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Effects of mannan oligosaccharide dietary supplementation on performances of the tropical spiny lobsters juvenile (*Panulirus ornatus*, Fabricius 1798)

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ABSTRACT

The effects of dietary mannan oligosaccharide (MOS) (Bio-Mos®, Alltech, USA) on the growth, survival, physiology, bacteria and morphology of the gut and immune response to bacterial infection of tropical rock lobsters (*Panulirus ornatus*) juvenile were investigated. Dietary inclusion level of MOS at 0.4% was tested against the control diet (trash fish) without MOS inclusion. At the end of 56 days of rearing period, a challenged test was also conducted to evaluate the bacterial infection resistant ability of the lobsters fed the two diets. Lobster juvenile fed MOS diet attained 2.86 ± 0.07 g of total weigh and 66.67 ± 4.76% survival rate which were higher ($P < 0.05$) than the lobsters fed control diet (2.35 ± 0.14 g total weight and 54.76 ± 2.38% survival rate, respectively) thus providing the higher ($P < 0.05$) specific growth rate (SGR) and average weekly gain (AWG) of lobsters fed MOS diet. Physiological condition indicators such as wet tail muscle index (Tw/B), wet hepatosomatic index (Hiw) and dry tail muscle index (Td/B) of the lobsters fed MOS supplemented diet were higher ($P < 0.05$) than that of the lobsters fed the control diet. Bacteria in the gut (both total aerobic and *Vibrio* spp.) and gut's absorption surface indicated by the internal perimeter/external perimeter ratio were also higher ($P < 0.05$) when the lobsters were fed MOS diet. Lobsters fed MOS diet were in better immune condition showed by higher THC and GC, and lower bacteraemia. Survival, THC, GC were not different among the lobsters fed either MOS or control diet after 3 days of bacterial infection while bacteraemia was lower in the lobsters fed MOS diet. After 7 days of bacterial infection the lobsters fed MOS diet showed higher survival, THC, GC and lower bacteraemia than the lobsters fed the control diet. The experimental trial demonstrated the ability of MOS to improve the growth performance, survival, physiological condition, gut health and immune responses of tropical spiny lobsters juveniles.

1. Introduction

For the past decades, antibiotics have been practicing at the subtherapeutic concentrations in the animal feed because of their possible effects on, survival, feed utilization and weight gain [1] which are the most important concerns in aquaculture production. However, the stimulation of antibiotic on the development of resistant bacteria in both animal and humans has been the subjects of controversy [2]. Restriction or ban of the antibiotic used as additives in fish and crustacean feed has prompted interest in developing the alternative strategies as health promoter and disease control. Thus, in the recent years, there has been a heightening research on dietary supplementation in which various health promoting compound have been studied [3]. Those compound can be classified as immunonutrients and immunostimulants with the differences between the two relates to their mechanisms of action. One group of immunostimulants showing beneficial effects in terrestrial and aquatic animals is referred as prebiotics [4].

Among the most common prebiotics, mannan oligosaccharide (MOS) has been recently receiving heightening application in aquaculture. Since the first use of MOS in aquaculture, there has been increasing the number of studies demonstrating their ability to increase the survival, growth performance and control of the potential pathogens of fish and crustacean. This prebiotic has also been demonstrated to benefit the gut health by improved absorption and immune modulation in the target species. The effective mechanism of MOS results in higher performance in term of survival, growth and bacterial resistant ability of common carp (*Cyprinus carpio*), rainbow trout (*Salmo gairdneri irideus* G.) [5], channel catfish (*Ictalurus punctatus*) [6], rainbow trout (*Oncorhynchus mykiss*) [7]. The profitable of MOS inclusion in the diet on performance of crustacean has also proved for green tiger prawn (*Penaeus semisulcatus*) [2] and recently, Sang et al. [8] reported that dietary supplementation at 0.2–0.4% of MOS resulted in higher

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resistant ability to bacterial infection and stressors by improving the immune response of marron (*Cherax tenuimanus*).

The culture of tropical spiny lobsters (*Panulirus ornatus*) is becoming an important aquaculture industry in the Asia-Pacific and Caribbean regions [9,10]. Annual production of rock lobster in Vietnam alone worth was US$90 millions at the farm gate in 2005 [11]. The existing farming practices are based on rearing of the wild collected puerulii and early juveniles to the desirable size (approximately 15 g total weight) before the growth out phase. Exclusive feeding of the lobster on fresh fishery bycatch is unsustainable due to competition pressures cause by declining inshore fishery catches, poor food conversions and negative environmental impacts [9–13]. High mortality was observed at seed rearing stages (only 43% for lobsters less than 0.5 g [14]) because of the high vulnerability of young lobster to the environmental stressors. One of the obstacles to sustainable aquaculture of rock lobster is lack of the suitable dietary supplementation that will enable seed to be reared to growth out phase size with high survival and good health status. While there are considerable studies on nutrient requirement in order to develop suitable pellet as alternative to trash fish as food for commercial farming of rock lobster [15–17], the use of additives such as immunostimulants to benefit the lobsters industry is poorly reported and there is no information available on the use of MOS to the lobster culture. Hence, the objective of this study was to evaluate the effects of dietary inclusion of MOS on (1) survival and growth performance; (2) physiological status; (3) gut morphology and bacterial count (4) survival and immune response to bacterial infection of early juvenile rock lobster.

2. Material and methods

2.1. Culture system

Twelve fiberglass rectangular blue color tanks (500 × 800 × 1000 mm, 400 L capacity) were used as cultural units in the experiment. Sufficient dead corals and bricks with holes were placed in each tank to provide shelters for the lobsters. Each tank was supplied with 300 L mechanical filtered seawater. The water in each tank was continuously aerated and independently filtered through abiological seawater filter system at a rate of approximately 5 L min^{-1}.

2.2. Experimental animals

Lobster juveniles were supplied by commercial lobsters juvenile farm at Nha Trang bay, Vietnam (109°12′53.28″E, 12°17′20.70″N) and shipped to the Institute of Oceanography, Nha Trang Vietnam. A hundred and thirty two lobsters were randomly distributed into twelve culture tanks, so that each tank received eleven animals. The lobsters were then acclimated to the culture conditions for 1 week. During the acclimation period, the lobsters were fed with grinded trash fish twice daily at 8:00 and 17:00 h till satiation. Uneaten food and feces were siphoned out before next feeding.

2.3. Reparation of the test diet

Trash fish (fish and prawn at the ratio 2:1) from the local market was used as the basal food for lobsters. Trash fish was washed and thoroughly mixed with 0.4% in weight of mannan oligosaccharide (MOS) (Bio-Mos®, Alltech, USA). The mixed ingredient was grinded using a grinder to obtain the pasty mix. The trash fish with no MOS supplementation was also grinded and used as control diet. The pasty mixes were packed and store in the freeze. Before feeding, the foods was taken out from the freeze and defrosted in the room temperature for 1 h.

2.4. Experimental design

After acclimation, the lobsters (1.28 ± 0.01 g total weight) from different tanks were merged together and then randomly distributed to each tanks at the density of 10 animals/tank. Each experimental diet was randomly assigned to six tanks, giving six replicates per diet. The food was initially provided at the rate of around 30% body weight daily divided into two lots and then fed to lobsters at 8:00 and 17:00 h. Uneaten food and feces were siphoned before next lot of food was provided. The amount of water lost during siphoning was added into each tank to retain the water level. The lobsters were reared for 8 weeks. Water quality parameter such as temperature, salinity and pH were monitored daily using thermometer, refractometer and pH meter, respectively. The number of lobster in each tank was checked every two weeks by removing the shelter and counting. The individual weight was measured to two decimal places using a balance SHIMADZU AW 220 (LabCommerce Inc, USA) every two weeks. Physiological parameters, gut bacteria (total bacteria and *Vibrio* spp.) and morphology and immunological parameters (Total haemocyte count, granular cell, bacteraemia) were measured at the week 8 of culture period.

At the end of the culture period, a bacteria challenge trial was conducted to evaluate the response of the lobsters to bacteria infection. Stock solution of *Vibrio* spp. (isolated from milky diseased lobsters) was obtained from the Research Institute for Aquaculture No.3 at Nha Trang, Vietnam. The concentration of stock solution was approximately 0.50 × 10^6 cfu/mL.

Bacterial challenge trial was initiated on day 60 of the feeding trial. Before the challenge trial, eighteen lobsters of each previous diet fed test were randomly distributed to three culture tank, giving three replicates per diet. The lobsters were then injected through the base of the fifth thoracic leg with 20 μL bacteria stock solution. During the challenge trial, the lobsters were also fed with their original diets. The infected lobsters were monitored for survival, total haemocyte count, granular cell and bacteraemia after 1, 3 and days of injection.

2.5. Data collection

2.5.1. Growth and survival

The survival rate in each tank was measured using the following formula: survival rate (%): $S = 100 \times (n_t/n_0)$, where: S is the survival rate; n_t is the number of lobsters at time t and n_0 is the number of lobsters at the commencement.

Growth rate was calculated and expressed as specific growth rates (SGR) and average weekly gain (AWG) according to the following equation: SGR (% day^{-1}) = 100 × (ln W_e − ln W_s)/d, AWG (g $week^{-1}$) = (W_f − W_o)/wk where W_s and W_e are the weights of the lobsters at the start and end of the growth period, respectively, and d and wk are the number of days and weeks, respectively, in the growth period.

2.5.2. Physiological condition parameters

The organosomatic indices of the lobsters including wet hepatosomatic index (Hiw), wet tail muscle index (Tw/B), hepatopancreas moisture content (HM%), tail muscle moisture content (TM%), dry hepatosomatic index (Hid) and dry tail muscle index (Td/B) as the indicator of physiological condition were measured by applying the established methods [18].

2.5.3. Immunological parameters

Total haemocyte count (THC), granular cell proportion (GC) and bacteraemia assessment were conducted as per the established procedure for rock lobsters [19]. The base of the fifth thoracic leg of each lobsters from each culture tank was cleaned with 70% alcohol.