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Research Article

An Evaluation of Yeast Culture Supplementation in the Diet of *Pseudobagrus ussuriensis*: Growth, Antioxidant Activity, Nonspecific Immunity, and Disease Resistance to *Aeromonas hydrophila*

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An 8-week feeding trial was conducted to evaluate the effects of dietary yeast culture (YC) supplementation on growth performance, antioxidant activity, nonspecific immunity, and disease resistance of *Pseudobagrus ussuriensis* (average initial weight 6.01 ± 0.01 g). Four isonitrogenous and isolipidic diets were formulated to contain 0 (Y0), 10 (Y1), 20 (Y2), and 30 (Y3) g/kg YC, respectively. After the feeding experiment, the challenge test of injecting *Aeromonas hydrophila* was executed. Results showed that appropriate YC supplementation level in the diet could improve growth performance, digestive enzyme activities, nonspecific immunity capacity, antioxidant capacity, and disease resistance of *P. ussuriensis*. And the highest weight gain, feed intake, specific growth rate, and IGF-1 gene expression level were observed in fish fed the Y2 diet. The activities of protease and amylase in intestine in fish fed the Y2 diet were enhanced compared with that in fish fed the Y0 diet significantly ($P < 0.05$). Simultaneously, fish fed the Y2 diet had significantly higher serum lysozyme activity and significantly lower serum alanine amino transferase activity ($P < 0.05$). Dietary 20 g/kg YC supplementation increased the activity of catalase and total antioxidant capacity in liver and reduced malondialdehyde content in the liver and intestine of *P. ussuriensis* significantly ($P < 0.05$). Fish fed the Y2 diet had the highest disease resistance under the condition of *A. hydrophila* challenge ($P < 0.05$). The quadratic regression analysis based on weight gain against dietary YC levels indicated that the appropriate dietary YC supplementation level is 13.4 g/kg diet.

1. Introduction

Fisheries play a critical role in making sure about food security and employment to millions of folks worldwide. Fishmeal (FM) is a highly nutritious and appropriately palatable protein source for fish feed formulation. However, due to increasing demand, unstable supply, and high price of the FM with the expansion of aquaculture, there is an increasing demand for searching more potential alternative protein sources in aquafeeds [1, 2]. Yeast culture (YC) is one kind of complicated yeast fermentation product, which has great prospects as a potential protein source to replace fish meal in aquiculture [3, 4]. Growth studies have confirmed that YC can improve growth performance of many fish species; for instance, the research on juvenile largemouth bass (*Micropterus salmoides*) fed high-starch diet showed dietary 30 g/kg YC supplementation could improve the growth performance, liver function, and intestinal barrier [5]. Liu et al. [6] observed that grass carp

(*Ctenopharyngodon idellus*) fed diet with YC (120-160 g/kg) significantly increased the growth performance.

YC have the ability to activate immune response, modulate intestinal microflora, and resist certain pathogenic bacteria and have been considered as potential "antibiotic alternatives" [7]. Some surveys found that the abovementioned effects may be attributed to the various functional elements in YC such as β-glucan, mannan-oligosaccharides, and digestible proteins [8, 9]. Previous reports have demonstrated that those elements take an important part of enhancing the immune function of different kinds of animals [10–13]. Zhang et al. [4] indicated that dietary YC addition could enhance the resistance to *Aeromonas hydrophila* in gibel carp (*Carassius auratus gibelio* CAS III). Moreover, Torrecillas et al. [14] found that mannan-oligosaccharides, one kind of yeast products constitute part, could facilitate antigen processing to activate immune response and then improve the host's health capability.

Pseudobagrus ussuriensis is an important economic fish in China and East Asia [8]. It is very popular among consumers due to its high nutritional value. However, due to the large-scale and intensive fish farming, the shortage of fishmeal is prominent. To date, several studies have reported feasibility in replacing fishmeal with different proteins, including corn gluten meal [15], rapeseed meal [16], cottonseed meal [17], meatand bone meal [18] as well as mussel (*Cristaria plicata*) meat meal [19] in *P. ussuriensis*. But there is limited message regarding the potential role of dietary YC as partial FM substitute on growth performance and healthy status for *P. ussuriensis*. Thus, the aim of the present study is to evaluate the effects of dietary YC as partial FM substitute on growth performance, nonspecific immunity, and antioxidant capacity as well as disease resistance of *P. ussuriensis* through an 8-week feeding experiment with different level of YC and an *A. hydrophila* challenge test after feeding experiment.

2. Materials and Methods

2.1. Experimental Diet and Fish Preparation. Four isonitrogenous and isolipidic diets were formulated: a control diet and three experimental diets (recorded as Y0, Y1, Y2, and Y3, respectively) with 10, 20, and 30 g/kg of YC inclusion. And the YC was provided by the Beijing Enhalor Institute of Biotechnology (Beijing, China). *Saccharomyces cerevisiae* strain (Patent No. 201210349633.4) was obtained from the cooperative research production between the Institute of Microbiology Chinese Academy of Sciences (Beijing, China) and Beijing Enhalor Institute of Biotechnology (Beijing, China), and its growth conditions were pH at 6.5, temperature at 30 ± 1°C, and dissolved oxygen at 20-60%. The compositions of the diets used in feeding trials are given in Table 1. The diets were produced as shown in the former study in our lab and stored at -20°C [8]. *P. ussuriensis* were provided by the Fisheries Research Institute of Harbin Academy of Agricultural Sciences (Harbin, China). Fish were acclimated using the Y0 diet for 2 weeks prior to the experiment to adapt to the diet and environment. 480 healthy and unwounded fish with homogeneous size were selected and divided into 4 groups (Y0, Y1, Y2, and Y3), containing 4 repetitions each group. These fish were placed, respectively, in 16 aquariums (1.0 × 0.5 × 0.6 m, water depth about 0.4 m) with 30 fish per aquarium. The total body weight of the fish in each aquarium was measured, and the average weight was calculated as 6.01 ± 0.01 g. Subsequently, fish were maintained in circulated, aerated, and filtered fresh water, and an 8-week feeding trial was conducted.

Table 1: Diets composition (g/kg dry matter).

Ingredients	Y0	Y1	Y2	Y3
Fish meal	280	270	260	250
Soybean meal	300	300	300	300
Cottonseed meal	110	110	110	110
Corn gluten meal	70	70	70	70
Wheat meal	164	164	164	164
Soybean lecithin	10	10	10	10
Soybean oil	33	33	33	33
Vitamin premix and mineral premix[a]	10	10	10	10
Choline	3	3	3	3
$Ca(H_2PO_4)_2$	20	20	20	20
Yeast culture	0	10	20	30
Proximate composition (g/kg dry matter)[b]				
Dry matter	932.7	926.0	928.0	931.0
Crude protein	458.3	453.5	455.5	450.4
Crude lipid	77.7	78.0	76.4	76.0
Gross energy (kJ/g)[c]	184.9	185.7	186.0	180.0
Ash	121.3	120.1	115.3	118.4

[a]Vitamin premix (IU or mg/kg dry diet): retinol (V_A) 3000 IU; cholecalciferol (V_D) 1500 IU; tocopherol (V_E) 40 mg; menadione (V_K) 4.5 mg; thiamin (V_{B1}) 8 mg; riboflavin (V_{B2}) 8.5 mg; pyridoxine (V_{B6}) 6.5 mg; cyanocobalamin (V_{B12}) 0.02 mg; nicotinic acid 45 mg; nicotinamide 45 mg; D-Ca pantothenate 17 mg; inositol 40 mg; biotin 0.15 mg; folic acid 1.3 mg; antiscorbic acid 110 mg. Mineral premix (mg/kg dry diet): copper 6.5 mg; iron 45 mg; selenium 0.35 mg; zinc 70 mg; manganese 8.5 mg; magnesium 100 mg; cobalt 1 mg; iodine 1.2 mg. [b]Proximate composition were measured values. [c]Gross energy was determined using an adiabatic bomb calorimeter (Parr 6300, USA).

2.2. Feeding Trial. Fish were artificially fed to apparent satiation thrice daily (07:00, 13:00, and 19:00). The trial was conducted under steady pH (6.5-8.5), ammonia-N (<0.1 mg/L), temperature (22 ± 2°C), water flow rate (2.5 L/min), dissolved oxygen (>5 mg/L), and a light: dark cycle of 12:12 h condition.

2.3. Sampling and Chemical Analysis. At the end of the experiment, the fish were starved for 24 hours. The fish were then euthanized with eugenol (1 : 12 000) anesthetic. The total number and body weight of fish in each aquarium were measured to calculate the growth performance and nutrient utilization. Three fish were randomly selected from each aquarium, and the body length and body weight were measured, and the weight of isolated muscle, viscera, intestine, and liver tissue were determined [17]. Caudal vein blood samples were collected by heparinized syringes from five fish per repetition, and plasma was collected after centrifuged

(3000 rpm, 10 min, 4°C; Microfuge 22R centrifuge, Beckman Coulter, USA) and stored at -80°C. Another five fish per repetition were randomly obtained, and the liver, intestine, and muscle were immediately frozen with liquid nitrogen and stored at -80°C. This study was performed under the guidelines approved by the Animal Care and Use Committee of the Northeast Agricultural University, China.

The digestive enzymes' activities (amylase, lipase, and protease) were measured as described by Luo et al. [19] with the kits (No.: C016, A054, A080, respectively; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The liver and intestine samples were homogenized in 0.9% (*w*/*v*) NaCl solution on the ice and then centrifuged at 3000 rpm (Microfuge 22R centrifuge, Beckman Coulter, USA) for 10 min. The approach used in this investigation is similar to that used by other researchers [8, 19]. The activities of catalase (CAT), SOD, and T-AOC and the content of malondialdehyde (MDA) in hepatic and intestinal tissues were measured with the reagent kits (No.: A007, A001, A015, A003, respectively; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The total protein content was determined by Coomassie brilliant blue staining method (No: A045; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum and liver as well as lysozyme (LZM) and alkaline phosphatase (AKP) in serum were also determined using the kits from the Nanjing Jiancheng Bioengineering Institute (No.: C009, C010, A050, A059, respectively; Nanjing, China).

2.4. IGF-1 Gene Expression. The method of IGF-1 gene expression was acted in accordance with those previously described in literatures of our laboratory [15, 16]. The primer sequences were displayed in Table 2, and β-actin was chosen as an internal reference. Total RNA was isolated from the muscle and liver samples using the Trizol method (TransGen Biotech Co., Ltd., Beijing, China), and then, cDNA was synthesized by PrimeScript® RT reagent Kit (Takara Biomedical Technology Co., Ltd., Beijing, China). Quantitative PCR (20 μL) was conducted using TransStart® Green qPCR SuperMix kit (TransGen Biotech Co., Ltd., Beijing, China) on Applied Biosystems® 7500 real-time PCR system (USA). Briefly the real-time PCR began with 30 s at 94°C, followed by 40 cycles at 94°C for 5 s and 60°C for 30 s. The gene expression levels were calculated with $2^{-\Delta\Delta CT}$ method [20].

2.5. Bacterial Challenge. The detailed description of *A. hydrophila* used in this study and the preparation was presented by our preliminary experiments [17]. The sample of graded doses of *A. hydrophila* was carefully injected into enterocoelia to explore 96 h LD50, and results found that 3.48×10^3 CFU per fish was the 96 h LD_{50}. At the end of the feeding trial, a total of 160 fish of all groups (40 fish from each group) were peritoneal injected with 0.1 mL per fish of bacterial suspension of *A. hydrophila* (3.48×10^4 CFU/mL). During challenge trial, the regulation condition was the same as feeding experiment. The cumulative mortality was observed and recorded for 7 days.

2.6. Calculations and Statistical Analysis. The proximate composition of diets, feed ingredients, and fish samples were analyzed using the standard methods of AOAC [21]. The data was presented as the means ± SE. All data were subjected to a one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. There was significant difference at the $P < 0.05$. All calculations were performed using SPSS 20.0 for Windows (SPSS, Inc., USA).

The following variables were calculated:

$$\begin{aligned}
\text{Weight gain (WG, \%)} &= 100 \times \frac{(W_t - W_0)}{W_0},\\
\text{Feed intake}\left(\text{FI, \%day}^{-1}\right) &= 100 \times \frac{\text{dry feed intake}}{[(W_t + W_0)/2 \times t]},\\
\text{Specific growth rate}\left(\text{SGR, \%day}^{-1}\right) &= 100 \times \frac{(\ln W_t - \ln W_0)}{t},\\
\text{Protein efficiency ratio (PER)} &= \frac{\text{wet weight gain}}{\text{crude protein intake}},\\
\text{Survival rate (SR, \%)} &= 100 \times \frac{\text{final fish number}}{\text{initial fish number}},\\
\text{Feed conversion ratio (FCR)} &= \frac{\text{feed consumption}}{\text{weight gain}},\\
\text{Condition factor}\left(\text{CF, g} \cdot \text{cm}^{-3}\right) &= 100 \times \frac{\text{body weight}}{(\text{body length})^3},\\
\text{Hepatosomatic index (HSI, \%)} &= 100 \times \frac{\text{liver weight}}{\text{whole body weight}},\\
\text{Viscera somatic index (VSI, \%)} &= 100 \times \frac{\text{viscera weight}}{\text{whole body weight}},
\end{aligned} \tag{1}$$

where W_0 is the initial body weight (g), W_t is the final body weight (g), and t is the feeding duration (day).

3. Results

3.1. Growth Performance. The body weight and length of *P. ussuriensis* were measured after feeding YC diet with different levels for 56 days, and the growth performance was evaluated. It was found in this study that dietary YC supplementation could promote WG, SGR, and PER and reduce FCR of *P. ussuriensis* except for dietary 30 g/kg YC supplementation level. The best growth performance was observed in the Y2 group (Table 3). FI was obviously affected by dietary YC supplement, and fish in Y2 group had the highest FI value ($P < 0.05$) (Table 3). Besides, no significant difference was observed in HSI, VSI, CF, and survival rate among all groups ($P > 0.05$), although fish in Y2 group showed the lowest survival (94.4%) (Table 3). Furtherly, the IGF-1 gene expression in the muscle (Figure 1(a)) and liver (Figure 1(b)) were meaningfully elevated in Y1 and Y2 groups compared to Y0 and Y3 groups ($P < 0.05$). Based on the polynomial curve analysis of WG among all groups, Figure 2 suggests that the optimal level of YC supplementation for *P. ussuriensis* was 13.4 g/kg.

Table 2: Primers sequence.

Primer names	Sequence (5′-3′)	Accession number	Annealing temperature (°C)
IGF-1-F	TTATTTCAGCAAGCCAACAGGC	KX257402.1	58
IGF-1-R	ACGGATCTTGGAGCTTTACCAG		
β-Actin-F	CCTCCGTCTGGATTTGGCTG	Bu et al. [15]	60
β-Actin-R	TCAAGGGCGACGTAGCAGAG		

Table 3: Growth parameters of *P. ussuriensis* fed diet with different levels of YC.

Parameters	Y0	Y1	Y2	Y3
Initial body weight (g)	6.01 ± 0.02	6.01 ± 0.01	6.02 ± 0.01	6.00 ± 0.01
Final body weight (g)	13.59 ± 0.11^{b}	13.82 ± 0.12^{b}	14.94 ± 0.13^{a}	12.36 ± 0.08^{c}
Weight gain (%)	126.10 ± 2.36^{b}	129.95 ± 2.38^{b}	148.29 ± 1.56^{a}	107.11 ± 1.64^{c}
Feed conversion ratio	1.24 ± 0.03^{b}	1.18 ± 0.01^{c}	1.19 ± 0.01^{bc}	1.30 ± 0.01^{a}
Specific growth rate (% day^{-1})	1.46 ± 0.02^{b}	1.49 ± 0.02^{b}	1.62 ± 0.01^{a}	1.30 ± 0.01^{c}
Feed intake (% day^{-1})	1.49 ± 0.02^{b}	1.44 ± 0.03^{bc}	1.57 ± 0.01^{a}	1.41 ± 0.02^{c}
Survival rate (%)	96.67 ± 1.92	98.89 ± 1.11	94.44 ± 2.94	95.83 ± 3.15
Protein efficiency ratio	1.70 ± 0.04^{bc}	1.79 ± 0.02^{a}	1.78 ± 0.02^{ab}	1.65 ± 0.02^{c}
Hepatosomatic index (%)	2.30 ± 0.01	2.31 ± 0.24	1.98 ± 0.10	2.20 ± 0.21
Viscera somatic index (%)	11.38 ± 0.73	10.70 ± 1.11	10.14 ± 0.12	10.68 ± 0.32
Condition factor (g cm-^{3})	0.97 ± 0.09	0.96 ± 0.09	0.86 ± 0.03	1.02 ± 0.04

Different superscripts within a line denote significant difference ($P < 0.05$). Data presented as mean ± SE.

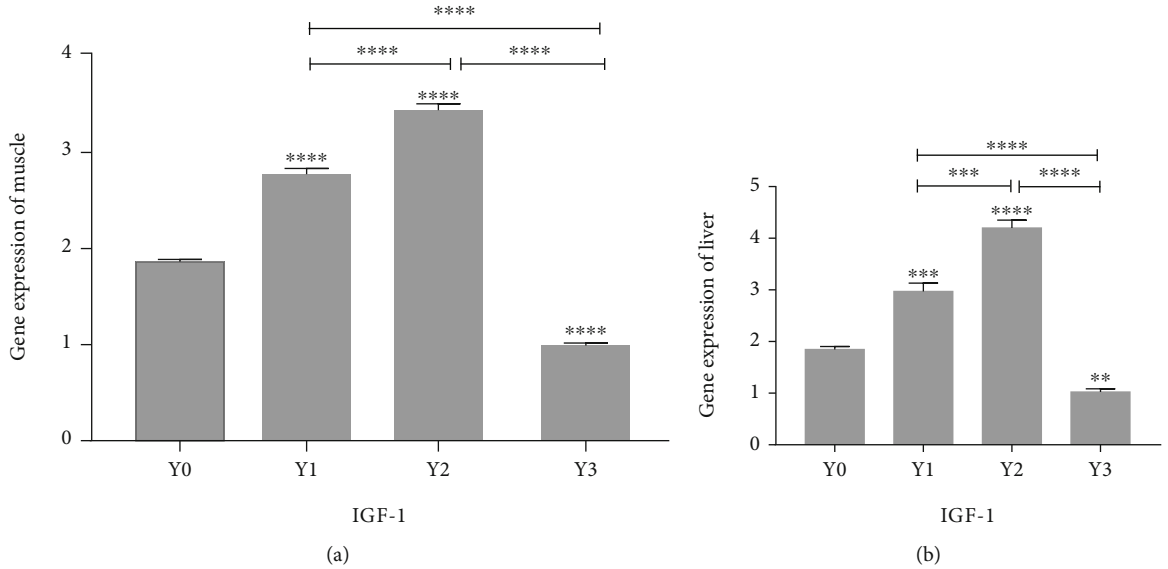

Figure 1: IGF-1 expression in liver and muscle of *P. ussuriensis* fed with test diets for 56 d ($n = 4$). Bars with asterisks are significantly different from the control (** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$).

3.2. Digestive Enzyme Activity in Intestine. There is a close correlation between the activity of digestive enzymes and feed digestion and absorption capacity. Fish fed with Y1 and Y2 diets showed significantly higher protease and amylase activities than fish in Y0 group ($P < 0.05$) (Figures 3(b) and 3(c)). However, lipase activity showed no obvious differences among all groups ($P > 0.05$) (Figure 3(a)).

3.3. Immune and Antioxidative Parameters. The effects of dietary YC supplementation on nonspecific immune and

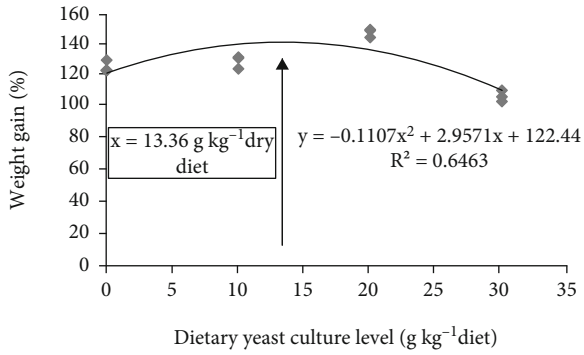

Figure 2: Polynomial curve model analysis based on weight gain against dietary YC levels for *P. ussuriensis* fed with test diets for 56 d.

antioxidant parameters could be used to evaluate the pathologic and nutritional status of *P. ussuriensis*. The immune (LZM) and antioxidative parameters (T-AOC, SOD, CAT, and MDA) were influenced significantly in the fish fed with 20 g/kg YC supplemented diet. The results revealed that the hepatic and intestinal CAT activities and T-AOC values were enhanced in dietary YC supplementation groups compared with the Y0 group, particularly in Y2 group which the highest T-AOC level was found ($P < 0.05$) (Table 4). The intestine MDA content was dramatically decreased in Y1 and Y2 groups compared with the control group ($P < 0.05$) (Table 4). The lowest value of hepatic MDA was observed in Y2 group, and the lowest hepatic SOD activity was found in Y3 group ($P < 0.05$) (Table 4). The significantly increased intestinal SOD and hepatic AST activities were observed in Y1 and Y2 groups ($P < 0.05$) (Table 4 and Table 5). The hepatic ALT and serum LZM activities were significantly raised in Y2 group compared with Y0 group ($P < 0.05$) (Table 5 and Figure 4(b)). There was significant reduction of serum ALT activity in Y2 group compared to Y0 group ($P < 0.05$) (Table 5). But no remarkable differences were obtained in serum AKP and AST activities among four groups ($P > 0.05$) (Table 5 and Figure 4(a)). The results in this section indicated that dietary 20 g/kg YC level could enhance nonspecific immunity and antioxidation capability of *P. ussuriensis*.

3.4. Challenge Test. At the ninth week after feeding, 30 fish were challenged with 96 h LD50 dose of *A. hydrophila* (3.48×10^4 CFU/mL), and each fish was injected 0.1 mL *A. hydrophila* suspension. Figure 5 showed that feeding *P. ussuriensis* with different levels of YC enhanced its resistance to disease caused by *A. hydrophila*, and Y2 group had the highest cumulative survival rate among all groups ($P < 0.05$).

4. Discussion

YC are recently viewed as an exploitable replacement of fishmeal, with promising results in Pacific white shrimp (*Litopenaeus vannamei*) and gibel carp [3, 4, 22]. Stephen et al. [22] found that 10 g/kg and 20 g/kg YC addition in shrimp diet improved growth performance and decreased FCR significantly. Similarly, Chen et al. [23] stated that 10 g/kg hydrolyzed yeast supplementation improved juvenile Nile tilapia's (*Oreochromis niloticus*) growth performance. Conversely, Zhang et al. [4] revealed that there was no influence in growth performance between different levels of dietary YC in gibel carp. It could be extrapolated from previous results that YC may have different effects on growth performance for different aquatic animal species. In the present study, the significantly higher growth performance was found in Y2 group by increasing FBW, WG, SGR, and FI as well as reducing FCR of *P. ussuriensis*. In addition, the inferior growth performance was observed in Y3 group compared with any other group. Similar results occurred in previous studies, that is, with the increase in the amount of yeast added, the weight gain of aquatic animals first increased and then remained stable or even decreased [24]. This may be due to the presence of nucleotides and indigestible polysaccharides (for example, β-glucans) in the yeast composition, which can act as antinutritional factors when used in high concentrations. This impairs the animal's digestive capacity and full nutrient utilization, which is then reflected in reduced livestock growth performance [25]. In our study, *P. ussuriensis* fed 30 g/kg YC diet had the lower FBW, WG, and FI than fish fed control diets. It revealed that dietary YC supplemental level of 30 g/kg is too high to be utilized effectively for *P. ussuriensis*. IGF-1, a hormone produced primarily by the liver which can be secreted into the blood, has influence on organismal growth, development, and anabolic protein [15, 26]. The IGF-1 gene expression level is one kind of indicator to appraise growth performance [16, 27]. Our results revealed that with the YC supplementation level rising, liver and muscle IGF-1 gene expression levels showed the "low-high-low" trend, and the highest level was observed in Y2 group, indicating that IGF-1 was positively correlated with the growth parameter.

The term "digestive enzymes" has been used to refer to enzymes which can enhance nutrient digestion and absorption. High-digestive enzyme activities could promote nutrition digestion and absorption, thereby affecting growth performance directly [28, 29]. Research showed that the activities of digestive enzymes (lipase, protease, and amylase) in aquatic animals could be affected by diet formula [28, 30]. Ran et al. [31] noted that the trypsin activity of Nile tilapia fed with yeast was improved. Moreover, it has been reported that the trypsinase and amylase activities of *L. vannamei* were enhanced by yeast extract, but the lipase activity was reduced [32]. In the current study, the significant difference was observed in protease activity between the Y2 and Y0 groups. Fish fed Y1 and Y2 diets had significantly higher amylase activity compared with fish fed Y0 and Y3 diets. These results were consistent with growth performance, implying that YC might increase growth performance by affecting digestive enzymes activities.

Oxidant stress, the state of imbalance between oxidation and antioxidation, plays a crisis element in disease development, such as metabolic and viral diseases [33–35]. T-AOC as an important antioxidant index could reflect the state of body oxidative stress [36]. Organisms can produce antioxidant enzymes (SOD and CAT, for example) to prevent oxidative stress [37]. Simultaneously, MDA is a lipid peroxidation

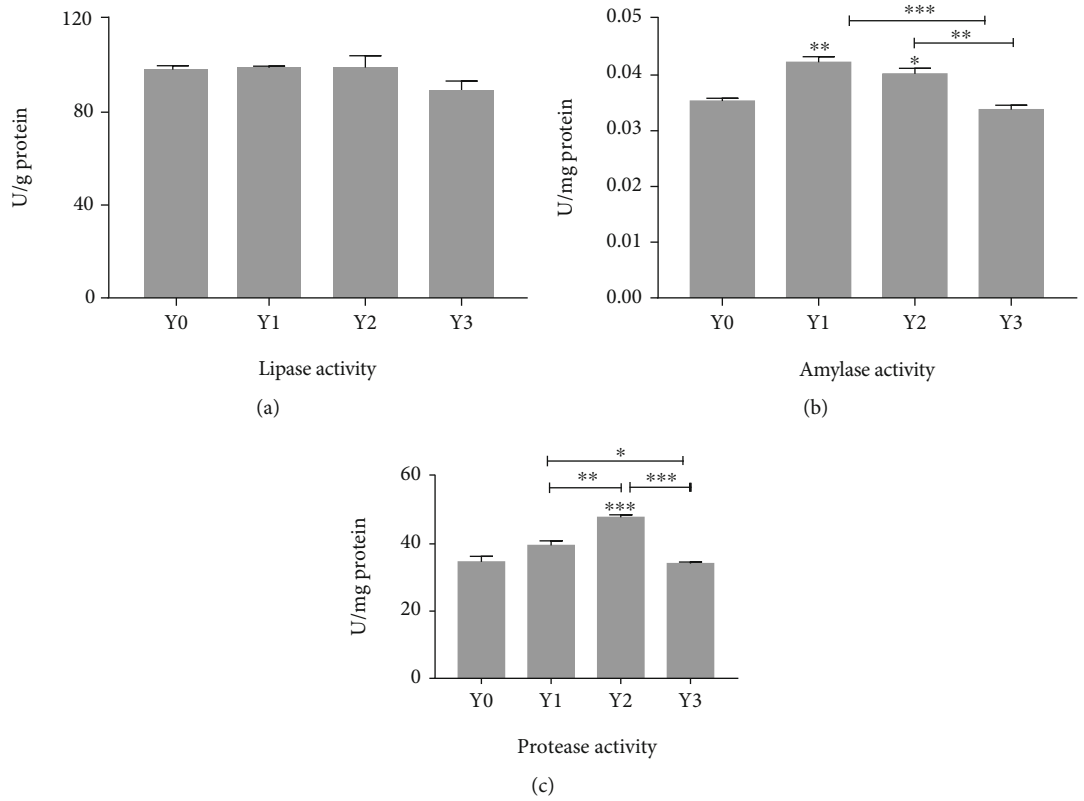

Figure 3: Digestive enzymes activities (amylase, lipase, and protease) in intestine of *P. ussuriensis* fed with test diets for 56 d ($n = 4$). Bars with asterisks are significantly different from the control ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$).

Table 4: Effect of dietary YC on liver and intestine antioxidant parameters of *P. ussuriensis*.

Parameters	Y0	Y1	Y2	Y3
Liver				
Catalase (U mg^{-1})	6.41 ± 0.63^{c}	9.41 ± 0.24^{a}	8.04 ± 0.21^{b}	7.13 ± 0.17^{bc}
Superoxide dismutase (U mg^{-1})	113.28 ± 5.84^{a}	117.81 ± 7.58^{a}	126.41 ± 1.65^{a}	80.26 ± 2.92^{b}
Total antioxidant capacity (U mg^{-1})	0.42 ± 0.03^{c}	0.78 ± 0.05^{b}	1.57 ± 0.12^{a}	0.54 ± 0.05^{c}
Malondialdehyde (nmol mg^{-1})	1.16 ± 0.15^{ab}	0.83 ± 0.05^{bc}	0.76 ± 0.06^{c}	1.24 ± 0.11^{a}
Intestine				
Catalase (U mg^{-1})	14.39 ± 1.13^{c}	18.42 ± 0.70^{b}	23.16 ± 1.53^{a}	15.41 ± 0.83^{bc}
Superoxide dismutase (U mg^{-1})	195.63 ± 3.55^{c}	230.03 ± 5.03^{b}	287.37 ± 6.41^{a}	181.13 ± 2.71^{c}
Total antioxidant capacity (U mg^{-1})	0.31 ± 0.03^{b}	0.39 ± 0.02^{ab}	0.52 ± 0.07^{a}	0.35 ± 0.05^{b}
Malondialdehyde (nmol mg^{-1})	7.07 ± 0.09^{a}	5.80 ± 0.31^{b}	4.83 ± 0.22^{c}	7.10 ± 0.35^{a}

Data represented as mean ± SE of triplicate aquariums. Means within rows with different superscript letters differ ($P < 0.05$).

Table 5: Effect of dietary YC on alanine aminotransferase and aspartate aminotransferase activities in serum and liver of *P. ussuriensis*.

Parameters	Y0	Y1	Y2	Y3
Serum				
Alanine aminotransferase (U gprot^{-1})	34.85 ± 3.32^{a}	40.05 ± 3.06^{a}	21.44 ± 1.45^{b}	32.56 ± 2.70^{a}
Aspartate aminotransferase (U gprot^{-1})	86.74 ± 7.31^{ab}	88.20 ± 7.44^{ab}	66.92 ± 10.36^{b}	97.62 ± 4.74^{a}
Liver				
Alanine aminotransferase (U gprot^{-1})	200.54 ± 8.93^{b}	204.86 ± 12.31^{b}	257.66 ± 6.97^{a}	167.75 ± 2.17^{c}
Aspartate aminotransferase (U gprot^{-1})	156.57 ± 3.67^{b}	182.73 ± 7.83^{a}	193.93 ± 6.24^{a}	144.22 ± 3.17^{b}

Data represented as mean ± SE of triplicate aquariums. Means within rows with different superscript letters differ ($P < 0.05$).

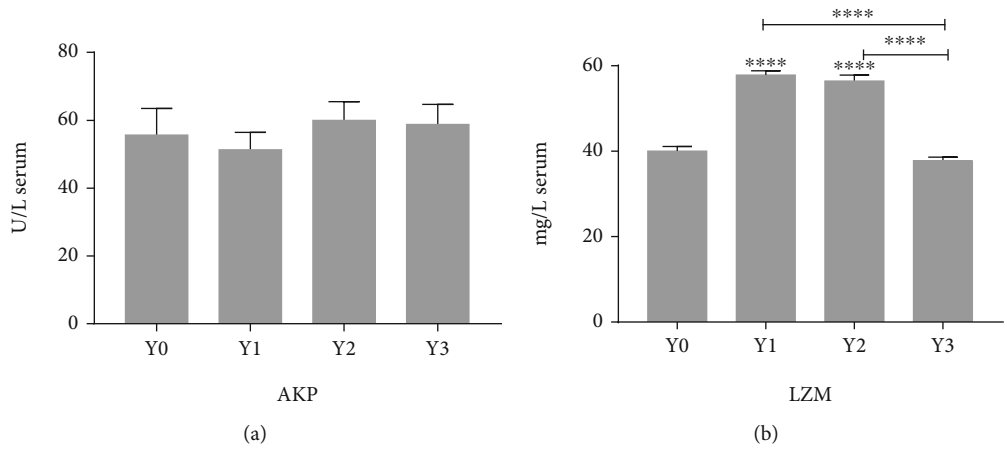

Figure 4: Serum immune related parameters of *P. ussuriensis* (AKP: alkaline phosphatase, LZM: lysozyme; $n = 4$) fed with test diets for 56 d. Bars with asterisks are significantly different from the control ($^{****}P < 0.0001$).

marker; its production will impair cell structure and function [38]. Yeast product applications were reported to enhance antioxidant capacity of some species such as Nile tilapia [39], blunt snout bream (*Megalobrama amblycephala*) [40], and *L. vannamei* [22]. Andriamialinirina et al. [39] stated that juvenile Nile tilapia fed 10 g/kg yeast hydrolysate showed significantly higher hepatic SOD, CAT activities, and lower MDA content than the control group. Our data suggested that the diet with 20 g/kg YC addition could enhance the activities of T-AOC, SOD, and CAT and reduce the value of MDA in liver and intestine of *P. ussuriensis* compared with the diet without YC addition. But dietary 30 g/kg YC addition increased the MDA content and decreased the activities of SOD and T-AOC significantly in liver of *P. ussuriensis* compared with dietary 20 g/kg YC addition. As we know, yeasts contain higher levels of nucleic acids and indigestible polysaccharides [25], but too high levels of nucleic acids and polysaccharides cannot be safely metabolized in fish to generate harmful hydroxyl radicals, which can destroy antioxidant system and contribute to oxidative stress [41, 42]. The results in the present study manifested that the proper YC supplementation (20 g/kg) in diet would enhance the antioxidase activities, thereby inhibiting the activity of oxygen free radicals and preventing oxidative damage; however, excessive YC addition may lead to an adverse impact for antioxidant capacity.

As an essential organ for the body, the liver has wide range of functions such as digestion, metabolism, immunity, and detoxification [33, 43]. AST and ALT, as the sensitive markers of liver integrity, can provide the assessment of animals' liver damage [44]. The ALT and AST content raised in serum are related to impair of hepatocytes, and its organelles caused by oxidative stress with endogenous antioxidant enzymes such as SOD and CAT values decreased [45–47]. It was noticed that YC had protective effect to the health of *P. ussuriensis*, and fish fed the 20 g/kg YC diet showed the lower serum AST and ALT activities and higher hepatic ALT and AST activities. An implication of this is the possibility that YC may help hepatic cell metabolism without negative effects on liver cells considering the truth of that serum ALT and AST activities rise when hepatocyte membrane permeability enhanced at the condition of liver cells damaged [40]. The results denoted that YC has certain prevention and treatment effects on liver lesions for *P. ussuriensis*, so that fish could better adapt to the complex environment, ultimately the culture efficiency being improved.

It is universally acknowledged that immunological parameters are useful tools to investigate health status of aquatic animals. *A. hydrophila* is one kind of important bacterium causing huge mortality of farmed fish worldwide [38, 48]. Zhang et al. [4] discovered that dietary YC addition improved disease resistance of *A. hydrophila* in gibel carp. Similarly, Stephen et al. [22] found that shrimps fed YC reduced mortality after *Vibrio harveyi* challenge, and the lowest mortality was found in the 20 g/kg yeast hydrolysate group with a survival rate of 70%. The results of this trial found that YC supplementation could obviously increase the survival rate of *P. ussuriensis* exposed to *A. hydrophila*. Lysozyme can hydrolyze bacteria cell wall's peptidoglycans which make it becoming a momentous bacteriolytic component in immune system [49]. Our study found that fish in 10 g/kg and 20 g/kg YC groups had obviously enhanced serum lysozyme activity than fish in 0 g/kg and 30 g/kg YC groups. β-Glucans present in the yeast cell walls can stimulate innate immune system, increasing nonspecific responses. However, excess yeast could lead to immune hyperstimulation, showing decreased lymphocyte numbers, which may be associated with the changes in metabolism and oxidative stress due to the intake of large amounts of polysaccharides and nucleic acids [24, 25]. Our results indicated that dietary proper YC supplementation enhanced the immunity of juvenile *P. ussuriensis*, and 20 g/kg was the suitable level. Also, Stephen et al. [22] found that *L. vannamei* fed diet 20 g/kg YC reached the peak for LZM content in serum. The highest survival rate was found in 20 g/kg YC group after *A. hydrophila* challenge in the present study; presumably, for one thing, the improved activity of LZM could further lyse the bacteria; for another, the enhanced SOD and CAT activities in liver and intestine produced synergistic effect to remove excessive free radicals and raised the body's disease resistance. Furtherly, this could be explained by the fact that β-glucan, MOS, and nucleotide from dietary YC

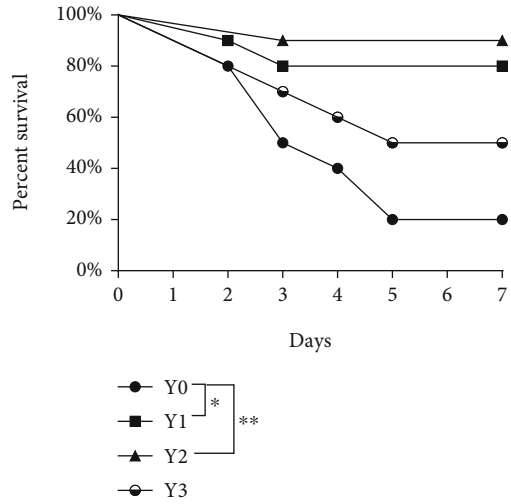

Figure 5: Survival rate of *P. ussuriensis* fed with test diets after challenge with *A. hydrophila* for 7 days ($n = 4$). Bars with asterisks are significantly different from the control ($^*P < 0.05$, $^{**}P < 0.01$).

supplementation may have favorable impact on immunity of aquatic animal [4, 50]. It is known that AKP plays significant roles in regulate nonspecific immunity, nutrient metabolism, and signal transduction [51, 52]. However, the present study showed that dietary YC supplementation had no significant influence on the AKP activity among all the groups. As the symbolic innate immunity factors, LZM and AKP could help body degrade exogenous substances [53, 54]. Our results showed that dietary YC addition improved LZM activity but did not affect AKP activity, indicating that the effect of YC on nonspecific immunity comes from the action of LZM rather than AKP.

This experiment is the first attempt to use yeast culture instead of fishmeal in the feed of *P. ussuriensis*, and our results showed that fish fed diet with YC supplemented levels from 10 to 20 g/kg had better growth performance, digestive enzyme activities, nonspecific immunity capacity, antioxidant capacity, and bacterial resistance; quadratic regression analysis based on weight gain against dietary YC levels indicated that the appropriate dietary YC supplementation level is 13.4 g/kg diet.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest

We declare no conflicts of interest related to the submitted work.

Authors' Contributions

Xuying Hou, Liujian Sun, and Zhiqiang Li contributed equally to this work.

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