Effects of Yeast Hydrolysate on Growth Performance, Plasma Biochemical Indices, and Liver Tissue Health in Largemouth Bass (Micropterus salmoides) Postprint

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Abstract

The present study was conducted to investigate the effects of dietary yeast hydrolysate supplementation on growth performance, plasma biochemical indices, and liver tissue health in largemouth bass (Micropterus salmoides). A total of 160 largemouth bass with an initial body weight of $(28.50\$\pm0.01)gwererandomlydividedinto2groupsandfeddietscontaining0(YH0group,asthecontrol)and5g/kg$ $0.05),buthadnosignificanteffectonothergrowthperformanceindicators(P >$ $0.05).Theplasmaalkalinephosphatase(AKP)activityintheYH5groupwassignificantlyhigherthanthatintheY$ $0.05),whiledietarysupplementationof5g/kgyeasthydrolysateproducednosignificanteffectonotherplasmabio$ $0.05).Analysisoftherelativeexpressionlevelsofgenesrelatedtoliverinflammationandapoptosisfactorsinlarge$ gene in the liver of the YH5 group showed a downward trend compared with the YH0 group (P>0.05), and there were no significant differences in the relative expression levels of α-smooth muscle actin (α-SMA), tumor necrosis factor-α (TNF-α), and caspase family genes in the liver among groups (P>0.05). Fatty infiltration and fibrotic phenotype were observed in the liver tissues of fish in the YH0 group, and the fibrotic tissues displayed abundant activated caspase-3 signals, indicating that the severity of apoptosis under this phenotype was higher than that in fatty liver and normal liver. No fibrotic phenotype was observed in the liver tissues of fish in the YH5 group. These results indicate that dietary supplementation of 5 g/kg yeast hydrolysate can promote protein deposition in largemouth bass and reduce the risk of hepatic fibrosis.

Full Text

Effects of Yeast Hydrolysate on Growth Performance, Plasma Biochemical Indices and Hepatic Tissue Health of Largemouth Bass (Micropterus salmoides)

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Abstract: This study investigated the effects of dietary yeast hydrolysate on growth performance, plasma biochemical indices, and hepatic tissue health in largemouth bass. One hundred sixty largemouth bass with an initial body weight of $(28.50\$\pm0.01)gwererandomlydividedintotwogroupsandfeddietssupplementedwith0(YH0group,asc$ $0.05)butdidnotsignificantlyaffectothergrowthperformanceindices(P > 0.05).Plasmaalkalinephosphatase(AKP)activityintheYH5groupwassignificantlyhigherthanintheYH0grou$ $0.05),whileotherplasmabiochemicalindicesshowednosignificantdifferences(P > 0.05).Analysisofhepaticinflammatoryandapoptosis-relatedgeneexpressionrevealedthattherelativeexpressi$ tended to be downregulated in the YH5 group compared to YH0 (P>0.05), with no significant differences observed in α-smooth muscle actin (α-SMA), tumor necrosis factor-α (TNF-α), or caspase family genes (P>0.05). Histological examination revealed fatty infiltration and fibrosis in YH0 group livers, with abundant activated caspase-3 signals indicating more severe apoptosis in fibrotic livers compared to fatty or normal livers. No hepatic fibrosis was observed in the YH5 group. These findings indicate that dietary supplementation with 5 g/kg yeast hydrolysate promotes protein deposition and reduces the risk of hepatic fibrosis in largemouth bass.

Keywords: largemouth bass (Micropterus salmoides); yeast hydrolysate; growth; hepatosis; apoptosis

Largemouth bass (Micropterus salmoides), also known as California perch, belongs to Perciformes, Centrarchidae, and Micropterus. Due to its rapid growth, disease resistance, low-temperature tolerance, delicious taste, and rich nutritional value, it has become one of the major freshwater aquaculture species in China. However, feeding artificial compound feeds often causes varying degrees of liver disease and anorexia in largemouth bass, leading to reduced growth performance, and the industry still relies heavily on fresh small fish. Liver disease has become a key factor limiting the use of artificial feeds, with potential causes including oxidative stress, high levels of digestible carbohydrates (>19%)

in feeds, and mycotoxins. Fish have low glucose utilization capacity, and high-carbohydrate diets cause excessive hepatic glycogen accumulation and persistent hyperglycemia, which can induce liver lesions.

Yeast hydrolysate (YH) is produced from fresh brewer' s yeast through modern bioengineering processes including impurity removal, autolysis, enzymatic hydrolysis, and spray drying. It is rich in nucleic acids, small peptides, cell wall polysaccharides (immune polysaccharides), free amino acids, and B vitamins. Amino acids and small peptides are easily digestible and demonstrate significant advantages in animal protein nutrition, making yeast hydrolysate an ideal functional protein source. This study investigated the effects of dietary yeast hydrolysate on hepatic tissue health in largemouth bass through analysis of growth performance, protein deposition rate, plasma biochemical indices, liver histopathology, and related gene expression.

1.1 Experimental Fish

Largemouth bass were purchased from Foshan Sanshui Baijin Aquatic Seedling Co., Ltd. in May 2016. Prior to the experiment, fish were acclimated in the culture system for one week and fed acclimation diets during this period.

1.2 Experimental Diets

A low-fishmeal basal diet (representing commercial largemouth bass feeds containing over 40% fishmeal) served as the control. The experimental diet was formulated by adding 5 g/kg yeast hydrolysate (provided by Zhuhai Tianxiangyuan Biotech Holding Co., Ltd.) to the basal diet, designated as YH0 and YH5 respectively. Ingredients were ultrafine pulverized, thoroughly mixed, and extruded using a twin-screw extruder (Yang Gong Machinery TSE65) to produce pelleted diets, which were air-dried and stored at -20°C.

The composition and nutrient levels of experimental diets are presented in Table 1 . The vitamin premix provided the following per kg of diet: VA 20 mg, VB1 10 mg, VB2 15 mg, VB6 15 mg, VB12 8 mg, VE 400 mg, VK3 20 mg, VD3 10 mg, niacinamide 100 mg, VC phosphate calcium (35%) 1,000 mg, inositol 200 mg, calcium pantothenate 40 mg, biotin 2 mg, folic acid 10 mg, corn gluten meal 150 mg, CuSO4 · 5H2O 10 mg, FeSO4 · H2O 300 mg, ZnSO4 · H2O 200 mg, MnSO4 · H2O 100 mg, KIO3 80 mg, Na2SeO3 10 mg, CoCl2 · 6H2O 5 mg, NaCl 100 mg, and zeolite powder 695 mg. The main nutrients of yeast hydrolysate were: crude protein 46.8%, amino acids (16 kinds) 40.6%, β-glucan 17.8%, mannan 10.5%, and nucleic acid 2.01% (data from China National Analytical Center, Guangzhou). Nutrient levels were measured values, with dry matter based on air-dry basis and others on dry matter basis.

1.3 Grouping and Culture Management

The experiment was conducted in an indoor recirculating aquaculture system at the National Aquafeed Safety Assessment Station (Nankou, Beijing).

Healthy largemouth bass with uniform size [average initial body weight (28.50$±0.01)*g*]*wererandomlystockedinto*0.26*m*^{3}$ conical culture barrels. Two dietary groups were established (YH0 and YH5), each with four replicate barrels containing 20 fish per barrel. The 10-week feeding trial involved apparent satiation feeding twice daily at 08:00 and 16:00. Water quality parameters were maintained as follows: dissolved oxygen >7.0 mg/L, total ammonia nitrogen <0.3 mg/L, pH 7.5-8.5, and temperature (23$±$1)°C.

After the 10-week growth trial, fish were fasted for 24 h before weighing and recording feed intake and survival numbers for growth index calculation. Four fish per barrel were randomly selected to measure body length, body weight, viscera weight, and liver weight for morphological index calculation. Six fish per barrel were randomly selected, anesthetized with chlorobutanol, and blood was collected from the caudal vein using sodium fluoride-potassium oxalate anticoagulant. Plasma was separated by centrifugation at 4°C and 4,000 r/min for 10 min and stored at -80°C for subsequent analysis.

1.4 Index Determination

1.4.1 Growth Indices Growth indices were calculated as follows: Survival rate (SR, %) = 100×Nt/N0; Weight gain rate (WGR, %) = 100×(Wt-W0+Wd)/W0; Specific growth rate (SGR, %/d) = 100×(lnWt-lnW0)/t; Feed conversion ratio (FCR) = C/(Wt+Wd-W0); Feeding rate (FR, %) = 100×C/{[(W0+Wt+Wd)/2]/t}; Protein deposition rate (PDR, %) = 100×(Wt×Wtp-W0×W0p)/(Wf×Wfp). Where N0 is initial fish number; Nt is final fish number; W0 is initial total fish weight (g); Wt is final total fish weight (g); Wd is dead fish weight (g); C is feed intake (g); Wtp is final whole-body crude protein content (%); W0p is initial whole-body crude protein content (%); Wf is feed amount (g); Wfp is feed crude protein content (%); and t is experimental duration (d).

1.4.2 Morphological Indices Morphological indices were calculated as: Condition factor (CF, g/cm^3) = average body weight/average body length^3; Hepatosomatic index (HSI, %) = 100×liver weight/body weight; Viscerasomatic index (VSI, %) = 100×viscera weight/body weight.

1.4.3 Routine Nutrient Composition Moisture, crude ash, crude protein, crude lipid, and gross energy in feeds were determined by 105°C atmospheric drying (GB/T 6435-2006), 550°C incineration (GB/T 6438-2007), Kjeldahl nitrogen determination (GB/T 6432-1994), total fat extraction (GB/T 6433-2006), and oxygen bomb calorimetry, respectively.

1.4.4 Plasma Biochemical Indices Plasma total cholesterol (TC), triglyceride (TG), glucose (GLU), total bile acid (TBA), malondialdehyde (MDA) contents, and alkaline phosphatase (AKP), alanine transaminase (ALT), aspartate

transaminase (AST) activities were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute) according to manufacturer instructions.

1.4.5 Liver Histological Sections Sixteen fish per group were randomly selected, and liver tissues (0.5 cm$×0.5*cm*×$0.5 cm) were fixed in 4% paraformaldehyde for 24 h. After dehydration and clearing, tissues were paraffin-embedded and sectioned at 7 m thickness. Liver sections were stained using three methods: hematoxylin-eosin staining, Sirius Red collagen-specific staining, and activated caspase immunofluorescence staining. Apoptotic cells were visualized with green fluorescence (Alexa Fluor 488, goat anti-rabbit) and nuclei with DAPI. Images were captured using a confocal microscope (Leica DM2500).

1.4.6 Liver Inflammatory and Apoptosis-Related Gene mRNA Extraction, Reverse Transcription and Expression Analysis Total liver RNA was extracted using the miRNeasy Mini Kit (TaKaRa). RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo), with OD260/OD280 ratios between 1.9-2.1. Reverse transcription was performed using the PrimeScript RT Reagent Kit (Bio-Rad). Real-time quantitative PCR was conducted as follows: 95°C for 30 s; 35 cycles of 95°C for 5 s, annealing temperature (Tm, specific for each primer as shown in Table 2) for 20 s, and 72°C for 40 s (CFX96 Real-Time System, Bio-Rad). The reaction mixture contained: SYBR Premix Ex Taq II 12.5 L, PCR Forward Primer (10 mol/L) 1.0 L, PCR Reverse Primer (10 mol/L) 1.0 L, RT product (cDNA) 2.0 L, and nuclease-free water to 25 L. cDNA samples were diluted 4-fold in series. Relative gene expression was calculated using the 2-ΔΔCt method. Primer sequences for inflammatory and apoptosis-related genes are listed in Table 2 .

1.5 Statistical Analysis

All data are presented as mean±SE. Independent-sample t-tests were performed using SPSS 20.0 software, with $P<0.05$ indicating significant differences.

2.1 Effects of Yeast Hydrolysate on Growth Performance of Largemouth Bass

The effects of yeast hydrolysate on growth performance are shown in Table 3 . Results indicated that except for significantly higher protein deposition rate in the YH5 group ($P<0.05$), no significant differences were observed in other growth performance indices between groups ($P>0.05$).

2.2 Effects of Yeast Hydrolysate on Plasma Biochemical Indices of Largemouth Bass

Plasma biochemical indices are presented in Table 4 . No significant differences were found in plasma TG, TC, GLU, MDA, TBA contents, or AST and ALT

activities between groups (P>0.05). However, plasma AKP activity in the YH5 group was significantly higher than in the YH0 group (P<0.05).

2.3 Effects of Yeast Hydrolysate on Liver Histology and Inflammatory/Apoptosis-Related Gene Expression

Liver histological phenotype statistics are summarized in Table 5 . With 16 samples per group, all livers exhibited varying degrees of damage, classified as normal liver, fatty liver (vacuolated cells), or fibrotic liver. The YH0 group showed 1 case of severe fibrosis, 7 cases of fatty liver phenotype, and 8 normal samples, while the YH5 group exhibited no fibrotic samples.

Liver histological sections are shown in Figure 1 [Figure 1: see original paper]. HE staining revealed extensive necrosis in fibrotic livers, while fatty liver samples showed numerous vacuolated cells with absent or displaced nuclei. Sirius Red staining demonstrated abundant red collagen signals in fibrotic samples, confirming fibrotic tissue, whereas normal and fatty liver samples showed low collagen signals. Fibrotic tissues exhibited high activated caspase-3 signals, indicating significantly greater apoptosis severity compared to fatty or normal livers. Gene expression results (Figure 2 [Figure 2: see original paper]) showed that TGF-$ $1 expression tended to be downregulated in the YH5 group compared to YH0 (P>0.05), with no significant differences in α-SMA, TNF-α, or caspase family genes between groups (P>0.05).

3.1 Effects of Yeast Hydrolysate on Growth Performance

This study demonstrated that dietary supplementation with 5 g/kg yeast hydrolysate did not significantly affect survival rate or weight gain rate but significantly improved protein deposition rate in largemouth bass. Oliva-Teles et al. found that partial replacement of fishmeal with 30% brewer' s yeast hydrolysate improved feed efficiency and protein deposition in European sea bass (Dicentrarchus labrax), while 50% replacement showed no negative effects on growth. Rumsey et al. reported that increasing yeast extract supplementation enhanced nitrogen deposition in rainbow trout (Oncorhynchus mykiss). These findings suggest that yeast hydrolysate supplementation can improve protein deposition, likely due to its rich content of amino acids and small peptides. Studies have shown that small peptide supplementation significantly improves protein retention efficiency in fish. Dietary proteins not only provide amino acids for nitrogen deposition but also release bioactive small peptides during digestion that affect digestion, absorption, and amino acid utilization. The yeast hydrolysate used in this study contained high levels of amino acids (40.6%), β-glucan (17.8%), and mannan (10.5%). Research indicates that dietary amino acid supplementation can improve whole-body protein content in largemouth bass. Glucans, mannans, and nucleotides can enhance intestinal microbial composition and tissue structure, promote nutrient absorption, and improve immunity and growth performance, thereby affecting protein metabolism. The func-

tional components of different yeast hydrolysates vary, with mannans, glucans, and nucleotides being primary functional substances besides essential amino acids. Yu et al. determined the optimal yeast cell wall supplementation level for Japanese seabass as 500 mg/kg, providing effective glucan and mannan levels of 140 and 120 mg/kg, respectively. In this study, 5 g/kg yeast hydrolysate provided β-glucan and mannan at 890 and 525 mg/kg, respectively, suggesting that effective component content is crucial for rational application of yeast products in fish feeds.

3.2 Effects of Yeast Hydrolysate on Plasma Biochemical Indices

Blood biochemical indices provide important information for assessing nutritional status, metabolism, and disease diagnosis in fish. This study showed no significant differences in plasma TG, TC, GLU contents, or AST activity between groups, with all values within reference ranges. TC and TG reflect cholesterol and lipid absorption/metabolism status. The absence of significant differences in TC, TG, and GLU indicates that 5 g/kg yeast hydrolysate supplementation did not significantly affect lipid or glucose metabolism. MDA, as a final product of lipid peroxidation, reflects reactive oxygen species levels and oxidative stress degree. No significant differences in plasma MDA content suggest that yeast hydrolysate supplementation did not cause oxidative damage.

Elevated plasma TBA content and AKP activity together indicate cholestasis. While TBA content showed no significant differences between groups, the isolated increase in AKP activity in the YH5 group may be associated with obstructive jaundice. Under normal physiological conditions, plasma ALT and AST activities are low but increase significantly when hepatocytes are damaged, with elevation degree correlating with damage severity. Increased ALT and AST activities serve as indicators of liver damage and chronic hepatitis. This study showed no significant differences in TBA content or ALT and AST activities between groups. Although AKP activity was significantly higher in the YH5 group, no other indicators of bile acid metabolism disorders were observed, warranting further investigation into whether yeast hydrolysate supplementation causes cholestasis.

3.3 Effects of Yeast Hydrolysate on Liver Histology

Histopathological analysis revealed varying degrees of liver damage across all groups, with the control group showing one case of hepatic fibrosis. The universal liver damage may be related to relatively high dietary carbohydrate levels. Xu et al. reported that dietary starch levels exceeding 10% can cause liver lesions in largemouth bass, while Tan et al. found that 15-23% dietary carbohydrate primarily affected visceral organ relative weight and liver nutrient composition.

Tissue fibrosis is generally considered a consequence of failed normal wound healing. Following tissue injury, new connective tissue formation requires fibrob-

last activation, proliferation, and migration to the wound site. Fibroblasts at injury sites differentiate into myofibroblasts that highly express α-SMA. While fibroblasts promote wound healing, persistent myofibroblast presence leads to tissue fibrosis. Tissue repair involves interactions between pro-fibrotic and anti-fibrotic cytokines, including TGF-β and TNF-α. The pro-inflammatory factor TNF-α is expressed in macrophages during tissue repair and may promote hepatic fibrosis, as increased TNF-α-positive cells have been observed in liver tissues of hepatocellular carcinoma patients. Hepatic fibrosis results from chronic inflammatory reactions triggered by persistent infection, autoimmune reactions, chemical injury, tissue damage, and oxidative stress, which increases mitochondrial permeability and promotes hepatocyte damage and fibrosis. TNF-α can induce hepatocyte apoptosis and cause liver injury or cancer, while TGF-$ 1$inhibitsgrowthanddifferentiationofvariouscelltypes, regulatesimmuneandinflammatoryresponses, and
$inflammatoryresponsesandisconsideredanti-inflammatory.StudieshaveshownthatTGF-$ $1
downregulation inhibits hepatic stellate cell activation and fibrosis progression.

The caspase family maintains homeostasis by regulating apoptosis and inflammatory responses. Apoptotic pathways include extrinsic and intrinsic pathways. Extrinsic apoptosis is activated by ligand binding to death domains, such as TNF-α superfamily members and their receptors, activating caspase-8 and caspase-10 to initiate apoptosis via caspase-3. Intrinsic apoptosis involves mitochondrial pathways where oxidative stress induces mitochondrial dysfunction, leading to ROS enhancement, lipid peroxide accumulation, and cytochrome c release. Caspase-9 is activated by various cellular stresses, and activated caspase-9 subsequently activates caspase-3 to initiate hepatocyte apoptosis and fibrosis. In this study, the lack of significant differences in inflammatory and apoptotic pathway gene expression between groups may be attributed to only one sample showing clear apoptotic signals and consistent fatty liver incidence between groups without extensive apoptotic cells. The downregulated trend of TGF-$ $1 expression in the YH5 group may explain the absence of fibrotic samples.

4 Conclusion

Dietary supplementation with 5 g/kg yeast hydrolysate in largemouth bass feeds promotes protein deposition and shows a trend toward reducing hepatocellular fibrosis risk.

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Note: Figure translations are in progress. See original paper for figures.