Fish & Shellfish Immunology 54 (2016) 516–522

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

The effects of feeding with synbiotic (*Pediococcus acidilactici* and fructooligosaccharide) enriched adult *Artemia* on skin mucus immune responses, stress resistance, intestinal microbiota and performance of angelfish (*Pterophyllum scalare*)

Mahmood Azimirad [a, *], Saeed Meshkini [b, **], Nasrollah Ahmadifard [a], Seyed Hossein Hoseinifar [c]

[a] Department of Fisheries, Faculty of Natural Resources, Urmia University, Urmia, Iran
[b] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[c] Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form
28 April 2016
Accepted 2 May 2016
Available online 3 May 2016

Keywords:
Synbiotic
Artemia franciscana
Angelfish
Skin mucus
Stress resistance

ABSTRACT

The aim of this study was to evaluate the effects of feeding on synbiotic (*Pediococcus acidilactici* and fructooligosaccharide) enriched adult *Artemia franciscana* on skin mucus immune responses, stress resistance, intestinal microbiota and growth performance of **angelfish** (*Pterophyllum scalare*). Three hundred and sixty fish with initial weight 3.2 ± 0.13 g were randomly divided into twelve aquaria (50 L) assigned to four groups in triplicates. Fish were fed for 7 weeks with dietary treatments, including treatment 1: feeding adult *Artemia* without enrichment (control group), treatment 2: feeding adult *Artemia* enriched with lyophilised probiotic *P. acidilactici* (700 mg L^{-1}), 3: feeding adult *Artemia* enriched with prebiotic fructooligosaccharide (FOS) (100 mg L^{-1}), group 4: feeding adult *Artemia* enriched with synbiotic (*P. acidilactici* (700 mg L^{-1}) + FOS (100 mg L^{-1})). Skin mucus immune responses (lysozyme activity, total Immunoglobulin and protease), stress resistance against environmental stress (acute decrease of temperature and increase salinity), intestinal microbiota as well as growth indices were measured at the end of feeding trial. *Artemia* enriched with synbiotic significantly improved growth performance compared to other treatments ($P < 0.05$). The highest weight gain and specific growth rate (SGR) was observed in synbiotic fed fish ($P < 0.05$). Compared to the other treatments, the population of lactic acid bacteria was significantly higher in the intestinal microbiota of fish fed synbiotic supplemented diet ($P < 0.05$). In the environmental stress challenge test, the maximum resistance to abrupt decrease of temperature (17 °C) or elevation of salinity (12 g per liter) was observed in the synbiotic treatment. Also, the total immunoglobulin and lysozyme activity level of skin mucus was significantly elevated in fish fed *Artemia* enriched with synbiotic ($P < 0.05$). These results revealed that feeding **angelfish** with synbiotic enriched *Artemia* was more effective than singular enrichment with probiotics or prebiotics.

1. Introduction

During the recent years, aquaculture has made significant advances in the production of a wide range of aquatic organisms, both for human consumption and as ornamental species [1–3]. One of the most popular freshwater fish species in the aquarium trade industry is the **angelfish** *Pterophyllum scalare*, a great economic potential cichlid native to the Amazon that has adapted throughout the world [4]. *P. scalareis* considered as an omnivorous fish since in nature fed, on planktons, insects and crustaceans, plants and worms. In captivity, the common live larval food used for growing is limited to macro-zooplankton such as nauplii and adult stages of *Artemia* [5,6]. This species is grown in intensive and semi-intensive systems, where its nutritional requirements are met with artificial

* Corresponding author.
** Corresponding author.
E-mail addresses: mahmoodazimirad@gmail.com (M. Azimirad), s.meshkiniy@urmia.ac.ir (S. Meshkini).

http://dx.doi.org/10.1016/j.fsi.2016.05.001
1050-4648/

diets and *Artemia* [7]. In spite of the importance of nutrition in the development of ornamental fish breeding, research and development in this area is limited.

In recent years the use of probiotics in aquaculture is extremely become prevalent and can overcome many of the problems associated with bacterial diseases [8–10]. The use of probiotics as a food supplement for farm animals goes back to the 1970s [11–15]. Various types of microalgae (*Tetraselmis*), yeasts (*Phaffia, Saccharomyces*), Gram-positive bacteria (*Bacillus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Micrococcus, Streptococcus* and *Weissella*) and Gram-negative bacteria (*Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas* and *Vibrio*) have been studied as probiotics [16,17]. The ambiguities in the use of probiotics such as the non-guaranteed viability of the probiotics in the gastrointestinal tract, necessity of competition of introduced probiotic with commensal microbiota, the ability to form the mass as well as the long-term sustainability of the masses, caused the researchers gain to the idea of prebiotic [18,19].

The prebiotics are selectively fermented by potentially beneficial bacteria groups (e.g LAB) and results in increased numbers and dominance of these beneficial bacteria in the intestinal tract [20]. Researches in these field have shown that non-digestible oligosaccharides such as inulin and fructooligosaccharide are among the most promising prebiotics [21,22]. Combined administration of probiotics species with appropriate prebiotics (synbiotic) as a substrate to increase dominance and sustainable growth of probiotics bacteria has been suggested, due to the inability of probiotic species to form stable masses and maintain dominance in the gut microbiota [23]. There are relatively limited studies regarding administration of synbiotics in aquaculture specially in case of ornamental fish culture. However, the results of those studies revealed positive effects on physiology and immunity [8,23–27].

Artemia has been widely used in the culture of ornamental fishes due to the high nutritional value, the proper size and the possibility of enrichment [28]. *Artemia* can be used as the carrier of particles used in aquaculture such as nutrients (fatty acids, vitamins, etc.), antimicrobial substances, vaccines and probiotics [29]. Administration of live, beneficial and non-pathogenic bacteria in the culture medium or *Artemia* culture can have positive effects on cultured fish species by improving the intestinal microbiota, eliminating harmful bacteria and improving the nutritional value of *Artemia* [30]. The number of bacteria in the *Artemia* exponentially increases at the time of *Artemia* hatching and enrichment processes by nutrients [31]. It also has been observed that in the early stages of development in fish larvae, the increase in the number of bacteria in the intestinal microflora of fish, mainly associated with the bacteria in live food [32]. It can be concluded that with increasing the number of opportunistic bacteria in the fish intestine, mortality becomes more in the intensive culture of early life stages of fish and control of bacterial population in the live feed may lead to higher survival rates of fish larvae and profitability in hatcheries [33].

Considering there is a gap in the existing knowledge regarding the effects of synbiotic enriched adult *Artemia* with on ornamental fish health status and performance, the present study was conducted to investigate the effects of dietary administration of pre-, pro- and synbiotic on mucosal immune response, stress resistance, intestinal microbiota and growth performance of **angelfish** (*P. scalare*).

2. Material and methods

2.1. Live feed culture conditions and enrichment process

Artemia (*Artemia franciscana*) cysts were obtained from Great Salt Company, USA. Chorionic layer of cysts were separated by the use of sodium hypochlorite during decapsulation. Hatching of decapsulated cysts was performed through the use of cone-shaped container with a volume of 120 litters and sea water (salinity of 30 g per litter). Cysts were incubated with a density of 5 g per litter at 30 °C with 2000 lux lighting conditions and vigorous aeration [34].

Artemia nauplii were transferred to culture environment after hatching. The culture environment was cone-shaped plastic containers (150 L) aerated by aeration pipes connected to the central pump. Nauplii were fed during the first few days by spirulina algae (*Spirulina platensis*) powder, and thereafter fed with mixture of rice bran, baker's yeast and spirulina [35]. Feeding was performed three times a day with an interval of 4 h. Stocking density was three nauplii per ml and culture period was 20 days to reach sexual maturity [28]. During this time, all physical and chemical parameters were measured and recorded daily. Physiochemical factors such as water temperature, salinity, dissolved oxygen, light and pH during culture period were monitored and maintained at 28.69 °C, 32 g L^{-1}, 7.75 mg L^{-1}, 1500 lux and 7.88, respectively.

Commercial probiotic used in this experiment was prepared from Tak Gene Company (Pedi-guard® Tehran, Iran) contains 1×10^{10} CFU g^{-1} *Pediococcus acidilactici*. Prebiotic, fructooligosaccharide (Raftilose P95) was supplied from Orafti Company, Belgium.

For enrichment of adult *Artemia* by synbiotic, combinations of probiotics and prebiotics were used in accordance with the following table. Thus, for the suspension preparation, first a ratio of 0.1:10 lecithin and water at 40 °C were poured into a clean and dry beaker and were mixed using an electric mixer. Then the rapeseed oil was added to this solution and was mixed very well by mixer. The ratio of lecithin, oil and water in suspension was 0.1, 1 and 10, respectively. To evaluate the diameter of oil particle, some samples were poured on slide and were observed under light microscope [36]. 150 ml was separated from prepared suspension, 700 mg probiotic, *P. acidilactici* and 100 mg of prebiotic, fructooligosaccharide were transferred to the beaker and were uniformed with an electric mixer, then mix in 2 litters of seawater and adult *Artemia* with the number of 4000 was placed inside the container [37,38] (Table 1).

2.2. Live feed microbiology

Amount of 100 ml (containing 0.5 g of adult *Artemia*) were collected using a sterile pipette in each of the mentioned time and were transferred to a filter with a mesh size of 300 μm, then in order to elimination of bacteria in the external surface of *Artemia* body, were washed for 60 s in a salt solution, Benzalkolium chloride (0.1%) and again were washed with sterile water and after that, water of samples was taken after a while [32]. The sterile samples were weighted and transferred to sterile porcelain mortar. Levels of lactic asid bacteria were analysed following the techniques employed by Hoseinifar et al. [23]. After the homogenization of samples using a sterile saline solution (0/87% w/v), dilutions of 10^{-1}–10^{-7} were prepared. From prepared dilutions, under sterile conditions, the volume of 0.1 mm was removed and was transmitted to the culture medium MRS (for determine the number of lactic acid bacteria) and was spread on surface of the plate. The incubation of plates was conducted for 3–5 days in an incubator at a temperature of 30 °C and under aerobic conditions. After the incubation period, the bacteria were counted, and recorded according to the logarithm of the colony unit (the number of bacterial colonies grown on culture medium × dilution coefficient^{-1}) per gram of *Artemia* [32]. Bacteria *P. acidilactici* was investigated and identified based on apparent characteristics, gram staining and also some standard biochemical tests such as phenol red, citrate, indole, motion and methyl red [39].