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Short communication

Effects of dietary β − 1,3 − glucan on the growth, survival, physiological and immune response of marron, *Cherax tenuimanus* (smith, 1912)

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ABSTRACT

Six isonitrogenous and isocalorific diets supplemented with five different levels of beta −1,3 − glucan (0.08%, 0.1%, 0.2%, 0.4% and 0.8%) were formulated and tested for marron (*Cherax tenuimanus*) growth, survival, organosomatic indices, osmoregulatory capacity and immunological parameters (total and differential haemocyte counts, haemolymph clotting time and bacteraemia). The sixth diet without any beta − 1,3 − glucan was used as a control. Each diet was provided to 18 marron (0.47 ± 0.02 g initial weight) replicated 3 times in individual 250 L fiberglass cylindrical tanks. Each tank was provided with a biological filtration recirculating water system. After 84 days of culture, the survival and yield were higher in the marron fed 0.1% beta glucan supplemented diet. The different levels of beta glucan did not alter any of the physiological parameters of marron. However, dietary supplementation with beta glucan resulted in significantly higher ($P < 0.05$) total haemocyte count (THC) and granular cells. The bacteraemia rank was lower in all diets having beta glucan supplemented with more than or equal to 0.1% compared to the control and 0.08% beta glucan supplemented diets. Results suggest that dietary beta − 1,3 − glucan at a minimum concentration of 0.1–0.2% can improve the immune system of marron.

1. Introduction

Microbial disease is a major threat to the sustainability of aquaculture [1]. For microbial pathogen resistance, invertebrates are entirely dependent on non-specific immune mechanisms to cope with infection as they lack the specific immunological "memory" that is found in fish and warm-blooded animal [2]. The use of specific biological compounds (immunostimulants) that enhance immune responses of the target organisms, rendering animals more resistant to disease may be an excellent preventive tool against infections by pathogenic organisms [3].

The distribution of marron (*Cherax tenuimanus*) in Western Australia has been extended as far east as Esperance and as far north as Geraldton and global interest in marron farming has led to the species being introduced into South Africa, Zimbabwe, Japan, USA, China and the Caribbean as well as several Australian states [4]. Like all freshwater crayfish, marron can have several small epibionts attached to their exoskeleton and gills. Two such epibionts are *Epistylis* and *Temnocephala*. These are symptomatic of poor water quality and result in poor growth rates, particularly in unaerated ponds containing excessive organic matter. This infection reduced appeal of marron to consumer [4]. Although there is no report on the losses in marron aquaculture caused by bacterial infection, the threat to consumers of organism infected marron needs to be considered.

Beta − 1,3 − glucan, soluble carbohydrates from the cell walls of yeast *Saccharomyces cerevisiae,* is known to have a potent stimulatory effect on the immune system of mammals, fish and crustaceans. In aquaculture, β − 1,3 − glucan have successfully been used to enhance the resistance of fish and crustacean against bacterial or viral infection [5]. In culture of crustaceans, the addition of beta glucan through diet significantly enhanced the survival and the resistance of postlarvae; juveniles [6,7] and adult of *Penaeus monodon* [8] to *Vibrio damsela*, *Vibrio harveyi* and white spot syndrome virus infection. Survival and the responses against vibriosis infection of *Macrobrachium rosenbergii* postlarvae are also improved by applying β − 1,3 − glucan [9]. However, the effect of this compound on the growth performance and immune response of freshwater crayfish have not been investigated and there is a need to verify the dosage rate and application strategy for freshwater crayfish for the most effective application of β − 1,3 − glucan. In addition to traditional performance indicators of feed additives such as growth and survival, measures of crustacean health can include physiological parameters such as organosomatic indices, moisture content and osmoregulartory capacity [10]. Moreover, immune–physiological parameters such as total haemocyte count (THC), proportion

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of granular cells, bacteraemia and haemolymph clotting time have also been used as indicators of immunological and health status of crustacean [11–15]. The aim of this experiment was to determine the suitable levels of dietary β − 1,3 − glucan by investigating the effects of dietary supplementation of various levels of β − 1,3 − glucan on the survival, growth, physiological and immunological parameters of marron.

2. Materials and methods

2.1. Test diets

Beta glucan (BG) (Beta-Mune™ brand, 100% German long chain Beta −1,3 − D − Glucan) was tested at five supplemented level of 0.08% (D2), 0.1% (D3), 0.2% (D4), 0.4% (D5) and 0.8% (D6) against a control diet (D1). All feed ingredients and supplements used in this trial were supplied by Specialty Feeds Pty Ltd, Western Australia. Proximate compositions of the ingredients and supplements were used as a basis to formulate the all diets using the software FeedLIVE version 1.52 (Table 1).

2.2. Experiment design

Juvenile marron (0.47 ± 0.02 g total weight and 7.47 ± 0.25 mm carapace length) were supplied by Aquatic Resource Management Pty Ltd, Western Australia and transported to the Curtin Aquatic Research Laboratory (CARL). The marron were placed in the tanks provided with aerated, recirculating filtered freshwater and acclimated to the culture conditions for 2 weeks. During the acclimation period, the marron were fed with a commercial diet supplied by Enviroplus, Australia (26% protein, 47–50% carbohydrate, 9% fats and 8.9% ash) at the rate of 3% body weight per two days.

Plastic cylindrical tanks (800 mm diameter, 500 mm high, 250 L capacity) were used for the experiment. Sufficient PVC pipes and oyster net of appropriate sizes were placed in each tank to provide shelters for the marron. Each tank was provided with a biological filtration recirculating water system. The water in the system was filtered through both mechanical and biological filtration at a rate of approximately 3 L min^{-1}.

Eighteen culture tanks were used for the trial which lasted for 12 weeks. The marron from the acclimation tanks were randomly distributed among the culture tanks at a density of 18 marron per tank. Each random block of three tanks was supplied one of the above diets so that each diet was represented in three replicates. The formulated diets were provided at the rate of 5–6% body weight every two days in all tanks. Uneaten food and faeces was siphoned out prior to feeding and sufficient water was added to maintain 200 L in each tank. Water quality parameters including pH, total ammonium, nitrite and nitrate were monitored weekly using pH meter (WP-80, TPS Pty Ltd, Brisbane) and chemical test kits (Aquarium Pharmaceuticals, Inc). Surviving marron were counted every two weeks and the weight of marron was measured to two decimal places using an electronic balance (SHIMADZU AW 220) every four weeks.

Table 1
Test diet formulations for marron.

Ingredients	D1 (%)	D2 (%)	D3 (%)	D4 (%)	D5 (%)	D6 (%)
Fish oil	3.2	3.2	3.2	3.23	3.23	3.23
Soybean meal	10.15	10.15	10.14	10.14	10.14	10.11
Fishmeal	33.78	33.8	33.83	33.83	33.86	34.01
Wheat flour	49.35	49.25	49.21	49.08	48.85	48.33
Ascorbic acid	0.05	0.05	0.05	0.05	0.05	0.05
Betaine	1.2	1.2	1.2	1.2	1.2	1.2
Calcium ascobate	0.02	0.02	0.02	0.02	0.02	0.02
Premix	0.15	0.15	0.15	0.15	0.15	0.15
Cholesterol	0.25	0.25	0.25	0.25	0.25	0.25
Wheat starch	1.85	1.85	1.85	1.85	1.85	1.85
β − 1,3 − D − glucan	0	0.08	0.1	0.2	0.4	0.8
Total	100	100	100	100	100	100

2.3. Data collection

The survival rate in all tanks was determined using the following formula: survival rate (%): $S = 100 \times (n_t/n_o)$, where: S is the survival rate; n_t is the number of marron at time t and n_o is the number of marron at the commencement. Yield was measured as the total weight of all marron in each tank.

Organosomatic indices analysis: The organosomatic indices of the marron including hepantosomatic index (Hiw), wet tail muscle index (Tw/B), hepatopancreas moisture content (HM%) and tail muscle moisture content (TM%), dry hepatosomatic index (Hid) and dry tail muscle index (Td/B) were measured by established method [10].

The osmoregulatory capacity of the marron was determined by the method of Sang and Ravi [10].

Total haemocyte count (THC), granular cells (GCs) and bacteraemia were conducted as per the established procedure for Western rock lobster [12].

2.4. Data analysis

All statistical analyses were performed using SPSS version 15. Results were presented as means ± SE (Standard error). ANOVA (analysis of variance) and LSD (Least significant difference) post hoc tests were used to determine significant differences between growth, survival, physiological and immunological parameters of the marron fed different diets. Results were judged as significant at $P < 0.05$.

3. Results

3.1. Growth and survival

After 84 days of culture, survival of marron was significantly different when fed with D2, D3, D4 and D5 diets compared to D1 (Fig. 1). There was no significant difference in the final weight of the

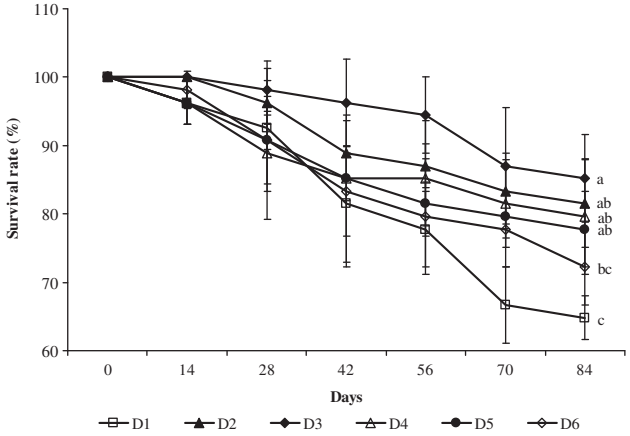

Fig. 1. Survival of marron fed different beta glucan supplemented diets (Mean ± S.D.). Different letters denote significant differences ($P < 0.05$) in mean value.