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Original Article

Effect of maitake mushroom *Grifola frondosa* on the growth performance of post-larvae of white leg shrimp, *Litopenaeus vannamei* infected with *Vibrio harveyi*

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Abstract

This study was conducted to evaluate the supplementation effect of maitake mushroom, *Grifola frondosa* in diet on growth and survival of white leg shrimp, *Litopenaeus vannamei* post larvae in a closed recirculating culture system. The experimental shrimps were fed with control diet (D-Con) and treatment diet that was supplemented with 2% of dried maitake mushroom powder (D-MM) which contained 336 mg of β-glucan / 100 g diet for one week before they were artificially infected with *Vibrio harveyi* at a concentration of 1.0 x 10^7 CFU/*ml* and thereafter for another 3 weeks. The results revealed that the percentage of body weight gain, daily growth index and survival rate were higher and feed conversion ratio was better in shrimps fed with D-MM diet than those fed with D-Con diet. Moreover, the number of *Vibrio* spp. in the hepatopancreas of shrimp fed the D-MM diet was significantly lower ($P < 0.05$) than that of the D-Con diet group. This study suggests that the addition of dried maitake mushroom powder in the diet can improve the growth, survival rate and feeding performance of the white leg shrimp post larvae and also helped to control the number of *Vibrio* spp. at minimal in the animals.

Keywords: *Litopenaeus vannamei*; maitake mushroom, *Grifola frondosa*; *Vibrio* spp.

1. Introduction

Maitake, *Grifola frondosa* is an edible mushroom that can be found widely growing in clusters at the bottom of trees throughout the temperate regions including Japan, China, Europe, Canada, United States and Australia [1]. Currently, it is artificially mass-cultivated in in-door facilities. Maitake are rich in minerals (calcium, potassium, and magnesium), vitamins (B1, B2, C, D) and Niacin. They also contain fibers, lipids and amino acids which are important for physiological functions.

Maitake mushroom is one of the major popular mushrooms in Japan and it is found to contain β-glucan [2]. Both the Japanese and Chinese communities use the mushroom in traditional medicine to enhance immune system. Maitake mushroom has been reported effective in regulating blood glucose, blood pressure and insulin level [3,4] hence beneficial for diabetic patients [5,6]. Recently, maitake mushroom has been widely reported useful in the treatment of cancer patients [7, 8, 9, 10]

In aquaculture, β-glucan has been reported to enhance immune system of culture animals against many bacterial diseases. Figueras *et al.* [11] have reported that the β-glucans from yeast (*Saccharomyces cerevisae*) was effective in improving the immune response of turbot (*Scophthalmus maximus*) against *Vibrio damsela*. It was also noticed that oral administration of β-glucan improved the growth and resistance of *Penaeus monodon* to *Vibrio alginolyticus* [12].

Moreover, β-glucan was found to enhance antibody response of Atlantic salmon (*Salmo salar*) to *Aeromonas salmonicida* [13]. In this paper, we report the effects of maitake mushroom supplementation in diet which contained β-glucan on the growth and survival of white leg shrimps post larvae, *Litopenaeus vannamei* infected with *Vibrio harveyi*.

2. Materials and Methods

Two experimental diets were prepared; a control diet (D-Con) without maitake mushroom and a treatment diet supplemented with 2% of freeze-dried maitake mushroom powder (D-MM) which contained 336 mg of β-glucan/100 g of diet. These diets were produced by Higashimaru Co., Ltd in Kagoshima, Japan, while freeze-dried maitake mushroom powder was supplied by Maitake Yukiguni Co., Ltd. Nigata, Japan. The composition and proximate

Table 1. Composition and proximate analysis of the experimental diets (%)

Ingredients	D-Con	D-MM
Squid meal	44.0	44.0
Brown fish meal	23.0	23.0
Dextrin	8.0	8.0
Pollack liver oil	4.5	4.5
Corn starch	3.0	3.0
Green algae powder	2.0	0.0
Cholesterol	1.0	1.0
n-3HUFA	0.5	0.5
Soybean lecithin	1.0	1.0
Mineral mix*1	4.0	4.0
Vitamin mix*2	2.4	2.4
Activated gluten	5.0	5.0
Na-citrate	0.3	0.3
Na-succinate	0.3	0.3
Attractant*3	1.0	1.0
Dried maitake mushroom*4	0.0	2.0
Total	100.0	100.0
Proximate composition (% dry weight)		
Crude protein	49.8	49.9
Crude fat	14.6	14.6
Crude ash	10.2	10.2
Moisture	9.9	9.8

*1 K_2HPO_4, 0.9; $Ca_3(PO_4)_2$, 1.3; $MgSO_4$-$7H_2O$, 1.4; Na_2HPO_4-$2H_2O$ 0.4 (g/100g diet).
*2 p-aminobenzoric acid, 27.06; biotin, 1.08; inositol, 1082.43; niacin, 108.24; Ca-panthothenate, 162.37; pyriodoxine-HCl, 32.48; riboflavin, 21.64; thiamine-HCl, 10.82; menadione,10.85; β cartene, 25.98; α tocopherol, 54.12; calciphero 3.23; cyanocobalamine,0.23; folic acid, 2.17; choline chloride,1621.37; L-ascorbyl-2-phosphate Mg, 44.94 (mg/100g diet).
*3 proline, 0.2; alanine, 0.2; betanine, 0.3; glutathione, 0.1; glutamate-Na, 0.4 (mg/100g diet).
*4 beta-glucan, 16.8 g/100g dried maitake mushroom powder

Table 2. Composition of dried maitake mushroom powder

Composition	Dry weight
Moisture	2.3%
Protein	19.3%
Lipid	2.6%
Fiber	13.9%
Ash	4.8%
Glucide	57.1%
β -glucan	16.8%
Phosphorus	556mg/100g
Iron	2.06 mg/100g
Calcium	2.2 mg/100g
Sodium	2.3 mg/100g
Potassium	2.26%
Magnesium	91.5 mg/100g
Retinol	Not detected (<0.01 mg/100g)
Total carotene	Not detected (<0.02 mg/100g)
Thiamine (Vitamin B1)	0.82 mg/100g
Riboflavin (Vitamin B2)	1.2 mg/100g
Total ascorbic acid (Vitamin C)	Not detected (<1 mg/100g)
Vitamin D	1370 IU/100g
Niacin	45.1 mg/100g
Lead	Not detected (<0.05 ppm)
Zink	48.4 ppm

analyses of the D-Con and D-MM diets are presented in Table 1. Both diets contained about 49.8% and 14.6% of dietary protein and lipid, respectively. Meanwhile, the compositions of freeze-dried maitake mushroom powder are given in Table 2.

The post-larvae of shrimp (PL5) used in the experiment were obtained from a private shrimp hatchery in Kota Kinabalu, Sabah, Malaysia. The shrimps were conditioned in an aerated 500 L tank between 26 and 28°C for 2 weeks prior to the experiment. During this conditioning period, the shrimps were fed with the D-Con diet twice a day at 10% of body weight. Then healthy shrimp (initial body weight 0.022±0.000g) were distributed randomly into aquarium at the stocking density of 50 individuals per aquarium and reared in closed recirculating culture system. The D-Con and D-MM diets were fed to triplicate groups of shrimp twice a day at 10% of total biomass. One week after feeding with the experimental diets, the shrimps from each aquarium were artificially infected with pathogenic *Vibrio harveyi* strain VHJR7 [14]. The artificial infection was conducted by immersing the shrimps in 1.0 x 10^7 CFU/*ml* of bacterial suspension for 40 minutes. After the immersion, the shrimps were removed and returned to respective aquarium and continued rearing for another 3 weeks..

The recirculating culture system used consisted of 12 L aquarium for shrimp rearing that was connected to an 8L filter aquarium. Pondprotect® (Novozymes Biologicals Inc., USA) consisted of two nitrifying bacteria (*Nitrosomonas eutropha* and *Nitrobacter winogradski*) was introduced into each filter aquarium at the rate of 0.3 mg/L at the beginning of the study. Excess feed and feces were removed by siphon before each feeding.

During the experiment, shrimps in each group were individually weighed every 7 days. At the end of the experiment, five shrimps were sampled from each aquarium to examine the present of *Vibrio* spp. in the shrimp. After rinsing with sterilized seawater, the hepatopancreas of the shrimp was removed and homogenized using pestle and mortar. The homogenate was diluted up to 10^{-7} dilution using sterilized PBS (pH 7.4). Then, 0.1 ml of the dilution was aseptically spread onto thiosulfate citrate bile salts agar plates and incubated at 37℃. Bacterial colonies formed were then counted and recorded after 48 hours of incubation.

Salinity, water temperature and pH of each aquarium were measured daily using refractor meter (New S-100, ATAGO, Japan) and pH meter (TPX-90, Tokoh Chemical, Japan). Salinity was controlled at 30 psu with the addition of distilled water once a week. Ammonia nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and dissolved oxygen (DO) levels were analyzed every two weeks using HACH methods (methods 8155, 8507, 8192; DR2000, HACH, USA) and DO meter (HI9828 HANNA, Italy), respectively.

All experimental data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (SPSS Version 17 software, SPSS Inc., Chicago, IL).

Table 3. The growth, survival and feeding performance of *L. vannamei* fed with control (D-Con) and maitake mushroom supplemented diet (D-MM)

Growth performance	D-Con	D-MM
Initial body weight (BW)(g)	0.022 ± 0.000	0.022 ± 0.000
Final BW (g)	0.20 ± 0.004	0.23 ± 0.028
Body weight gain (%)*[1]	825.76 ± 18.37	957.58 ± 127.79
Daily growth index (%)*[2]	0.654 ± 0.01	0.75 ± 0.10
Feed conversion ratio*[3]	1.98 ± 0.64	1.85 ± 0.18
Survival rate (%)*[4]	67.77 ± 15.75	89.00 ± 8.54

Values are means ± standard deviation (n = 3)
*[1] Body weight gain (%) = (Final BW (g) - Initial BW (g)) / Initial BW (g) x 100
*[2] Daily growth index (%) = (Final BW (g) - Initial BW (g)) / days x 100
*[3] Feed conversion ratio = Feed consumed (g) / wet weight gained (g)
*[4] Survival rate (%) = Final number of shrimp/Initial number of shrimp x 100

3. Results

The growth, survival and feeding performance of *L. vannamei* shrimp fed with the D-Con and D-MM diets are shown in Table 3. This study showed that the percentage of weight gain, daily growth index and survival rate were higher, and feed conversion ratio was better in shrimps fed with D-MM diet than the shrimp fed with D-Con diet. Furthermore, the number of *Vibrio* spp. in the hepatopancreas was significantly lower ($P< 0.05$) in the D-MM group than in the D-Con group (Figure. 1). However, there was no significant difference on the number of *Vibrio* spp. in the rearing water in both treatment and control groups. The water temperature, pH, salinity, NH_3-N, NO_2-N, NO_3-N and DO in both groups over the 28 days was ranged 24.4-26.8℃, 8.18-8.55, 30-34 psu, 0.02-0.07 ppm, 0.035-0.262 ppm, 0.2-13.8 ppm and 5.0-6.7 ppm, respectively.

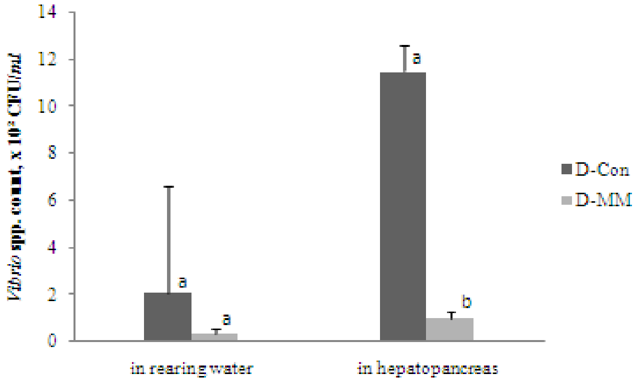

Figure 1. *Vibrio* spp. count in the rearing water and in the hepatopancreas of *L. vannamei* fed with control diet (D-Con) and maitake mushroom diet (D-MM) after 28 days of experiment.

Same alphabet within the same type of sample is not significantly different ($P> 0.05$).

4. Discussion

The use of maitake mushroom in aquaculture is still scarce. The β-glucans contained in the mushroom may provide excellent stimulatory effects to aquatic animals. Figueras *et al.* [11] have reported that the β-glucans from yeast (*Saccharomyces cerevisae*) were effective in improving the immune response of turbot (*Scophthalmus maximus*) against *Vibrio damsela*. It was also reported that oral administration of β-glucan improved growth and resistance of *Penaeus monodon* against *V. alginolyticus* [12]. Bao *et al*. [15] and Encarnacion *et al*. [16] reported that β-glucan and ergothioneine extracted from edible mushroom *Flammulina velutipes* were shown effective in preventing shrimp meat from becoming black due to bacterial propagation. Adachi *et al.* [17] also reported that β-glucan and ergothioneine contained in *F. velutipes* enhanced immune system of Kuruma shrimp, *Marsupenaeus japonicus*.

The nutrient composition of the freeze-dried maitake mushroom powder used in this study showed that it contained 168 mg/g of β-glucan in the form of non-digestible polysaccharide. This could be the reason behind the high survival of *L. vannamei* against *V. harveyi* infection in this study. The low number of bacterial count in the hepatopancreas of the shrimps received mushroom supplemented feed than in the control shrimps also suggests the beneficial effects of maitake mushroom. We believed that these beneficial effects must have been contributed by the high amount of β-glucan contained in the mushroom.

It was also observed that the shrimps fed with D-MM diet exhibited improved growth than the shrimps received the D-Con diet. This was similar to the finding of Bai *et al.* [18] and López *et al.* [19] and agreed with the studies in several fish species [20] and shrimps [21]. It is not clear how β-glucan improves growth but it was suggested that it induces local intestinal inflammatory response and increase resistance against pathogens [18, 22]. According to Hai and Fotedar [23], *P. latisulcatus* fed with β-1,3-D-glucan exhibited larger intestine surface which enable the shrimp absorbed more nutrients than shrimps fed with β-1,3-D-glucan free diet. The β-glucan was also reported to enhance the growth of white spot syndrome virus (WSSV)-infected black tiger prawn, *P. monodon* [24]. In this study shrimps fed with mushroom-supplemented diet exhibited high survival rate than the shrimps fed with mushroom-free diet. This could have been the result of immunostimulatory effect of β-glucan contained abundantly in the maitake mushroom. β-glucan also has been reported to enhance the haemocyte phagocytic activity, cell adhesion and superoxide anion production in black tiger prawn brooders [25].

β-glucan is not only advantageous to aquaculture animals but have also reported beneficial to cancer patients [26, 27]. Konno [8] proposed that the β-glucan from maitake mushroom can inhibit or destroy cancer cells. Moreover, studies have also shown that maitake mushroom helps control blood sugar, high blood pressure, reduce cholesterol and increase insulin sensitivity [3,4].

5. Conclusions

In this present study, maitake mushroom supplement in diet promoted the growth and survival of white leg shrimp, *L. vannamei* in a recirculating culture system. This mushroom supplemented diet also enhanced the resistance of the shrimp against pathogenic *V. harveyi* infection.

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