

Effects of Dietary Active Yeast Preparation on Growth, Immunity, and Disease Resistance of Sea Cucumber Postprint

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Date: 2017-10-11T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary active yeast preparation on the growth, immunity, and disease resistance of sea cucumbers. A single-factor experimental design was adopted, in which six experimental diets were formulated by supplementing commercial compound feed with 0 (control), 0.025%, 0.050%, 0.100%, 0.200%, and 0.400% active yeast preparation, respectively, and fed to sea cucumbers with an average initial body weight of $(4.0 \pm 0.1)g$ for 6 weeks. Each diet had three replicates, with 15 sea cucumbers per replicate. After the 6-week culture experiment, 10 sea cucumbers were selected from each replicate, and each sea cucumber was injected with 10^6 CFU/mL. The cumulative mortality within 14 days after *Vibrio splendidus* challenge was observed and recorded. The results showed that dietary supplementation of active yeast preparation significantly improved sea cucumber growth ($P < 0.05$), with the highest specific growth rate observed at the supplementation level of 0.200%. After dietary supplementation of active yeast preparation, catalase (CAT) activity in sea cucumber coelomic fluid increased significantly ($P < 0.05$); when the active yeast preparation supplementation level was 0.050%–0.400%, lysozyme (LZM) activity in sea cucumber coelomic fluid was significantly higher than that of the control group ($P < 0.05$); when the active yeast preparation supplementation level was 0.200%–0.400%, acid phosphatase (ACP) activity in sea cucumber coelomic fluid was significantly higher than that of the control group ($P < 0.05$); when the active yeast preparation supplementation level was 0.400%, superoxide dismutase (SOD) activity in sea cucumber coelomic fluid was significantly higher than that of the control group ($P < 0.05$). Dietary supplementation of active yeast preparation reduced the cumulative mortality of sea cucumbers 14 days after challenge, with the lowest cumulative mortality observed at the supplementation level of 0.200%. Therefore, under the conditions of this experiment, dietary supplementation of active yeast preparation could promote sea cucumber growth and enhance immunity and disease resistance. Based on comprehensive analysis of sea

cucumber growth, immunity, disease resistance, and practical production conditions, the appropriate supplementation level of active yeast preparation in sea cucumber feed is considered to be 0.200%-0.400%.

Full Text

Effects of Dietary Active Yeast Products on Growth, Immunity and Disease Resistance of Sea Cucumber (*Apostichopus japonicus* Selenka)

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Abstract: This experiment investigated the effects of dietary active yeast products on the growth, immunity, and disease resistance of sea cucumber (*Apostichopus japonicus* Selenka). Using a single-factor design, six experimental diets were formulated by supplementing a commercial formula feed with 0% (control), 0.025%, 0.050%, 0.100%, 0.200%, and 0.400% active yeast products. These diets were fed to sea cucumbers with an average initial body weight of $(4.0 \pm 0.1)g$ for six weeks. Each diet had three replicates with 15 sea cucumbers per replicate. After the six-week feeding trial, 10 sea cucumbers from each replicate were selected and injected intramuscularly with *0.1mL* of *Vibriosplendidus* * suspension (1.5×10^9 CFU/mL). The cumulative mortality was observed and recorded over 14 days post-challenge. Results showed that dietary supplementation of active yeast products significantly enhanced the specific growth rate (SGR) of sea cucumbers ($P < 0.05$), with the highest SGR observed at 0.200% supplementation. Active yeast products significantly increased catalase (CAT) activity in coelomic fluid ($P < 0.05$). Lysozyme (LZM) activity was significantly higher than the control group at supplementation levels of 0.050%-0.400% ($P < 0.05$). Acid phosphatase (ACP) activity was significantly elevated at 0.200%-0.400% supplementation ($P < 0.05$). Superoxide dismutase (SOD) activity was significantly higher than the control only at 0.400% supplementation ($P < 0.05$). Active yeast products reduced the cumulative mortality of challenged sea cucumbers after 14 days, with the lowest mortality occurring at 0.200% supplementation. These findings indicate that dietary active yeast products can promote growth and enhance immunity and disease resistance in sea cucumbers. Based on comprehensive analysis of growth performance, immune responses, disease resistance, and practical production considerations, the optimal dietary supplementation level of active yeast products for sea cucumbers is 0.200%-0.400%.

Keywords: active yeast products; sea cucumber; growth; immunity; disease resistance

Introduction

Apostichopus japonicus belongs to the phylum Echinodermata, class Holothuroidea, and genus *Apostichopus*. Widely distributed along the Asian coast between 35°N and 44°N, it is a typical temperate species. Sea cucumbers are rich in mucopolysaccharides, collagen, various trace elements, and vitamins essential for human health. They are reputed to improve intelligence and vision, nourish the kidneys, delay aging, prevent arteriosclerosis, exhibit anti-tumor properties, and enhance human immunity, earning them recognition as the foremost delicacy among seafood products. Due to their exceptional nutritional, medicinal, and economic value, market demand for sea cucumbers has continuously increased, driving rapid development of the aquaculture industry.

With the rapid expansion of high-density sea cucumber farming and deteriorating aquaculture environments, disease outbreaks have become frequent and represent a major constraint to the healthy and sustainable development of the industry. Sea cucumber diseases are diverse, highly damaging, and often occur suddenly. Antibiotic-based control measures lead to bacterial resistance and drug residues, posing significant risks to product quality and safety. To address these challenges, developing green antibiotic alternatives and immunostimulants to enhance immunity and disease resistance has become a priority in sea cucumber disease prevention and control, representing a current research hotspot.

Active yeast products, commonly used as probiotic feed additives, are widely applied in ruminant, livestock, and aquaculture production. Yeast cells are extremely rich in protein and contain 16 amino acids, 14 minerals, 17 vitamins (excluding vitamins A, C, and E), and trace elements such as selenium, iron, zinc, and chromium. Yeast cell walls are primarily composed of carbohydrates (35%–60%), mainly yeast polysaccharides including mannan oligosaccharides and glucans. Yeast also contains various pigments and unidentified growth-promoting factors, making it an excellent nutritional source for farmed animals. These special nutrients can stimulate immune system defenses, reduce production of intestinal toxic substances, and enhance immune function. However, research on the effects of active yeast products on sea cucumber growth, immunity, and disease resistance has not been reported to date.

Therefore, this study aimed to investigate the effects of different dietary levels of active yeast products on the growth, immunity, and disease resistance of sea cucumbers, providing a scientific basis for the application of active yeast products in formulated feeds for echinoderms.

Materials and Methods

1.1 Materials The feeding trial was conducted at the Aoshanwei Laboratory of the Germplasm Resources Research Center, Marine Biology Institute of Shandong Province, from October to November 2015. Healthy juvenile sea cucumbers bred by the center, with an initial body weight of $(4.0 \pm 0.1)g$, were used in the experiment. The commercial formula feed was purchased from Qingdao Longger Biotech Co., Ltd., containing inactive yeast cells and fermentation products with a viable count of 1.0×10^{10} CFU/g. The *Vibrio splendidus* used in the challenge test was provided by the Shandong Provincial Key Laboratory of Mariculture Disease Prevention and Control.

1.2 Experimental Design and Management Sea cucumbers were randomly divided into six groups with three replicates per group (15 individuals per replicate). Each replicate was reared in a 45 cm \times 35 cm \times 30 cm plastic container for six weeks. The control group was fed the commercial formula feed, while five treatment groups received feeds supplemented with 0.025%, 0.050%, 0.100%, 0.200%, and 0.400% active yeast products, respectively. Diets were thoroughly mixed before feeding. Sea cucumbers were fed once daily at 16:00. During the trial, water salinity was maintained at 30.2–31.4, pH at 8.1 ± 0.2 , and temperature at 17–20 °C. Continuous micro-aeration was provided, and water was completely exchanged daily with temperature and salinity differences of less than 0.5 °C and 0.5, respectively.

1.3 Sample Collection and Analysis Before the experiment, feeding was stopped for one day, and initial body weight was measured after blotting surface moisture with absorbent paper to avoid measurement errors. After six weeks, feeding was again stopped for one day, and final body weight and survival were recorded to calculate specific growth rate (SGR). Five sea cucumbers were randomly sampled from each container to collect coelomic fluid for immune parameter analysis. Activities of superoxide dismutase (SOD), catalase (CAT), acid phosphatase (ACP), and lysozyme (LZM) in coelomic fluid were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer's instructions with a spectrophotometer. The remaining sea cucumbers in each group were used for the challenge test.

1.5 Calculation Formulas Survival rate, specific growth rate, and cumulative mortality were calculated as follows:

- Survival rate (%) = $100 \times N_t/N_0$
- Specific growth rate (%/d) = $100 \times (\ln W_t - \ln W_0)/t$
- Cumulative mortality (%) = $100 \times D_t/D_0$

Where N_0 and N_t represent initial and final numbers of sea cucumbers; W_0 and W_t represent initial and final body weights; t represents experimental days; and D_0 and D_t represent initial and cumulative dead individuals during challenge.

1.6 Statistical Analysis Data are expressed as mean \pm standard error (n=3). One-way ANOVA and Tukey's test were used for statistical analysis, with $P < 0.05$ considered statistically significant.

Results

2.1 Effects on Growth Performance As shown in Table 2, after six weeks, all five treatment groups exhibited significantly higher specific growth rates (SGR) compared to the control group ($P < 0.05$). The SGR increased with supplementation levels from 0.025% to 0.200%, reaching its maximum at 0.200% (3.06 times that of the control). When supplementation increased to 0.400%, SGR decreased slightly but remained 2.50 times higher than the control. Survival rates were 100% in both control and treatment groups. These results demonstrate that dietary supplementation with 0.025%-0.200% active yeast products significantly improves SGR and effectively promotes growth in sea cucumbers.

2.2 Effects on Immune Parameters in Coelomic Fluid Immune parameters in coelomic fluid were measured after six weeks of feeding (Table 3). SOD activity increased at 0.025%-0.200% supplementation but showed no significant difference from the control ($P > 0.05$), while 0.400% supplementation resulted in the highest SOD activity, significantly higher than the control ($P < 0.05$). CAT activity was significantly elevated in all treatment groups ($P < 0.05$), with the highest activity (3.67 U/mL) observed at 0.400% supplementation (1.96 times the control). ACP activity showed no significant difference at 0.025%-0.100% ($P > 0.05$) but was significantly higher at 0.200%-0.400% ($P < 0.05$), peaking at 1.04 U/dL in the 0.400% group. LZM activity did not differ significantly at 0.025% ($P > 0.05$) but was significantly elevated at 0.050%-0.400% ($P < 0.05$), reaching 0.5 g/mL in the 0.400% group. These findings indicate that active yeast products at 0.025%-0.400% can enhance SOD, CAT, ACP, and LZM activities in sea cucumber coelomic fluid.

2.3 Effects on Disease Resistance Following artificial challenge with *Vibrio splendidus*, control group sea cucumbers began showing disease symptoms after four days, including reduced activity, body shrinkage, swollen mouthparts, and visceral ejection. Ulcers appeared on the abdomen after five days, progressing to extensive epidermal necrosis with mucus secretion by day seven, ultimately leading to death. Treatment groups showed delayed onset and fewer affected individuals. Mortality in the 0.025% group began after eight days, with deaths increasing daily thereafter. After 14 days, the control group cumulative mortality reached 56.67%, significantly higher than all treatment groups. Among treatments, 0.025% supplementation showed the highest mortality (30.00%), while 0.200% supplementation resulted in the lowest mortality (6.67%) (Figure 1 [Figure 1: see original paper]). These results demonstrate that dietary active yeast products effectively enhance resistance to *Vibrio splendidus* infection,

reducing both morbidity and mortality.

Discussion

3.1 Effects on Growth Yeast cells are rich in protein, organic matter, and minerals, containing 16 amino acids, 14 minerals, 17 vitamins (excluding A, C, and E), and trace elements such as selenium, iron, zinc, and chromium. Yeast cell walls contain 35%-60% carbohydrates, primarily yeast polysaccharides (mannan oligosaccharides and glucans), along with various pigments and unidentified growth-promoting factors, making yeast an excellent nutritional source. Numerous studies have demonstrated that yeast-based feed additives effectively promote growth in aquatic animals, significantly improving specific growth rates and reducing feed conversion ratios in grass carp, flounder, catfish, gibel carp, and Pacific white shrimp. Research has also shown that dietary yeast selenium significantly enhances sea cucumber SGR, while active dry yeast promotes growth in Chinese mitten crabs. The present study confirms that 0.025%-0.400% active yeast products significantly improve sea cucumber SGR. This effect may be attributed to the comprehensive nutritional profile and growth-promoting factors in yeast products, enhanced digestive enzyme activities (amylase, protease, lipase) improving nutrient absorption, and probiotic effects where yeast competes with pathogenic microorganisms for oxygen and nutrients, inhibits pathogen adhesion to intestinal mucosa, maintains intestinal health, and reduces disease occurrence.

3.2 Effects on Immunity Sea cucumbers possess both cellular and humoral immunity, with non-specific immune parameters playing irreplaceable roles in defense against pathogens. CAT and SOD are primary antioxidant enzymes that scavenge reactive oxygen species and protect against oxidative damage, playing crucial roles in anti-aging and biomolecular protection. The present study found that 0.025%-0.400% active yeast products significantly increased CAT activity, with SOD activity significantly elevated only at 0.400% supplementation, indicating enhanced antioxidant capacity. ACP is a marker enzyme of macrophage lysosomes that hydrolyzes phosphate esters on foreign surfaces under acidic conditions, modifying bacterial surface molecules to accelerate phagocytosis and clearance, thus holding biological significance in disease resistance and immune response. ACP activity was higher in all treatment groups, with significant differences at 0.200%-0.400% supplementation, suggesting that appropriate yeast supplementation enhances phosphorus metabolism and growth while improving phagocytic clearance of foreign substances. LZM is an alkaline protease widely present in tissues and body fluids that dissolves bacteria or acts as an opsonin, representing a major component of non-specific immunity in invertebrates. Previous studies have demonstrated that dietary active dry yeast increases serum LZM activity in Chinese soft-shelled turtles and flounder. The present study similarly found that 0.025%-0.400% active yeast products enhanced LZM ac-

tivity in sea cucumber coelomic fluid, with significant differences at 0.050%-0.400% supplementation.

These results indicate that active yeast products effectively enhance non-specific immunity in sea cucumbers, likely due to β -glucans and mannan oligosaccharides. β -glucans can directly bind and activate macrophages, enhancing phagocytosis and cytokine release. Mannan oligosaccharides recognize, adhere to, and exclude pathogenic microorganisms, interfering with colonization of harmful bacteria in the intestine, reducing bacterial diseases, increasing cytokine release, and adsorbing toxins. Dietary β -glucans and mannan oligosaccharides have been shown to effectively improve immunity and disease resistance in aquatic animals.

3.3 Effects on Disease Resistance Studies have demonstrated that yeast-derived feed additives positively impact disease resistance in aquatic animals. Research on Chinese soft-shelled turtles and mitten crabs has shown that dietary active dry yeast enhances disease resistance. Artificial pathogen challenge is a common method for evaluating disease resistance, and *Vibrio splendidus* is the causative agent of skin ulcer syndrome in sea cucumbers, posing a serious threat to aquaculture. The challenge test results showed that active yeast products significantly reduced cumulative mortality in sea cucumbers, indicating enhanced resistance to *Vibrio splendidus* infection. This effect correlates with improved non-specific immune parameters, suggesting that yeast products enhance pathogen resistance by boosting immunity. The lowest mortality (6.67%) was observed at 0.200% supplementation.

In conclusion, dietary active yeast products improve specific growth rate, enhance LZM, SOD, CAT, and ACP activities in coelomic fluid, and effectively increase immunity and disease resistance in sea cucumbers, demonstrating potential as a safe and efficient feed additive. Based on comprehensive analysis of growth, immunity, and disease resistance, the optimal dietary supplementation level of active yeast products for sea cucumber culture is 0.200%-0.400%.

References

- [1] LI Chenglin, SONG Aihuan, HU Wei, et al. Analysis of current status and sustainable development strategies of sea cucumber aquaculture industry in Shandong Province[J]. *Progress in Fishery Sciences*, 2010, 31(4): 126-133.
- [2] WANG Jiqiao, TIAN Xiangli. *Advances in Sea Cucumber Aquaculture Biology*[M]. Beijing: Ocean Press, 2012: 3-13.
- [3] WANG Yingeng, RONG Xiaojun, LIAO Meijie, et al. *Solutions for Healthy Sea Cucumber Culture and Disease Prevention*[M]. Beijing: China Agriculture Press, 2014: 1-2.

- [4] YANG Hongsheng, ZHOU Yi, ZHANG Tao. *Sea Cucumber Biology: Theory and Practice*[M]. Beijing: Science Press, 2014: 8-9.
- [5] HU Youjun, LIN Yingcai, ZHENG Li, et al. Effects of active yeast on growth performance and immune function in early-weaned piglets[J]. *Chinese Journal of Animal Nutrition*, 2003, 15(4): 49-53.
- [6] WANG Ling, PU Wanxia, ZHAXI Yingpai, et al. Effects of active yeast preparation on diarrhea rate, growth performance and intestinal pH in early-weaned piglets[J]. *Chinese Journal of Veterinary Drug*, 2008, 42(9): 1-5.
- [7] NAN Ruiqing. Effects of active yeast and amylopectin on growth and blood physiological-biochemical indices in calves[D]. Master's thesis. Jinzhong: Shanxi Agricultural University, 2013.
- [8] QIU Yan. Effects of three microecological preparations on growth performance, physiological function and intestinal mucosa of grass carp (*Ctenopharyngodon idellus*)[D]. Master's thesis. Suzhou: Soochow University, 2010.
- [9] WEN Jun. Effects of compound probiotics and yeast culture on growth, immunity and disease resistance of flounder (*Paralichthys olivaceus*)[D]. Master's thesis. Qingdao: Ocean University of China, 2007.
- [10] ROBINETTE H R, YOUNG C, MENG Fanyi. Effects of yeast culture on growth performance of channel catfish (*Ictalurus punctatus*) fingerlings[J]. *Feed Industry*, 2011, 32(6): 25-27.
- [11] XU Lei, LIU Bo, XIE Jun, et al. Effects of yeast culture on growth, blood biochemistry and immunity of gibel carp (*Carassius auratus gibelio*)[J]. *Jiangsu Agricultural Sciences*, 2010(6): 371-374.
- [12] SU Xionggao, LI Xiaoqin, LENG Xiangjun, et al. Effects of yeast culture and *Bacillus* on growth, protease activity and immune performance of Pacific white shrimp (*Litopenaeus vannamei*)[J]. *Marine Fisheries*, 2012, 34(2): 168-176.
- [13] ZHANG Qin, MAI Kangsen, ZHANG Wenbing, et al. Effects of dietary selenium yeast and vitamin E on growth, immunity and disease resistance of sea cucumber (*Apostichopus japonicus*)[J]. *Chinese Journal of Animal Nutrition*, 2011, 23(10): 1745-1755.
- [14] CHEN Changfu, YAO Juan, XIAO Zhimeng, et al. Effects of active dry yeast on growth and disease resistance of Chinese mitten crab (*Eriocheir sinensis*)[J]. *Journal of Yangtze University (Natural Science Edition)*, 2005, 2(5): 24-26.
- [15] LIAO Canqing, CHEN Zhongping, WANG Jianlin, et al. Effects of active yeast and its culture on growth performance and immune function in piglets[J]. *China Feed*, 2015(13): 42-43.
- [16] BAI Xiaoting. Research and application of yeast products in feed[J]. *China Feed*, 2005(2): 8-10.

- [17] WANG Jiqiao, WANG Zhixiang, ZHANG Kai, et al. Effects of dietary methionine selenium on survival, growth and immune indices of juvenile sea cucumber (*Apostichopus japonicus*)[J]. *Journal of Dalian Ocean University*, 2012, 27(2): 110-115.
- [18] QIN Bo, CHEN Siqing, CHANG Qing, et al. Effects of dietary cellulase on growth performance, digestive capacity and non-specific immunity of juvenile sea cucumber (*Apostichopus japonicus*)[J]. *Chinese Journal of Animal Nutrition*, 2014, 26(9): 2698-2705.
- [19] SONG Jian, HE Zhou, CHENG Long, et al. Effects of dietary alfalfa meal on growth, body composition and immune enzymes of juvenile sea cucumber (*Apostichopus japonicus*)[J]. *Acta Prataculturae Sinica*, 2015, 24(2): 208-214.
- [20] LIU Zongying. Effects of active dry yeast on non-specific immune function and disease resistance of aquatic animals[D]. Master's thesis. Wuhan: Huazhong Agricultural University, 2005.
- [21] XU Hejin, FENG You, LIU Ding, et al. β -glucan and its application in shrimp production[J]. *Feed Review*, 2013(3): 28-31.
- [22] FENG You, ZHONG Wenbiao, XU Hejin, et al. Application of mannan oligosaccharide in aquatic animal production[J]. *Feed Review*, 2013(5): 51-54.
- [23] YU H H, HAN F, XUE M, et al. Efficacy and tolerance of yeast cell wall as an immunostimulant in the diet of Japanese seabass (*Lateolabrax japonicus*)[J]. *Aquaculture*, 2014, 432: 217-224.
- [24] MESHARAM S J, MURTHY H S, ALI H, et al. Effect of dietary β -glucan on immune response and disease resistance against *Aeromonas hydrophila* in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879)[J]. *Aquaculture International*, 2015, 23(2): 439-447.
- [25] TORRECILLAS S, MAKOL A, CABALLERO M J, et al. Effects on mortality and stress response in European sea bass, *Dicentrarchus labrax* (L.), fed mannan oligosaccharides (MOS) after *Vibrio anguillarum* exposure[J]. *Journal of Fish Diseases*, 2012, 35(8): 591-602.
- [26] NIE Qin, YANG Fan, YI Jianhua, et al. Application of yeast-derived biological feed in aquaculture[J]. *China Feed*, 2015(15): 35-40.
- [27] YU Huanhuan, ZHOU Wenhao, ZENG Hong, et al. Research progress on application and mechanism of yeast culture in aquatic animals[J]. *Feed Industry*, 2015, 36(18): 25-29.

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