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Effects of dietary yeast hydrolysate on the growth, antioxidant response, immune response and disease resistance of largemouth bass (*Micropterus salmoides*)

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ABSTRACT

A 56-day growth trial was conducted to investigate the effects of dietary yeast hydrolysate on the growth performance, antioxidation, immune response and resistance against *Aeromonas hydrophila* in largemouth bass. Four experimental diets were prepared with yeast hydrolysate levels of 0% (Y0), 1.5% (Y1.5), 3.0% (Y3.0) and 4.5% (Y4.5). Each diet was randomly assigned to triplicate 150-L tanks and each tank was stocked with 30 largemouth bass (initial body weight, IBW = 7.71 ± 0.02 g). A challenge test was carried out after the feeding trial by injecting *A. hydrophila* intraperitoneally for 4-day observation. The results showed that the FBW and WGR in Y1.5 group were significantly higher than those in Y0 group ($P < 0.05$) and the feed conversion ratio (FCR) got the lowest value in Y1.5 group. And the hydrolysate supplement significantly increased the 4-day cumulative survival rate after the bacterial challenge ($P < 0.05$). The plasma malondialdehyde was lower in the yeast hydrolysate supplement groups in both pre- and post-challenge test ($P < 0.05$), while the plasma C3 increased ($P < 0.05$). In post-challenge test, the plasma superoxide dismutase (SOD) and catalase (CAT) activities increased in the Y1.5 and Y3.0 groups respectively ($P < 0.05$), and plasma lysozyme in Y1.5 group and the plasma IgM in Y3.0 group were higher than those in others respectively ($P < 0.05$). For the q-PCR results, in post-challenge test, the hepatic *hep2* expression level in Y1.5 and Y4.5 groups were both significantly higher than those in others ($P < 0.05$), as well as *il-8* in Y3.0 group. The spleen *hif-1alpha* and *tgf-beta1* expression levels in Y4.5 group were all significantly lower than those in others ($P < 0.05$), while the *gilt* was significantly higher ($P < 0.05$) in the post-challenge test. And the expression levels of spleen *tnf-alpah1* in Y1.5 and Y3.0 groups and *il-8* in Y3.0 group were all significantly higher than those in other groups ($P < 0.05$) in the post-challenge test. The head kidney *gilt* expression level was significantly higher in the yeast hydrolysate supplement groups compared with the Y0 group ($P < 0.05$), and the head kidney *il-8* expression level in Y1.5 group was significant higher than those in other groups in post-challenge test ($P < 0.05$). The present results indicated dietary yeast hydrolysate improved the antioxidant ability and enhanced the immune response of largemouth bass without negative effect on growth. And 1.5% or 3.0% of dietary yeast hydrolysate was recommended for largemouth bass based on the present results.

1. Introduction

As an important native carnivorous freshwater fish species in lakes and small rivers of North America [1], largemouth bass (*Micropterus salmoides*) has been widely cultured in many countries and has become one of the economically valuable species with rapid production growth in China [2], because of its rapid growth, tender flesh and high nutritional value and so on. However, infectious diseases and oxidative stress are more and more serious in largemouth bass farming. Recent years, immunomodulatory additives have been considered an effective method for improving the immune status and heath condition of cultured animals [3–5].

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Yeast products are widely used as supplements in aquatic feeds due to their relatively high protein, energy, and micronutrient contents [6]. And some studies have explored the effects of replacement of fish meal with yeast products on aquaculture animals. It was found that 50% of fish meal protein could be replaced with brewer's waste with no adverse effects on growth and feed utilization for Nile tilapia (*Oreochromis niloticus*) [7] and sea bass (*Dicentrarchus labrax*) [8]. Another study also indicated that replacing 45% of fish meal with brewer's yeast could improve the growth performance and immune response of the Thai panga (*Pangasianodon hypophthalmus* × *Pangasius bocourti*) [9]. Additionally, up to approximately 45% of the fish meal in the diet of Pacific white shrimp (*L. vannamei*) can be replaced by yeast extract in the presence of supplemental fish oil, phosphorus and calcium [10]. These studies prove that yeast products can be effective ingredients in aquafeed.

Besides, yeast products not only are rich in protein and amino acids but also contain various immunomodulatory compounds such as β-glucans, mannan oligosaccharides (MOS), chitin and nucleic acids [11–13]. It has been reported that the intraperitoneal injection of β-glucan, which was extracted and purified from *Saccharomyces cerevisiae* enhanced the non-specific and specific immune responses of carp (*Cyprinus carpio*) to bacteria challenge by *A. hydrophila* [14]. And another study indicated that dietary β-1, 3 glucan binding protein and selenium nanowire enhanced the growth performance and immune status of carp (*Cyprinus carpio*) to protect against *A. hydrophila* infection [15]. Mannan oligosaccharides are another important immunomodulatory compounds, and the 0.5% dietary yeast oligonucleotide could positively influence non-specific immune response and resistance of juvenile hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) to *Streptococcus iniae* [16]. It has been reported that mannan oligosaccharides could improve the growth performance of rainbow trout (*Oncorhynchus mykiss*) in net cage (42 days) as well as in raceways (90 days), and it also enhanced the survival and immune status of the experimental fish [17].

Accordingly, yeast products such as yeast, yeast culture and yeast hydrolysate could be used as additives to add in diet directly. There was a study showed that 4% dietary yeast culture could evidently improve the antioxidant and immune status without negative effects on growth in Gibel carp (*Carassius auratus gibelio* CAS III) [12]. In addition, in Jian carp (*Cyprinus carpio* var. Jian), 3% dietary yeast hydrolysate induced a much better growth performance, activated complements via an alternative complement pathway and the classical complement pathway and improved the protection against *A. hydrophila* [18]. Another study reported that brewer's yeast positively influenced the growth performance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) as well as its resistance to *Streptococcus iniae* infection. In addition, fish fed a diet with 2% brewer's yeast were found to have a better antioxidant status in this study [19]. However, the reasonable dietary yeast products dosage should be tested for the best effects. When the dietary yeast hydrolysate exceeded 3%, the growth performance of Jian carp was decreased with the increasing supplement [18]. These studies indicated that the appropriate dietary yeast products can improve the health status of aquaculture animals by regulating their immune systems, and it will support the potential use of yeast products in aquaculture.

Yeast hydrolysate is a hydrolysate of yeast cells derived by acids enzymes or other method of hydrolysis methods [20]. In the present study, the yeast was obtained by the liquid fermentation method, which used the *Saccharomyces cerevisiae* as strain. Then the collected yeast (*Saccharomyces cerevisiae*) began to autolyze and use the protease, peptidase and nuclease to catalyze and hydrolyze. Finally, the yeast hydrolysate was obtained after condensing and drying. The yeast hydrolysate contains much more free immune polysaccharides, amino acids and small peptides, which are easy to digest and immunostimulatory, than yeast culture [21]. Nevertheless, there are few studies have investigated the health-improving effects of yeast hydrolysate in aquatic animals, especially in largemouth bass culture. Therefore, the objectives of the present study were to evaluate the effects of dietary yeast hydrolysate on growth performance, blood antioxidant and immune response indices, immune and antioxidative genes expressions and resistance against *A. hydrophila* infection in largemouth bass.

Table 1
Formulation and compositions of experimental diets.

Ingredient (%)	Y0	Y1.5	Y3.0	Y4.5
Fishmeal[a]	35.00	35.00	35.00	35.00
Soybean meal	18.00	18.00	18.00	18.00
Casein	16.14	15.26	14.38	13.46
Wheat flour	8.00	8.00	8.00	8.00
Tapioca	11.90	11.90	11.90	11.90
Yeast hydrolysate[b]	0.00	1.50	3.00	4.50
Fish oil	4.54	4.54	4.54	4.54
Kelp meal	2.00	2.00	2.00	2.00
Vitamin premix[c]	0.10	0.10	0.10	0.10
Mineral premix[d]	0.15	0.15	0.15	0.15
Monocalcium phosphate	2.00	2.00	2.00	2.00
Choline chloride (50%)	0.20	0.20	0.20	0.20
Methionine	0.15	0.15	0.15	0.15
Cellulose	1.82	1.20	0.58	0.00
Proximate composition				
Moisture (%)	9.24	10.17	10.39	10.67
Crude protein (% DM)	45.70	46.18	46.01	46.01
Crude lipid (% DM)	5.99	6.21	5.93	6.00
Ash (% DM)	9.89	10.17	10.35	10.39

[a] Fishmeal: from Coland Feed Industry (Wuhan, Hubei, China).
[b] Yeast hydrolysate: from ANGEL YEAST Co. Ltd. (Yichang, Hubei, China).
[c] Vitamin premix (mg kg^{-1} diet): Vitamin A 10; Vitamin B_1 6; Vitamin B_2 5; Vitamin B_6 7.5; Vitamin B_{12} (1%) 4; Niacinamide 50; Ascorbyl calcium phosphate (35%) 500; Calcium pantothenate 20; Biotin (2%) 1; Folic acid 5; Vitamin E (50%) 200; Vitamin K_3 10; Vitamin D_3 5; Inositol 100; Corn protein powder 75.
[d] Mineral premix (mg kg^{-1} diet): $CuSO_4 \cdot 5H_2O$ 10; $FeSO_4 \cdot H_2O$ 300; $ZnSO_4 \cdot H_2O$ 200; $MnSO_4 \cdot H_2O$ 100; KIO_3 (10%) 80; Na_2SeO_3 (10% Se) 67; $CoCl_2 \cdot 6H_2O$ (10% Co) 5; NaCl 100; Zeolite 638.

2. Materials and methods

2.1. Experimental diets

In the present study, four isonitrogenous (46% crude protein) and isolipidic (6% crude lipid) diets were formulated and their chemical composition is shown in Table 1. The yeast hydrolysate supplement levels were 0% (Y0), 1.5% (Y1.5), 3.0% (Y3.0) and 4.5% (Y4.5) respectively. The yeast hydrolysate derived from *Saccharomyces cerevisiae* was obtained from ANGEL YEAST Co. Ltd. (Yichang, Hubei, China). The nutrient contents of the yeast hydrolysate are listed in Table 2. All ingredients of each diet were ground through a particle size 100 mesh,

Table 2
The contents of nutrients in yeast hydrolysate (%).[a]

Chemical composition	%
Moisture	4.01
Crude protein	51.26
Crude lipid	3.02
Crude fiber	0.1
Ash	6.57
N free extract	35.04
Functional composition	
Glucan	14.43
Mannan oligosaccharides	11.86
Acid soluble protein	49.13
Small peptides	37.49
Free amino acids	11.64
Nucleic acid	12.17

[a] Data from ANGEL YEAST Co. Ltd. (Yichang, Hubei, China).

completely mixed and then extruded into 2 mm diameter pellets under the following extrusion conditions: feeding section (90 °C/5 s), compression section (150 °C/5 s) and metering section (120 °C/4 s) using a twin-screwed extruder (Jinan Dingrun Machinery CO., LTD., Jinan, Shandong, China). The pellets were dried using an oven (60 °C) and then stored at 4 °C and kept out of the sun for 8 weeks.

2.2. Fish and feeding trial

All experimental animal care protocols were approved by the ethics committee of the Institute of Hydrobiology, Chinese Academy of Sciences. The largemouth bass were collected from Wuhan Bairui Biotechnology CO., LTD., (Wuhan, Hubei, China). Four weeks prior to the feeding trial, all fish were acclimated in 3 fiber glass cylinders (1325 L) and fed up to satiation twice a day at 8:30 and 18:30 with a commercial feed. The feeding trial was conducted at an indoor recirculating system. At the beginning of the trial, all fish were fasted for 24 h. Apparently healthy and similar size fish (initial body weight: 7.71 ± 0.02 g) were randomly distributed into 12 fiber glass cylinders (150-L) at a density of 30 fish per tank. Triplicate tanks were randomly assigned to each diet. During the trial, fish were fed up to apparent satiation twice a day at 8:30 and 18:30 for 56 days. The water flowing rate into each tank was approximately 2000 ml min^{-1}. The photoperiod was 12 h light: 12 h dark with the light period from 8:00 to 20:00. The water temperature was recorded daily and maintained at 28.5 ± 1.5 °C. Ammonia-N, dissolved oxygen and pH were monitored once every week, and the concentration of ammonia nitrogen was recorded below 0.4 mg kg^{-1}. The dissolved oxygen was more than 6 mg L^{-1}, and the pH was 6.8–7.0.

2.3. Challenge test

The single colony of *A. hydrophila* used in the challenge test was kindly supplied by Fish Diseases Laboratory, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, Hubei, China). It was originally isolated from Gibel carp. The bacteria were grown on brain heart infusion (BHI) agar plates and then isolated and cultured in BHI broth at 30 °C for 6 h and centrifuged at 3500 g for 10 min to harvest the bacteria. Before the challenge test, a preliminary experiment was conducted to determine the 4-day LC50 (the dose of *A. hydrophila* could kill 50% of the test largemouth bass) by intraperitoneal injection of *A. hydrophila* at 0.01 ml g^{-1} body weight in four graded doses (2×10^6, 2×10^7, 2×10^8, 2×10^9 CFU ml^{-1}) at 29 °C. The result showed that the 4-day LC50 was 2×10^8 CFU ml^{-1}. At the end of the feeding trial, 12 tanks of fish (n = 16 per tank) were injected intraperitoneally with 0.01 ml g^{-1} body weight 2×10^8 CFU ml^{-1} *A. hydrophila* at the same water temperature. Survival rate of fish was recorded for 4-day after bacterial challenge. The survival rate was calculated as follows: survival rate (%) = (number of fish which survived/initial number of fish) × 100.

2.4. Sample collection

At the end of the feeding trial, all fish from each tank were anesthetized with MS-222 (50 mg L^{-1}) and weighed after fasted overnight, 2 fish per tank were randomly sampled for blood, liver, spleen and head kidney tissues, and the remaining fish were put back into the original tanks. 12 h after challenge test, 2 live fish per tank were randomly sampled for blood, liver, spleen and head kidney tissues to supervise immunological parameters. Blood samples were collected from the caudal vein by heparinized syringes. Then, the blood samples were centrifuged at 3000 g for 15 min to obtain plasma and stored at - 80 °C for further analysis. After blood sampling, the liver, spleen and head kidney were dissected on ice and stored at - 80 °C.

2.5. Biochemical composition

Diets were analyzed for proximate composition according to the method of the Association of Official Analytical Chemists (AOAC) [22]. The moisture content of the samples was tested by oven drying at 105 °C to a constant weight. Ash content was determined by incineration in a muffle furnace at 550 °C for 12 h. A Kjeltec 8400 Analyzer Unit machine (FOSS Tecator, Haganas, Sweden) was used to measure crude protein content. Crude lipid content was determined through ether extraction using a Soxtec system (Soxtec™ 2005, FOSS Tecator, Haganas, Sweden).

2.6. Plasma immunological and antioxidant assays

The activities of plasma superoxide dismutase (SOD), catalase (CAT), and lysozyme (LZM) were determined using commercial kits according to the manufacturer instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). And the plasma malondialdehyde (MDA), immune globulin M (IgM) and complement 3 (C3) were tested using commercial kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.7. Quantitative real time PCR analysis

The qPCR primers were designed using the National Center for Biotechnology Information (NCBI) primer BLAST service or from Yu et al. [26] and are listed in Table 3. Total RNA in tissues of liver, head kidney and spleen of largemouth bass were extracted using Trizol reagent according to the instruction of manufacturer (Invitrogen, Carlsbad, CA, USA). The quality of RNA was assessed by agarose gel electrophoresis. And the quantity of RNA was detected with the NanoDrop® ND-2000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The total RNA was then reverse-

Table 3
Primer sequences for real-time PCR.

Gene	Accession no.	Forward primer (5′ – 3′)	Reverse primer (5′ – 3′)	Product length (bp)	Tm (°C)	PCR efficiency[a/b/c]
β-actin	KJ669298.1	TTCACCACCACAGCCGAAAG	TCTGGGCAACGGAACCTCT	179	57	2.010/1.966/2.045
hep-1	EU502754.1	GCTCTGCCGTCCCATTCAC	GCCACAGCCCTTGTTACCC	191	57	2.030/—/—
hep-2	EU502755.1	CTGGAGGAGCCAATGAGCG	CAGCCATAAATTCGACAGTAGGT	254	57	1.979/—/—
hif-1α	KY952764	CAGAGGACCTGTTGAATCGTT	TTGTAGATGACAGTGGCTTGG	178	57	1.966/2.022/1.983
tnf-α	*	CTTCGTCTACAGCCAGGCATCG	TTTGGCACACCGACCTCACC	161	63	—/2.050/2.041
tgf-β1	*	GCTCAAAGAGAGCGAGGATG	TCCTCTACCATTCGCAATCC	118	57	—/1.990/1.953
gilt	KR270996.1	CCTACCTGGGTCTTGCTGAAT	ACACAGGTCTTGGCTTCCTTG	230	57	—/1.952/2.090
il-8	*	CGTTGAACAGACTGGGAGAGATG	AGTGGGATGGCTTCATTATCTTGT	112	57	1.968/2.002/2.036
il-10	*	CGGCACAGAAATCCCAGAGC	CAGCAGGCTCACAAAATAAACATCT	119	57	2.025/1.990/2.001

Note: [a]Liver, [b]Spleen, [c]Kidney.
"—": It is not measured in this tissue.
*: Form Yu et al. [27].

Table 4
Effects of dietary yeast hydrolysate on the growth performance of largemouth bass (Means ± SEM).

	Y0	Y1.5	Y3.0	Y4.5
IBW[a], g	7.77 ± 0.02	7.72 ± 0.03	7.69 ± 0.06	7.68 ± 0.02
FBW[b], g	30.58 ± 1.67[a]	34.88 ± 0.92[b]	33.03 ± 0.61[ab]	31.54 ± 0.62[ab]
WGR[c], %	294.2 ± 21.80[a]	352.13 ± 13.41[b]	329.71 ± 10.91[ab]	310.75 ± 7.17[ab]
FCR[d], %	96.84 ± 4.40[a]	92.22 ± 2.09[a]	100.43 ± 2.35[ab]	108.91 ± 1.74[b]

[a] IBW: initial body weight.
[b] FBW: final body weight.
[c] WGR: Weight gain rate (%) = (total final body weight – total initial body weight)/total initial body weight × 100.
[d] FCR: Feed conversion ratio (%) = feed intake/(total final body weight – total initial body weight) × 100.

transcribed with an M-MLV FirstStrand Synthesis Kit (Invitrogen, Shanghai, China). The obtained cDNA was stored at −20 °C. Quantitative real time PCR was performed on LightCycle 480 II system (Roche, Basel, Switzerland). Each sample was run in duplicate, and the relative expressions were calculated according to Vandesompele et al. [23].

2.8. Statistical analysis

All data were statistically analyzed with SPSS 19.0 and subjected to one-way ANOVA analysis. Duncan's multiple range test was used to detect the significance of differences of mean values among different feed treatments. Differences of data between pre- and post-challenge test groups were estimated using a paired-samples T-test. The significance level was set at $P < 0.05$.

3. Results

3.1. Growth performance and post-challenge cumulative survival

The growth performance of largemouth bass fed with diets containing different levels of yeast hydrolysate is presented in Table 4. After 56-day feeding trial, the final body weight (FBW) and weight gain rate (WGR) of the largemouth bass were higher in the yeast hydrolysate supplement groups, and the those in Y1.5 group was significantly higher than those in others ($P < 0.05$). The feed conversion ratio (FCR) was significantly lower in the Y1.5 and Y0 groups than that in the other groups, and it had the lowest value in the Y1.5 group. It indicated that there was no negative effect on the growth of largemouth bass when fed with diets containing yeast hydrolysate.

The cumulative survival rate of largemouth bass after the challenge test with *A. hydrophila* is shown in Fig. 1. Compared to the Y0 group, the 4-day cumulative survival rates of largemouth bass of the yeast hydrolysate supplement groups were significantly higher ($P < 0.05$). The 4-day cumulative survival rate was 37.50 ± 0.00% in the Y4.5 group, followed by 35.33 ± 5.45% in the Y3.0 group, 29.17 ± 2.08% in the Y1.5 group and 12.50 ± 3.61% in the Y0 group.

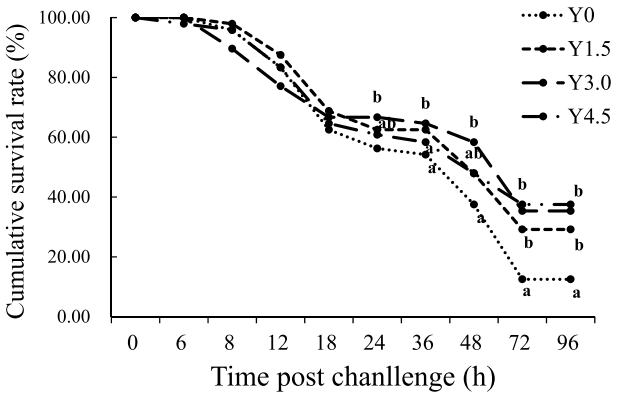

Fig. 1. Effects of dietary yeast hydrolysate on post-challenge survival of largemouth bass after infection with *A. hydrophila*. The cumulative survival rate of largemouth bass was recorded and analyzed. The equation is expressed as survival rate (%) = (final number of fish survivor/initial number of inoculated fish) × 100. Values of four groups with different lowercase letters at a specific timepoint indicate difference at $P < 0.05$ (n = 16).

3.2. Plasma immunological and antioxidant assays

The plasma MDA content and activities of plasma SOD and CAT of fish pre-challenge and 12 h post-challenge test are shown in Fig. 2. The results showed that the plasma MDA levels of the Y0 and Y3.0 groups in 12 h post-challenge were significantly higher than those in pre-challenge ($P < 0.05$), and the plasma MDA level was significantly lower in the yeast hydrolysate supplement groups in both the pre- and post-challenge tests ($P < 0.05$) (Fig. 2A). The plasma SOD activities of the Y0, Y1.5 and Y4.5 groups in 12 h post-challenge were significantly higher than those in the pre-challenge ($P < 0.05$), and it was higher in the yeast hydrolysate supplement groups in post-challenge test, in which the plasma SOD activity of the Y1.5 group was significantly higher than that of the Y0 group ($P < 0.05$) (Fig. 2B). And the plasma CAT activities of the Y3.0 and Y4.5 groups in 12 h post-challenge were significantly higher than those in pre-challenge ($P < 0.05$), and it was higher in the Y3.0 and Y4.5 groups post-challenge test, in which the plasma CAT activities in Y3.0 group was significantly higher than those in Y0 and Y1.5 groups ($P < 0.05$) (Fig. 2C).

In addition, the plasma LZM activity, plasma IgM levels and C3 levels of fish pre-challenge and 12 h post-challenge test are shown in Fig. 3. The results showed that the plasma LZM activities of the Y1.5 and Y3.0 groups in 12 h post-challenge were significantly higher than that in pre-challenge ($P < 0.05$), and it was higher in Y1.5 and Y3.0 groups in 12 h post-challenge test, in which the Y1.5 group was significant higher than that in the Y0 and Y4.5 groups ($P < 0.05$) (Fig. 3A). The plasma IgM level of the Y3.0 group in the 12 h post-challenge was significantly higher than that in pre-challenge ($P < 0.05$), and the plasma IgM level of the Y3.0 group was also significantly higher than that in the other groups in 12 h post-challenge ($P < 0.05$) (Fig. 3B). Meanwhile, the plasma C3 level of the Y1.5 group in 12 h post-challenge was significantly higher than that in pre-challenge ($P < 0.05$), and it was significantly higher in the yeast hydrolysate supplement groups in both pre- and post-challenge tests, compared with that in the Y0 group ($P < 0.05$) (Fig. 3C).

3.3. Immune and antioxidant related genes expression

The expressions of immune- and antioxidant-related genes in hepatic tissue are shown in Fig. 4. The results indicated that the expression levels of liver *hep1*, *hep2*, *hif-1alpha*, *il-8*, and *il-10* in 12 h post-challenge were all significantly higher than those in pre-challenge ($P < 0.05$). There was a significant increase in hepatic *hep2* in the Y1.5 and Y4.5 groups in the12 h post-challenge ($P < 0.05$) (Fig. 4B), while there was no significant difference in hepatic *hep1* among groups in the 12 h post-challenge (Fig. 4 A). There was no significant change in hepatic *hif-1alpha* and *il-10* in any group in either pre- or post-challenge (Fig. 4C and E). However, it is worth noting that the hepatic *il-8* of the

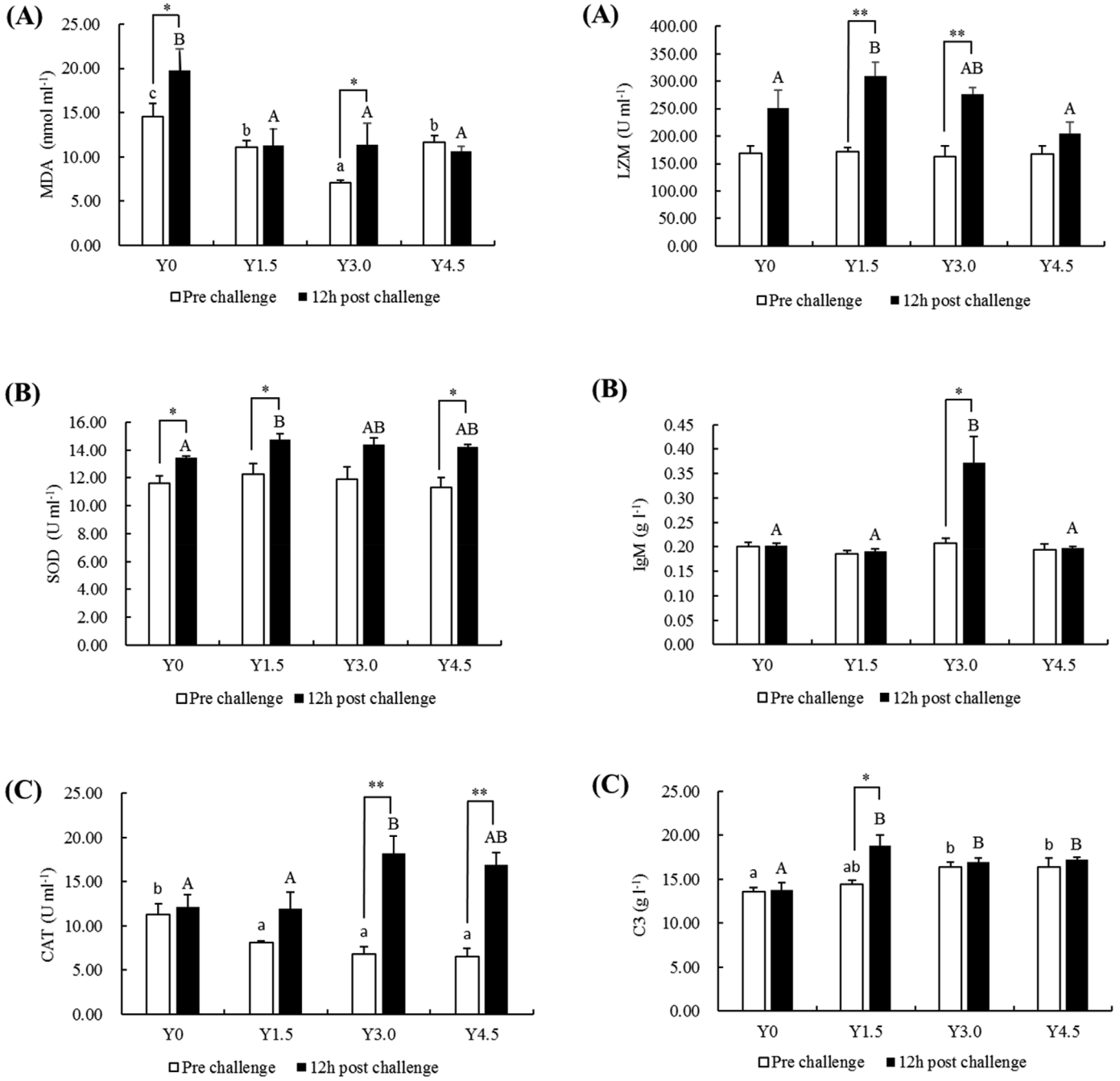

Fig. 2. Effects of dietary yeast hydrolysate on plasma MDA level (A), SOD activity (B) and CAT (C) activity of largemouth bass at the end of feeding trial (pre-challenge) or 12 h post-challenge with *A. hydrophila*. Data are indicated as mean ± SEM (n = 6). Bars with different lowercase letters mean significant changes among groups pre-challenge ($P < 0.05$). Bars with different capital letters mean significant changes among groups 12 h post-challenge ($P < 0.05$). Bars with * mean significant changes pre and 12 h post-challenge at the same group ($P < 0.05$). Bars with ** mean excellent significant changes pre- and 12 h post-challenge at the same group ($P < 0.01$).

Fig. 3. Effects of dietary yeast hydrolysate on plasma lysozyme (A), IgM (B) and C3 (C) level of largemouth bass at the end of feeding trial (pre-challenge) or 12 h post-challenge with *A. hydrophila*. Data are indicated as mean ± SEM (n = 6). Bars with different lowercase letters mean significant changes among groups pre-challenge ($P < 0.05$). Bars with different capital letters mean significant changes among groups 12 h post-challenge ($P < 0.05$). Bars with * mean significant changes pre and 12 h post-challenge at the same group ($P < 0.05$). Bars with ** mean excellent significant changes pre- and 12 h post-challenge at the same group ($P < 0.01$).

Y3.0 group was significantly increased compared with that of the other groups in the 12 h post-challenge (Fig. 4D and F).

The expressions of immune and antioxidative genes in the spleen are shown in Fig. 5. Compared with the pre-challenge levels of expression, the expression of spleen *gilt* was significantly increased in the Y1.5 and Y4.5 groups in the12 h post-challenge ($P < 0.05$), and the spleen *gilt* expression of the Y4.5 group was significantly higher than that in the other groups both pre- and post-challenge ($P < 0.05$) (Fig. 5B). A). The expression of spleen *hif-1alpha* was significantly increased in all four groups in the 12 h post-challenge ($P < 0.05$) compared with the expressions in pre-challenge (Fig. 5 B). Additionally, the spleen *hif-1alpha* and *tgf-beta1* expression levels in the Y4.5 group were significantly lower than those in the other groups both pre- and post-challenge ($P < 0.05$) (Fig. 5B and C). Significant post-challenge increases in *tnf-alpha1* expression were only observed in the Y3.0 and Y4.5 groups ($P < 0.05$), and it is worth noting that the expression levels of *tnf-*

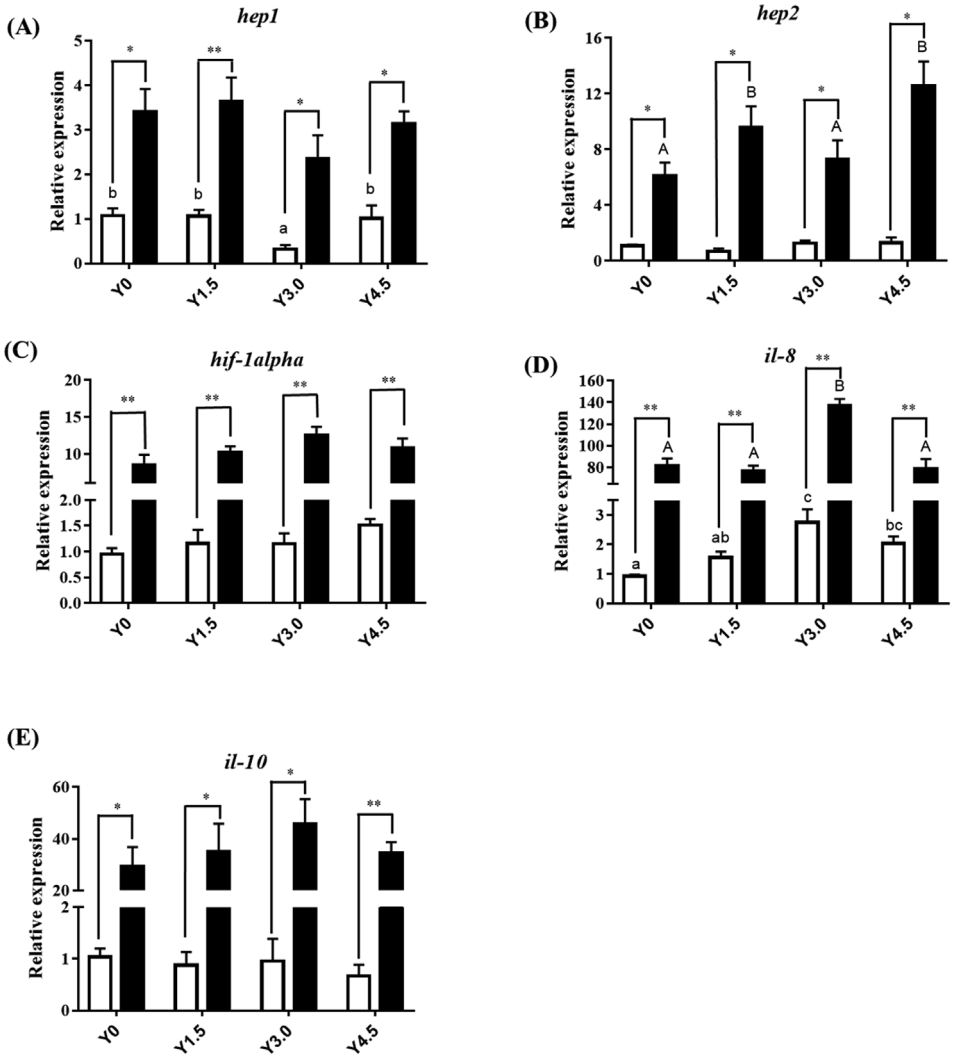

Fig. 4. Gene expressions of *hep1* (A), *hep2* (B), *hif-1alpha* (C), *il-8* (D) and *il-10* (E) in hepatic of largemouth bass fed with yeast hydrolysate-containing diets at the end of feeding trial (pre-challenge) or 12 h post-challenge with *A. hydrophila*. Data are indicated as mean ± SEM (n = 6). Bars with different lowercase letters mean significant changes among groups pre-challenge ($P < 0.05$). Bars with different capital letters mean significant changes among groups 12 h post-challenge ($P < 0.05$). Bars with * mean significant changes pre and 12 h post-challenge at the same group ($P < 0.05$). Bars with ** mean excellent significant changes pre- and 12 h post-challenge at the same group ($P < 0.01$).

alpha1 in the Y1.5 and Y3.0 groups were significantly higher than those in the Y0 group in the 12 h post-challenge ($P < 0.05$) (Fig. 5D). For the interleukin genes expressions, the *il-8* expression levels in the four groups were all significantly increased in the 12 h post-challenge ($P < 0.05$) (Fig. 5E) compared with the pre-challenge expression levels. The *il-8* expression levels in the Y1.5 and Y3.0 groups were significantly increased in the pre- and post-challenge, respectively ($P < 0.05$) (Fig. 5E). There was no significant change in *il-10* expressions in the four groups between pre- and post-challenge (Fig. 5F).

The expressions of immune and antioxidative genes in the head kidney are shown in Fig. 6. The results showed that all of the quantitative genes in the four groups were significantly up-regulated in the post-challenge compared with pre-challenge ($P < 0.05$), except *tnf-alpha1* in the Y4.5 group (Fig. 6A–F). The *gilt* expressions of the yeast hydrolysate supplement groups were significantly higher than that in the Y0 group both pre-challenge and post-challenge ($P < 0.05$) (Fig. 6A). The *hif-1alpha*, *tnf-alpha1* and *il-10* expression levels in the yeast hydrolysate supplement groups were significantly lower than those in the Y0 group in the post-challenge ($P < 0.05$), and these values were the lowest in the Y3.0, Y4.5 and Y4.5 groups respectively (Fig. 6B, D and F). There was no significant difference in *tgf-beta1* among the four groups in either pre- or post-challenge (Fig. 6C). However, the *il-8* expressions in the Y1.5 group were significantly increased compared with that in the other groups both pre- and post-challenge ($P < 0.05$) (Fig. 6E).

4. Discussion

Yeast products is the general name of brewer's yeast, yeast culture and yeast hydrolysate. There are some differences in the compounds and production technologies of them. Brewer's yeast is a kind of fungus which is the second largest by-product originated by the food industry. It is rich in protein (35–60% dry basis) and includes all the essential amino acids [20]. And yeast culture is a compound which is produced by yeast through anaerobic fermentation in certain conditions. It contains inactive yeast cells, yeast cell contents, fermented culture medium and extracellular metabolites [24]. However, yeast hydrolysate is a compound of catalyzed and hydrolyzed yeast which is obtained from the liquid fermentation products of *Saccharomyces cerevisiae*. And the yeast soluble matters in it aren't extracted and it contains more than 35% crude protein.

In the present study, the growth-promoting effects of dietary brewer's yeast hydrolysate were confirmed in largemouth bass, which are similar to the previous studies on yeast and its hydrolysate [12,13,18,19]. Particularly, 1.5% and 3.0% dietary yeast hydrolysate improved the blood antioxidant and immune response indices, immune gene expressions and the resistance of body against *A. hydrophila* infection in largemouth bass.

Growth performance is an important aspect for evaluating the effects of feed additives [8,17,25]. Represented by the FBW, WGR and FCR of largemouth bass in yeast hydrolysate supplement groups were higher than those in the control, especially those of the Y1.5 group,

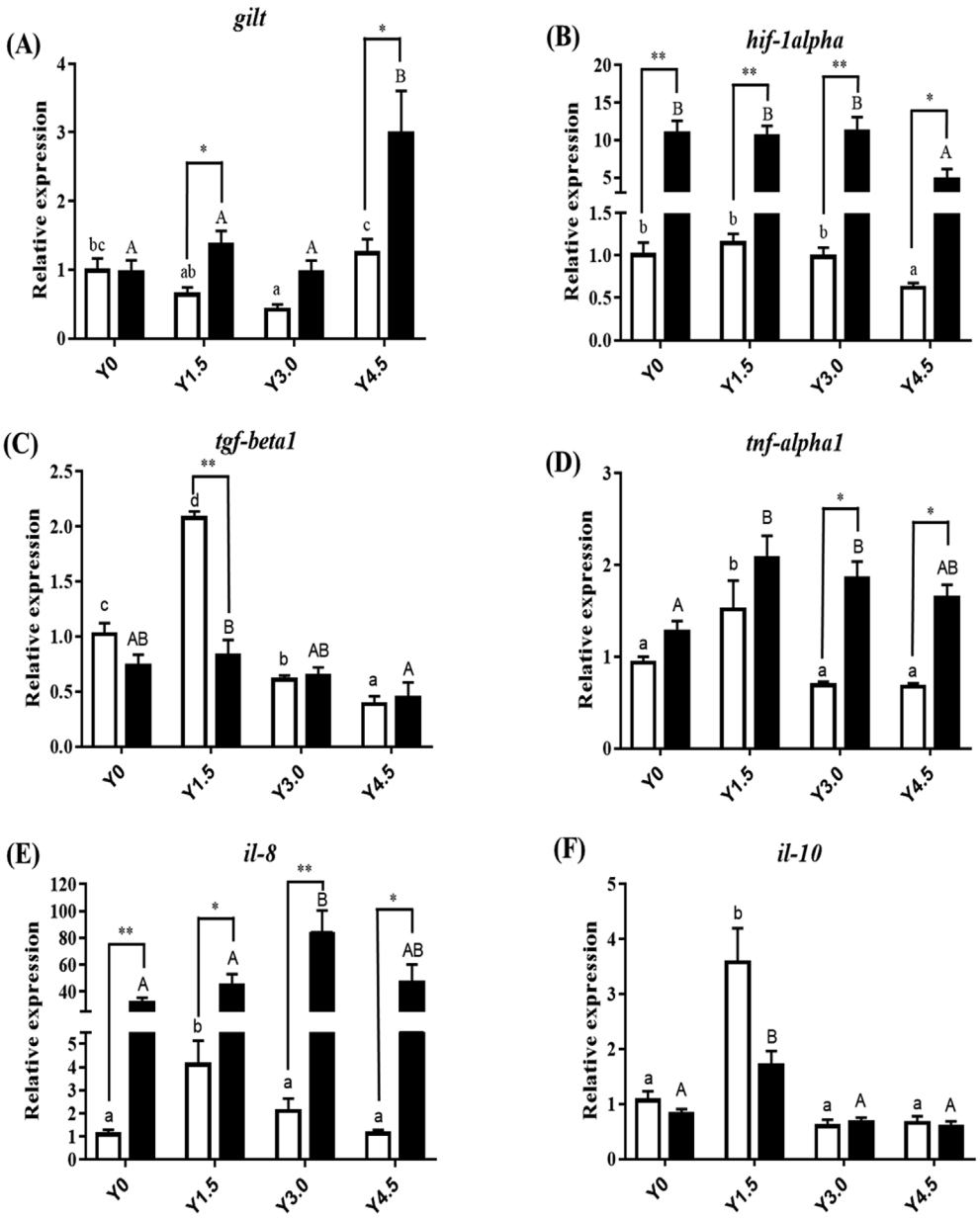

Fig. 5. Gene expressions of *gilt* (A), *hif-1alpha* (B), *tgf-beta1* (C), *tnf-alpha1* (D), *il-8* (E) and *il-10* (F) in spleen of largemouth bass fed with yeast hydrolysate-containing diets at the end of feeding trial (pre-challenge) or 12 h post-challenge with *A. hydrophila*. Data are indicated as mean ± SEM (n = 6). Bars with different lowercase letters mean significant changes among groups pre-challenge ($P < 0.05$). Bars with different capital letters mean significant changes among groups 12 h post-challenge ($P < 0.05$). Bars with * mean significant changes pre and 12 h post-challenge at the same group ($P < 0.05$). Bars with ** mean excellent significant changes pre- and 12 h post-challenge at the same group ($P < 0.01$).

which were significantly higher than others ($P < 0.05$) in the present study. The growth results were similar to some previous studies. In Pacific white shrimp, 1% yeast hydrolysate supplement increased the WG and SGR, but decreased the FCR significantly [13]. Additionally, a 3% dietary yeast hydrolysate supplement increased the FBW and WG of juvenile common carp, however, it did not affect the FCR [18]. While in fry Nile tilapia, *Oreochromis niloticus* (L.), 1 g kg^{-1} bakers' dietary yeast (*Saccharomyces cerevisiae*) could increase the WG and SGR of the experimental fish [26]. Combined with these previous studies, the present results indicate that the 1.5% dietary yeast hydrolysate could promote the growth performance of largemouth bass.

Antioxidative ability is usually used to reflect the health conditions of aquatic animals [27–29]. The results related to blood antioxidants also indicated dietary yeast hydrolysate supplement elevated the antioxidative status of the experimental largemouth bass. Both of the SOD and CAT have an important role in maintaining the free radical balance and reducing oxidative damage. Concretely, SOD is one the important antioxidative enzymes in antioxidant defense system, which can neutralize excessive reactive oxygen species (ROS) produced in the metabolism process [30]. The present results showed that the plasma SOD activities were increased in the yeast hydrolysate supplement groups in the post-challenge test, in which the plasma SOD activity in the Y1.5 group was significantly higher than that in the Y0 group ($P < 0.05$). CAT is another key antioxidative enzyme, which reduces H_2O_2 to H_2O and O_2 [31]. The present results showed that the plasma CAT activities were higher in the Y3.0 and Y4.5 groups in the post-challenge test, in which the plasma CAT activities in the Y3.0 group was significantly higher than that of Y0 and Y1.5 groups ($P < 0.05$). Meanwhile, MDA is a metabolite derived from lipid peroxidation, and the MDA levels are always used to evaluate the degree of lipid peroxidation and oxidative stress in fish [32]. The plasma MDA levels were significantly lower in the yeast hydrolysate supplement groups than those in the control in both the pre- and post-challenge test ($P < 0.05$). A study in Gibel carp found that 4% dietary yeast culture could improve the plasma SOD activities [12], meanwhile 3% yeast hydrolysate supplement increased the liver antioxidant capacities of the juvenile Jian carp [33]. The present plasma antioxidant results indicated that dietary yeast hydrolysate supplement improved the body antioxidant status of the largemouth bass, which is consistent with the conclusions of the previous studies in aquatic animals [12,32].

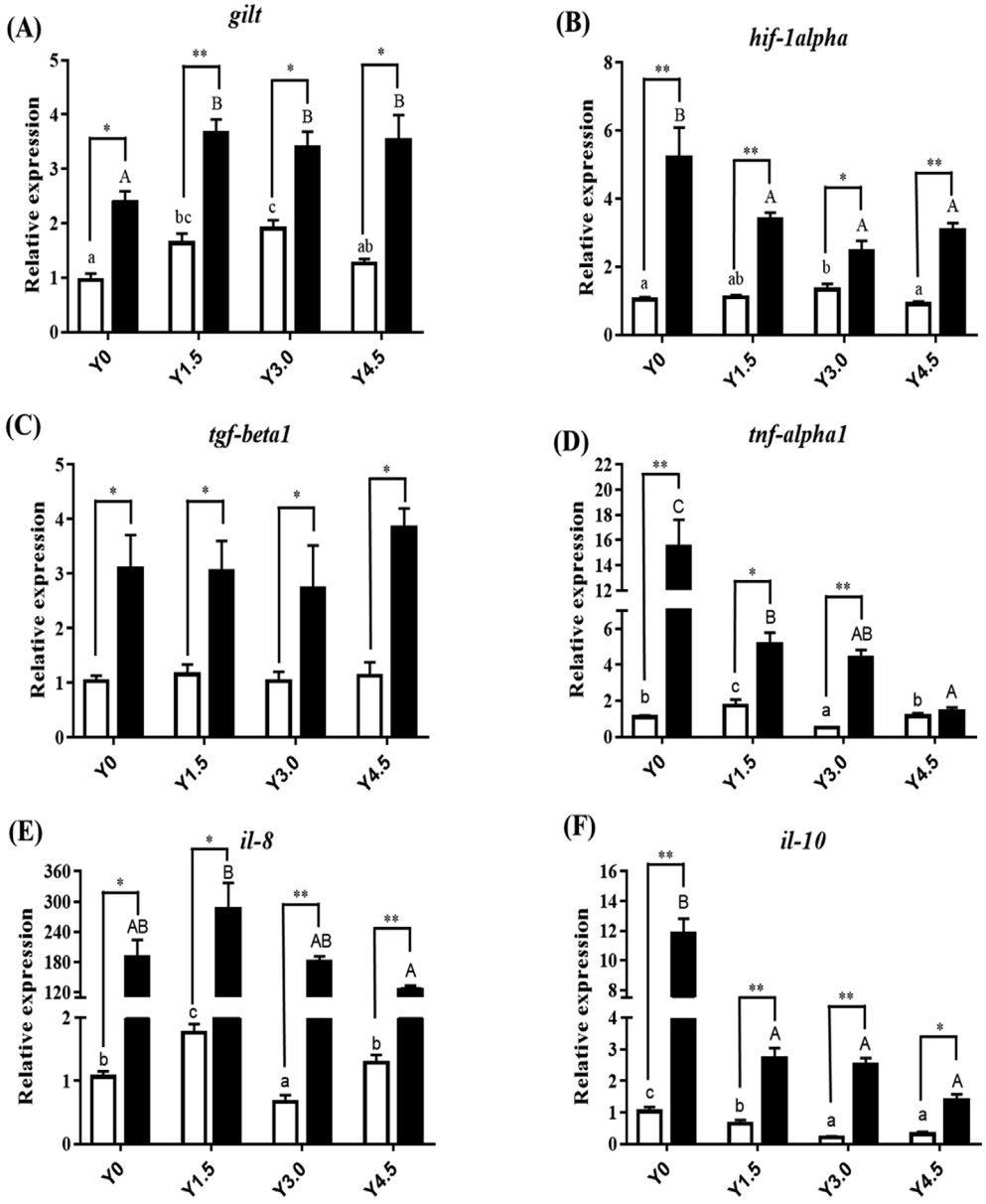

Fig. 6. Gene expressions of *gilt* (A), *hif-1alpha* (B), *tgf-beta1* (C), *tnf-alpha1* (D), *il-8* (E) and *il-10* (F) in head kidney of largemouth bass fed with yeast hydrolysate-containing diets at the end of feeding trial (pre-challenge) or 12 h post-challenge with *A. hydrophila*. Data are indicated as mean ± SEM (n = 6). Bars with different lowercase letters mean significant changes among groups pre-challenge ($P < 0.05$). Bars with different capital letters mean significant changes among groups 12 h post-challenge ($P < 0.05$). Bars with * mean significant changes pre and 12 h post-challenge at the same group ($P < 0.05$). Bars with ** mean excellent significant changes pre- and 12 h post-challenge at the same group ($P < 0.01$).

Gamma-interferon-inducible lysosomal thiol reductase (GILT) is expressed in antigen-presenting cells (APCs) such as monocytes/macrophages, B cells, and bone marrow derived dendritic cells, and it can also be induced by interferon-γ (IFN-γ) in fibroblasts and endothelial cells [34,35]. And GILT is involved in regulating the endocytic MHC pathway, cellular redox state, inhibiting T cell activation, and neutralizing extracellular pathogens, suggesting that it is also a host factor for some bacterial pathogens. It has also been reported that GILT inhibition of T cells might be related to increasing the expression and activity of superoxide dismutase (SOD), reducing active oxygen level [36]. The full-length cDNA sequence and structure of largemouth bass GILT have been identified, and high expression levels were found in the spleen and head kidney [37]. In the present study, the increased spleen *gilt* expression level was only observed in the Y4.5 group in the post-challenge test. However, the significant up-regulation of the head kidney *gilt* expression levels was found in the three yeast hydrolysate supplement groups both in the pre- and post-challenge tests. And the head kidney *gilt* expression levels in the post-challenge test were significantly higher than those in the pre-challenge test. It is speculated that GILT exhibited a tissue-specific function and promoted the SOD activity mainly in head kidney in this experiment.

Besides, hypoxia-inducible factors are also involved in oxidative stress [38]. Hypoxia-inducible factor-1 (HIF-1) was first discovered as a binding factor of the hematopoietic growth factor erythropoietin under hypoxic conditions, and the hypoxia-induced antioxygen process genes share a common mode of transcriptional regulation depending on activation of HIF-1 [39]. The availability of HIF-1 is mainly determined by oxygen-destructible HIF-1alpha [40]. The previous study in largemouth bass showed that the increased expression of *hif-1alpha* is involved in the oxidative stress, and the decreased activities of SOD and CAT is also related to *hif-1alpha* [28]. In the present study, the up-regulations of liver, spleen and head kidney *hif-1alpha* expressions were found in the post-challenge test compared to the pre-challenge test. And the significantly decreased expression of *hif-1alpha* were observed in the spleen of the Y4.5 group and in the head kidney of yeast supplement groups in the post-challenge test. These results were in keeping with the plasma antioxidant indexes, especially the increased plasma SOD activity in the post-challenge test.

Lysozyme activity is an important innate immunity index in fish and is ubiquitous in all kinds of living organisms. It can also protect against

certain Gram-positive bacteria and some Gram-negative bacteria by destroying β-1, 4-glycosidic bond of the cell wall between *N*-acetylmuramic acid and *N*-acetyl glucosamine. It is also known to be opsonic in nature and activates the complement system and phagocytes [27,41]. In the present study, the increased plasma lysozyme levels were observed in the Y1.5 and Y3.0 groups in the post-challenge test ($P < 0.05$). This is well agreement with the results in juvenile Jian carp, which was 3.0% dietary yeast hydrolysate supplement [18]. Meanwhile, the complement system is also important for innate immune system, and C3 is a common one from them [42]. The present study showed that the yeast hydrolysate supplement increased the plasma C3 level both in pre- and post-challenge tests ($P < 0.05$), and it was similar to a study in juvenile Jian carp in which 3% dietary yeast hydrolysate enhanced the plasma C3 [18]. In addition, IgM plays a critical role in the response to antigenic challenge in aquatic animals [43]. It is not only the major antibody of primary response but a key part of the adaptive immune response of fish [44]. And the present study showed that there was only one obvious increase of plasma IgM in the Y3.0 group of post-challenge test ($P < 0.05$). And it was reported that the 4% yeast culture supplement increased the plasma IgM of Gibel carp [12]. These results related to plasma immunity, combined with the results in plasma antioxidation and related genes expressions, indicate that the appropriate yeast hydrolysate supplement can improve the blood health status and organism antioxidative ability of the largemouth bass.

Hepcidin is originally expressed in liver and exhibits antifungal activity and antibacterial activity in human beings [45]. The expression of hepcidin is induced by infection, inflammation anemia and hypoxia [46,47]. There are two hepcidin genes, *hep-1* and *hep-2*, have been identified in largemouth bass, and both of them mainly express in liver [48]. In the present study, the obvious up-regulations of liver *hep-1* and *hep-2* were observed in the post-challenge test of all groups, which indicated the immunity reaction took place in liver. However, only the *hep-2* expression levels of Y1.5 and Y4.5 groups were significantly increased compared with those in the Y0 group in the post-challenge test. And the previous study also found that *hep-2* was more sensitive than *hep-1* when largemouth bass was exposed to pathogenic bacteria [47]. These results suggest that dietary yeast hydrolysate can stimulate the hepcidin genes expressions to resist bacteria invasion in largemouth bass, and the *hep-2* may play a more important role in this process.

Tumor necrosis factor-alpha1 (TNF-alpha1) and interleukin-8 (IL-8), two of the important proinflammatory cytokines and good markers of inflammatory responses, are involved in host defense against microbial pathogens [49,50]. IL-8, also known as chemokine (C-X-C motif) ligand 8 (CXCL8), is a member of the CXC chemokine family and is produced by several cell types in response to inflammatory stimuli [51]. In addition, TNF-alpha has been reported to increase with the expression of IL-8 [52]. In the present study, the spleen *tnf-alpha1* expressions were significantly up-regulated in the Y1.5 and Y3.0 groups, while the head kidney *tnf-alpha1* expressions were significantly down-regulated in the yeast hydrolysate supplement groups and it get the lowest value in the Y4.5 group in the post-challenge test. Compared with the pre-challenge, the post-challenge transcription levels of *il-8* were all increased in the liver, spleen and head kidney. And it showed the similar changing pattern with *tnf-alpha1* expression levels in spleen and head kidney respectively. The differences in the proinflammatory cytokines expression pattern between spleen and head kidney may be due to the tissue specificity and may also be associated with the tested time and antigen species of challenge test [5,27,53].

Meanwhile, transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) are two anti-inflammatory cytokines [50,54]. There is a study showed that the higher increase of TGF-beta is involved in the controlled inflammation by IL-10 [55]. And TGF-beta1 is an important isoform of TGF-betas and has been proved to related to oxidative stress triggered inflammation [26,53]. In the present study, the post-challenge *il-10* expression levels in liver and head kidney as well as the *tgf-beta1* expression level in head kidney were all up-regulated in 4 groups compared to pre-challenge. And the *il-10* expression levels of yeast hydrolysate supplement groups were significantly lower than those of the Y0 group in head kidney, while there was no difference among treatments in liver. Nevertheless, there was no post-challenge up-regulation of *tgf-beta1* or *il-10* expression in spleen compared to the pre-challenge. Besides, there are some interactions among these proinflammatories or anti-inflammatories. A previous study illustrated that the TNF-alpha can induce MSCs (mesenchymal stem cells) to suppress inflammation by increasing TGF-beta and IL-10 [54]. And it has been demonstrated that IL-10 reduces inflammatory responses and the expression of proinflammatory cytokines such as TNF-α [56]. It is hypothesized that in the present study the proinflammatory effect mainly occurred in the spleen of largemouth bass, while the anti-inflammatory effect mainly occurred in the head kidney in the present experiment. These results suggested the dietary yeast hydrolysate can decrease the inflammation of the head kidney rather than spleen in largemouth bass.

In summary, the present results indicated that dietary inclusion of yeast hydrolysate significantly improved the antioxidant ability and enhanced the immune response of largemouth bass without negative effect on the growth. Additionally, 1.5% or 3.0% of dietary yeast hydrolysate was recommended for the juvenile largemouth bass based on the growth, antioxidant and immune response. And the present study will support the potential use of yeast hydrolysate in aquaculture.

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