kumar et al., 2018; Niu et al., 2014). For instance, CHO accounts for approximately 90 % of muscle (Kumar et al., 2018; Tsape et al., 2010). In the present study, yeast culture increases CHO level, especially in SYC (2 %) group. This suggests that the increased CHO in the SYC (2 %) group indicate muscle build-up in the shrimp. TG are the main form in which shellfish's store fatty acids (Huang et al., 2020). The essential nutrients contribution by TG provide energy which facilitates cell division in the shrimp tissues (Chen et al., 2019a, b, c; Xiong et al., 2018). In the present study, the hemolymph TG concentration in the shrimps fed SYC treatments were enhanced than the control group. However, high concentration of TG was observed in the shrimp fed SYC (2 %) than SYC (1 %), indicating that dietary yeast culture diets can support cell division in shrimp to promote growth, reproduction and moulting (Kumar et al., 2018). Yeast cells can utilize a wide range of carbon sources in the presence of GLU to suppresses molecular activities involved in the use of alternate carbon sources as well as it represses respiration and gluconeogenesis (Kayikci and Nielsen, 2015). Through gluconeogenesis pathway, the cell can sense extracellular GLU levels and use this to regulate GLU uptake, thereby triggering GLU repression (Kayikci and Nielsen, 2015). Yeast culture represses/suppresses the GLU through gluconeogenesis; however the present study showed a slight decrease in GLU of the SYC (2 %) than the other groups. This implies that, the SYC (2 %) utilized the GLU as a form of energy or fuel to maintain energy homeostasis. In addition, GLU level in SYC (2 %) can be influenced by nutritional and hormonal factors which are very important for maintaining GLU homeostasis; primarily dependent on the regulation of activity and expression of key enzymes involved in the glycolysis and gluconeogenesis pathways (Pilkis and Granner, 1992). The GLU level in the SYC (2 %) depicts glycogenolytic activity thereby

enhancing protein/amino acids synthesis.

AST and ALT are hepatopancreas – specific enzymes that promotes gluconeogenesis from amino acids (Sakyi et al., 2020). They are found within some tissues such as hepatopancreas and muscle cells (Kim et al., 2008). When studying aquatic organisms, it is very important to establish whether experimental procedures such as handling, sampling, and other physical stressors may affect physiological responses (Iwama et al., 2004). The releases of these AST and ALT enzymes are as result of hepatocellular injuries which increases the levels of these enzymatic activities in the hemolymph (Ebrahimi et al., 2017; Kim et al., 2008). The current data indicate increased levels of AST and ALT in the control group than the SYC groups. This may be as result of physical stressors which causes hepatocellular injuries in the control group. Also, the levels of these enzymes in the hemolymph indicate the extent to which hepatopancreas cells are damaged (Sakyi et al., 2020). Contrarily, the current study indicates a significant decreased in AST and ALT in the SYC groups which implies yeast culture improved hepatopancreas from cellular injury (Table 5). Similarly, in a previous study, reduced levels of ALT and AST were found in *L. vannamei* fed yeast culture diet groups (Ayiku et al., 2020). We can speculate that, SYC groups reduce the hepatopancreas damages and improved physiological responses than the control group.

Oxidative stress occurs when there is an imbalance between the generation and elimination of reactive oxygen species (ROS) and could increase shrimps vulnerability to disease conditions (Sheikhzadeh et al., 2012; Yuan et al., 2019). Antioxidant enzymes including SOD and CAT are responsible for protecting the host against ROS that induce oxidative stress. In the present study, increased SYC supplementation groups (SYC, 2 %) significantly improve the activities of SOD and CAT which was similar with the results on *L. vannamei* (Yang et al., 2010) and Chinese mitten crab (Zhang et al., 2019) (Table 5). MDA in the present study decreased in the SYC groups. MDA reflects diminishing lipid peroxidation which may be as a result of generation of excess reactive oxygen species (Zhao et al., 2014). Our findings suggest that increased in antioxidant activities (SOD and CAT) enhanced the antioxidative capability and the gut innate immunity (Zhang et al., 2019). Currently, there is limited information on how antioxidative status is being influenced by yeast culture, its constituent, and its specific mechanisms. We can conclude that SYC (2 %) influence positive antioxidant activities than the other groups.

The present study examined the innate immune parameters (LZM, ACP, AKP, and PO) in the hemolymph of shrimp. Before challenge, significant levels of ACP and AKP were maintained in the SYC groups while the LZM levels slightly increased in the SYC groups than the control group. After 12 h injection, the innate immune parameters (LZM, ACP, and AKP) were highly increased in the SYC (2 %) group. These immune parameters aided in improving innate immune systems of marine shellfish against pathogenic infection and exhibited phagocytic activities in removing foreign bodies (Liu et al., 2019; Wang et al., 2019; Xing et al., 2002; Zhao et al., 2012). Additionally, the enzymatic activities of ACP and AKP are produced by phagocytes and are innate immune promoters. Furthermore, the yeast cell wall possesses a structure known as β-glucan, which has particular receptors on phagocytic cells and also bind the receptor molecules on the surface of distributing and tissue phagocytes (Abu-Elala et al., 2013). The binding elevate the activities of the phagocytes in engulfing, killing, and breakdown bacteria (Abu-Elala et al., 2013). The activities of phagocytes play a vital role in antibacterial defenses. For instance, existing research prove β-glucan supplemented diets improve immune response in *L. vannamei* (Zhao et al., 2012). Therefore, the SYC groups exhibited strong immunity and disease resistance than the control group. PO is an important component of innate immune defense for crustaceans which includes shrimps (Li et al., 2009). The enzyme, PO normally activates melanin synthesis pathway; this produce cytotoxic byproducts that kills microorganisms (Mucklow et al., 2004; Palmer et al., 2011). In the present study, the PO activities were slightly enhanced in shrimp fed SYC diets before challenge. After 12 h of *V. harveyi* injection, shrimps fed SYC diets significantly increased the activities of PO. The activities of PO and its immunocompetence have several direct benefits on the shrimp body to identify and defend against foreign pathogens through cell-cell communication (Jin et al., 2018; Johansson and Soderhall, 1989). Shrimp with increased levels of PO activity are less vulnerable to microorganism infection (Zhang et al., 2012). Besides, the increase PO activities in SYC diets group after *V. harveyi* challenge can be attributed to immune stimulatory effects of glucan in shrimp (Chiu Yang, 2014; Sakai, 1999). Furthermore, β-glucan improved the cytotoxic activities of macrophages, lymphocytes and natural killer cells in aquatic animal immune system (Chang et al., 2013a, b; Chiu Yang, 2014; Engstad et al., 1992) and promoted proPO in the lymphatic fluids of crustaceans, leading to a defensive bactericidal effect and phagocytosis, in addition to promotion of hemolymph coagulation (Cerenius and Söderhäll, 2011; Chiu Yang, 2014; Soderhall, 1981). Increased PO activity in the present study also agree with Chang et al. (2013a, b), who reported that yeast culture enhanced innate immune responses of shrimps against infections. Hence, the yeast culture diets helped in the immune responses of shrimp after challenge.

The treatment significantly affected intestinal bacteria composition using dietary yeast culture supplemented diet fed to shrimp. The SYC (2 %) group had higher α-diversity indices (OTUs, ACE, Chao, and Shannon indices) compared to the control group. At the phylum level, SYC (2 %) group had high abundance of Proteobacteria followed by Bacteroidetes and Actinobacteria than the control group. The abundance of Bacteroidetes was observed which improve the nutrient uptake and digestion of shrimp (Amoah et al., 2019; Hou et al., 2017). Previous studies reported that Actinobacteria (*Actinomyces*) enhance the production of cellulose degrading bacterial (Liu et al., 2018; Tremaroli and Bäckhed, 2012; Ye et al., 2014), which maintain intestinal homeostasis in the SYC groups (Binda et al., 2018). Increased Bacteroidetes and Actinobacteria can improve the intestinal microflora composition to enhance the health of shrimp. Proteobacteria are usually dominant bacteria phylum in aquatic environment and in the intestine of the aquatic animals. The current study identified genus of Proteobacteria, *Vibrio*, causative agents of Vibriosis in marine environments (Jiang et al., 2020) which cause tissue pathology to different degree or death. The SYC (2 %) group significantly decreased the abundance of *Vibrio* in the intestine of the shrimp than the control group. Our findings suggest that the SYC group contain some chemical substances such as β-glucan, chitin, and mannan-oligosaccharides which prevented the *Vibrio* colonization (Zhou et al., 2018). Other research studies reported that these substances are able to improve the mechanism of the immune system or have suppressive effects on pathogens and diseases (Dalmo and Bøgwald, 2008; Flint et al., 2008; Liu et al., 2014; Ringø et al., 2012; Wu et al., 2018). In addition, there was an increase in the abundance of *Pseudoalteromonas* genus which serve as probiotics because they produce digestive enzymes such as protease, amylase, phospholipases and extracellular materials which help in preventing settlement and metamorphosis of pathogenic bacteria (Amoah et al., 2019). We can conclude that SYC (2 %) group influenced the intestinal microbiota and improve immunity than the control group.

Intestine of an animal plays a major function in nutrient digestion and absorption as well as providing immune barrier against disease infection (Zhu et al., 2012). Intestinal morphology and histological evaluation are known to be critical which can be influenced by dietary nutrient absorption and also showed immune blockages against pathogenic infection. The exposure of intestinal villi surface has direct correlation with nutrient absorption (Wang et al., 2017). The longer and wider villi exhibit higher absorption of nutrient, increased nutrient uptake and may improve growth performances. Contrarily, impairment such as decreased villi length and width affects nutrient utilization which result in poor growth performances of shrimp and increase diseases infection (Farhangi, 2001). Although, there was no change observed in muscle thickness, significant differences were observed in

relation to villus height and width of shrimp fed with SYC groups in the present study. Previous studies demonstrated that mannan oligosaccharides as a component in yeast culture have the capacity to increase villus length in *L. vannamei* and European lobster (Daniels et al., 2010; Zhang et al., 2012). The results of our study suggest that yeast culture can increase villus height and length, therefore enhancing intestinal surface area for more nutrient absorption. In fact, the present data demonstrates SYC (2 %) treatment groups could stimulate the growth of villus length and width in the intestine and increased growth performance of shrimp. However, there are limited studies on the effect of yeast culture on intestinal morphological development in shrimp. To increase our understanding, further studies are required in this regard.

In aquatic animals, survival rate after challenge with bacteria is usually used in determining its immune responses to resist diseases (Deng et al., 2006). The non-specific immune system of the shrimp plays a major role in shielding shrimp from bacterial infection. In the present studies, increase survival rates was observed in the shrimp fed SYC groups after being challenge with *V. harveyi.* However, the SYC (2 %) had the highest survival rate followed by SYC (1 %) and control group (0 %). Studies have reported that inclusion of yeast culture fed diets to shrimp after challenged with *V. harveyi* and *V. alginolyticus* increased survival rate (Burgents et al., 2004; Chang et al., 2013a, b). The abundance of these chemical substances such as β-glucan, chitin, and mannan-oligosaccharides in the SYC groups exhibit immunostimulatory effects (Hassan, 2011) after pathogenic bacteria challenge which increased their survival. Additionally, post-challenged shrimp after being fed with β-glucan supplement had decreased cumulative mortalities than the untreated group (Chang et al., 2011; Ma et al., 2019). Therefore, the influence of yeast culture on bacterial disease resistance suggests administration of 2 % yeast culture may effectively improve shrimp resistance to *V. harveyi* infection.

The present study demonstrated that dietary yeast culture supplementation can be beneficial for increasing growth performance, antioxidant activities, immune enzyme activities, intestinal heath and disease resistance of *L. vannamei*. Also, SYC groups improved the physiological and metabolic responses. Based on the above results, SYC (2 %) yeast culture supplementations in practical diet are recommended for *L. vannamei*. Furthermore, the SYC groups influenced the intestinal microbiota which enhanced the immune system of the *L. vannamei*.

6. Author contributions

Liu Hong-yu conceived and designed the experiment. Stephen Ayiku and Shen Jianfei carried out field experiment. Stephen Ayiku carried out the laboratory work. Bei-ping Tan, Xiao-hui Dong drafted and proof read the manuscript.

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Declaration of Competing Interest

All the authors declare no conflict of interests.

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