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Effects of dietary live and heat-inactive baker's yeast on growth, gut health, and disease resistance of Nile tilapia under high rearing density

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ABSTRACT

In this study, the effects of baker's yeast as probiotics was evaluated in Nile tilapia reared at high density. Juvenile tilapia were distributed to tanks at high density (436 fish/m^3) and fed with basal diet (CK) or diets supplemented with live (LY) or heat-inactivated yeast (HIY). Another group of fish reared at low density (218 fish/m^3) and fed with basal diet was also included (LowCK). After 8 weeks of feeding, growth, feed utilization, gut microvilli morphology, digestive enzymes, and expressions of *hsp70* and inflammation-related cytokines in the intestine were assessed. Intestinal microbiota was investigated using 16S rRNA gene pyrosequencing. Fish were challenged with *Aeromonas hydrophila* to evaluate disease resistance. High rearing density significantly decreased the growth, feed utilization, microvilli length, and disease resistance of fish (CK versus LowCK). Moreover, the intestinal *hsp70* expression was increased in fish reared at high density, supporting a stress condition. Compared to CK group, supplementation of live yeast significantly increased gut microvilli length and trypsin activity, decreased intestinal *hsp70* expression, and enhanced resistance of fish against *A. hydrophila* (reflected by reduced intestinal alkaline phosphatase activity 24 h post infection). The gut microbiota was not markedly influenced by either rearing density or yeast supplementation. Heat-inactivated yeast (HIY) didn't display the beneficial effects observed in LY except an increase in gut trypsin activity, suggesting the importance of yeast viability and thus secretory metabolites of yeast. In conclusion, live baker's yeast may alleviate the negative effects induced by crowding stress, and has the potential to be used as probiotics for tilapia reared at high density.

1. Introduction

Tilapia is one of the most extensively cultured species in aquaculture with great economic importance, and the production of tilapia has increased intensely to meet the growing global demand for fishery products [1]. China is the biggest country in tilapia production, accounting for about 55% of the total production in the world. In aquaculture systems, high stocking densities have been used to increase productivity. However, intensive production systems of fish can cause different types of chronic stress, which can affect the physiological homeostasis, growth rate, reproductive performance, and immune system to produce fish that are more susceptible to diseases [2].

Probiotics have received much attention as novel dietary supplements in aquaculture [3–5]. Probiotics have been used in aquaculture to promote growth, modulate the immune system, inhibit pathogen, and increase stress tolerance [6–9]. Moreover, studies have demonstrated an improvement in tolerance to crowding stress by dietary probiotics in gilhead seabream [10] and Senegalese sole [11]. Baker's yeast, *Saccharomyces cerevisiae*, has been used as probiotics in various aquatic species. Beneficial effects of baker's yeast as probiotics include growth promotion, enhancement of innate immune response, as well as protection against pathogens [12–17]. In our previous study, dietary

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supplementation of baker's yeast, *Saccharomyces cerevisiae*, improved the growth performance and gut microvilli morphology of Nile tilapia reared in normal stocking density. Also, it reduced *hsp70* expression, down-regulated intestinal inflammation status, and protected the host against infection by *Aeromonas hydrophila* [18]. Moreover, in the same study, comparison of live and heat-inactivated yeast (devoid of secretory metabolites as secretion was suggested to rely on viability) showed that secretory metabolites of yeast didn't play major roles in the growth promotion and disease protection effects of yeast, while they were the major contributor toward the improved gut morphology, relieved stress status, and reduced intestinal inflammation of Nile tilapia [18]. Nevertheless, the potential role of baker's yeast in crowded stress situations has rarely been evaluated in fish, neither has the contribution of yeast secretory metabolites in fish at crowded conditions been investigated.

In this study, we investigated the beneficial effects of baker's yeast on tilapia reared in high density condition. Live baker's yeast was prepared and supplemented to basal diet. A group supplemented with heat-inactivated yeast was also incorporated to investigate the contribution of secretory metabolites of yeast in fish under high density condition. The effect of live and heat-inactivated baker's yeast on growth performance, gut morphology, digestive enzyme activity, immune response, intestinal microbiota, as well as disease resistance of Nile tilapia reared under high density was evaluated.

2. Materials and methods

2.1. Experimental diets

The formulation and chemical composition of the experimental diets (%) are presented in Table 1. The live baker's yeast was obtained as a commercial preparation Actisaf® (Lesaffre, France). Heat-inactivated yeast was prepared by incubating live yeast at 80 °C for 30 min. The inactiveness was confirmed by culturing on YPD agar, and there were no yeast colony growing on the plates. The basal diet was supplemented with live yeast or its heat-inactivated counterpart. The procedures of feed preparation have been described by Huang et al. [19]. Briefly, the dietary ingredients were blended with water (100 ml water per 1 kg diet) to form a paste which was then passed through a meat grinder equipped with a 3-mm die to obtain uniform pellets. The pelleted diets were air-dried till the moisture was lower than 10%. The levels of *S. cerevisiae* in diets supplemented with live yeast were verified by pellet homogenization, serial dilution and spreading of the dilutions on YPD plates. All the diets were stored in plastic bags at 4 °C until use. The viability of the supplemented live yeast during storage was confirmed by spread plate method described above.

Table 1
Formulations and chemical compositions of the experimental diets (%).

Ingredients	CK/LowCK	LY	HIY
Fish meal[a]	5.00	5.00	5.00
Corn gluten meal	4.00	4.00	4.00
Soybean meal	28.00	28.00	28.00
Cottonseed meal	10.00	10.00	10.00
Rapeseed meal	20.00	20.00	20.00
Wheat flour	24.00	24.00	24.00
Soybean oil	4.40	4.40	4.40
Zeolite powder	1.48	1.38	1.38
CMC[b]	0.20	0.20	0.20
Vitamin C phosphate	0.050	0.050	0.050
Mineral Premix	0.20	0.20	0.20
Vitamin Premix[c]	0.20	0.20	0.20
Lysine sulphate[d]	0.19	0.19	0.19
Calcium methionine hydroxy	0.28	0.28	0.28
$Ca(H_2PO_4)_2$	2.00	2.00	2.00
Live yeast	0	0.10	0
Heat-inactivated yeast	0	0	0.10
Crude protein	32.36	32.36	32.36
Crude lipid	6.83	6.83	6.83
Crude ash	5.50	5.50	5.50
Crude fiber	6.84	6.84	6.84

[a] Domestic fish meal. Tianshen Corporation, Tangshan, China.
[b] Carboxymethylcellulose sodium, as feed adhesive.
[c] Vitamin premix (g/kg): thiamine, 0.438; riboflavin, 0.632; pyridoxine·HCl, 0.908; *d*-pantothenic acid, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B-12, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; retinyl acetate, 0.677; cholecalciferol, 0.116; *dl*-α-tocopherol-acetate, 12.632.
[d] Mineral premix (g/kg): $CoCl_2 \cdot 6H_2O$, 0.074; $CuSO_4 \cdot 5H_2O$, 2.5; $FeSO_4 \cdot 7H_2O$, 73.2; NaCl, 40.0; $MgSO_4 \cdot 7H_2O$, 284.0; $MnSO_4 \cdot H_2O$, 6.50; KI, 0.68; Na_2SeO_3, 0.10; $ZnSO_4 \cdot 7H_2O$, 131.93; Cellulose, 501.09.

2.2. Fish and rearing conditions

All experimental and animal care procedures were approved by the Feed Research Institute of Chinese Academy of Agricultural Sciences Animal Care Committee, under the auspices of the China Council for Animal Care (Assurance # 2012 ZZGCC01). MS-222 was used as the anaesthetic. Nile tilapia, *Oreochromis niloticus* (L.), fingerlings were obtained from a local aquaculture farm in Beijing, China. The fish were acclimatized in plastic aquaria (50 × 30 × 38 cm) in a recirculation system (at 0.5 L/min) for at least 2 weeks before the feeding trial. After the acclimatization, fish with similar size (9.8 ± 0.04 g) were randomly chosen and distributed into 12 tanks at a density of 24 fish per tank (436 fish/m^3). Fish were fed with basal diet (CK) or diets supplemented with live (LY) or heat-inactivated yeast (HIY), with each diet randomly assigned to four tanks. Another 4 tanks were loaded with fish at 12 fish per tank (218 fish/m^3) and fed with basal diet (LowCK). The fish were handfed to apparent satiation three times daily (8:00, 11:30 and 17:30). The rearing conditions were same as described by Huang et al. [19]. The feeding trial was conducted for 8 weeks.

2.3. Growth performance and sampling

After 8 weeks feeding, all the fish were batch weighed and counted after 24 h starvation, and then feeding schedules continued at least 5 days before sampling. Four fish were randomly sampled from each tank and anaesthetised with MS-222 (50.0 mg/l). The intestine and head kidney were sampled. For three of the sampled fish, a piece of the midgut (~1 cm) was cut and gently agitated three times in PBS (pH 7.2) for 1 min to remove the digesta for intestinal mucosal morphology investigation; the rest of midgut was washed the same way and used for RT-PCR analysis. The hindgut was used for analysis of autochthonous and allochthonous microbiota [18]. The whole intestine from another fish was immediately frozen in liquid nitrogen and stored at −80 °C for digestive enzyme activity assay [20]. Survival rate % (SR), Weight gain (WG), Feed conversion ratio (FCR) were calculated as described previously [18].

2.4. Digestive enzyme activity in intestine

The whole gut of fish were homogenized in ice-cold PBS and centrifuged at 30,000 *g* for 30 min at 4 °C [20]. The supernatant was collected. The digestive enzyme activity (amylase, lipase, trypsin) was tested by kits following the manufacturer's specifications (Nanjing Jiancheng Bioengineering Institute, China; Kit C016-1, A054, A080-2 for amylase, lipase, and trypsin, respectively). The enzymatic activity was expressed as U/mg protein. The concentration of total protein was measured using a BCA Protein Assay Kit