



Evaluation of the Impacts of Long-Term Enriched *Artemia* with *Bacillus subtilis* on Growth Performance, Reproduction, Intestinal Microflora, and Resistance to *Aeromonas hydrophila* of Ornamental Fish *Poecilia latipinna*

Nasrollah Ahmadifard¹ · Vahid Rezaei Aminlooi¹ · Amir Tukmechi² · Naser Agh³

© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

The present study investigated the effect of enriched *Artemia* with *Bacillus subtilis* on growth performance, reproductive factors, proximate composition, intestinal microflora, and resistance to *Aeromonas hydrophila* of ornamental fish, *Poecilia latipinna*. Using a completely randomized design, the experiment included three groups. The first group was fed with commercial food without any probiotic. The second group was fed with unenriched *Artemia*, and the last group consumed long-time enriched *Artemia* with *Bacillus subtilis*. The bacteria *B. subtilis* with a density of 1×10^5 CFU mL⁻¹ was added daily to *Artemia* culture medium. The total microflora and *Bacillus subtilis* counts were significantly increased in enriched *Artemia* compared to the unenriched group ($P < 0.05$). In fish fed groups, growth factors did not show any significant difference ($P > 0.05$). The maximum relative fecundity (28.65 ± 2.52 egg number g⁻¹), fry production (62.93 ± 4.6 individual per female), and fry survival ($70.97 \pm 1.56\%$) obtained in the third group were found to be significantly more than those in the first and the second groups. Moreover, intestinal bacterial count for *Bacillus* revealed that the higher concentration of bacteria was significantly related to the third group (6.24 ± 0.11 log CFU g⁻¹) ($P < 0.05$). Maximum protein and fat contents were observed in fish fed with *Bacillus*-enriched *Artemia*; however, no significant difference was found between control and unenriched *Artemia* groups ($P > 0.05$). The highest amount of ash was observed in fish fed with commercial food without any probiotic ($P < 0.05$). At the end of the feeding period, each of the three groups along with positive group (oxytetracycline 100 mg kg⁻¹ of commercial food) was exposed to *A. hydrophila* (BCCM5/LMG3770) bacteria intraperitoneally. Based on the results, the lowest cumulative mortality was significantly found in group three ($68.75 \pm 3.6\%$) and positive group ($62.5 \pm 7.0\%$) compared to control and unenriched *Artemia* groups ($P < 0.05$). Hence, *B. subtilis* with a concentration of 1×10^5 CFU mL⁻¹ during the period of *Artemia* culturing can improve the reproductive parameters, intestinal microflora, and resistance to pathogenic bacteria of *Poecilia latipinna*.

Keywords *Aeromonas hydrophila* · *Artemia* · *Bacillus subtilis* · *Poecilia latipinna* · Probiotics · Reproduction

Introduction

Ornamental fish is important for the development of aquaculture in developing countries. Reproduction and cultivation of

the ornamental fish species have increased dramatically and have received attention in a number of studies. Considering that the economic value of the ornamental fish is high, to support their sustainability, it is important to study various aspects of ex situ cultivation of broodstock in order to avoid relying on animals in the wild. With over 2500 species, freshwater aquarium fish trade is the biggest section of fish industry. New methods for the cultivation and reproduction of ornamental fish have been investigated worldwide in the recent decades [1].

Health and nutrition of the ornamental fish are of paramount importance in ornamental fish trade. Probiotics are microorganisms enhancing fish health via microbial balance of the host gut [2]. Probiotics have no negative effects [3], using several mechanisms such as production of essential digestive

✉ Nasrollah Ahmadifard
n.ahmadifard@urmia.ac.ir

¹ Department of Fisheries, Faculty of Natural Resources, Urmia University, P.O. Box: 46414-356, Urmia, West Azerbaijan, Iran

² Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, West Azerbaijan, Iran

³ Department of Artemia, Urmia Lake Research Institute, Urmia University, Urmia, West Azerbaijan, Iran