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The Effects of Different Concentrations of Probiotic *Bacillus* spp. And Different Bioencapsulation Times on Growth Performance and Survival Rate of Persian Sturgeon (*Acipencer persicus*) Larvae

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Abstract: A 3 × 3 factorial experiment was conducted for four weeks to determine the effects of different concentrations of probiotic *bacillus spp*. and different bioencapsulation times on Persian sturgeon larvae survival rate and growth parameters. Daphnia with three concentrations of bacteria, 1×10^7, 2×10^7 and 3×10^7 bacteria per milliliter in suspension of broth at 3 time of bioencapsulation (10, 5 and 3 hours) were bioencapsulated and sturgeon larvae were fed by them. Sturgeon larvae were fed 30 percent of their body weight for 5 times a day. At the end of the experiment we found that different times and different concentrations of bacteria could effect on growth parameters in Persian sturgeon larvae. Persian sturgeon larvae in different treatments fed bioencapsulated daphnia differ significantly in conversion efficiency ratio (CER), specific growth rate (SGR), food conversion ratio (FCR), condition factor (CF) and Daily growth coefficient (DGC) ($P<0.05$). Survival of all groups was no significantly different after 28 days. The result indicated that different times of bioencapsulation and different concentrations of bacteria could influence on growth parameters in Persian sturgeon larvae ($P<0.05$).

Key words: Probiotic % Bioencapsulation % *Daphnia magna* % Growth % Survival % *Acipencer persicus*

INTRODUCTION

The word probiotic is constructed from the Latin word pro (for) and the Greek word bios (life). The definition of a probiotic differs greatly depending on the source, but the first generally accepted definition was proposed as "…a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance" [1].

Animal gut microflora consists of hundreds of different bacterial strains, [2] able to promote digestion and absorption of nutrients, to increase body resistance to infectious diseases [3], to yield positive affects on growth and to improve general animal welfare [4]. FAO has now designated the use of probiotics as a major means for the improvement of aquatic environmental quality [5]. 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 [6], growth [7], stress resistance [8], immunosystem enhancement [9, 10] and finally general welfare [11, 12].

Most studies on the effects of probiotics on cultured aquatic animals have emphasized a reduction in mortality or the improved resistance against putative pathogens [13]. However, the beneficial effects are sometimes temporal, depending on the time of exposure [14]. As most fish contain a specific intestinal microbiota established at the juvenile stage [15], the colonization of probiotics to fish intestines requires adequate probiotics presented in ambient microbial community (MC) and their interaction with MC should not be neglected. In aquaculture, captive

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rearing conditions generally can be sources of stress, triggering high mortality, mainly during larval rearing. 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 [16-19].

Aquatic probiotics have been defined as live microbial supplements that can modulate microbial communities and improve microbial balance, thus providing benefits to the host [20]. Furthermore, probiotic *Bacillus* species have been shown to improve digestive enzyme activities, growth and survival of crustaceans [21-24]. The beneficial effects of these probiotics include higher growth and feed efficiency, prevention of intestinal disorders and pre-digestion of anti-nutritional factors present in the ingredients. Also, existing literature on probiotics usually focused on resistance to some aquatic pathogens such as *Vibrio spp.* [25, 26], *Amyloodinium ocellatum* [27] and *Carnobacterium sp.* These studies mainly focused on effects of probiotics on enhancement of survival and nutritional parameters such as feed efficiency and feed conversion ratio. *Bacillus* can act positively on cultured organisms by enhancing survival and growth [28], by stimulating the digestive [29]. 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 [30, 31]. Concurrently, *Bacillus* species can be found in marine environment and are part of the microflora of several marine species [32]. little studies had been carried out to incorporate probiotics into a freahwater species common carp, *C. carpio* [33]. As stated above, the application of probiotics in aquaculture as the environment friendly treatments has been also increasing rapidly [34] and some papers were associated with the effect of probiotics in fish and other marine organisms [35]. Probiotics is usually defined as live microbial feed supplements, that are administered in such a way as to enter the gastrointestinal tract and to be kept alive; this beneficially affects the host animal by improving its intestinal microbial balance and in turn its health [36].

Although, beneficial effects of probiotics are well known in aquaculture, there is no information about best times of bioencapsulation and best concentrations of bacteria for enrichment by *Daphnia*. Therefore, this study was designed to we found best time and concentration of probiotics bioencapsulationt for *Daphnia*. The present study examined the effects of concentrations probiotic *Bacillus spp*. and enrichment times on growth factors and survival rate in Persian sturgeon *Acipencer persicus* larvae via feeding by bioencapsulated *Daphnia magna*.

MATERIALS AND METHODS

Experimental Materials and Animals: The probiotic *Bacillus* was prepared from Protexin Co (Iran-Nikotak). The three species of probiotic *Bacillus* as bacterial blend under the commercial title of Protexin aquatic were used for bioencapsulation of *Daphnia magna*. The blends of probiotic bacillii (*Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus circulans*) from suspension of spores with special media were provided. Three concentrations of bacterial suspension, 1×10^7, 2×10^7 and 3×10^7 bacteries per milliliter (CFU ml^{-1}) were provided by Protexin Co and the colony forming unit (CFU) of probiotic bacillii were tested by microbial culture in Tryptic Soy Agar (TSA).

The *Daphnia magna* and persian sturgeon larvea were obtained from intensive production ground ponds of the center of sturgeon culture of Marjani (Iran). *D.magna* is a important live food that were used as a vector to carry probiotic bacillus to digestive tract of *Acipencer persicus* larvae.The *Daphnia magna* with density of 5 g live *Daphnia* $litter^{-1}$ was enriched with *Bacillus circulans*, *Bacillus subtilis* and *Bacillus licheniformis* in density of 1×10^7, 2×10^7 and 3×10^7 bacteries per milliliter for 10, 5 and 3 hours in suspension of broth.

Experimental Setup: Twenty-seven 40-L plastic tanks (with water circulation) with three replicates for experimental treatments were used. This experiment was conducted in a completely randomized design with nine treatments (treatment 1-9). Ten-day old Persian sturgeon (Acipenser persicus) Larvae (initial weight: 74.9±0.89 mg) were obtained from the center of sturgeon culture of Marjani (Iran) and cultured for 28 days. The density of fish larvae in per tank were 71 larvae. Persian sturgeon larvae in experimental treatments were fed 30 percent of their body weight for 5 times in a day (2.00, 7.00, 12.00, 17.00 and 22.00). Water quality parameters of input water to rearing system were monitored each week throughout the experimental. The water temperature was 19.46±1.23°C, pH was 7.85±0.26 and water oxygen level was maintained above 7.65 ± 0.55 mg l^{-1} during the experiment by setting electrical air pump (by a single filtration unit).

Treatment one, two and three fed by bioencapsulated *Daphnia* by 1×10^7, $2\times10^7$and 3×10^7 bacteria per milliliter in

10 hours, respectively. Treatment four, five and six fed by bioencapsulated *Daphnia* by 1×10^7, 2×10^7 and 3×10^7 bacteria per milliliter in 5 hours, respectively. Treatment seven, eight and nine fed by bioencapsulated daphnia by 1×10^7, 2×10^7 and 3×10^7 bacteria per milliliter in 3 hours. The water circulation was stopped in all tanks for 2 h after every application of feed to allow the larvae to ingest the *Daphnia*. Sturgeon larvae were fed 30 percent of their body weight for 5 times a day.

Sample Collection: Fish were individually weighted at the beginning and at the end of the experiment. Before distributing fish to the experimental tanks (in the beginning of exogenous feeding), 30 fish were sampled from the holding tank for biometry. In the termination of experiment, 55 larvae from each tank were sampled and the final weight and length of body were measured.

Data Analysis: The results were presented as means±Sd. Two-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using SPSS program.

RESULTS

The feeding and growth parameters of Persian sturgeon larvae are presented in Table 1. Laboratory based growth trials indicated that time of enrichment and concentrations of bacterial suspension effected on growth rate of the larvae (Table1). Significant difference was observed for FCR between the treatment groups ($p<0.05$). After 28 days, there was significant difference between the final body weights of groups, although the survival in each group had no significantly difference with different concentration of probiotics and different times of bioencapsulation. The mean final weight of experimental treatment group T6 was significantly higher than other groups ($p<0.05$). The values of specific growth rate (SGR), condition factor (CF) and Daily growth coefficient (DGC) in all groups treated with probiotics at all concentrations and time were significantly different. Also, mean values of FCR were significantly different among treatment groups. Different concentration of probiotics and different times of bioencapsulation had no significant positive effect on survival in experimental groups ($p<0.05$). The highest weight gain and lower FCR was obtained in experimental treatment T6.

Table 1 Growth parameters and survival of Persian sturgeon (*Acipencer persicus*) larvae in experimental treatments

Treatments / Parameters	T1 Bioencapsulated *Daphnia magna* with 1×10^7 CFU/ ml at 10 hours	T2 Bioencapsulated *Daphnia magna* with 2×10^7 CFU/ ml at 10 hours	T3 Bioencapsulated *Daphnia magna* with 3×10^7 CFU/ ml at 10 hours	T4 Bioencapsulated *Daphnia magna* with 1×10^7 CFU/ ml at 5 hours	T5 Bioencapsulated *Daphnia magna* with 2×10^7 CFU/ ml at 5 hours	T6 Bioencapsulated *Daphnia magna* with 3×10^7 CFU/ ml at 5 hours	T7 Bioencapsulated *Daphnia magna* with 1×10^7 CFU/ ml at 3 hours	T8 Bioencapsulated *Daphnia magna* with 2×10^7 CFU/ ml at 3 hours	T9 Bioencapsulated *Daphnia magna* with 3×10^7 CFU/ ml at 3 hours
IW(mg)	74.9±0.89	74.9±0.89	74.9±0.89	74.9±0.89	74.9±0.89	74.9±0.89	74.9±0.89	74.9±0.89	74.9±0.89
FW(mg)	617.04 ± 17.40^{d}	642.98 ± 57.88^{d}	640.63 ± 19.01^{d}	915.43 ± 73.33^{b}	892.30 ± 55.75^{bc}	1032.67 ± 83.35^{a}	798.23 ± 94.21^{c}	863.57 ± 62.27^{bc}	817.29 ± 61.35^{bc}
CF	0.537 ± 0.012^{ab}	0.527 ± 0.013^{abc}	0.530 ± 0.010^{abc}	0.492 ± 0.014^{d}	0.500 ± 0.011^{cd}	0.427 ± 0.018^{d}	0.507 ± 0.021^{bcd}	0.489 ± 0.022^{d}	0.492 ± 0.012^{d}
CER(%)	43.91 ± 1.96^{d}	46.89 ± 6.66^{d}	46.65 ± 2.27^{d}	80.28 ± 9.84^{b}	77.73 ± 6.01^{bc}	95.64 ± 11.40^{a}	6.01 ± 12.28^{c}	73.71 ± 7.17^{bc}	68.74 ± 7.81^{bc}
FCR	4.92 ± 0.15^{a}	4.73 ± 0.50^{a}	4.71 ± 0.15^{a}	3.1 ± 0.27^{bc}	3.27 ± 0.21^{bc}	2.8 ± 0.25^{bc}	3.73 ± 0.49^{b}	3.39 ± 0.26^{b}	3.60 ± 0.29^{b}
SGR(% BW day-1)	7.36 ± 0.11^{d}	7.51 ± 0.32^{d}	7.49 ± 0.09^{d}	8.79 ± 0.26^{b}	8.66 ± 0.33^{bc}	9.24 ± 0.27^{a}	8.23 ± 0.36^{c}	8.55 ± 0.35^{bc}	8.23 ± 0.26^{c}
DGC(%)	1.5 ± 0.03^{d}	1.54 ± 0.08^{d}	1.46 ± 0.13^{d}	1.92 ± 0.08^{b}	1.88 ± 0.09^{bc}	2.06 ± 0.09^{a}	1.75 ± 0.11^{c}	1.85 ± 0.10^{bc}	1.76 ± 0.08^{c}
SURVIVAL(%)	94.28 ± 4.28^{a}	96.18 ± 2.18^{a}	98.09 ± 2.16^{a}	99.88 ± 0.68^{a}	97.65 ± 2.31^{a}	99.79 ± 0.84^{a}	99.52 ± 0.82^{a}	95.12 ± 3.59^{a}	95.36 ± 3.45^{a}

Groups with different alphabetic superscripts differ significantly at $p<0.05$

DISCUSSION

The current study demonstrated different times and concentrations of bacteria for enrichment *Daphnia* whit commercial *Bacillus spp*. was significantly difference in growth parameters among experimental treatments. Similar findings have previously been documented in preliminary trials on larval *H. gammarus* [37]. Also, this result indicated that different times and concentrations of bacteria not differed significantly in survival. Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to control potential pathogens and to increase the growth rates and welfare of farmed aquatic animals [38-40]. Here, we reported, for the first time, an enhancement of the growth rate of the *Acipencer persicus*, one of the most important native species for the Iran. All the probiotic-resulted in an increase of Final weight, showing that the addition of probiotics increased the growth performance of shrimps. Similar results were reported for Indian carp (*Labeo rohita*) [41].

Supplementation of trout starter diet with the proper density of commercial *Bacillus* probiotic could be beneficial for growth and survival of rainbow trout fry [42]. The *B. circulans*, *B. subtilis* and *Bacillus pamilus*, isolated from the gut of Rohu, have extracellular protease, amylase and cellulose and play an important role in the nutrition of Rohu fingerlings [43]. Here, we studied the effects of a combination of *Bacillus sp.* (*Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus circulans*) at different concentrations and different times of enrichment on growth performance in Persian sturgeon larvae. There was significant difference in growth parameters rate among the treatment groups with different concentrations of probiotics and different time of enrichment (Table 1). This indicated that the quantity of probiotics and time of bioencapsulation is important factors promoting the growth performance of larvae. In the present study, different concentrations of probiotics had significantly different effects on growth parameters; also different times of enrichment had significantly different effects on growth parameters. The best body weight and SGR and FCR were obtained in experimental treatments that larvae fed by daphnia that enriched in 5 hours and lowest growth performance obtained in treatment T1. In this study we found that the best time for bioencapsulation of *Daphnia* whit bacillus is 5 hours that we think 3 h for enrichment is low time because *Daphnia* don't had enough time for caring bacteria, also 10 h is much time because *Daphnia* excrete bacteria after feeding bacteria thus in this study we found that 5 h is best time for enrichment. Effect of concentrations of probiotics in different treatments was different and also we think being effective from time of enrichment that showed in Table 1. This result indicated that always growth performance and survival rate don't increase whit increasing concentrations of probiotics, similar results were reported in feeding bluga larvae via bioencapsulation *bacillus spp.* [18].

In conclusion, research aimed to determine best time of bioencapsulation time *Daphnia* with *Bacillus spp*. and best concentration of bacteria. The results presented in this study that enrichment time and concentrations of *Bacillus* spp. did show significant difference in the growth rate, furthermore we found that different enrichment time and concentrations of *Bacillus* spp. had no significantly difference on survival.

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