Fish & Shellfish Immunology 70 (2017) 621–627

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Dietary Immunogen® modulated digestive enzyme activity and immune gene expression in *Litopenaeus vannamei* post larvae

Hamed Kolangi Miandare [a, *], Ali Taheri Mirghaed [b], Marjan Hosseini [a], Nastaran Mazloumi [c], Ashkan Zargar [b], Sajad Nazari [a, d]

[a] Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[b] Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[c] Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES), Department of Agriculture and Water Resources, Canberra, Australia
[d] Cold-water Fishes Genetic and Breeding Research Center, Iranian Fisheries Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Yasouj, Iran

ARTICLE INFO

Article history:
Received 1 April 2017
Received in revised form
12 September 2017
Accepted 16 September 2017
Available online 19 September 2017

Keywords:
White shrimp
Immunogen
Prebiotic
Immune system

ABSTRACT

Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) is an important economical shrimp species worldwide, especially in the Middle East region, and farming activities of this species have been largely affected by diseases, mostly viral and bacterial diseases. Scientists have started to use prebiotics for bolstering the immune status of the animal. This study aimed to investigate the influence of Immunogen® on growth, digestive enzyme activity and immune related gene expression of *Litopenaeus vannamei* post-larvae. All post-larvae were acclimated to the laboratory condition for 14 days. Upon acclimation, shrimps were fed on different levels of Immunogen® (0, 0.5, 1 and 1.5 g kg^{-1}) for 60 days. No significant differences were detected in weight gain, specific growth rate (SGR) and food conversion ratio (FCR) in shrimp post-larvae in which fed with different levels of Immunogen® and control diet. The results showed that digestive enzymes activity including protease and lipase increased with different amounts of Immunogen® in the shrimp diet. Protease activity increased with 1.5 g kg^{-1} Immunogen® after 60 days and lipase activity increased with 1 and 1.5 g kg^{-1} Immunogen® after 30 and 60 days of the trial respectively ($P < 0.05$), while amylase activity did not change in response to different levels of Immunogen® ($P > 0.05$). The expression of immune related genes including, prophenoloxidase, crustin and g-type lysozyme increased with diet 1.5 g kg^{-1} Immunogen® ($P < 0.05$) while expression of penaeidin gene increased only with experimental diet 1 g kg^{-1} of Immunogen®. These results indicated that increase in digestive enzymes activity and expression of immune related genes could modulate the Immunogen® in the innate immune system in *L. vannamei* in this study.

1. Introduction

Shrimp farming is one of the most important economic aquaculture activities worldwide. However, the shrimp industry is very susceptible to diseases and low level of feed conversion ratio [1,2]. Different pathogens such as protozoa, fungi, bacteria and virus has known as important causes of diseases in farming ponds and natural environment [3,4]. Traditionally, chemical component were used for infection pathogens treatment in shrimp farms; however, the potential development of antibiotic-resistant bacteria, the emergence of antibiotic in host animals and potential harm to human and environmental make the use of antibiotics undesirable [5,6].

Innate immune system plays a key role in protection of the organism against pathogens [8]. In an attempt for improving the immune system of animals against different types of bacterial infection, environmentally friendly food additives such as prebiotic and probiotic have been used for improving the immune system in crustaceans [7–11]. Prebiotics are known as non-digestible food ingredient that stimulate growth rate and control the activity of one or limited numbers of bacterial colony in body of the host animal [12]. It is recommended that modifying the bacterial populations in the gastrointestinal tract (GIT) of shrimp and other aquatic

* Corresponding author. Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan 35 University of Agricultural Sciences and Natural Resources, Gorgan 36, Iran.
E-mail address: hkolangi@gau.ac.ir (H.K. Miandare).

https://doi.org/10.1016/j.fsi.2017.09.048
1050-4648/

organisms may results in weight gains (WG) and improvement of health status in the farmed animals. These modifications are known as symbionts or commensals in the shrimp's GIT [13].

Immunogen® is a commercial prebiotic, which includes β-glucan and mananoligosaccharides (MOS), derived from *Saccharomyces cerevisiae* yeast cell wall [14]. MOS and β-glucan have beneficially affected both specific and non-specific immune mechanism of animal [15,16,and17]. It is reported that administration of Immunogen® in diet of both warm water and cold water fish species can improve their growth rate, immune response via regulating the immune related gene expression and enhance disease resistant [e.g. common carp (*Cyprinus carpio*) [14]; rainbow trout (*Oncorhynchus mykiss*) [18]. However, some previous researchers have found that inaccurate administration level of prebiotic can have a negative influence on growth rate of the animal [19,20,and21]. Despite several studies on the effect of prebiotic as a dietary supplement on growth performance and immune system of fish, shellfish and crustaceans, there is still limited information about the effects of Immunogen® on innate immune responses in shrimps. Hence, in this study the effects of Immunogen® on growth performance, digestive enzymes activity and immune related gene expressions in *L. vannamei* during 30 and 60 days experimental period were examined.

2. Materials and method

2.1. Shrimp culture and feeding trial

Two hundred post–larval of shrimp (*L. vannamei*) were collected from the Science Research Station of Gomishan, Gorgan, Iran and transported to Gorgan University of Agricultural Sciences and Natural Resources laboratory. Shrimps were acclimated for 14 days in fiberglass tanks at the laboratory condition (20.27 ± 1.21 ppt salinity and 25.84 ± 1.64 °C temperature). Upon adaption shrimp were weighed (0.54 ± 0.06 g) and randomly assigned in equal to 12 fiberglass tanks (400 L, 1 m^2 round bottom area) in four groups with three replicates. During the 60 days feeding trial the first group was fed un-supplemented food as a control and other three groups were fed on different Immunogen® (Provided by Soroush Radian, Co. Tehran, Iran) concentrations (0.5, 1and 1.5 g kg^{-1}) (all groups at feeding rate of 5% body weight) (see Table 1).

Table 1
Dietary formulation and chemical composition (%).

Ingredient	
Fish meal	220
Soybean	340
Wheat flour	250
Wheat gluten	50
Squid	30
Shrimp	20
Fish oil	40
Lecithin	10
Vitamin complex[a]	20
Mineral complex[b]	20
Dry matter	922
Crude protein	433
Crude lipid	83
Crude fiber	36
Crude ash	84

[a] Vitamin contains (kg^{-1}dry weight), vitamin A: 50.000 MIU, vitamin D3: 10 MIU, vitamin E: 130 g, vitamin K3: 10 g, vitamin B1: 10 g vitamin B2: 25 g, vitamin B6: 16 g, vitamin B12: 100 mg, niacin: 200 g, pantothenic acid: 56 g, folic acid: 8 g, biotin: 500 mg, antioxidant: 0.2 g, Anti-cake: 20 g.

[b] Mineral premix, contains (kg^{-1}dry weight): calcium phosphate 397 g; calcium lactate 327 g; ferrous sulphate 25 g; magnesium sulphate 137 g; potassium chloride 50 g; sodium chloride 60 g; potassium iodide 150 mg; copper sulphate 780 mg; manganese oxide 800 mg; cobalt carbonate 100 mg; zinc oxide 1.5 g; sodium selenite 20 mg.

2.2. Biometry and growth parameters assay

At the end of the feeding trial, post-larvae were weighed to calculate the growth performance. The weight gain, food conversion ratio (FCR), and specific growth rate (SGR) for different treatments were calculated according the following formula [22];

Weight gain (%) = [(Final body weight (g) − Initial body weight (g)) / Initial body weight (g)] * 100

Specific growth rate (SGR %) = [(Final bodyweight − Initial body weight) / Experiment period]*100

Food conversion ratio (FCR) = Total feed intake (g) / Total wet weight gain (g)

2.3. Sample preparation and determination of intestinal digestive activity

For intestinal digestive activity determination, intestinal of three shrimp were collected and homogenized by adding sterile 0.8% saline solution to prepare 10% (W: V) homogenates. Homogenates were centrifuged at 1000 × g for 8 min at 4 C. The supernatants were rapidly used for digestive enzyme activity analyses, which were performed using a spectrophotometer. Digestive enzymes (e.g. protease, amylase and lipase) activity in the intestinal of *L. vannamei* were measured using the protease, amylase and lipase commercial kits respectively (Sigma-Aldrich Co, USA) following by the manufacturer's instruction (Bio-RAD, USA). After 30 and 60 days post trial three shrimps from each group were randomly taken and the hepatopancreas was collected. The tissue samples were immediately placed in liquid nitrogen and stored at −80 °C until RNA extraction and evaluated the expressions of Lys, Cru, Pen-3a, proPo and β-actin.

2.4. RNA extraction and cDNA synthesis

Hepatopancreas RNA was extracted using RNAx Plus (CinnaGen, Iran). The 50–100 mg hepatopancreas was homogenized in 1.0 ml RNAxPlus reagent (Sinaclon; Iran) and left at room temperature for 15 min. Following steps for RNA extraction were conducted as described by Panigrahi et al. [23]. Then, the quantity and concentration of RNA was measured by spectrophotometer at 260/280 nm. The RNA quality was measured by electrophoresis on a 1.5% agarose gel and staining with ethidium bromide. First-strand cDNA synthesis by SuPrime Script RT Premix (2X) cDNA Synthesis Kit (GeNet BIO Inc; Daejeon, South Korea) following the protocol suggested by the company.

2.5. Quantitative real-time PCR

Real-time PCR analysis was performed using an iCycler (Bio-Rad) with SYBR Green qPCR Master Mix and the gene-specific primers. 100 nM of each forward and reverse specific primers, 10 ng of cDNA template and nuclease free water to final volum of 20 μl were used and all thermal profile of PCR reaction mixtures was carried out after Miandare et al. [2]. The primers used for