

## The Potential of *Daphnia magna* Bioencapsulated with Probiotics Bacilli on Growth and Feeding Parameters of Persian Sturgeon (*Acipenser persicus*) Larvae

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**Abstract:** *Daphnia magna* was used as a vector to carry probiotic bacillus to digestive tract of Persian sturgeon (*Acipenser persicus*) larvae. *Daphnia magna* with three concentrations of bacteria,  $1 \times 10^7$ ,  $2 \times 10^7$  and  $3 \times 10^7$  bacteria per milliliter (CFU ml<sup>-1</sup>) in suspension of broth at 5 hours was bioencapsulated and was fed by Persian sturgeon larvae. The control treatment was fed on unbioencapsulated *Daphnia magna*. The final body weight and specific growth rate (SGR) in experimental treatments had significant difference in comparison with control treatment ( $p < 0.05$ ). The probiotic bacillus had significant positive effects on conversion efficiency ratio (CER), Daily growth coefficient (DGC), food conversion efficiency (FCE) and thermal growth coefficient (TGC) in comparison with control treatment ( $p < 0.05$ ). Also, Food conversion ratio (FCR) significantly decreased ( $p < 0.05$ ) while protein efficiency ratio (PER) and lipid efficiency ratio (LER) significantly increased ( $p < 0.05$ ). The maximum of protein productive value (PPV) were obtained in treatment of T2 (the Persian sturgeon larvae were fed on bioencapsulated *Daphnia magna* by  $2 \times 10^7$  CFU L<sup>-1</sup>). Also, Lipid productive value (LPV) was significantly increased in experimental groups ( $p < 0.05$ ). This study showed that *bacillus spp* had high efficiency in growth performance and survival rate of *Acipenser persicus* larvae.

**Key words:** Digestive tract • Suspension • Thermal growth coefficient • Conversion efficiency ratio  
• Survival rate

### INTRODUCTION

The *Daphnia magna* is common live food organisms used for the rearing of marine fish larvae. These have been considered as possible vectors for the delivery of different substances, such as nutrients and probiotics. Intensive rearing of marine fish larvae suffers from heavy mortalities, which may be attributed to bacteria introduced in the rearing system with live food [1]. Optimization of microbial compositions and load in live food during the process of bioencapsulation is one of the most important concerns in aquaculture, as it can reduce the heavy mortalities which often occur during the rearing of fish larvae [2]. In the last decade, the scientific community

carefully examined roles and effects of probiotics in aquaculture as an alternative to antimicrobial drugs, demonstrating positive effects on fish survival [3], growth [4], stress resistance [5,6], immunosystem enhancement [7,8] and finally general welfare [9,10].

The use of natural prophylactic supplements in place of chemotherapeutics in aquaculture has received a great deal of attention in the past decade; such preventive products include probiotics. These biotics can be applied through external bathing or dietary supplementation and have been demonstrated to improve growth performance, feed utilization, digestibility of dietary ingredients, disease resistance and stimulate the immune response of aquatic animals [11-14].

Probiotics can be defined as live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance [15]. Most studies on the effects of probiotics on cultured aquatic animals have emphasized a reduction in mortality or the improved resistance against putative pathogens [16]. However, the beneficial effects are sometimes temporal, depending on the time of exposure [17].

Several studies demonstrated these positive effects using a single or two probiotic strains and just few studies described the effects of a mixture of probiotics in fish and shrimp aquaculture [18]. Concurrently, *Bacillus* species can be found in marine environment and are part of the microflora of several marine species [19]. Few studies had been carried out to incorporate probiotics into a freshwater species common carp, *C. carpio* [20] and crustaceans, Indian white shrimp *Fenneropenaeus indicus* [21] and shrimp *Penaeus vannamei*, [22] based on growth performances and digestive enzyme activities.

The use of probiotic bacteria has been suggested as an important strategy to accomplish reproducible outputs through biocontrol in cultivation systems for marine fish larvae and crustaceans [23]. The bacterial flora in the larval gut originates from the bacteria associated with the eggs, the water in the rearing tanks and the live food [24]. The gut of marine fish larvae is rapidly colonized by bacteria during the first days after hatching. Members of this pioneer community that colonize the gut at an early stage may acquire a competitive advantage compared with bacteria introduced at a later stage [25]. Successful colonization in digestive system of larvae involves competition with the established microflora for attachment sites and nutrients. The species composition of the intestinal microflora of fish larvae can be influenced at an early stage of development, when few, if any, bacteria are present in the larval gut, by addition of specific bacterial strains to the live food or the water [26].

Live food (as *Daphnia magna*) have been used as vectors for delivering compounds of diverse nutritional and/or therapeutic value to larval stages of aquatic animals [27], a process known as bioencapsulation. *Daphnia magna* are able to graze bacteria [28]. The number of bacteria accumulated in live food during bioencapsulation depends on the concentration of the bacterial suspension and the bacterial strain applied [29,30]. *Bacillus spp.* has been used as a probiotic and diet additive for various animals. It has been observed to be capable of enhancing growth parameters [31] of various fish species and thus may serve as an excellent health promoter for fish culture.

The live bacterial additives may have a positive effect on the host organism by improving the growth parameters and feeding efficiency. An incubation of live food organisms in a bacterial suspension consisting of one or several probiotic strains is a possible approach to carrying of beneficial bacteria into digestive tract of fish larvae. There is little information about the bioencapsulation of *Daphnia magna* by probiotic bacilli and thus present study was conducted to evaluate the potential of the effects of different levels of the beneficial probiotic bacilli in bioencapsulation of *Daphnia magna* on the exploitation of nutrient composition of this live food by Persian sturgeon (*Acipenser persicus*) larvae for promotion growth parameters. This study was performed in center of Marjanii sturgeon culture in Iran.

## MATERIALS AND METHODS

Ten-days old Persian sturgeon (*Acipenser persicus*) larvae with initial weight of  $74.9 \pm 0.9$  mg were obtained from Hatchery of Marjanii sturgeon center, Golestan, Iran. The blend of probiotic *Bacillii* (*B. licheniformis*, *B. subtilis* and *B. circulans*) under the commercial title of Protexin aquatic was prepared from Protexin Co (Iran-Nikotak). The *Daphnia magna* was cultured in earthly ponds in Marjanii sturgeon center. Three concentrations of bacillus suspension ( $1 \times 10^7$ ,  $2 \times 10^7$  and  $3 \times 10^7$  bacteria per milliliter in suspension of broth) were provided.

The newly caught *Daphnia magna* from pond, were collected on a 120mm-pore-size sieve, washed with fresh water thoroughly and were bioencapsulated (incubated) at the density of 5 g/liter under condition of  $29 \pm 1^\circ\text{C}$ , illumination (2000Lux), salinity of 0.5ppt and aeration [28]. After 5 h, the bioencapsulated *Daphnia magna* was collected on a 120mm-pore-size sieve, washed with fresh water and was used as a live food and vector to carry bacteria to digestive system of *Acipenser persicus* larvae. Twelve fiberglass tanks (capacity of 40 liters) with three replicates for experimental treatment and control were used. In experimental treatments of T1, T2 and T3 the Persian sturgeon larvae were fed by bioencapsulated *D. magna* by  $1 \times 10^7$ ,  $2 \times 10^7$  and  $3 \times 10^7$  of bacteria per milliliter in suspension of broth, respectively. The fish were transferred to twelve 40-liter circular fiberglass tanks. The density of fish larvae in per tank was  $71 \text{ fish tank}^{-1}$ . Sturgeon larvae were fed based on the 30% of their body weight for five times a day at 2.00, 7.00, 12.00, 17.00 and 22.00 with bioencapsulated *Daphnia magna* in experimental treatments and unbioencapsulated *Daphnia magna* in control treatment, respectively.

Each rearing tank was supplied with running fresh water which had been filtered through the special cotton filter (flow rate: 1 L min<sup>-1</sup>). Water quality parameters from every tank were monitored each week throughout the experimental. The water temperature was 19.46±2.05°C, pH was 7.6-8.3 and water oxygen level was maintained above 7.5 mg l<sup>-1</sup> during the experiment by setting electrical air pump. In the termination of experiment, fifty larvae from each tank were sampled and the total weight and length of body were measured.

Proximate composition of *Daphnia magna*, fish carcass (final of experiment) were analysed using the standard procedures described by the Association of Official Analytical Chemists [32]. crude protein (nitrogen 6.25) by micro-Kjeldahl digestion and distillation after acid digestion using a Kjeltec 1026 Distillation Unit together with a Tecator Digestion System (Tecator, Sweden); lipid was determined by extracting the residue with 40-60°C petroleum ether for 7-8 h in a Soxhlet apparatus. In the termination of experiment, 50 larvae from each tank were sampled and the final weight and length of body were measured and Thirty fish from each tank were sampled at the termination of the feeding experiment, homogenized and analysed for crude protein, crude lipid (on wet weight

basis) following the aforementioned methods. Some growth and feeding parameters of the fish were calculated based on the data of carcass analysis and biometry of *Acipenser persicus* larvae. The growth parameters and feeding parameters of the studied fish were calculated on the data of carcass analysis.

Treatment one, two and three fed by bioencapsulated *Daphnia* by 1×10<sup>7</sup>, 2×10<sup>7</sup> and 3×10<sup>7</sup> bacteria per milliliter at 5 hours, respectively. Also, larvae of control fed by unbioencapsulated *Daphnia*. The water circulation was stopped in all tanks for 2 h after every application of feed to allow the larvae to ingest the *Daphnia*.

Results analyzed by one-way ANOVA and significant different as determined by Duncan test. All statistical was performed using the software SPSS 15.0 for Windows.

## RESULTS

The growth parameters of Persian sturgeon larvae in experimental treatments and control were showed in table 1. Final body weight (FBW) in experimental treatments of larvae had significant difference in comparison with control treatment (p<0.05). The highest FBW (mg) was obtained in experimental treatment of T2.

Table 1: The growth parameters and survival rate of Persian sturgeon larvae in different treatments.

Parameters	Treatment			
	Control Unbioencapsulated <i>Daphnia magna</i>	T1 Bioencapsulated <i>Daphnia magna</i> with 1×10 <sup>7</sup> CFU/ ml	T2 Bioencapsulated <i>Daphnia magna</i> with 2×10 <sup>7</sup> CFU/ ml	T3 Bioencapsulated <i>Daphnia magna</i> with 3×10 <sup>7</sup> CFU/ ml
Initial weight (mg)	74.9±9.71	74.9±9.71	74.9±9.71	74.9±9.71
Final body weight (mg)	391.67±55.24 <sup>c</sup>	798.43±73.33 <sup>b</sup>	863.30±55.75 <sup>ab</sup>	817±67±83.35 <sup>ab</sup>
Specific growth rate (% BW day <sup>-1</sup> )	5.54±0.12 <sup>c</sup>	8.23±0.26 <sup>b</sup>	8.55±0.33 <sup>ab</sup>	8.23±0.27 <sup>b</sup>
Daily growth coefficient (%)	1.04±0.02 <sup>c</sup>	1.75±0.08 <sup>b</sup>	1.85±0.09 <sup>ab</sup>	1.76±0.09 <sup>b</sup>
Thermal growth coefficient (%)	5.30±0.12 <sup>c</sup>	9.01±0.4 <sup>b</sup>	9.41±0.44 <sup>a</sup>	9.05±0.45 <sup>b</sup>
Conversion efficiency ratio	20.80±0.92 <sup>c</sup>	66.01±9.84 <sup>b</sup>	73.71±6.01 <sup>ab</sup>	68.74±7.40 <sup>ab</sup>
Food conversion efficiency	11.49±0.54 <sup>c</sup>	26.08±2.37 <sup>b</sup>	29.12±2.11 <sup>ab</sup>	27.16±3.49 <sup>ab</sup>
Feed conversion ratio	8.48±0.33 <sup>a</sup>	3.73±0.27 <sup>b</sup>	3.39±0.21 <sup>b</sup>	3.6±0.25 <sup>b</sup>
Survival	79.99±5.71 <sup>b</sup>	99.88±0.68 <sup>a</sup>	97.65±2.31 <sup>a</sup>	99.79±0.84 <sup>a</sup>
Protein productive value (g)	1.5±0.24 <sup>c</sup>	3.25±0.49 <sup>b</sup>	3.88±0.68 <sup>a</sup>	3.66±0.23 <sup>ab</sup>
Lipid productive value (g)	0.13±0.00 <sup>d</sup>	0.25±0.05 <sup>c</sup>	0.30±0.03 <sup>b</sup>	0.36±0.01 <sup>a</sup>
Protein gain (g)	0.22±0.06 <sup>c</sup>	0.47±0.06 <sup>a</sup>	0.52±0.04 <sup>a</sup>	0.49±0.07 <sup>a</sup>
Energy retained as protein (PD.KJ day <sup>-1</sup> )	5.27±1.27 <sup>b</sup>	11.27±2.19 <sup>a</sup>	12.35±3.21 <sup>a</sup>	11.65±2.97 <sup>a</sup>
Protein efficiency ratio (g)	9.31±2.19 <sup>b</sup>	19.08±2.39 <sup>a</sup>	20.84±4.08 <sup>a</sup>	19.71±0.211 <sup>a</sup>
Lipid efficiency ratio (g)	15.13±3.31 <sup>b</sup>	31.02±6.79 <sup>a</sup>	33.87±4.36 <sup>a</sup>	32.03±4.04 <sup>a</sup>

The *probiotics bacillus* had significant positive effects on the Specific growth rate (SGR), Food conversion efficiency (FCE), Thermal growth coefficient (TGC) and Daily growth coefficient (DGC) in comparison with control treatment ( $p < 0.05$ ). The maximum of SGR ( $\% \text{ BW day}^{-1}$ ), TGC (%) and DGC (%) were obtained in treatment of T2. The *bacillus spp* in experimental treatments whereas the Persian sturgeon larvae were fed by bioencapsulated *Daphnia magna* with this probiotic bacillus, significantly increased the Conversion efficiency ratio (CER) ( $p < 0.05$ ). The survival rate was significantly ( $p < 0.05$ ) increased in T1 ( $99.88 \pm 0.68\%$ ), T2 ( $97.65 \pm 2.31\%$ ) and T3 ( $99.79 \pm 0.84\%$ ) in comparison with control treatment ( $79.99 \pm 5.71\%$ ). In experimental treatments the Food conversion ratio (FCR) decreased in comparison with control treatment ( $p < 0.05$ ). The best results in this trial obtained in experimental treatment where Persian sturgeon larvae were fed with bioencapsulated *Daphnia magna*.

The best results in this trial obtained in experimental treatment whereas Persian sturgeon larvae were fed with bioencapsulated *Daphnia magna*. The maximum of Protein productive value ( $3.88 \pm 0.49$ ) was showed in treatment of T2.

The results indicated that the *bacillus spp* significantly promoted levels of Protein productive value (PPV) and Lipid productive value (LPV) in experimental treatments in comparison with control treatment ( $p < 0.05$ ). The level of Protein efficiency ratio (PER) and Lipid efficiency ratio (LER) had significant difference in experimental treatments in comparison with control ( $p < 0.05$ ).

## DISCUSSION

The incorporation of probiotics via live food constitutes a very important potential tool for supplying probiotics to the larvae. *D. magna* is one of the most important live foods that were used as a vector to carry bacteria to digestive system of *A. persicus* larvae, while the most studies about the probiotics concerned with bioencapsulations by *Artemia* and rotifer. Bioencapsulation of *Daphnia* by *bacillus spp* and other probiotics were not reported. This study highlights the effects of probiotics bacilli on the enhancement of growth of *A. persicus* larvae. The beneficial influence of *bacillus spp* on growth parameters and survival rate of *Acipenser persicus* larvae, were completely observed. All the probiotic treatments resulted in growth performances better than of control treatment while the treatment of T2 had higher than all groups. The best performance of fish in terms of growth performance and

feed utilization efficiency was recorded at the enrichment *D. magna* with  $3 \times 10^7$  bacteria per milliliter in suspension of broth. Then this concentration of bacteria was recognized the best level for process of bioencapsulations of *D. magna* by *bacillus spp* in feeding of *Acipenser persicus* larvae.

Some reports have shown that probiotics bacillus has been recognized to have potential as a substitute for live food in the production of certain fish or as a potential replacement for fish meal and potential of probiotic [22]. Bagheri *et al.* [34] found that supplementation of trout starter diet with the proper density of commercial bacillus probiotic could be beneficial for growth and survival of rainbow trout fry. This finding agrees with our results. Ghosh *et al.* [35] indicated that the *B. circulans*, *B. subtilis* and *Bacillus pumilus*, isolated from the gut of Rohu, have extracellular protease, amylase and cellulase and play an important role in the nutrition of Rohu fingerlings. In experimental trials, the *bacillus spp* optimized the feed consumption of *A. persicus* larvae. In the probiotic experimental treatments the FCR with the present of bacteria significantly decreased. The other feeding parameters as DGC, TGC and FCE significantly increased. Similar effects had been reported for other fishes to increase considerably with the use of probiotic in the diet [36].

In this study different results of growth parameters were obtained from using different levels of probiotics bacillus in enrichment of *D. magna*. The growth parameters of FBW, CER in probiotic trials of T2 had the highest levels in comparison with control. The positive effect of *Bacillus spp.* were observed by Gatesoupe [37] in using *Bacillus toyoi* on turbot (*Scophthalmus maximus* Linnaeus 1758), Swain *et al.* [38] in Indian carps that improved the growth factors and feeding performance and Ghosh *et al.* [39] on the Rohu. The SGR significantly increased in experimental treatments and so survival rate of experimental treatment significantly increased in comparison with control.

In treatment T2, the Persian sturgeon larvae was fed by bioencapsulated *Daphnia magna* in level of  $2 \times 10^7$  CFU  $\text{ml}^{-1}$  of suspension of broth, had the best results in growth parameters. The results of the present experiment highlighted that Persian sturgeon larvae effectively could with *bacillus spp* show high efficiency in promotion of growth and feeding performance and survival rate.

The best results was showed by *Daphnia magna* bioencapsulated via  $2 \times 10^7$  bacteria per milliliter in suspension of broth. The result indicated that always growth performance and survival rate don't increase with increasing concentrations of probiotics, similar results

was reported in feeding bluga larvae via bioencapsulation *bacillus* spp. [40]. Perhaps *Daphnia magna* has a limited capacity for carrying bacteria to larval digestive tract.

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