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Effects of dietary hydrolyzed yeast (*Rhodotorula mucilaginosa*) on growth performance, immune response, antioxidant capacity and histomorphology of juvenile Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

An 8 weeks feeding experiment was conducted to evaluate the effects of dietary supplementation with hydrolyzed yeast (HY) (*Rhodotorula mucilaginosa*) on growth performance, hematological parameters, immune response and antioxidant ability of juvenile Nile tilapia. Five isonitrogenous and isolipidic diets (32% protein and 4% lipid) with different levels (0%, 0.125%, 0.25%, 0.5%, 1%) of HY were formulated. Each diet was randomly assigned to quadruplicate groups of fish (initial body weight 19.1 ± 0.01 g). Results indicated that significantly higher specific growth rate (SGR) and lower feed conversion rate (FCR) were obtained in fish fed 1% HY diet than that of fish fed 0% HY diet ($P < 0.05$). Fish fed 0.25% HY diet showed the lowest value of hepatopancreas somatic indices (HSI) and significantly lower than that of fish fed 0% HY diet ($P < 0.05$). Meanwhile, protein and ash in the whole-body content of fish fed 1% HY diet was significantly higher than that of fish fed 0%–0.5% HY diets. Serum immunological parameters showed that the lysozyme (LZM) activity and Complement C3 content were significantly increased by dietary supplementation of 0.5%–1% HY ($P < 0.05$). However, dietary supplementation with 0.125%–1% HY significantly decreased the activity of myeloperoxidase (MPO) ($P < 0.05$). Antioxidant status in serum and liver was significantly enhanced by dietary supplementation of 0.25%–1% HY through the remarkably improved superoxide dismutase (SOD) activity both in serum and liver, the raised total antioxidative capacity (T-AOC) of serum as well as the notably reduced malondialdehyde (MDA) content in the liver ($P < 0.05$). However, T-AOC in the liver was not significantly influenced among all diet treatments ($P > 0.05$). Villi height and intraepithelial lymphocytes (IEFs) of mid-intestine were significantly higher in fish fed 0.5%–1% HY diets ($P < 0.05$). The challenge test demonstrated the enhanced protection against *Streptococcus iniae* strain by the obtained higher cumulative survival rate. In conclusion, dietary supplementation of 1% HY could maintain the better growth performance, nutrient composition as well as immune response and antioxidant capacity for juvenile Nile tilapia.

1. Introduction

The expanding development of aquaculture in the last decades satisfied the demands of high-quality proteins for humans. However, inferior fish fries, nutritional deficiency, poor water quality, bacterial and viral diseases as well as other factors hinder the further development of the aquaculture industry [1]. Rapid growth of pathogen bacteria caused by high-stocking and routine handling resulted in the deterioration of aquatic environment, which could have negative effects on the growth profile or be fatal for the fish [2]. In order to settle the problems caused by intensive culture, antibiotics and chemicals (disinfectants, antifoulants, pesticides and vaccines) were considered the effective ways. Whereas, indiscriminate use of antibiotics and chemicals resulted in the disease resistance of some pathogenic bacteria and increased concerns about residuals in fish tissues [3,4].

The alternative strategies to decrease outbreaks of disease currently focus on the administration of vaccine, probiotics and prebiotics. Prebiotics are considered as promising candidates due to their non-digestible property and beneficial effects on the growth and immunity of aquatic animals [5]. Numerous studies have demonstrated that yeast

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or yeast cell wall isolated from *Saccharomyces cerevisiae* could have a positive influence on the growth performance and immune status of fish and shrimp [6–11]. Besides, polysaccharides come from the other sources of yeast, fungi, plants, herbs, algae are equally reported to strengthen defense and provide protection against potentially severe or lethal conditions [5,12]. The observed immunological function of the above substances or products may be related to the natural existed β-glucan and mannan oligosaccharides (MOS). Diverse branch structures and indigestible property of β-glucan plays a vital role in the immune modulator response when it goes through the gastrointestinal tract of fish [13]. When β-glucan binds itself to several types of leukocyte receptors, such as toll-like receptors and pattern recognition receptors, it results in the release of interferon and cytokine, activation of macrophages, neutrophils, natural killer cells as well as the production of antibody, complements and lysozyme in the consequence of the enhanced protection against microbials [12]. MOS, in terms of the character of prebiotic and immune modulation effects, could effectively impede the colonization of harmful bacterial in the intestine and modulate cellular and humoral immune response by affecting proinflammatory cytokines through intracellular cascade signal system [14]. The underlying mechanisms may be attributed to the secretion of mucus, improvement of gut epithelial integrity, modulation of microbiota and stimulation of immune response [15].

Nile tilapia is a freshwater species of high commercial values that gained favorable assessment because of its fast growth, high acclimation ability to different circumstance, high yield and delicious meat. However, *Streptococcus iniae*, the most lethal pathogen, especially in China, could cause massive mortality in critical seasons and bring severe economic loss to this industry [16].

Rhodotorula mucilaginosa, has been identified as permanent residential strain in the gut microbiota of some wild fish that developed their own immunostimulant components by resorting to commensal microflora [17]. what's more, the pivotal role of this strain in comprising yeast core gut microflora of wild and reared carnivorous fish has also been documented in the previous investigation [18]. However, to the author's knowledge, this yeast species is rarely administrated in aquaculture industry. Relevant investigations are available in very limited source [19]. Hence, the application of this yeast species in various aquatic animals deserves to be evaluated.

In brief, the purpose of the current study is to evaluate the effects of dietary hydrolyzed yeast (*Rhodotorula mucilaginosa*) on growth, immune response, antioxidant capacity and disease resistance of juvenile Nile tilapia.

2. Materials and methods

2.1. The preparation for hydrolyzed yeast (Rhodotorula mucilaginosa) and feed

The yeast (*Rhodotorula mucilaginosa*) was supplied by South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. An intermixture was obtained by mixing dried yeast powder with 4 × (w/v) purified water. Ultrasonication for 30 min was followed (500v, start: pause = 2:1) (Sonics; America) and pH of the miscible liquids was subsequently adjusted to 5.0 with 0.001 mol/L hydrochloric acid and sodium hydroxide, respectively. Afterwards, an enzymatic hydrolysis process was carried out for 18 h at 55 °C by the addition of 0.2% (w/v) papain (Dongxiao food additives technology corporation, Shandong, China). Continued to centrifuge the pre-hydrolysate at 4 °C (8000 rpm) for 15 min and collected the precipitants. A vacuum freeze-drying machine was available to remove extra moisture of the product until constant weight. The accumulated products were ground into powder before drying in an oven at 55 °C for 4 h. The proximate composition, including β-glucan, mannan oligosaccharides (MOS), and amino acid content, was listed in Table 1 and the analysis process was conducted by the Food Industry Research Institute of Guangdong Province (Guangzhou, China).

Table 1
Proximate composition of hydrolyzed yeast.

Hydrolyzed Yeast			
Essential amino acids (%)		Non-essential amino acids (%)	
Methionine	0.26	Glutamic acid	1.83
Valine	0.86	Glycine	1.06
Lysine	0.87	Alanine	1.09
Isoleucine	0.71	Aspartic acid	1.29
Phenylalanine	0.70	Tyrosine	0.36
Leucine	1.10	Proline	0.75
Threonine	0.71	Serine	0.79
Histidine	0.81		
Arginine	0.67		
Proximate composition (%)			
Moisture	8.7		
Crude protein	17		
β-Glucan	24.3		
mannan oligosaccharides (MOS)	14.2		

Five practical diets with levels of 0, 0.125%, 0.25%, 0.5%, 1% hydrolyzed yeast (HY) were formulated, the formulation of the diets was nutritionally comprehensive for the fish, and proximate composition analysis of the diets was shown in Table 2. Fish meal, soybean meal, rapeseed meal and peanut meal were utilized as the main protein source, the requirement of lipid was satisfied by soy oil and soy lecithin, the demand for amino acid was supplied by commercial crystalline methionine and lysine in diets. Briefly, all the dry ingredients including the HY were precisely weighed, and mixed by a Hobart mixer (A-200T Mixer bench Model unit, Russell Food Equipment, Ottawa, Canada). Lipids and purified water were subsequently added to mix with dried ingredients. 1.5 mm and 2.5 mm diameter puffed pellets were produced by using a puffing apparatus (Institute of Chemical Engineering, South China University of Technology, Guangdong, China), the pellets were

Table 2
Formulation and composition of experimental diets (%).

Ingredients	HY0	HY1	HY2	HY3	HY4
Fish meal	2	2	2	2	2
Soybean meal	18	18	18	18	18
Rapeseed meal	32	32	32	32	32
Wheat flour	24.99	24.865	24.74	24.49	23.99
Rice bran meal	4.5	4.5	4.5	4.5	4.5
Peanut meal	10	10	10	10	10
a-starch	3	3	3	3	3
Monocalcium phosphate	2	2	2	2	2
Soy oil	1	1	1	1	1
Soy lecithin	1	1	1	1	1
Hydrolyzed Yeast	0	0.125	0.25	0.5	1
Vitamin and Mineral mixture[a]	1	1	1	1	1
Choline chloride (50%)	0.2	0.2	0.2	0.2	0.2
Yttrium (III)-Oxide (Y_2O_3)	0.01	0.01	0.01	0.01	0.01
Ascorbic acid Polyphosphate	0.1	0.1	0.1	0.1	0.1
DL-Methionine	0.1	0.1	0.1	0.1	0.1
L-lysine hydrochloride (78%)	0.1	0.1	0.1	0.1	0.1
Proximate composition					
Dry matter	91.1	90.56	90.42	90.08	90.72
Crude protein	32.64	32.71	32.79	32.31	32.38
Crude lipid	4.16	4.28	4.19	4.08	4.18
Ash	6.97	7.14	6.88	7.05	6.99

HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

[a] Vitamin and Mineral mixture (IU or mg g^{-1} of diet): vitamin A, 6000; vitamin D3, 5600; vitamin E, 0.04; vitamin K3, 10; vitamin B1, 9; vitamin B2, 18; vitamin B6, 12; vitamin B12, 0.04; vitamin C, 140; niacin, 70; biotin, 0.16; folic acid, 3.2. D-calcium pantothenate, 40; Magnesium, 100; iron, 70; manganese, 13.3; iodine, 2.24; copper, 10.5; zinc, 56; selenium, 0.3; cobalt, 1.75. Vitamin and mineral mixture were supplied by Ashare corporation, Guangzhou, China.

air-dried to approximately 10% moisture and stored −20 °C until used.

2.2. Experimental fish and feeding trial

Approximate 800 juveniles of Nile tilapia were obtained from a local hatchery (Guangzhou, China) to perform the investigation in the nutrition laboratory of aquatic animals (Sun Yat-sen university, Guangzhou, China). Prior to the experiment, fish were acclimated at an indoor circulating aquarium for two weeks and fed the completed diet without HY. After the acclimation period, 600 fish, similar size and without visual injury or signs of disease, were selected uniformly and distributed randomly into 20 fiberglass tanks (200 L, 30 fish per tank, 4 replicates per diet) with an initial body weight at 19.1 ± 0.01 g. Fish were fed two times daily at 9:00 and 16:00 till satiation for 8 weeks. Uneaten feed was aspirated by siphoning manually after 45 min' feeding and dried to record the feed intake. Fresh water was filtered through coral sand and biochemical cotton. Sufficient oxygen was provided by a lower pressure blower continually.

During the feeding trail, water temperature, pH and dissolved oxygen were measured daily, total ammonia nitrogen was measured weekly. Temperature and pH of the rearing water were staying at 28 ± 2 °C, pH 7.6 ± 0.2 respectively, the dissolved oxygen was more than 6.5 mg L^{-1}. The ammonia nitrogen was lower than 0.01 mg L^{-1}, natural photoperiod was used during the trial.

2.3. Sampling and chemical analysis

At the end of the feeding trial, all fish were fasted for 24 h after the last feeding. All surviving fish from each tank were weighed and counted to calculate the final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR) and survival rate. 12 fish per tank were randomly selected and anesthetized with MS-222 (Sigma, St Louis, MO, USA) for the sampling. Accordingly, three fish were used to analyze the whole body composition. Afterwards, six another fish were used to take the blood samples with heparinized syringes centrifuged (3500 g, 10min) at 4 °C, plasma was gathered and stored at −80 °C until analyzed. The blood drained fish was dissected to obtain viscera, liver, mesenteric fat and dorsal muscle. The remained three fish were subsequently gathered to obtain liver and midgut. The fresh liver was detached and immediately frozen in liquid nitrogen and stored at −80 °C until used. The midgut was fixed in Bouin's solution for later histopathological analysis.

Frozen liver samples were slightly defrosted on ice for several seconds before slicing with sterile scissors. 0.5 g integral liver tissue was selected and weighed for the homogenization with 9 × (w/v) iced phosphate buffer solution (0.1 mol/L, pH 6.4) by using Homogenizer (Jingxin Industrial Development Corporation, Shanghai, China). The obtained homogenates were subsequently centrifuged (4000 rpm, 10 min) at 4 °C and supernatants were collected and kept at −80 °C until used.

Moisture, crude protein, crude lipid, and ash in diets, muscle and whole body of Nile tilapia were determined according to the methods of AOAC [20]. All samples were dried in an oven at 105 °C to a permanent weight for the determination of moisture. Crude protein (N × 6.25) was measured with an Auto Kjeldahl System (1030-Auto-analyzer; Tecator, Hoganas, Sweden) after acid digestion. Crude lipid was determined by the ether-extraction method with a Soxtec System HT (Soxtec System HT6; Tecator, Sweden). Ash content was measured by using a muffle furnace at 550 °C for 24 h.

2.4. Biochemical parameters

The activities of alkaline phosphatase (ALP) and the content of albumin (ALB), total protein (TP), cholesterol (CHO), triglyceride (TG), high density lipoprotein (HDL), lower density lipoprotein (LDL) in plasma were analyzed with an automatic biochemical analyzer (Hitachi 7170; DAICHI, Tokyo, Japan) and supplied kits (Sekisui Medical, Tokyo, Japan) in a clinical laboratory of the First Affiliated Hospital of Sun Yat-sen University.

2.5. Serum and liver immunological and antioxidant parameters

The activity of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), Lysozyme (LZM) and the content of malondialdehyde (MDA) were analyzed by following the protocols of the kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). T-AOC was detected with an enzymatic reaction by deoxidizing ferric iron to ferrous iron. SOD was measured by inhibiting superoxide anion produced by an oxidase reaction system. LZM was measured by its capacity to hydrolyze the peptidoglycan of bacteria's cytoderm. MDA was measured through a process with thiobarbituric acid at thermal and acid conditions to generate a chromogenic reaction. Immune and antioxidative related parameters of serum and liver were generally presented as U/ml, U/mg protein respectively.

Complement C3 was determined by following the protocol of the kit (Yilikang biotechnology corporation, Zhejiang, China). Briefly, 3 μl serum samples were well-mixed with 225 μl Tris buffered saline (100 mmol L^{-1}) and polyethylene glycol (40 g L^{-1}), incubated at 37 °C for 5 min and the absorbance value at 340 nm was read as A1, antibody of complement C3 and sodium azide (0.95 g L^{-1}) were subsequently added to cease the reaction, absorbance value was read as A2 at the same wavelength. Double distilled water and different standard complement C3 antigens were respectively used as control and reference.

Myeloperoxidase activity (MPO) was determined according to the method from Quade and Roth [21] with a slight difference. Briefly, 15 μl serum was well-mixed with 135 μl Hank's balanced salt solution without Ca^{2+} or Mg^{2+} (Sigma-Aldrich, USA) in 96-well plate. Then 50 μl of 3,3′, 5,5′-tetramethylbenzidine hydrochloride system (TMB, 1 mM, Sigma-Aldrich, USA) was added to the reaction. The color change reaction was stopped after adding 50 μl of 2 M sulfuric acid (H_2SO_4). The OD was read at 450 nm in a microplate reader with 96-well plates.

2.6. Intestinal histological examination

The same segment of middle intestine from two fish per tank was sheared and fixed in Bouin's solution for 24 h. The samples for histological slice were dehydrated and slightly transferred to an ethanol solution (70%). All fixed specimens were embedded in paraffin after dehydration in a graded series of ethanol. Sections of 5-μm thick were transversely cut and stained with hematoxylin and eosin (H&E). The 5 vertically well oriented height and width of villi per slice were measured under a color video camera (Nikon CCD) and a light microscope (Nikon Ni-U, Japan). The measurement of villus height and width as well as the quantity of the IEFs (intraepithelial lymphocytes) were completed by Image J_v1.8.0.

2.7. Bacteria and challenge study

The *Streptococcus iniae* used in challenge test was supported by the Key Laboratory for Aquatic Products Safety, SYSU, Guangzhou, China. It was originally isolated from the viscera of infected Nile tilapia. Before the challenge test, a preliminary experiment of verifying the median lethal dose (LC50) in 7 days was carried out by intraperitoneal injecting 0.2 ml of four graded doses (1×10^7, 5×10^7, 1×10^8, 5×10^8 CFU ml^{-1}) of *S. iniae* at 28 °C. OD values at 600 nm and plates-spread were used to quantify the *S. iniae*. The fish used in the preliminary test were remained fish after the distribution of the 600 fish. Results showed that 0.2 ml 1×10^8 CFU ml^{-1} was the 7-day LC50 dose. The remained fish after sampling stress were recovered with respective diet for around 2 weeks until the challenge day. The bacterial suspension of LC50 concentration of *S. iniae* was prepared in advance, 10 fish from each tank with three replicates for each diet were intraperitoneal

injected with 0.2 ml bacterial suspensions by using sterile syringes. Fish were starved during the challenge test and reared in a still water system same as the preliminary experiment. All the intraperitoneal injections were performed after MS222 treatment with proper concentration as to reduce stress, no dead fish was found on that day after the injection. For the following seven days, fish were carefully monitored and the mortality was recorded accordingly.

2.8. Calculations and statistical analysis

All the data were statistically analyzed by SPSS 23.0 (SPSS, Chicago, IL, USA). The data were firstly tested for homogeneity of variance. One-way ANOVA was then used for the analysis of the main effects when homogeneous variance exists. Duncan's multiple-range test was carried out to test the significant differences among all groups. The Kruskal-Wallis ANOVA test was administrated when inhomogeneous variance appeared. P values smaller than 0.05 were considered significant and the results are presented as means ± SEM (standard error).

3. Results

3.1. Growth performance

Growth performance of Nile tilapia (*Oreochromis niloticus*) was shown in Table 3. Results showed that significantly increased SGR and decreased FCR were obtained in fish fed 1% HY diet compared to fish in the HY0 group ($P < 0.05$). However, no significant difference was found in FW, WG and survival rate among all groups ($P > 0.05$). Fish fed 0.25% HY diet showed the lowest value of HSI and significantly lower than that of fish fed 0% HY diet ($P < 0.05$). VSI indices of fish in all treatments were lower than that of fish in HY0 group, while no significant difference was detected ($P > 0.05$).

3.2. Proximate carcass composition

The proximate composition of the whole body and muscle was shown in Table 4. Results showed that crude protein and ash content of whole body in fish fed 1% HY diet were significantly higher than that of fish fed 0% HY diet ($P < 0.05$). The moisture and lipid of the whole body were not significantly influenced ($P > 0.05$).

3.3. Biochemical parameters

The results of biochemical parameters were listed in Table 5. Plasma TP, ALB, CHO, TG, HDL and LDL content were not significantly difference among all diet treatments ($P > 0.05$). ALP activity of fish fed 0.125%–1% HY diets was significantly higher than that of fish fed HY0 diet ($P < 0.05$), and there was no significant difference in ALP activity among treatments ($P > 0.05$).

3.4. Immunological and antioxidant related parameters in serum

The serum immunological and antioxidant related parameters of tilapia were shown in Fig. 1. The results showed that the fish fed 0.25%–1% HY diets significantly increased the serum LZM activity compared to fish in HY0 group ($P < 0.05$). Complement C3 content in fish fed 0.5%–1% HY diets was remarkably higher than that of fish fed 0% HY diet ($P < 0.05$). However, fish fed 0.125%–1% HY diets significantly decreased the MPO activity ($P < 0.05$). In addition, the serum T-AOC and SOD activity were significantly enhanced in fish fed 0.25%–1% HY diets compared to fish in HY0 group ($P < 0.05$).

3.5. Antioxidant related parameters in liver

The antioxidant related parameters of liver in Nile tilapia were presented in Fig. 2. Results showed that fish fed 0.25%–1% HY diets significantly increased the SOD activity compared to fish in HY0 group ($P < 0.05$). The MDA content of liver in fish fed 0.25%–1% HY diets was remarkably reduced ($P < 0.05$). However, T-AOC activity of liver was not significantly different among all diet treatments ($P > 0.05$).

3.6. Intestine histology

The mid-intestine morphometric parameters of tilapia were presented in Table 6. Results showed that fish fed 0.5%–1% HY diets significantly increased villi height compared to fish in HY0 group ($P < 0.05$). The number of intraepithelial lymphocytes (IEFs) in fish fed 0.125%–1% HY diets was significantly increased than that of fish fed 0% HY diet ($P < 0.05$, Table 6 and Fig. 3). However, villi width was not significantly different among all diet treatments ($P > 0.05$).

Table 3
Growth performance, feed utilization and body condition indices of the juvenile Nile tilapia fed with different levels of hydrolyzed yeast for 8 weeks.

	HY0	HY1	HY2	HY3	HY4
IBW	19.14 ± 0.03	19.13 ± 0.03	19.15 ± 0.01	19.11 ± 0.02	19.12 ± 0.01
FBW	106.86 ± 1.91	106.95 ± 1.43	105.90 ± 1.59	106.78 ± 1.56	110.25 ± 0.42
WG	458.29 ± 9.77	459.12 ± 7.31	452.81 ± 7.93	458.76 ± 8.76	476.69 ± 2.45
SGR	3.01 ± 0.04a	3.07 ± 0.02 ab	3.05 ± 0.03 ab	3.06 ± 0.02 ab	3.13 ± 0.01b
FCR	1.18 ± 0.02b	1.15 ± 0.01 ab	1.15 ± 0.01 ab	1.14 ± 0.01 ab	1.12 ± 0.01a
Survival	96.67 ± 1.93	100.00 ± 0.00	100.00 ± 0.00	99.17 ± 0.83	99.17 ± 0.83
VSI	10.66 ± 0.26	10.52 ± 0.21	10.30 ± 0.24	10.11 ± 0.34	10.13 ± 0.26
HSI	1.75 ± 0.06b	1.57 ± 0.06 ab	1.54 ± 0.07a	1.57 ± 0.06 ab	1.63 ± 0.05 ab
IPF	1.71 ± 0.10	1.74 ± 0.12	1.80 ± 0.15	1.55 ± 0.12	1.69 ± 0.11
CF	3.75 ± 0.03	3.75 ± 0.04	3.73 ± 0.05	3.74 ± 0.04	3.73 ± 0.04

Data represent mean ± SEM of four replicates. Means in the same raw with different letters are significantly different ($P < 0.05$).
IBW, initial mean body weight (g/); FBW, final mean body weight (g/).
Weight gain (WG) (%) = 100 × (final mean weight - initial mean weight)/initial mean weight.
Specific growth rate (SGR, %day^{-1}) = 100 × (LnWt −LnWi)/t.
FCR, feed conversion rate = feed consumed (g, dry weight)/weight gain (g, wet weight).
Survival (%) = 100 × (final amount of fish)/(initial amount of fish).
VSI, Viscera somatic index = 100 × viscera somatic weight (g)/body weight (g).
HSI, Hepatopancreas somatic index = 100 × liver weight (g)/body weight (g).
IPF, Intraperitoneal fat ratio = 100 × intraperitoneal fat weight (g)/body weight (g).
CF, condition factor (g/cm^3) = 100 × body weight (g)/body length (cm) ^{3}.
Where Wi is the initial body weight (g) and Wt is the final body weight (g), and t is the experimental duration in days. For VSI, HIS, IPF and CF, n = 20.
HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

Table 4
The composition in Whole-body and muscle (%) of juvenile Nile tilapia fed with different levels of hydrolyzed yeast for 8 weeks.

	HY0	HY1	HY2	HY3	HY4
Whole body					
Moisture	73.14 ± 0.36	73.43 ± 0.27	73.66 ± 0.46	73.45 ± 0.23	73.38 ± 0.16
Crude protein	52.54 ± 0.17a	52.72 ± 0.13a	52.49 ± 0.58a	53.55 ± 0.39 ab	54.49 ± 0.24b
Crude lipid	26.27 ± 0.61	25.68 ± 0.66	25.93 ± 1.77	24.68 ± 1.19	24.22 ± 0.92
Ash	15.10 ± 0.20a	15.12 ± 0.26a	15.12 ± 0.30a	15.31 ± 0.21a	16.17 ± 0.33b
Muscle					
Moisture	76.07 ± 0.30	76.63 ± 0.14	76.15 ± 0.35	76.01 ± 0.17	76.00 ± 0.15
Crude protein	83.83 ± 0.24	83.61 ± 0.38	83.54 ± 0.34	84.39 ± 0.28	83.88 ± 0.17
Crude lipid	5.10 ± 0.43	4.70 ± 0.43	5.13 ± 0.37	5.17 ± 0.61	4.93 ± 0.20
Ash	5.61 ± 0.12	5.60 ± 0.02	5.61 ± 0.06	5.75 ± 0.08	5.69 ± 0.10

Data represent mean ± SEM of four replicates. Means in the same raw with different letters are significantly different ($P < 0.05$). HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

Table 5
Biochemical parameters of juvenile Nile tilapia fed with different levels of hydrolyzed yeast for 8 weeks.

	HY0	HY1	HY2	HY3	HY4
ALP (U/L)	14.75 ± 0.25a	18.33 ± 0.33b	16.67 ± 0.33b	17.50 ± 0.65b	16.67 ± 0.88b
TP (mmol L^{-1})	27.43 ± 0.52	27.13 ± 1.07	27.10 ± 1.37	26.80 ± 1.07	26.18 ± 1.20
ALB (mmol L^{-1})	8.55 ± 0.63	9.25 ± 0.46	9.33 ± 0.63	8.67 ± 0.64	8.88 ± 0.39
CHO (mmol L^{-1})	2.49 ± 0.18	2.63 ± 0.05	2.52 ± 0.10	2.43 ± 0.17	2.58 ± 0.20
TG (mmol L^{-1})	0.43 ± 0.10	0.59 ± 0.07	0.50 ± 0.02	0.48 ± 0.06	0.51 ± 0.04
HDL (mmol L^{-1})	0.58 ± 0.04	0.62 ± 0.02	0.65 ± 0.03	0.61 ± 0.01	0.61 ± 0.02
LDL (mmol L^{-1})	0.11 ± 0.01	0.13 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.11 ± 0.02

Data represent mean ± SEM of four replicates. Means in the same raw with different letters are significantly different ($P < 0.05$). TP, total protein; ALB, albumin; CHO, cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein. HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

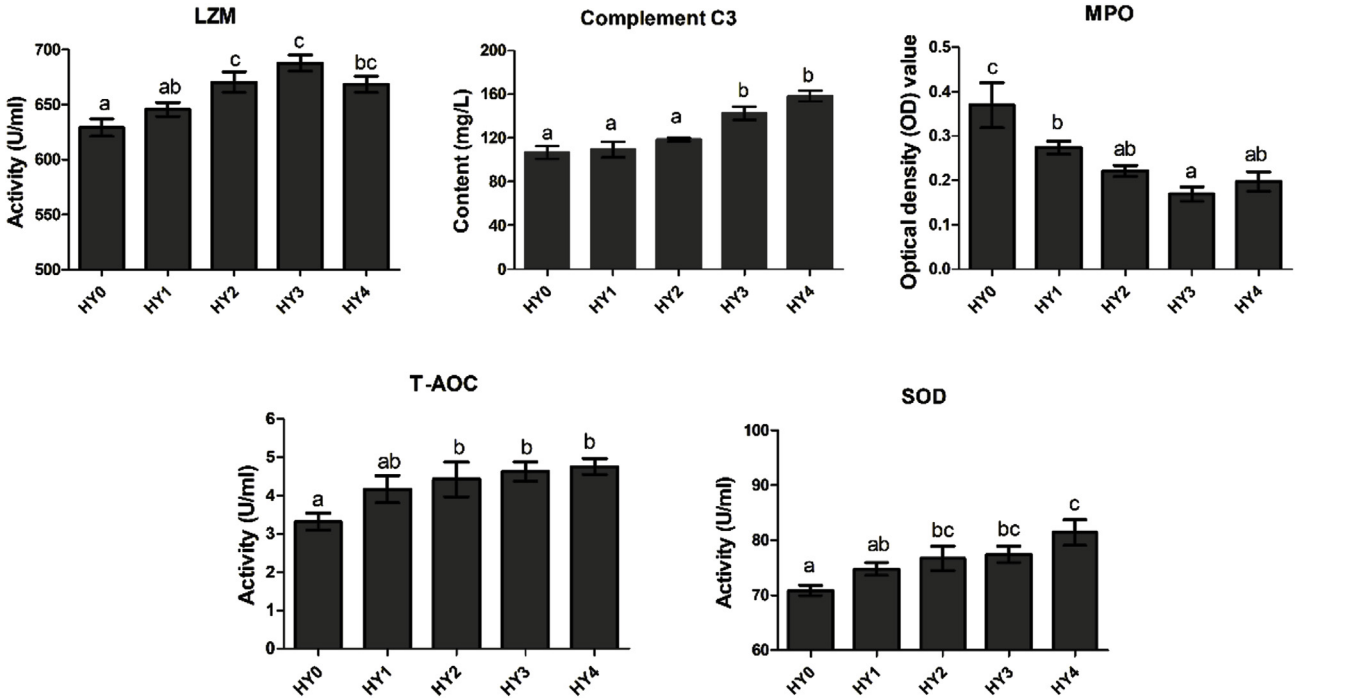

Fig. 1. Serum immune and antioxidative parameters (LZM, lysozyme; MPO, Myeloperoxidase; T-AOC, total antioxidant capacity; SOD, Superoxide dismutase) of Juvenile Nile tilapia fed with different levels of hydrolyzed yeast for 8 weeks. Data represent means ± S.E.M of four replicates. Bars with different lowercases are significantly different ($P < 0.05$). HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

3.7. Challenge experiment

The cumulative survival rate in juvenile tilapia challenged with *S. iniae* for 7 days was shown in Fig. 4. Result showed that feeding Nile tilapia with different levels of HY in diets improved its resistance to pathogen disease. All HY treatments of fish exhibited enhanced cumulative survival rate compared to fish fed 0% HY diet. The lowest survival rate of fish was shown in HY0 group (40%), while the highest value was found in fish fed 1% HY diet (73%).

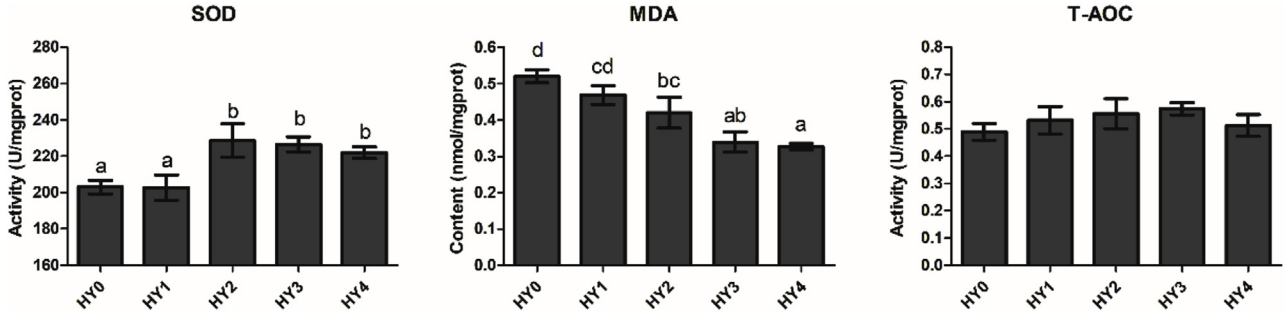

Fig. 2. Antioxidative indices (SOD, Superoxide dismutase, MDA, Malondialdehyde; T-AOC, total antioxidant capacity) of liver in Juvenile Nile tilapia fed with different levels of hydrolyzed yeast for 8 weeks. Data are presented as means ± S.E.M of four replicates. Different letters above a bar denote the significant difference between treatments ($P < 0.05$). HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

4. Discussion

The application of prebiotics in aquaculture has demonstrated that probiotics play a beneficial role in promoting growth and immunity of the host [5,12,14]. Thus, numerous studies have evaluated the effects of yeast [22,23], yeast nucleotides [24], yeast hydrolysate [25], yeast cell wall, yeast subcomponent alone or in combination [26–31] on growth performance of aquatic animals. The present study showed that supplementation with 1% HY (*R. mucilaginosa*) primally containing β-glucan and MOS improved growth performance by increasing SGR and reducing FCR of Nile tilapia. In agreement with our results, the increase of growth and feed utilization could also be found in some investigations of Nile tilapia [6,7,22,24,32], pacu (*Piaractus mesopotamicus*) [31,33], sea cucumber (*Apostichopus japonicus*) [34] and Pacific white shrimp (*Litopenaeus vannamei*) [10]. Li et al. [35] also evaluated the use of GroBiotic®-A (a commercial product containing partially autolyzed brewer yeast) in diets for juvenile hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) and significantly increased feed efficiency. Torrecillas et al. [29] reported that supplementation of 2% and 4% MOS in diet significantly increased the growth performance of European sea bass (*Dicentrarchus labrax*). The underlying mechanisms of the growth results currently observed may be related to the modulation of MOS on intestinal microflora. MOS resulted in the increased villus integrity and promoted efficiency of digestion and absorption [36,37] as well as the energy and building protein produced from β-glucan degenerated by glucanases in the digestive gland [38].

The body condition indices are useful indicators to assess nutritional and physiological status of fish [39]. The current study showed that all treatments of Nile tilapia exhibited lower HSI indices compared to fish in the HY0 group and fish treated with 0.25% HY obtained significantly decreased HSI indices. Similar results were also revealed in gilthead sea bream (*Sparus aurata*) fed with 0.2–0.4% MOS [40]. The reduced HSI indices may associate with the increased metabolism of fish, due to the enhancement of protein synthesis in the whole body and improved immunity resulted in the consumption of glycogen and lipid of hepatocytes.

The crude protein and ash content of whole body in tilapia were significantly affected by supplementation with 1% HY in diets. Similar to our results, Mohsen et al. [32] detected that supplementation of 1–5 g/kg live bakers' yeast in diet significantly improved the crude protein and ash content in Fry Nile tilapia. David et al. [22] showed that supplementation of yeast (*Saccharomyces cerevisiae*) in diet increased crude protein and ash content in juvenile Nile tilapia but without significant difference. Besides, that yeast can significantly increase the ash content of fry Nile tilapia was also reported [23]. The changes of crude and ash is generally related to the synthesis, deposition rate of protein, lipids, and carbohydrates in muscle and liver [21,33]. Therefore, the increase of protein and ash content in this study may be due to the improvement of nutrient digestibility, feed utilization rate and absorption function of gut on amino acid and minerals, because MOS can interact directly with enterocytes and cause an increased exchange rate of nutrients.

Alkaline phosphatase (ALP) is considered to be involved in some metabolic functions, such as growth, cell differentiation, protein synthesis, uptake and transport of nutrients as well as immune capacity [41,42]. Several studies reported that immunostimulants, such as yeast nucleotide and Vitamin C, could increase the ALP activity [43,44]. Thus, the remarkable increase of ALP activity in the present study may be indicated that HY supplementation could improve growth and immunity status.

The innate immune system is mainly composed of humoral and cellular components. Without any doubt, lysozyme and complement system are important elements in humoral immune response to protect against pathogen such as bacteria, virus [12]. Lysozyme has the capacity of hydrolyzing the link between *N*-acetylmuramic acid and *N*-acetylglucosamine with the consequence of destroying the cell wall structure of microorganisms [45]. In the present study, the fish fed 0.25%–1% HY diets obtained higher lysozyme activity compared to fish fed 0% HY diet. In line with our results, Rodriguez et al. [46] used a dose of 10 g/kg modified strain of *S. cerevisiae* to feed seabream (*Sparus aurata* L.) for 6 weeks and detected the increased serum lysozyme activity. The commercial product DVAQUA (product of fermentation of *S. cerevisiae*) was used to feed hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) for 8 weeks and increased serum lysozyme activity was reported [47]. A previous study has also reported a significantly higher serum lysozyme activity in the juvenile red sea bream (*Pagrus major*) fed 1 g/kg β-glucan diet [48]. Apart from lysozyme, complements also constitute a particularly important part for the immune system by directly killing microorganisms via different pathways [49]. Previous study reported that complement C3 content of hybrid tilapia fed different

Table 6
Histological measurement of mid-intestine of juvenile Nile tilapia fed with different levels of hydrolyzed yeast for 8 weeks.

	HY0	HY1	HY2	HY3	HY4
Villi height	463.18 ± 18.68a	483.78 ± 15.56a	509.67 ± 24.21 ab	574.98 ± 22.28bc	624.10 ± 31.30c
Villi width	44.03 ± 2.18	43.40 ± 1.77	47.41 ± 1.77	44.97 ± 3.76	45.47 ± 1.32
IEFs	6.50 ± 0.56a	11.86 ± 0.83b	15.00 ± 1.05c	15.33 ± 0.76c	35.40 ± 1.54d

Means in the same raw with different letters are significantly different ($P < 0.05$). The unit of villi height and villi width represents as um. IEFs means intraepithelial lymphocytes. HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

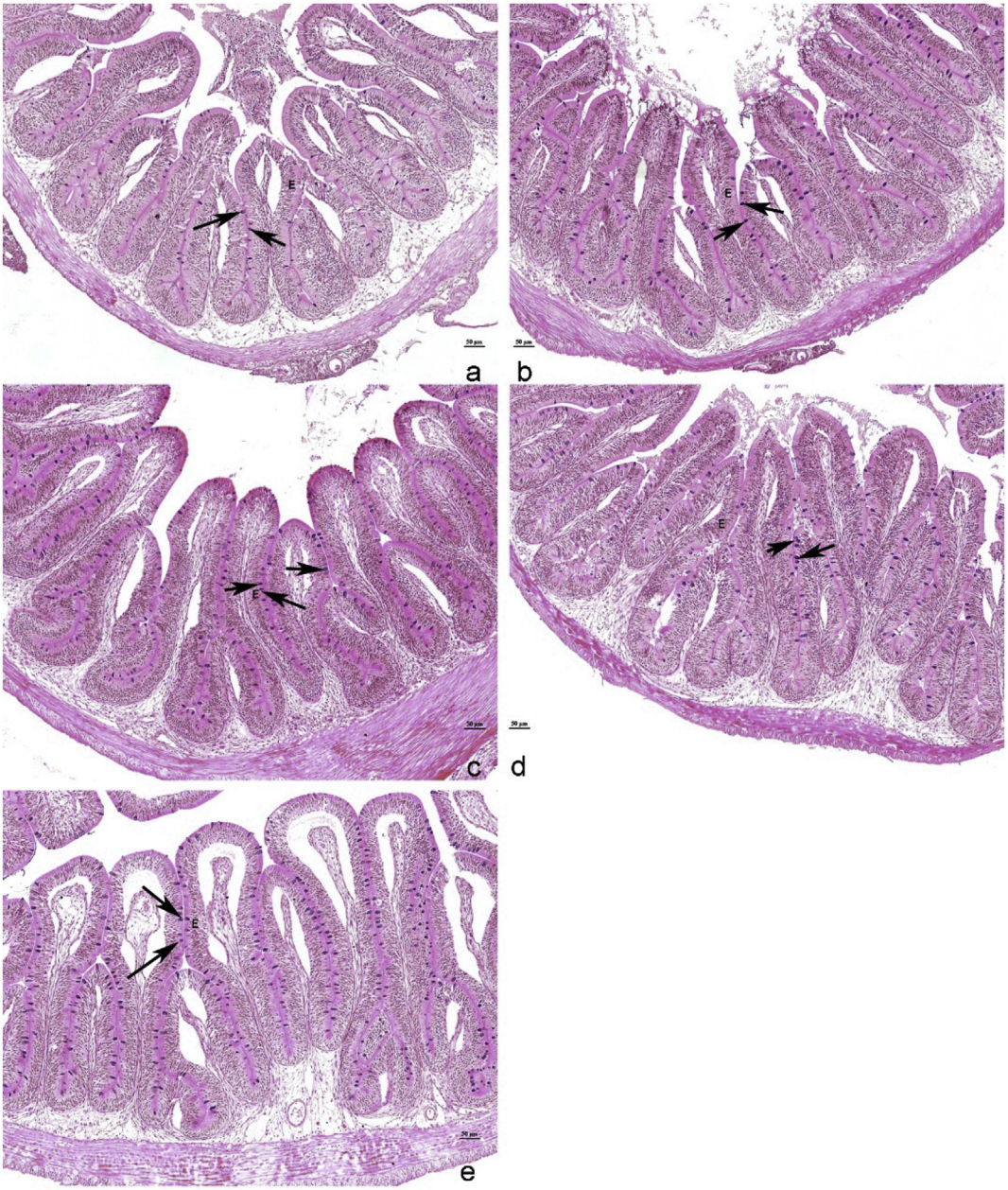

Fig. 3. Light micrographs (100X magnification) of mid-intestine of Nile tilapia fed 0% HY (a), 0.125% HY(b), 0.25% HY(c), 0.5% HY(d), 1% HY(e) for 8-week. Abbreviation E means enterocytes. IELs (intraepithelial lymphocytes) were exhibited with black arrows. Scale bars = 50 μm. HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

doses of commercial product DVAQUA was increased [47]. However, no significant effect was observed for the C3 content in Japanese sea bass (*Lateolabrax japonicus*) fed yeast cell wall (YCW) at doses of 250, 500, 1000, 2000 and 20,000 mg/kg [50]. In the present study, we observed that the supplementation of 0.5%–1% HY in diets could significantly enhance the Complement C3 content in the serum of Nile tilapia. The improved lysozyme and complement C3 in our results confirmed the protective effects of dietary supplementation of HY on the immune system of Nile tilapia. Thus, fish fed diets containing the HY could enhance protection against pathogens in the culture system. In addition, Myeloperoxidase (MPO) is a lysosomal protein produced by phagocytes, such as neutrophils, monocytes [12]. The antimicrobial ability is mainly reflected by producing hypochlorous acid through one of the oxidative radicals [51]. Yu et al. [50] reported the down regulated MPO values in Japanese seabass (*L. japonicus*) fed different levels of yeast cell wall. Whereas, Kumari et al. [52] found that MPO activity could be increased by yeast β-glucan in Asian catfish (*Clarias batrachus*). In the present investigation, serum MPO activity of Nile tilapia was decreased with the supplementation of HY in diets. The different results regarding the MPO activity may be due to the different immune response of different fish species to the identical or diverse stimulation. Undoubtedly, more studies are needed to address this issue.

Bacterial challenge test is generally recognized as an effective strategy to assess the immune status of fish. The addition of prebiotics or immunostimulants is generally reported to reduce the disease occurrence in fish [53,54]. El-Boshy et al. [55] indicated that mortality rate of Nile tilapia (*Oreochromis niloticus*) fed 10 g/kg β-glucan (*S. cerevisiae*) diet was significantly reduced when challenged with *Aeromonas hydrophila*. A similar result was also obtained by Yu et al. [50] who reported that Japanese seabass (*L. japonicus*) fed yeast cell wall at dose of 500 mg/kg and 20,000 mg/kg exhibited lower mortality rate when injected with *Aeromonas veronii*. Our results showed that fish fed 1% HY

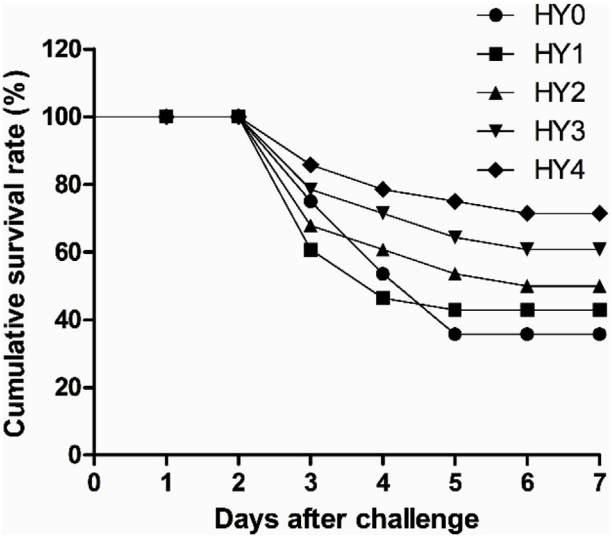

Fig. 4. The effect of hydrolyzed yeast levels on survival of Nile tilapia after challenge with *S. iniae* for 7 days. HY0, HY1, HY2, HY3 and HY4 represented groups containing 0%, 0.125%, 0.25%, 0.5% and 1% hydrolyzed yeast, respectively.

diet decreased mortality rate when challenged with a lethal *S. iniae*. The intensive protection against *S. iniae* in the current study could be confirmed by the enhanced lysozyme activity and complement C3 contents in the serum.

Respiratory burst, a bactericidal reaction implemented by macrophages and neutrophils that conventionally generate harmful substances such as superoxide anion, hydrogen peroxide, hydroxyl radical [12]. Therefore, antioxidant enzyme system plays an important role for the organism as it reduces oxidative stress or damage caused by reactive oxygen species (ROS). SOD is the one of the major antioxidant enzymes and T-AOC can be generally used to assess the antioxidant ability through non-enzymatic or enzymatic components in the organism [56]. Additionally, malondialdehyde (MDA), a product of lipid peroxidation reactions, is mostly utilized to assess the damage degree of cell structure and function through its concentration in serum or tissues [57]. Previous investigations have reported that the enhancement of antioxidant enzyme system of the organism correlated with a better health status and metabolic homeostasis for human or aquatic animals [58,59]. Prebiotics also reported the positive impact on the antioxidant enzyme system such as SOD or T-AOC, MDA, LPO (lipid peroxidation) in aquatic animals [9,60–62]. In the present study, the SOD activity was elevated both in serum and liver of Nile tilapia fed 0.25%–1% HY diets as well as the increased T-AOC activity in serum but not in the liver. The decreased MDA content of liver could also be observed in fish fed 0.25%–1% HY diets. Therefore, our results suggested that the damage comes from ROS could be recovered by the enhanced the antioxidant enzymes system induced by dietary HY supplements.

In general, anti-nutritional factors existed in plant-based protein, can induce moderate and severe enteritis in fish [63]. Considerations about whether diet cause inflammation or not as large varieties of plant-based protein were used in our formula. In the present study, no severe inflammation was detected and replaced with the better intestinal morphology in the mid-gut after 8 weeks HY supplements in diets. The digestive function of intestine commonly remains close relationship with gut development [64]. And study also acquiesced to the reality that higher villi stand for better absorption efficiency and healthier intestinal tracts in aquatic animals [65]. Anguiano et al. [66] found that villus height in midgut could be increased in hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed GroBiotic®-A (GBA) at 2% levels for 4 and 8 weeks. In the present study, villus height was noticed to be significantly increased by dietary HY supplements at 0.5%–1% levels and the number of IEFs (intraepithelial lymphocytes) increased significantly in all HY-treated fish, especially when 1% levels of HY was added to diet. Similar studies regarding the increased infiltration of IEFs were also reported in mice [67] receiving more than 25 mg of a β-(1,3)(1,6)-D-glucan per day and mirror carp (*Cyprinus carpio* L.) [68] fed with dietary high inclusion levels (1% and 2%) of MacroGard® (yeast β-glucan). The increase of IEFs comes from lamina propria was also reported in European sea bass (*D. labrax*) fed diets that included two levels of MOS (0 and 4 g/kg) for eight weeks [69]. The integrity of intestinal structure and function undertakes great importance for the organism. In the present study, the increase of villus height and the transported number of IELs in the mid-intestine epithelium not only promoted absorption ability but also improved the capacity of purging microbial antigens out of the intestine. However, their protection merits more investigations as the intestine is major infection routes for pathogens in fish [70,71].

In conclusion, this study assessed the application of hydrolyzed yeast (*R. mucilaginosa*) in Juvenile Nile tilapia. The results showed that supplementation of 1% hydrolytic yeast in diet could promote growth, enhance immunity and antioxidant capacity of Nile tilapia.

Authors' contributions

The authors thank the participants who gave their time to the trial. Jin Niu, Yong-Jian Liu and Li-Xia Tian contributed to the design of the study. Xian-Quan Chen carried out the experiment. Jia-Jun Xie, Zhi-Hao Zhang and Shi-Wei Xie analyzed the results and Xian-Quan Chen wrote the paper under the instruction of Jin Niu, Wei Zhao and the other authors. There are no conflicts of interest.

Conflicts of interest

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Sun Yat-sen University, Guangzhou, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

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