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Credit author statement

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**Yeast culture improved the growth performance, liver function,
intestinal barrier and microbiota of juvenile largemouth bass
(*Micropterus salmoides*) fed high-starch diet**

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Abstract:

The present study was conducted to investigate the effects of yeast culture on the growth, health and microflora of the juvenile largemouth bass fed high-starch diet. The experiment set three isonitrogenous and isolipidic diets, control (high-starch diet), HSY₁ (high-starch diet with 1% yeast culture) and HSY₃ (high-starch diet with 3% yeast culture). A feeding trial was conducted in largemouth bass juveniles for 8 weeks. The results indicated fish fed with 3% yeast culture not only could improve specific growth rate (SGR), but also significantly decreased hepatic lipid content, hepatic glycogen content, and hepatopancreas somatic index (HSI) compared with the control group ($p < 0.05$). The total superoxide dismutase (T-SOD) and catalase (CAT) activities of HSY₃ group significantly increased while malondialdehyde (MDA) content significantly reduced in liver compared with the control group ($p < 0.05$). Meanwhile, the mRNA expression levels of hepatic *Sod* and *Cat* were up-regulated ($p < 0.05$), and liver metabolism showed 111 metabolites were significantly changed in HSY₃ group, liver lipid metabolism pathway remarkably changed. Besides, the intestinal anti-inflammatory cytokines were significantly up-regulated, and the pro-inflammatory cytokines were significantly down-regulated as the inclusion of yeast culture ($p < 0.05$). Notably, HSY₃ group diet up-regulated the expression of *Zo-1*, *Claudin* and *Occludin* in intestine compared with the other groups ($p < 0.05$). Serum D-lactate (D-lac), diamine oxidase (DAO) and lipopolysaccharide (LPS) decreased significantly with the inclusion of yeast culture ($p < 0.05$). Furthermore, the abundance of probiotics (such as *Lactobacillus*, *Bacillus* and *Bifidobacterium*) increased significantly, and the abundance of intestinal potential pathogenic bacteria (*Plesiomonas*) decreased in HSY₃ group ($p < 0.05$). The phenotypic analysis showed that gram-negative bacteria significantly decreased while gram-positive bacteria increased in HSY₃ group ($p < 0.05$). All in all, this study revealed that supplementation of 3% yeast culture can improve the growth performance and the health of juvenile largemouth bass, and has the potential to be used as an effective synbiotics for *M. salmoides*.

Keywords: high-starch diet; non-target metabolomics; antioxidant capacity;

intestinal microbiota; *Micropterus salmoides*

1 Introduction:

It is well known that starch plays a very important role in aquatic animal feed, which act as an economical energy source [1], and has also the function of stabilizers and swelling agent. However, starch utilization is very low in many carnivorous fish [2, 3], and high starch diets can negatively affect fish growth [4], induce hepatic lipid accumulation [5], impair immune system [6], and dysregulation of intestinal flora in fish [7]. Therefore, how to solve the adverse effects of high starch in fish is an urgent problem that we need to explore.

In recent years, many probiotics have shown good efficacy in promoting growth [8], regulating immune system [9], and inhibiting potential pathogens [10]. Yeast culture (YC), as a member of probiotics family is a natural product produced from yeast fermentation, which contains proteins, polysaccharides (mainly β -glucans and mannan-oligosaccharides), lipids, vitamins, peptides, amino acids, nucleotides, organic acids, and antioxidants [11]. Yeast culture supplementation has a range of beneficial effects, including increased feed intake [12] and feed digestion [13], enhancement of overall immunity [14], disease resistance [15], modulation of the animal gut microbiota [16] and consequently improved animal performance [17]. Therefore, yeast culture has attracted extensive attention in aquaculture.

To increase stability of aquafeed, feed processor tend to increase the proportion of starches in the diet [18]. Thus, one of the key goals when feeding largemouth bass is to maintain the optimal starch for feed processing. High-yielding largemouth bass fed a

certain amount of starch in their diets can suffer the same problem. However, a high-starch diet can induce oxidative stress, disorders of liver function, suppress innate immunity, consequently inhibit the growth of *M. salmoides* [19]. Most importantly starch diets were inefficiently used as an energy source by *M. salmoides* juveniles [20]. Previous studies have demonstrated that yeast culture could improve growth performance, immune response, intestinal health, antioxidant capacity and disease resistant in aquatic animals [21, 22]. To solve the problem, yeast culture is supplemented to the diet of largemouth bass because of its role in health. We hypothesise that yeast culture supplementation still has beneficial effects on the largemouth bass growth. The objective of the study was to examine the effects of supplementing yeast culture to diets containing high proportions of starch in the diet on animal performance, health, and intestinal microbial communities of juvenile largemouth bass.

2 Materials and methods

2.1 Experimental diets

In the present study, three isonitrogenous (47.8% crude protein) and isolipidic (11.8% crude lipid) diets were formulated and their chemical composition is shown in Table 1. The yeast (*Saccharomyces cerevisiae*, microbial preservation registration number: CGMCC NO. 6120 2012.05.18) culture (from Beijing Enhelor International Tech Co., Ltd, Beijing, China)) supplement levels were 0% (control), 1% (HSY₁) and 3% (HSY₃) respectively. The yeast culture is fermented by single colony (including liquid fermentation and solid fermentation). The main protein source of the diet was

steam fish meal, domestic chicken meal, soybean meal, soybean protein concentrate, wheat gluten, the main fat source was fish oil, soybean oil (1:2), and the sugar source was cassava starch. The feed materials were crushed and screened by 245 μm , then mixed evenly by step-by-step enlargement method. Pellet feed with a particle size of 3 mm was prepared, which was naturally air-dried and baled and stored in a refrigerator at 4 $^{\circ}\text{C}$ for later use.

2.2 Fish and feeding trial

Largemouth bass juveniles were obtained from a breeding farm in Changshou, Chongqing, China. Prior to the start of the normal feeding experiment, fish was disinfected and placed in a cylinder with a volume of 400L for two weeks. During the period of temporary breeding, commercial feed (Guangzhou Jieda Feed Co. LTD, China) was used for domestication. Next, the largemouth bass with similar size and good health were randomly selected as experimental fish. All fish were fasted for 24 h. Apparently healthy and similar size fish (average initial body weight: 12.17 ± 0.02 g) were randomly distributed into 9 the blue circle cylinder (capacity: 225 L) at a density of 30 fish per tank (each group is assigned 3 tanks). During the growth period, the fish were fed 3 times per day (8:30, 13:30 and 18:30) with two rounds of feeding each time. The water source for aquaculture is fully aerated tap water. During aquaculture, the water temperature is $23.2\text{--}28.5$ $^{\circ}\text{C}$, dissolved oxygen ≥ 6.5 mg/L, ammonia nitrogen < 0.1 mg/L, nitrite < 0.01 mg/L, and pH was around 7.5. The photoperiod of culture was 12 h light and 12 h dark, and the illumination time was 08:00-20:00. Water is exchanged every two days and is a quarter the size of the aquarium.

2.3 Collection and preparation of samples

At the end of the breeding experiment, the fish were fasted for 24 h, and then weighed and counted separately after being anesthetized with 0.01% MS-222. The growth characteristics and survival rate of largemouth bass were calculated in each test tank. After feeding for 6 h, twelve fish (4 fish in each tank) were randomly selected from each experimental group. After being anesthetized with 0.01% MS-222, the blood sample was obtained from the caudal vein using the 1 mL syringe. After standing for 12 h, serum was obtained by centrifugation. Body length and weight were measured, viscera, liver and intestines were dissected on an ice tray, and weighed. The liver and intestinal tissues were collected and frozen in the -80 °C refrigerator with liquid nitrogen for the determination of tissue physiology and biochemistry. Intestinal fecal tissues of 2 fish were randomly collected from each tank, then put into 1.5 mL sterile test tube with RNA enzyme removed, frozen in liquid nitrogen, and placed in -80 °C refrigerator for microbiome sequencing. Complete livers of 2 fish were randomly collected from each tank and then placed into a 1.5 mL sterile tube with RNA enzyme removed. After freezing in liquid nitrogen, the samples were placed in a -80 °C refrigerator for liver metabolome sequencing.

2.4 Liver antioxidant ability and serum biochemistry

The liver was taken out at -80 °C, 1 g of tissue was accurately weighed, 2 enzyme-free beads and 9 mL double-steamed water were added into a 10 mL enzyme-free tube, homogenized, centrifuged (wuhan Seevicebio technologic Co. Wuhan, China) in a frozen centrifuge (4000 RPM) for 15 minutes, and the supernatant was extracted. The

10% tissue homogenate was obtained. The activities of total superoxide dismutase (T-SOD), catalase (CAT), malondialdehyde (MDA), and Glutathione peroxidase (GSH-Px) was determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). After the serum was removed from the -80 °C refrigerator and thaw naturally, the activity of D-lactate (D-lac), Diamineoxidase (DAO) and lipopolysaccharide (LPS) in the serum was measured using a commercial ELISA kit (Shanghai Bio-Technology Co., Ltd.). See the instructions of the kit for the operation method. Hepatic lipid was determined by chloroform methanol method. Hepatic glycogen was measured using a commercial kit.

2.5 RNA isolation, reverse transcription and mRNA levels analysis

Total RNA in liver and intestine was extracted using RNAiso Plus reagent (Takara, Japan) according to the manufacturer's instructions. Total RNA concentration and purity were determined using a Nano Drop 2000 ultramicrophotometer (Thermo Fisher, USA). The total RNA concentration was then 1000 µg/L by appropriate dilution. The total RNA extracted was then reverse-transcribed into cDNA using the reverse transcription kit Fastking-RT Supermix (Novoprotein, Shanghai, China). Real-time quantitative PCR (qPCR) system was 20 µL, including 1 µL upstream, 1 µL downstream primer (1 µM), 2 µL first strand cDNA, 10 µL 2×NovoStart® SYBR qPCR Supermix Plus, and 6 µL free water. The sequences of specific primers used are shown in Table 2. The quantitative instrument was real-time quantitative PCR (Bio-RAD-CFX96, USA). The procedure was kept at 95 °C for 1 min for 1 cycle, 95 °C for 20 seconds, 60 °C for 1min and a total of 40 cycles. After that, a fusion curve was performed to verify that there

was only one PCR product per PCR reaction. Ct values of cDNA were obtained by qPCR. The relative mRNA expression of target genes was normalized to the *β-actin* mRNA, and determined using the $2^{-\Delta\Delta ct}$ method [23]

2.6 UHPLC-QTOF-MS non-target metabolomics

Non-target metabolomics data were measured by biotech companies (Biomarker Technologies Co, LTD) and analyzed on the BMKCloud. The main instrument platforms used are high performance liquid chromatography (Waters UPLC Acquity I-Class PLUS), high resolution mass spectrometry (Waters UPLC Xevo G2-XS QToF) and chromatographic columns (Acquity UPLC HSS T3 1.8 μ m 2.1*100 mm, Waters). After the metabolites are extracted, the sample can be tested on the machine. In the machine parameters, the positive ion mode includes mobile phase A (0.1% formic acid aqueous solution), mobile phase B (0.1% formic acid acetonitrile anion) and injection volume (1 μ L). The flow rate was always maintained at 400 μ L/min. The separation was achieved using the following gradient: 0 min, 98% A, 2% B; 0.25 min, 98% A, 2% B; 10.0 min, 2% A, 98 % B; 13.0 min, 2% A, 98% B; 13.1 min, 98% A, 2% B; 15.0 min, 98% A, 2 % B. Parameters of mass spectrometry were as follows: low collision energy, 2 V; high collision energy range, 10~40 V, sweep frequency, 0.2 s. Parameters of ESI ion source were as follows: capillary voltage, 2000 V (+) and 1500 V (-); cone voltage, 30 V; ion source temperature, 150 $^{\circ}$ C; degree of desolvation temperature, 500 $^{\circ}$ C; backblow velocity, 50 L/h; flow rate of desolvation gas, 800 L/h. The original data collected by Masslynx V4.2 were processed by ProGenesis QI software for peak extraction and peak alignment. The identification was carried out based on the online

Metlin database of ProGenesis QI software and the self-built database of Biomarker Technologies Co, LTD.

Principal Component Analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) analysis were used to distinguish the differences between the two groups. Cluster analysis can visually show the data characteristics. By mapping the differential metabolites to KEGG database, we can obtain the enrichment metabolic pathways.

2.7 Intestinal microbiota

Intestinal microorganism index entrusts biological company (Biomarker Technologies Co, LTD) to determine, and the data results were analyzed on the BMKCloud. Total bacterial DNA were extracted from samples using the Power Soil DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's protocol. The V3 - V4 region of the 16S rRNA was amplified by PCR (forward primer, 5' - ACTCCTACGGGAGGCAGCA-3'; reverse primer, 5' - GGACTACHVGGGTWTCTAAT-3') and sequenced using a HiSeq platform (Illumina, USA) at Biomarker Technologies Corporation (Beijing, China). Sequences similarity $\geq 97\%$ were assigned to the same OTUs. The representative sequence of OTU can be compared with the microbial reference database to obtain the taxonomic information of the corresponding species for each OTU, and then the composition of sample communities can be counted at various levels (Phylum, Class, Order, Family, Genus, Species). Then, the QIIME software was used to generate the species abundance tables at different taxonomic levels, and the VennDiagram R package was used for

visualization [24]. Significant differences between groups were analyzed using Metastats [25]. The microbial alpha diversity index [26] was calculated, including Chao1, ACE, Shannon, Simpson and other indicators, and the OTU coverage rate (Sequencing depth) was also calculated. The BugBase tool was used for bacterial phenotypic prediction analysis [27].

2.8 Statistical analysis

All data were expressed as the mean value of 3 data + standard error (S.E.M) and analyzed using SPSS25.0 version. One-way ANOVA was used to analyze the differences between the experimental groups, and Tukey's multiple comparison was performed. The level of significance was set as $P < 0.05$. The graphics were drawn using GraphPad Prism7.0 (GraphPad Software Inc. USA).

3 Results

3.1 Growth performance

The growth performance and feed utilization of *M.salmoides* fed dietary yeast culture supplemental level under high-starch stress were presented in Table 3. In terms of final body weight, WG, and SGR, HSY₃ group was significantly higher than the control group ($p < 0.05$), but there was no statistical difference between HSY₁ group and the other groups ($p > 0.05$).

Hepatic lipid, hepatic glycogen and HSI was significantly decreased with the increase of dietary yeast culture supplemental level ($p < 0.05$, Table 3). In addition, CF was significantly increased with dietary yeast culture supplemental level increasing ($p < 0.05$), but compared with the control group, HSI in HSY₃ was significantly

decreased ($p<0.05$). In terms of body composition, adding yeast culture significantly increased crude protein and decreased crude ash ($p<0.05$).

3.2 Hepatic antioxidant capacity

The antioxidant capacity of *M.salmoides* liver was shown in Table 4. The activities of T-SOD and CAT significantly increased and the MDA content reduced as dietary fiber levels increase ($p<0.05$), and *Sod* and *Cat* gene (Fig. 1) in HYS₃ was significantly up-regulated compared with the control group ($p<0.05$).

3.3 Liver metabolome

In order to evaluate the metabolites of largemouth bass between the control and HSY₃, principal component analysis (PCA) and orthogonal projections to latent structures - discriminant analysis (OPLS-DA) were used to compare and analyze the samples (Fig. 4A) for PCA and (Fig. 4B) for OPLS-DA. The results showed a significant separation between the two groups. The Volcano Plot showed that 2,836 metabolites were produced in liver (Fig. 4C), of which 5 metabolites were significantly up-regulated and 106 metabolites were significantly down-regulated (“Fold change>1” and “VIP>1”, $P < 0.05$). Cluster analysis was performed on the 111 differential metabolites (Fig. 4D). Typical metabolites include dide-O-methylsimmondsin, (3 beta, 22 E, 24 R)-ergosta-4, 6, 8 (14), 22-tetraen-3-ol, (PC) (18:0/18:3 (9Z, 12Z, 15Z)), and squamocin L.

The KEGG annotation results of some differential metabolites can be seen in Table 6, which mainly included metabolism, human diseases, organismal systems and genetic information processing. To be specific, the up-regulated differential

metabolites were mainly enriched in alpha-linolenic acid metabolism (ko00592), choline metabolism in cancer (ko05231), arachidonic acid metabolism (ko00590), linoleic acid metabolism (ko00591), glycerophospholipid metabolism (ko00564), steroid hormone biosynthesis (ko00140) and cortisol synthesis and secretion (ko04927) pathways. Secondly, the down-regulated differential metabolites were mainly enriched in folate biosynthesis (ko00790), metabolic pathways (ko01100) and tryptophan metabolism (ko00380) pathways.

3.4 Serum biochemical

The serum biochemical parameters of *M.salmoides* are shown in Table 5. The serum levels of D-lac, DAO and LPS in HSY₃ were significantly lower than those in other groups ($p < 0.05$), and there was no significant difference between HSY₁ and the control ($p > 0.05$).

3.5 Intestinal physical and immune barrier

Intestinal expression levels of tight junction related genes are shown in Fig 2. The results showed that the mRNA levels of *Zo-1*, *Claudin* and *Occludin* increased significantly as the addition of yeast culture ($p < 0.05$). In addition, intestinal expression levels of anti-inflammatory cytokines (*TGF- β 1*) were significantly up-regulated (Fig. 3), and intestinal expression levels of pro-inflammatory cytokines (*IL-8*, *IL-1 β* , *TNF α*) were significantly down-regulated as the addition of yeast culture ($p < 0.05$).

3.6 Intestinal microbial

A total of 458,436 pairs of raw Reads were obtained by sequencing of the six samples. A total of 455,335 Clean Reads were generated after quality control and

splicing of double-ended Reads, and at least 58,137 Clean Reads were generated from each sample. With the addition of yeast culture, Shannon and Simpson index showed no significant difference, while OTUs, ACE and Chao1 index increased significantly ($p < 0.05$, Table 7).

The histogram of intestinal microbial species classification is shown in Fig 5. At the phylum level, *Proteobacteria* and *Firmicutes* were the dominant phylum of largemouth bass, followed by *Actinobacteria* and *Bacteroidetes*. With the increase of yeast culture, the abundance of *Proteobacteria* decreased from 51.90% to 33.37% while the abundance of *Firmicutes* increased from 28.09% to 39.14% (Fig. 5A). At the genus level, *Plesiomonas* (17.34%) and *Stenotrophomonas* (8.39%) were dominant genera in the control group while *Kroppenstedtia* (6.18%) and *Acetobacter* (5.19%) were dominant genera in HSY₃ group (Fig. 5B). Meanwhile, the difference of microbial community abundance (Fig. 6A) between the two groups was analyzed by Metastats software. We found that the abundance of *Plesiomonas* and *Stenotrophomonas* significantly decreased while the abundance of *Lactobacillus*, *Kroppenstedtia*, *Acetobacter*, *Bacillus*, *Virgibacillus* and *Bifidobacterium* significantly increased in HSY₃ group compared to the control group ($p < 0.05$).

The phenotypic prediction (Fig. 6B) showed that the HSY₃ group significantly increased the proportion of Gram-positive bacteria while decreased the proportion of Gram-negative bacteria, compared with the control group ($p < 0.05$). With the addition of YC, the potential pathogens were significantly reduced ($p < 0.05$).

4 Discussion

The current study demonstrated the benefit of dietary supplementation with yeast culture (YC) on the growth of *M. salmoides*. Similar result was observed in Nile tilapia (*Oreochromis niloticus* L.) [28]. As well, many researchers have reported that YC promotes nutrient absorption and improves the growth performance of weaned piglets [29], broiler [30] and lambs [31]. However, some studies showed that YC has no effect on growth of animals [32]. The different results may be mainly related to fish species, dietary composition and yeast additive form. In addition, growth enhancing effect of YC is dependent on dosage, duration of feeding, the route of administration and species [33]. The results of the present study exhibited that the growth-promoting effect of YC was related to doses of yeast culture, and supplementation of YC at 30.0 g kg⁻¹ was optimal for the growth of *M. salmoides* fed high-starch diets.

Liver is an important metabolic organ that may regulate various metabolism functions, when the hepatic injury may lead to these physiochemical function disorder. Previous studies have confirmed that a high-starch diet leads liver damage of largemouth bass [20]. The present study found that the protective effect of YC against high starch-induced liver damage in largemouth bass, which might be due to the up-regulation of related genes (*Cat* and *Sod*) and resulted in the increase of antioxidant enzymes activities. This is consistent with previous research on Atlantic salmon (*Salmo salar*) [34] and Ussuri catfish (*Pseudobagrus ussuriensis*) [21]. Other studies have also shown that yeast product can improve the antioxidant status and disease resistance of fish [35]. Some studies have indicated that yeast products are mainly composed of a

high content of polysaccharides, which have been reported to play a protective role against oxidative damage preserving the cellular integrity [36, 37]. Thus, it can be presumed that the protective effect of YC against high starch-induced liver damage in largemouth bass may be related to the mannose oligosaccharides (MOS), β -glucan and other antioxidants in YC.

In addition, using liver non-target metabolomics techniques, we found that phosphatidylcholine (PC) (18:0/18:3 (9Z, 12Z, 15Z)) was significantly upregulated in HSY₃ group compared to the control group. Studies have shown that decreased hepatic PC content or/and decreased ratio of hepatic PC to phosphatidylethanolamine (PE) have been linked to non-alcoholic fatty liver disease (NAFLD) [38]. The ratio of PC/PE is closely associated with severe fatty liver [39], which generally was regarded as the clinical evidence of liver health [40]. Noteworthy, in the present study, liver PC was upregulated 13.5 times in HSY₃ group, and (PC) (18:0/18:3 (9Z, 12Z, 15Z)) was significantly enriched in the lipid metabolism pathway in KEGG database. This suggested that YC supplementation can could positively influence the hepatic health of largemouth bass. Moreover, it was also observed that 3% YC supplement significantly reduced the liver glycogen and hepatic lipid of largemouth bass. These results suggest that the liver protection mechanism of YC may be related to the change of liver fat metabolism. This is well agreement with the results in in broiler chickens, which used yeast to ferment cottonseed meal to improve liver and abdominal fat metabolism [41]. As far as we know, this is the first attempt to explore the effects of YC on a non-target liver metabolome in largemouth bass and needs to be further studied for providing new

essential information.

Intestinal barrier represents the first line of defense against environmental pathogens in fish [42]. DAO, D-lac and LPS are three important markers of intestinal mucosal injury [43-45]. We found that YC reduced the DAO activity and the D-lac and LPS contents in the serum, indicating that it reduced intestinal permeability in largemouth bass. Intestinal permeability is mainly controlled by the tight junction (TJ) proteins between epithelial cells [46, 47]. Further studied suggested that YC promoted the expression of intestinal TJ proteins in vivo. When TJ structure is destroyed, the host initiates a series of protective mechanisms to restore the normal state. Using the high starch-treated fish model, we found that YC reduced starch-induced TJ breakdown by enhancing the expression of *Zo-1*, *Claudin* and *Occludin*. This agrees with study by Wang et al. [48] Who found that yeast products (contain yeast culture) significant also increased the expression of *Occludin* and *Zo-1* gene in the small intestinal epithelial. In addition, the efficiency of yeast supplementation for fish varies in relation to the dose of the components used in the diet. The supplementation of a higher dose of YC (3%) was observed to be more beneficial than the dose of 1% supplementation in largemouth bass. These evidences suggest that YC is beneficial to TJ barrier in vitro.

Importantly, the release of proinflammatory cytokines can destroy the integrity of intestinal TJ structure, which exacerbates intestinal permeability [49]. The cytokines in animals reflected systemic immune status to a certain extent. Yeast culture and their glycan components improve mucosal damage and inflammation in intestinal diseases [50]. Previous researches reported that yeast products (contain yeast culture) increased

the concentration of anti-inflammatory factor in the jejunum and ileum of weaned piglets [51], and downregulated the expression of proinflammatory cytokines via NF- κ B related pathway in Ussuri catfish [21]. The present study also found that YC significantly up-regulate the expression of anti-inflammatory cytokines (*TGF- β 1*) and down-regulate pro-inflammatory cytokines genes (*IL-8*, *IL-1 β* , *TNF α*) in the intestine. These findings maybe attribute to the immune polysaccharide of yeast cell wall, such as mannan oligosaccharides and β -glucans. Although several studies have shown that *IL-8* dysregulation can influence intestinal inflammation, the contribution of *IL-8* to increase the permeability of TJ remain unclear [52]. Therefore, the yeast culture should be conducted in future investigations to further explore the mechanisms of the anti-inflammatory effects on high starch-induced intestine damage. Intestinal homeostasis is a dynamic process regulated by many factors and can change at any time to achieve a balanced state. Because of the importance of intestinal barrier function, the regulation of TJ proteins involves a complex network that is affected by many factors. Our study provides a reference for YC to participate in the regulation of the integrity of the intestinal barrier in fish.

Probiotics are known to benefit gut healthy by suppressing the number of harmful microorganisms and promoting growth of beneficial bacterium [53]. In this investigation, the dietary YC inclusion altered intestinal microbial community in largemouth bass. We did not find the difference in the diversity of intestinal microbiota in groups of fish pretreated with YC. But administration of YC significantly increased the relative abundance of intestinal microbiota, which suggested that YC could improve

the stability of microflora. This observation is in accordance with the findings by Liu et al [54]. In contrast, [55] found that yeast supplementation on Nile Tilapia exerted a significant influence on the alpha-diversity of the autochthonous microbiota. This discrepancy is likely due to the intestinal microbiota variably depending on the environmental factors and the variety of host species. Furthermore, the most abundant two phyla *Firmicutes* and *Proteobacteria* were predominant in the intestine, being over 70% of the total bacteria. When treated with high starch, the abundance of *Proteobacteria* in the intestine increased sharply, while *Firmicutes* decreased, which is consistent with studies on largemouth bass [7, 56] and on golden pompano (*Trachinotus ovatus*) [57]. Intriguingly, after the addition of 3% YC, the abundance of *Proteobacteria* in the gut decreased, while the abundance of *Firmicutes* increased. *Proteobacteria* is a microbial signature of inflammation in the gut [58, 59]. *Firmicutes* can play a key role in host nutrition and metabolism through the synthesis of short-chain fatty acids (SCFA) [60, 61]. This partly suggested that adding 3% YC can improve intestinal flora and reduce the possibility of intestinal inflammation. Besides, the ratio of *Firmicutes/Bacteroidetes* increased from 5.02 to 6.98 in this study. The higher ratio of *Firmicutes* to *Bacteroides* reflect the stronger ability of nutrient transportation and utilization [61, 62]. It revealed that YC might enhance the digestion and absorption of nutrients, which led to the higher growth of largemouth bass.

Noteworthy, significant changes of the gut microbiota in different groups were also found at the genus taxonomic level. Our data show that high-starch stress results in significant increase of *Plesiomonas*, which is known as a potential opportunistic

pathogen, positively correlated with gut permeability [63, 64]. We hypothesize that *Plesiomonas* bacteria could be related to the disturbance in the TJ proteins expression, observed in control group. Conversely, treatment with YC in high-starch diet (HSY₃) prevented increase of potential pathogenic bacteria and destruction of intestinal barrier. The present results found significant decrease of potential pathogenic bacteria (*Plesiomonas*) and increase of potential beneficial bacteria (*Lactobacillus*, *Bacillus* and *Bifidobacterium*) in microbiota of largemouth bass from HSY₃ group vs the control group. Essential impact of the YC on microbiota during high-starch stress is manifested in elevated number of beneficial bacteria. Beneficial effects of these bacteria were shown in many studies containing in humans and animals. Thus, *Bifidobacterium* are known as a normal component of the gut microbiota and as probiotics for human and animal consumption [65]. *Lactobacillus* are showed to reduce the intestinal permeability and exert a protective effect on intestinal epithelium via inhibiting the regulation of immune cytokines [66]. *Bacillus* could regulate intestinal physiology and maintaining intestinal homeostasis [67, 68]. A large number studies have shown that these three strains have the potential function of secreting bile salt hydrolase (BSH) [69-71]. BSH forms free bile acids such as cholic acid (CA), chenodeoxycholic acid (CDCA) and muricholic acid (MCA) through conjugation and increases the rate of bile acid reabsorption into the liver. In addition, our results also showed that the relative abundances of *Kroppenstedtia* and *Acetobact* increased with the supplementation of YC. Knowledge of the biological functions of these two genera in the intestine reported in the literature so far is too little to explain the altered abundance of the genera in fish

with the supplementation of YC and thus warrants further studies. Further phenotypic analysis of BugBase confirmed the decline of potential pathogenic bacteria in fish with the supplementation of YC. Simultaneously, a decrease in the proportion of negative bacteria was also observed, which could explain the decrease of LPS in serum to some extent. It is not surprising that positive bacteria decline under a high-starch diet. Therefore, positive contribution of YC to the change of microbiota was testified in fish model. It is well-known that the intestinal microbial community is associated with animal performance, health, and immunity.

In conclusion, the present results revealed substantial effect of YC synbiotics in prevention of high starch-related complications. Oral treatment of fish with prebiotic in high-starch stress conditions protected disruption of liver and intestine homeostasis, enhanced antioxidant capacity, maintained expression of TJ proteins. We suggest that these effects are associated with beneficial modulation of the gut microbiota by prebiotic. However, the effect of YC depends on its concentration in the high-starch diet of juvenile largemouth bass, and the dose of YC in this study is recommended to be 3%. Our findings may provide new insights into the mechanism by which yeast culture regulates TJ proteins in fish.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Ethics statement

This study was conducted under the standard code of protocol for the Care and Use of Laboratory Animals in China. This research was approved by the Animal Ethics Committee of Southwest University (No. IACUC-20181015-12, 15 October 2018).

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Table 1

Composition and nutrient levels of test diets (air-dry basis)

Items	Groups		
	Control	HSY ₁	HSY ₃
<i>Ingredient (%)</i>			
Fish meal	35.00	35.00	35.00
Domestic chicken powder	10.00	10.00	10.00
Yeast culture	0	1.00	3.00
Soybean meal	9.90	9.90	8.90
Concentrated soy protein	15.00	14.00	13.00
Wheat gluten	3.00	3.00	3.00
Cassava starch	15.00	15.00	15.00
Fish oil	2.00	2.00	2.00
Soy lecithin	1.50	1.50	1.50
Soybean oil	4.00	4.00	4.00
Vitamin premix ^a	1.00	1.00	1.00
Mineral premix ^b	1.50	1.50	1.50
Choline chloride	0.50	0.50	0.50
Ca(H ₂ PO ₄) ₂	1.50	1.50	1.50
Vitamin C	0.05	0.05	0.05
Ethoxyquinoline	0.05	0.05	0.05
<i>Chemical composition (%)</i>			
Crude protein	47.76	47.67	47.69
Crude lipid	11.77	11.77	11.76
Ash	11.48	11.50	11.48

^a Vitamin premix (mg kg⁻¹ of diet): VA, 18; VD₃, 5; VE, 150; VC (350 g kg⁻¹), 500; VB₁, 16; VB₆, 20; VB₁₂, 6; VK₃, 18; riboflavin, 40; inositol, 320; calcium-D-pantothenate, 60; niacinamide, 80; folic acid, 5; biotin, 2; ethoxyquin, 100.

^b Mineral premix (mg kg⁻¹ of diet): Na, 30; K, 50; Mg, 100; Cu, 4; Fe, 25; Zn, 35; Mn, 12; I, 1.6; Se, 0.2; Co, 0.8.

Table 2

Primer sequences for real-time PCR

Genes	Primers	Sequence 5'-3'	GenBank
<i>Sod</i>	F ^a	CCACCAGAGGTCTCACAGCA	XM_038713969.1
	R ^b	CCACTGAACCGAAGAAGGACT	
<i>Cat</i>	F	TGGTGTTACGATGAGATGG	XM_038704976.1
	R	GGAGAAGCGGACAGCAATAGG	
<i>GSH-Px</i>	F	ATACCAAGTCTCCTTCCCTCTGT	XM_038715030.1
	R	CGTCCACCACTTTGCCATT	
<i>Zo-1</i>	F	ATCTCAGCAGGGATTCGACG	XM_038701018.1
	R	CTTTTGCGGTGGCGTTGG	
<i>Claudin-1</i>	F	CCAGGGAAGGGGAGCAATG	XM_038713307.1
	R	GCTCTTTGAACCAGTGCGAC	
<i>Occludin</i>	F	GATATGGTGGCAGCTACGGT	XM_038715419.1
	R	TCCTACTGCGGACAGTGTTG	
<i>IL-10</i>	F	CGGCACAGAAATCCCAGAGC	XM_038696252.1
	R	CAGCAGGCTCACAAAATAAACATCT	
<i>TGF-β1</i>	F	GCTCAAAGAGAGCGAGGATG	XM_038693206.1
	R	TCCTCTACCATTCGCAATCC	
<i>IL-8</i>	F	CGTTGAACAGACTGGGAGAGATG	XM_038704088.1
	R	AGTGGGATGGCTTCATTATCTTGT	
<i>IL-1β</i>	F	CGTGACTGACAGCAAAAAGAGG	XM_038696252.1
	R	GATGCCCAGAGCCACAGTTC	
<i>TNF-α</i>	F	CTTCGTCTACAGCCAGGCATCG	XM_038710731.1
	R	TTTGGCACACCGACCTCACC	
<i>β-actin</i>	F	GGACACGGAAAGGATTGACAG	XM_038695351.1
	R	CGGAGTCTCGTTTCGTTATCGG	

^a F: forward primer; ^b R: reverse primer.

Table 3

Effect of yeast culture on the growth performance and morphological measurements of *M.salmoides* induced by high-starch

Items	Groups		
	Control	HSY ₁	HSY ₃
<i>Growth performance</i>			
Initial body weight (g)	12.15±0.02	12.19±0.03	12.17±0.04
Final body weight (g)	83.33±1.17 ^a	84.83±1.07 ^{ab}	88.40±1.05 ^b
WG ^b (%)	591.60±8.83 ^a	595.70±6.95 ^{ab}	626.51±10.82 ^b
SGR ^c (%/d)	3.22±0.02 ^a	3.23±0.02 ^{ab}	3.30±0.02 ^b
PER ^d	1.89±0.03	1.84±0.05	1.85±0.03
FR ^e	2.75±0.05	2.82±0.08	2.84±0.05
FCR ^f	1.10±0.02	1.13±0.03	1.12±0.02
SR ^g (%)	100	100	100
<i>Morphological measurements</i>			
CF ^h (g/cm ³)	2.08±0.02 ^a	2.29±0.02 ^b	2.36±0.01 ^c
VSI ⁱ (%)	9.22±0.42	8.68±0.50	8.37±0.09
HSI ^j (%)	2.21±0.03 ^b	2.12±0.06 ^{ab}	1.99±0.05 ^a
Hepatic lipid (%)	3.28±0.28 ^b	3.07±0.17 ^b	2.21±0.05 ^a
Hepatic glycogen (mg/g)	97.65±1.09 ^b	97.39±0.62 ^b	91.56±0.52 ^a
<i>Body composition</i>			
Moisture (%)	70.86±0.01	70.99±0.15	71.10±0.11
Crude lipid (%)	6.88±0.44	6.76±0.16	6.29±0.14
Crude protein (%)	17.65±0.01 ^a	17.82±0.02 ^b	18.20±0.07 ^c
Crude ash (%)	4.43±0.02 ^c	4.29±0.04 ^b	4.07±0.03 ^a

^a Values (means ± S.E.M, n=3) in the same row with different letter superscripts were significantly different ($P<0.05$), while with the same or no letter superscripts were not significantly different ($P>0.05$). The same as below.

^b Weight gain rate (WG) = $100\% \times [\text{final weight (g)} - \text{initial weight (g)}] / \text{initial weight (g)}$.

^c Specific growth rate (SGR) = $100\% \times [\ln(\text{mean final weight}) - \ln(\text{mean initial weight})] / 56 \text{ days}$.

^d Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g).

^e Feeding ratio (FR) = $100\% \times F / [(W_t + W_0) / 2 \times t]$.

^f Feed conversion ratio (FCR) = total feed intake in dry basis (g) / weight gain (g).

^g Survival rate (SR) = $100 \times \text{final number} / \text{initial number}$.

^h Condition factor (CF) = $100\% \times \text{fish weight} / (\text{body length})^3$.

ⁱ Viscera somatic index (VSI) = $100\% \times \text{visceral weight} / \text{fish weight}$.

^j Hepatopancreas somatic index (HSI) = $100\% \times \text{liver weight} / \text{fish weight}$.

Table 4Effects of yeast culture on hepatic antioxidant capacity of *M.salmoides* induced by high-starch

Items	Groups		
	Control	HSY ₁	HSY ₃
T-SOD (U/mgprot)	518.95±14.08 ^a	568.56±8.80 ^b	582.08±8.20 ^b
CAT (U/mgprot)	16.53±0.54 ^a	16.93±0.28 ^{ab}	18.30±0.49 ^b
GSH-Px (U/mgprot)	41.11±2.43	43.74±1.65	44.67±1.91
MDA (nmol/ml)	1.22±0.05 ^b	1.21±0.06 ^{ab}	1.01±0.10 ^a

Table 5Effects of yeast culture on serum biochemical parameter of *M.salmoides* induced by high-starch

Items	Groups		
	Control	HSY ₁	HSY ₃
D-lactate (umoL/L)	528.59±10.59 ^b	513.46±13.10 ^b	388.95±8.15 ^a
Diamine oxidase (U/L)	22.51±0.42 ^b	22.48±0.49 ^b	15.27±2.35 ^a
Lipopolysaccharide (EU/L)	288.81±8.34 ^b	285.22±4.74 ^b	203.41±9.74 ^a

Table 6

Annotation results of differentially expressed metabolite KEGG in the control group and 3% yeast culture group (Control vs HSY₃, n=3)

Metabolites	up or down	Log ₂ FC	VIP	KEGG Pathway Annotation
Phosphatidylcholine	up	3.76	1.49	alpha-Linolenic acid metabolism(ko00592) Choline metabolism in cancer(ko05231) Arachidonic acid metabolism(ko00590) Linoleic acid metabolism(ko00591) Retrograde endocannabinoid signaling(ko04723) Glycerophospholipid metabolism(ko00564) Metabolic pathways(ko01100)
Cortexolone	up	2.95	1.38	Metabolic pathways(ko01100) Steroid hormone biosynthesis(ko00140) Cortisol synthesis and secretion(ko04927) Cushing syndrome(ko04934)
Cellobiose	down	-2.82	1.40	Starch and sucrose metabolism(ko00500) Metabolic pathways(ko01100) ABC transporters(ko02010)
5-Hydroxy-L-tryptophan	down	-1.29	1.35	Serotonergic synapse(ko04726) Metabolic pathways(ko01100) Tryptophan metabolism(ko00380)
4-Oxo-1-(3-pyridyl)-1-butanone	down	-1.87	1.45	Metabolism of xenobiotics by cytochrome P450(ko00980) Nicotinate and nicotinamide metabolism(ko00760) Chemical carcinogenesis(ko05204)
10-Formyltetrahydrofolate	down	-2.07	1.40	One carbon pool by folate(ko00670) Aminoacyl-tRNA biosynthesis(ko00970) Metabolic pathways(ko01100) Carbon metabolism(ko01200)
Indole	down	-1.46	1.31	Tryptophan metabolism(ko00380) Metabolic pathways(ko01100) Protein digestion and absorption(ko04974) Phenylalanine, tyrosine and tryptophan biosynthesis(ko00400)
Dihydrobiopterin	down	-1.41	1.41	Folate biosynthesis(ko00790) Metabolic pathways(ko01100)
Oxalic acid	down	-2.00	1.35	Metabolic pathways(ko01100) Glyoxylate and dicarboxylate metabolism(ko00630)

ADP-ribose	down	-1.82	1.35	Purine metabolism(ko00230)
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Table 7Effects of yeast culture on intestinal microbiota α diversity of *M.salmoides* (means $\pm$ S.E.M, n=3)

Items	Richness estimates			Diversity estimates		Coverage (%)
	OTUs	ACE	Chao1	Simpson	Shannon	
Control	583 $\pm$ 112 ^a	681 $\pm$ 85.2 ^a	677 $\pm$ 92.4 ^a	0.924 $\pm$ 0.05	6.16 $\pm$ 1.02	99.8 $\pm$ 0.00
HSY ₃	988 $\pm$ 2.52 ^b	1008 $\pm$ 2.66 ^b	1018 $\pm$ 2.45 ^b	0.989 $\pm$ 0.01	8.09 $\pm$ 0.09	99.8 $\pm$ 0.00

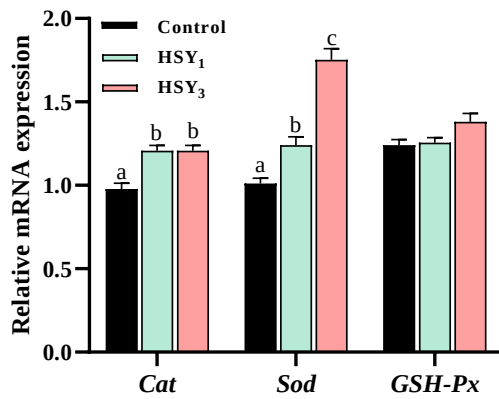


Fig. 1 Liver expression levels of antioxidant-related genes of juvenile largemouth bass fed the experiment diet. Values (means $\pm$ S.E.M, n=3) in each column with different superscripts have significant differences ($P < 0.05$). The same as below.

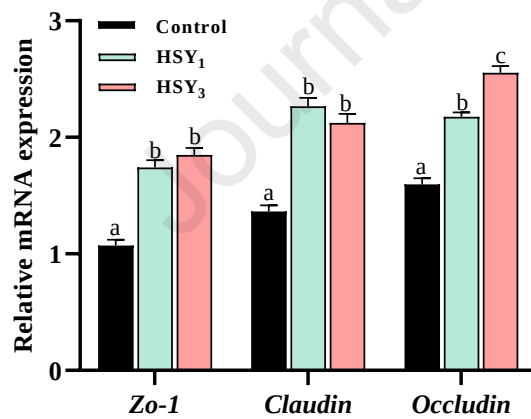


Fig. 2 Intestinal expression levels of tight junction-related genes in juvenile largemouth bass

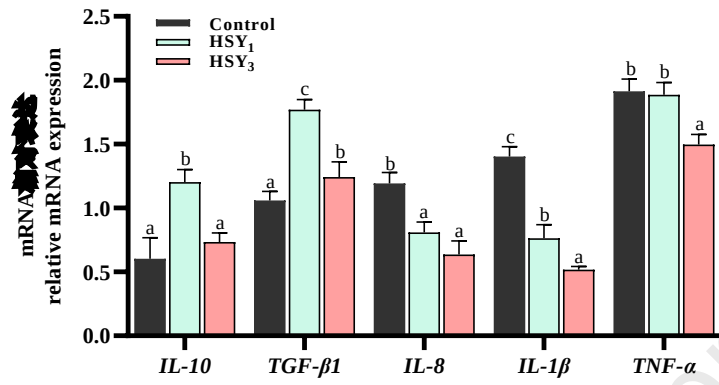


Fig. 3 Intestinal expression levels of inflammation-related genes in juvenile largemouth bass

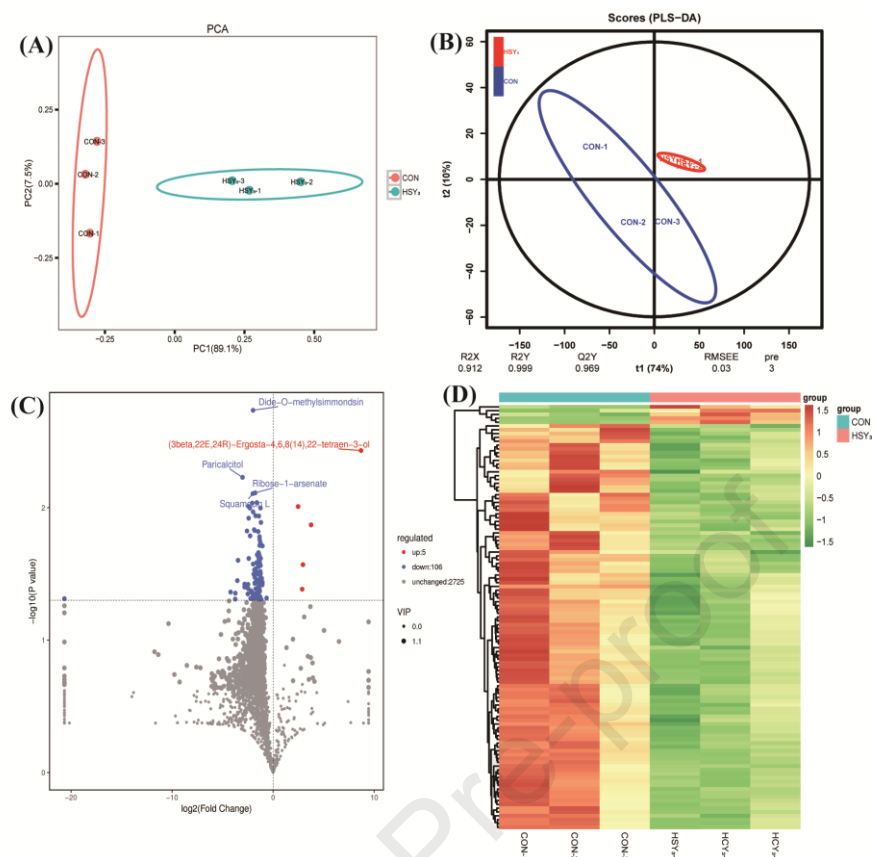


Fig. 4 The degree of difference between the control group and the HSY₃ group was shown in the untargeted metabolic. (A) Principal Component Analysis, The X-axis represents the first principal component, and the Y-axis represents the second principal component. The different colored ellipses represent 95% confidence intervals. (B) Orthogonal feeding Interests-discriminant analysis (OPLS-DA) was used to analyze the results. (C) Volcano Plot looks at the differences between the two groups of metabolites, and each dot represents a metabolite. The abscissa represents the group contrast ratio of each material change (with 2 logs base), and the vertical says the P value (in the logarithm of 10 at the bottom). Scatter represents OPLS - DA model to the size of the VIP value, the greater the scatter VIP value, the greater the screening of differentially expressed metabolites is more reliable. Blue dots represent downregulated and differentially expressed metabolites, red dots represent up-regulated and differentially expressed metabolites, and gray dots represent detected but not significantly different metabolites (D) Clustering analysis of the selected differential metabolites can further excavate the characteristics and obtain the differential metabolic clustering heat map.

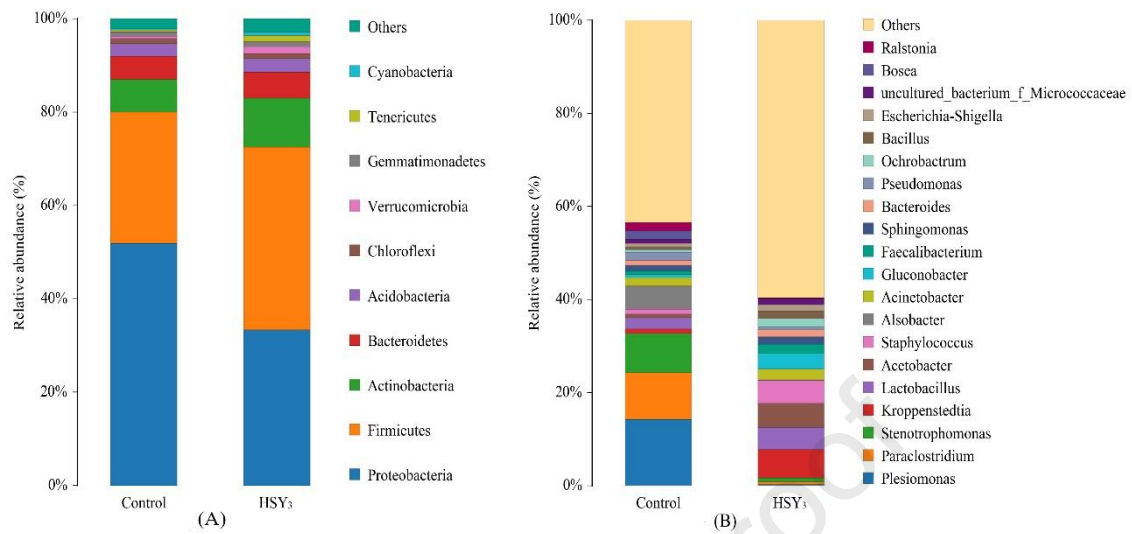


Fig. 5 Histogram of intestinal microbial species classification at phylum (A) and genus (B) levels in control group and HSY₃ group. The phylum level lists the top 10 most abundant (Based on relative abundance) bacterial phyla and the genus level lists the top 20 most abundant bacteria. Other phyla genera were all assigned as ‘Others’.

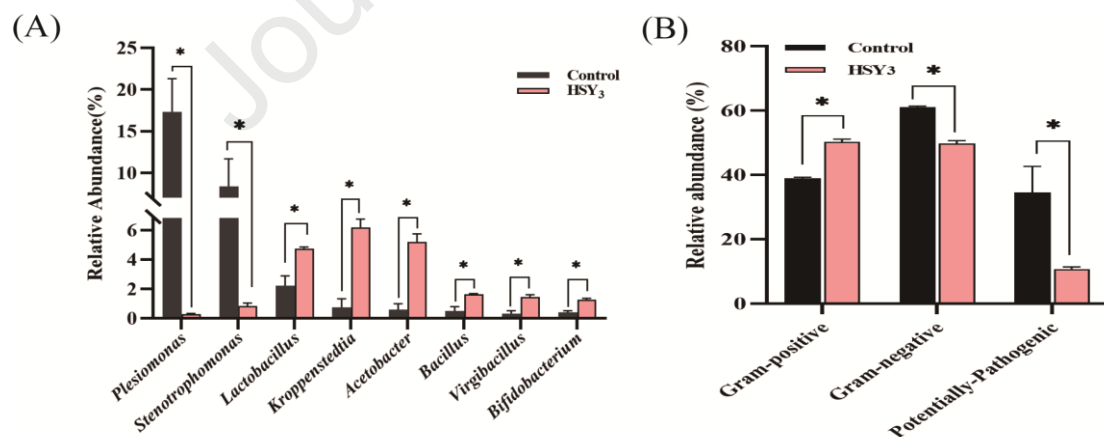


Fig. 6 (A) The differences in microbial community abundance (at genus level) between the two groups were analyzed by Metastats software. (B) BugBase phenotype analysis of control group and HSY₃ group. “*” represents a significant difference between the two groups ($P < 0.05$).

Highlights:

- The supplemental dietary 3% yeast culture could enhance growth performance of *M. salmoides* fed high-starch diet.
- The supplemental dietary 3% yeast culture could improve liver function of *M. salmoides* fed high-starch diet.
- The supplemental dietary 3% yeast culture could reinforce intestinal physical barrier and immune barrier of *M. salmoides* at high dietary starch levels.
- The supplemental dietary 3% yeast culture could regulate intestinal microbiota of *M. salmoide*.