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Immune related transcriptional responses and performance of *Litopenaeus vannamei* post-larvae fed on dietary probiotic PrimaLac®

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ABSTRACT

The present study investigated the effects of various levels of multi-strain probiotic on the immune related gene expression, digestive enzyme activity, growth performance, body chemical composition and survival of *Litopenaeus vannamei* post-larvae. After transferring post-larvae of *L. vannamei* to indoor conditions and subsequent acclimation to laboratory condition for 14 days, the shrimp were fed multi-strain probiotic at four different doses of 0, 0.25, 0.5 and 1.0 g kg^{-1} for eight weeks. Shrimp fed 0.5 and 1.0 g kg^{-1} probiotic PrimaLac® exhibited significantly ($p < 0.05$) higher weight gain, specific growth rate, body crude protein as well as lower FCR compared to other groups. Feeding on 0.5 and 1 g kg^{-1} dietary multi-strain probiotic significantly ($p < 0.05$) increased the level of body crude protein. Oral administration of 0.5 and 1.0 g kg^{-1} multi-strain probiotic significantly ($p < 0.05$) decreased body crude lipid and body moisture respectively. 30 days after feeding, protease, amylase and lipase activity increased in groups fed 0.5 and 1.0 g kg^{-1} probiotic PrimaLac®. However, on the 60th day, specific protease and amylase activity in all treatment groups were significantly higher than control group ($p < 0.05$) but lipase activity was higher ($p < 0.05$) in groups fed 0.5 and 1.0 g kg^{-1} multi-strain probiotic. Oral administration of 1.0 g kg^{-1} probiotic increased ($p < 0.05$) the level of prophenoloxidase and g-type lysozyme gene on day 30th and 60th after treatment. On day 30th and 60th, penaeidin gene expression was significantly higher in all treatment groups compared to the control group ($p < 0.05$). In general, findings of this study demonstrated that oral administration of 0.5 and 1.0 g kg^{-1} multi-strain probiotic improved the performance of the fish and increased the expression of immune related genes.

1. Introduction

Many penaeid shrimps are of high commercial value. In recent years, shrimp farming has grown rapidly, but the shrimp aquaculture industry yet faces several problems such as the low level of feed conversion ratio and outbreaks of different diseases [1]. Several infectious disease outbreaks in shrimp farms have resulted serious mortality and economic losses [2,3]. In natural environment and farming ponds different pathogens such as protozoa, fungi, bacteria and virus have been mentioned as important causes of disease outbreaks [3–5]. Physiological and immunological factors are key components in shrimps as in many other animals to cope against these pathogens. However, the immune system of shrimps shows lower levels of development compared to vertebrates. Shrimps are strongly dependent on innate immune system to combat against pathogens [6–8].

Several chemical compounds are used to treat infectious pathogens in shrimp farming. The use of these compounds is associated with issues such as the development of antibiotic resistant genes among opportunistic bacteria. Antibiotic residues can accumulate in animal tissue and environment subsequently passing to the consumer [9,10]. In addition, the use of antibiotics acts against normal micro flora of gastrointestinal tract and suppresses formation of the natural microflora of fish larvae as well as shrimp post-larvae [11,12]. Therefore, it has been suggested that use of alternative strategy, immune-stimulant and food additive such as prebiotic and probiotic can improve immune system and can prevent or minimize shrimp diseases. There is has been an increased

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interest in the use of probiotic to prevent or control pathogen outbreaks in shrimp farms [13–15].

Probiotics can have positive effect on host through various immune-related mechanisms [16]. PrimaLac® is a commercial multi-strain probiotic (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidium*). Previous studies have indicated the positive effects of dietary probiotic supplementation on shrimp immune system including positive effect of *Bacillus subtilis* on growth performance, survival and up-regulation of immune related genes in white leg shrimp (*Penaeus vannamei*) [17]. Oral administration of *Lactobacilus planetarium* also increased the level of immune related genes of *Litopenaeus vannamei* [18]. Growth performance and digestive enzyme activity in *P. vannamei* was also increased after photosynthetic bacteria and *Bacillus* sp. administration [19]. It was observed that digestive enzyme activity, survival and growth of Indian white shrimp *Fenneropenaeus indicus* fed *Bacillus* spp. were improved [20]. *Streptococcus phocae PI80*, *E. faecium MC13*, *Lactococcus garvieae LC149*, *B49* and one commercial probiotic (ECOFORCE) also increased the resistance of *Penaeus monodon* against vibriosis [21].

Although there are a variety of studies that investigated the effect of dietary probiotic supplementation on feed utilization, growth performance and immune system of fish and shellfish species, however, there is limited information regarding the effect of multi-strain probiotic on growth performance and immune related genes in shrimps. Therefore, the aim of the present study was to investigate the effect of commercial multi-strain probiotic on growth performance, body composition, survival and immune related genes in *L. vannamei* during a 60-day experimental period.

2. Material and methods

2.1. Feed preparation

The experimental diets were prepared based on previous formulation [22]. Ingredients and food composition are presented in Table 1. The control group was fed with basal diet and other groups were fed with diets supplemented with different levels of probiotic PrimaLac® (Star-Labs, USA) (0.25, 0.5 and 1.0 g/kg). Different strains of water soluble multi strain probiotic PrimaLac® include *L. acidophilus*, *L. casei*, *E. faecium* and *B. bifidium* were presented by manufacture.

2.2. Experimental design

Pacific white shrimp (*L. vannamei*) at post-larvae stages (number = 300) were obtained from the Science Research Station of Gomishan, Gorgan, Iran and was transferred to indoor laboratory condition. All post-larvae were acclimated to the experimental condition for 14 days. Then, they were weighed and randomly distributed to 12 fiberglass tanks and were assigned to four groups in triplicates. Each group was fed at 5% of body weight with its determined experimental diets four times per day (8 a.m., 12 p.m., 4 p.m., 8 p.m.) for 60 days. One group was assumed as control and fed with basal diet (un-supplemented feed) whilst other groups were fed with different doses of probiotic PrimaLac® including 0.25, 0.5 and 1.0 g kg^{-1} feed.

2.3. Biometry and growth parameters assay

At the end of the feeding trial, post-larvae were weighed to calculate the growth performance. The weight gain, feed conversion ratio (FCR), and specific growth rate (SGR) of different treatments were estimated according the following formula [17]. To calculate survival rate the number of mortality per each replicate were also recorded.

Table 1
Dietary formulation (g kg^{-1} dry matter) and proximate composition of basal diet.

Ingredient	Control	0.25	0.5	1.0
Treatments				
Fish meal	220	220	220	220
Soybean meal	340	340	340	340
Wheat flour	250	250	250	250
Wheat gluten	50	50	50	50
Squid meal	30	30	30	30
Shrimp meal	20	20	20	20
Fish oil	40	40	40	40
Lecithin	10	10	10	10
Vitamin complex	20	20	20	20
Mineral complex	20	20	20	20
Proximate composition (g kg^{-1} dryweight)				
Dry matter	922	922	922	922
Crude protein	443	443	443	443
Crude lipid	83	83	83	83
Crude fiber	36	36	36	36
Crude ash	84	84	84	84

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.
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.

$$\text{Weight gain (g/shrimp)} = \text{final weight (g)f mortality per each}$$

$$\text{Feed conversion ratio (FCR)} = \text{Total Feed Given (g)} / \text{Weight Gain (g)}$$

$$\text{Specific growth rate (SGR)} = (\{\ln\text{final wt}\}\text{grln initial wt}\} \times /\text{Days}) * 100$$

$$\text{Survival rate (\%)} = (\text{final numbers/initial numbers}) * 100$$

2.4. Body composition assay

Whole shrimp samples and experimental diet were analyzed for proximate chemical composition including the dry matter, crude protein, crude lipid, crude ash and fiber content based on standard method that previously described by the Association of Official Analytical Chemists [23]. All diet and whole shrimp samples were dried at 105 °C to constant weight to measure dry matter. Crude lipid was estimated based on ether extraction by using Soxhlet. Crude protein was calculated based on nitrogen levels (N × 6.25) using Kjeldahl and ash was determined by ignition at 550 °C.

2.5. Digestive enzymes assay

To measure digestive enzyme activity, the whole gastrointestinal track (GIT) of three shrimp per replicate was collected, polled, and homogenized in four fold 10 mM saline phosphate buffer pH 7. Total protease activity was estimated based on casein as the substrate which reacts with Folin's reagent [24]. Amylase activity was estimated based on starch as substrate and measures maltose released by hydrolysis of substrate reacting with 3,5-dinitrosalicylic acid based on method previously described by Wilchek and Bayer [25].