Effects of *Artemia* nauplii enrichment with a bacterial species (*Weissiella koreensis*) on growth performance and survival rate of stellate sturgeon larvae (*Acipenser stellatus*)

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Abstract

Artemia franciscana nauplii was enriched with a bacterial species *Weissiella koreensis* (lactic acid gram-positive) isolated from the alimentary tract of Stellate sturgeon (*Acipenser stellatus*), at 3 levels. Treatments included T_1: 6.9×10^4 colony-forming units (CFU) mL^{-1}, T_2: 6.9×10^5 CFU mL^{-1}, T_3: 6.9×10^6 CFU mL^{-1} and their effects were compared with the control diet (no probiotic were added). Each diet was fed to triplicate tanks. In total 3600 larvae were distributed in 12 fiberglass tanks (300 larvae per each tank contain 100 L of water). The larvae were fed, 60% of the body weight per day, with enriched *Artemia* nauplii, immediately after absorption of yolk sac, 6 times a day for 14 days. The results showed that, enrichment of *Artemia* nauplii with *Weissiella koreensis* in the T_1, T_2 and T_3 could significantly enhance feed conversion ratio (FCR), in comparison with control (P<0.05). Survival in T_3 and T_1 was significantly more than control (P<0.05). Also biomass increase, in T_3 was significantly more than control (P<0.05). It can be stated that, the T_3, in term of effect on growth performance was in a more favorable condition than others treatments and control.

Keywords: Probiotic, Enrichment, *Artemia* nauplii, Sturgeon, *Acipenser stellatus*, Larvae.

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Introduction

One of the important starter feeds used in larviculture is newly hatched nauplii of *Artemia* (Agh and Sorgeloos, 2005; Bahadir Koca *et al*., 2015). *Artemia* sp. is a common live feed used for the rearing of sturgeon fish larvae (Roozbehfar *et al*., 2012) and widely use in aquaculture due to the high nutritional value, the appropriate size and the enrichment possibility (Azimirad and Meshkini, 2017). It can be used as a carrier of particles in aquaculture such as nutrients (fatty acids, vitamins, etc.), antimicrobial substances, vaccines and probiotics (Yan Loh and Yien Ting, 2015; Azimirad and Meshkini, 2017). The process of incorporating some ingredients to the nauplii is termed as bioencapsulation (Jamali *et al*., 2012). Probiotics that are used for aquaculture include lactic acid bacteria, Bacillus bacteria, vibrio and yeasts (Jamali *et al*., 2012). Probiotics are live microbial food supplements (Seenivasan *et al*., 2012), which offer benefits to the host (Sahandi *et al*, 2012; Iranshahi *et al*., 2012; Pourgholam *et al*., 2017), through the formation of colonies in the intestine (Arığ *et al*., 2013). Also, probiotics could improve growth performance and feed conversion efficiency of fish (Ibrar *et al*., 2017). Changing the microflora of the intestine through probiotics plays an important role in increasing gowth and survival (Soltani *et al*., 2016). The gastrointestinal tract of the fish larvae, has no bacteria at birth and before the onset of feeding (Jafaryan *et al*., 2007). After that, bacterial flora is formed by the introduction of environmental bacteria through water and food and replacing it in the intestinal tract of fish (Jafaryan *et al*., 2007). There is no boundary between the microbial community inside and outside the fish (Ibrahem, 2013). The reason for this is that, there is a permanent effect between the environment and the fish (Ibrahem, 2013). The bacteria present in the aquatic environment affect the composition of the gut microbiota and vice versa (Ibrahem, 2013). In this situation, probiotics must dominate (Ibrahem, 2013).

It is also mentioned that, in the early stages of development of fish larvae, increasing the number of bacteria in the intestine microflora is mainly due to living bacteria in live food (Azimirad and Meshkini, 2017). With increasing population of opportunistic bacteria in the fish intestine, mortality increases in the early life stages of fish and control of the bacterial population in the live foods may lead to higher survival rates of fish larvae (Azimirad and Meshkini, 2017). The objectives of this study was to determine the effect of enrichment of *Artemia* nauplii with a probiotic species isolated from the same species of fish, on growth performance of larvae and determine the best level of probiotic for enrichment.

Material and methods

Hatching of Artemia cysts and enrichment

Hatching of decapsulated *Artemia franciscana* cysts was performed through the use of cone-shaped container with a volume of 100 L and with salinity of 30 g per L. Cysts were incubated at 30±1^{o}C with 2000 lux lighting conditions and vigorous aeration (Azimirad and Meshkini, 2017), with a density of 2 g per L. *Artemia franciscana* nauplii was enriched with a lactic acid gram-positive bacterial species (*Weissiella koreensis*), isolated from the alimentary tract of stellate sturgeon (*Acipenser stellatus*) at 3 levels. *Artemia* nauplii enrichment was performed 6 hours after hatching by adding bacteria to the amounts indicated (Ziaei-Nejad, 2014), in 20 L containers with severe aeration, 2000 lux lighting, and water temperature (30±1^{o}C) for 10 hours (Jamali *et al*., 2012).

Treatments and control group

Treatments included, Treatment 1 (T_1): 6.9×10^4 Colony-Forming Units (CFU) mL^{-1}, T_2: 6.9×10^5 CFU ml^{-1}, T_3: 6.9×10^6 CFU ml^{-1} and their effects were compared with the control diet (no probiotic were added). Each diet was fed to triplicate tanks.

Cultivation of fish larvae

For the cultivation of fish larvae, fiberglass tanks (103 cm×100 cm×50 cm size) were used and 100 L of water was poured into each tank. In total 3600 larvae with an average initial weight, initial length, initial biomass of 0.042±0.001 g, 12.6±0.3 g and 2.05±0.01 cm were distributed in 12 fiberglass tanks, respectively (300 larvae per each tank). The larvae were fed 60% of the body weight per day, with enriched *Artemia* nauplii, immediately after absorption of yolk sac, 6 times a day (Ghebanov and Galich, 2013) for 14 days. Some physico-chemical parameters of water were measured daily (Table.1).

Table 1: Average (Mean ± SD) of some physico-chemical parameters of water.

Mean ± SD	Parameters
Dissolved oxygen (mg/L)	8.28±0.16
Water temperature (Co)	20.01±0.53
Salinity (mg/L)	0
pH	7.42±0.19

Sample collection and analyses

Determining the weight and the number of larvae was performed 14 days after the start of study. For this purpose, larvae in each tank were counted and their average weight was determined. Survival rate, Weight gain, Specific growth rate, feed conversion ratio, Condition factor (Seenivasan *et al*., 2012), Daily growth rate, Specific growth rate (Sahandi *et al*., 2012), Biomass increase (Jamali *et al*., 2012) were determined using following formulations:

Survival rate (SR, %) = No. of live fish/ No. of fish initially introduced × 100

Biomass increase (BI, g) = Final fish biomass (g) – Initial fish biomass (g)

Body weight increase (BWI, %) = Final weight (g) – Initial weight (g)/ Initial weight × 100

Specific growth rate (SGR, g day^{-1}) = Final weight (g) - Initial weight (g)/ Days of experiment× 100

Feed conve rsion ratio (FCR) = Feed intake (g)/ Weight gain (g)

Condition factor (CF) = Fish weight (g)/Fish length (cm) ^{3}×100

Daily growth rate (DGR, g day^{-1}) = [(Final body weight–Initial body weight)/days of experiment].

Specific growth rate (SGR, %)=[ln final weight of fish – ln initial weight of fish)]/days of feeding ×100

Statistical analysis

This experiment was conducted based on a completely randomized design. All statistical analyses used the SPSS statistical package version 16.0. One-way analysis of variance (ANOVA) and Duncan's multiple comparison tests were used to identify significant variations at 0.95 confidence limits (P=0.05).

Results

Growth performance

The effects of enrichment of *Artemia franciscana* nauplii with the probiotic *Weissiella koreensis* on the growth performance of stellate sturgeon larvae are given in Table 2. Final weight in T_3 was higher than other treatments and control, but there was no significant difference between T_2, T_3 (0.181±0.03 g and 0.192 ±0.01 g respectively) and control (0.184±0.03 g) (P>0.05). Final length in T_3 (3.70±0.02 cm was more than other treatments and control (3.58±0.27), but there was no significant difference between T_3 and control (P >0.05).

Table 2: Growth parameters of *Acipenser stellatus* larvae under feeding of enriched *Artemia* sp. with different levels of a probiotic (*Weissiella koreensis*).

Parameters	**Treatments**			
	Control	**6.9×10^4 T_1**	**6.9×10^5 T_2**	**6.9×10^6 T_3**
Final Weight (g)	0.184±0.03^{ab}	0.179±0.01^{a}	0.181±0.03^{ab}	0.192±0.01^{b}
Final length (cm)	3.58±0.27^{abc}	3.60±0.02^{c}	3.47±0.17^{a}	3.70±0.02^{b}
Final biomass (g)	41.4±11.16^{a}	48.80±4.96^{a}	47.47±1.48^{a}	53.03±2.48^{b}
Survival (%)	74.70±13.08^{a}	90.63±10.60^{b}	88.33±12.10^{ab}	92.00±4.16^{b}
Biomass increase (g)	28.80±10.89^{a}	36.20±4.69^{a}	34.87±1.42^{a}	40.43±2.20^{b}
Feed conversion ratio (FCR)	3.98±1.19^{a}	2.96±0.35a^{b}	3.04±0.14a^{b}	2.62±0.09^{b}
Specific growth rate (SGR)	10.50±1.25^{a}	10.37±0.32^{a}	10.40±1.05^{a}	10.87±0.15^{a}
Body weight increase (BWI)	339.30±74.48^{ab}	327.83±20.06^{a}	332.03±62.35^{ab}	358.00±9.40^{b}
Daily growth rate (DGR) (g)	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.011±0.00^{a}
Condition factor (CF)	0.40±0.03^{ab}	0.38±0.02^{b}	0.43±0.02^{a}	0.38±0.01^{b}

Each value is mean ± SD of 3 individual observations. Different letters in each row mean significant difference (Duncan's multiple comparison tests, p<0.05).

Survival rate in T_1 and T_3 were significantly more than control (92.00±4.16%, 88.33±12.10%, 90.63±10.60% and 74.70±13.08% respectively) (P<0.05) (Fig. 1). Feed conversion ratio (FCR) in T_3 (2.62±0.09) was significantly lesser than control (3.98±1.19), but there was no significant difference between T_3, T_1 (2.96±0.35) and T_2 (3.04±0.14) (Fig. 2) (P<0.05). Also biomass increase, in T_3 (40.43±2.20 g) was significantly higher than other treatments and control, but there was no significant difference between T_1 (36.20±4.69 g), T_2 (34.87±1.42 g) and control (28.80±10.89 g) (P <0.05) (Fig. 3).

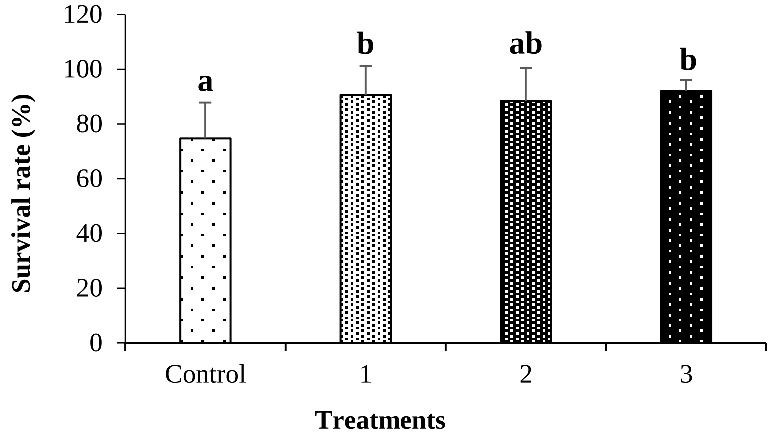

Figure 1: Survival rate in different treatments.

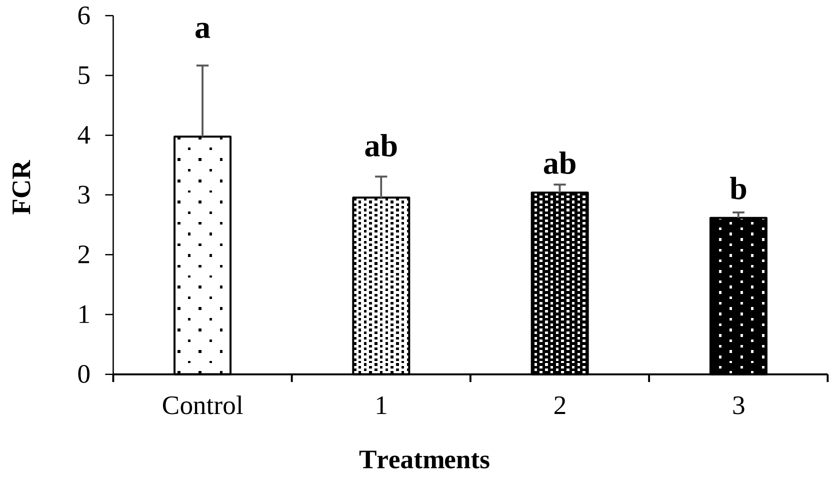

Figure 2: FCR values in different treatments.

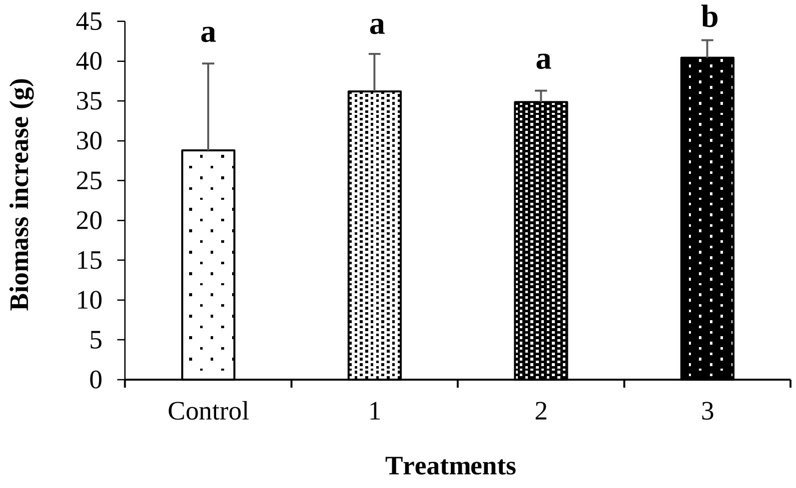

Figure 3: Biomass increase (g) in different treatments.

Specific growth rate (SGR) in T_3 (10.87±0.15) was significantly higher than other treatments and control (10.50±1.25), but there was no significant difference between T_2, T_1 and control (P<0.05). Condition factor (CF) in T_2 was more than others treatments and control, but there was significant difference between T_2, T_1 and T_3 (P <0.05). There was no statistically significant difference in terms of daily growth rate (DGR) between control and other treatments (P <0.05). Body Weight Increase (BWI) in T_3 was more than other treatments and control, but had no significant difference with control treatment (P <0.05). Condition factor in T_2 was more than other treatments and control. But, it was significantly more than T_1 and T_3 (P <0.05). Treatment 3, in term of effect on growth parameters was in a more favorable condition than others treatments and control.

Discussion

Nauplii of *Artemia* sp. are well used for Sturgeon larviculture (Jafari *et al*., 2012). *Artemia* nauplii has used its own energy sources in the first 6 to 8 hours after hatching, so there is no need for other food sources (Ziaei-Nejad, 2014). During this time, *Artemia* Nauplii has the smallest size and high storage energy; therefore, it has the highest nutritional value for larvae (Ziaei-Nejad, 2014). So, in the present study, *Artemia* nauplii enrichment was carried out 6 hours after the cyst hatching. In the present study, fish larvae fed with *Artemia* nauplii enriched with *Wessiello korensis* at 6.9×10^6 CFU/mL (T_3), showed increased of growth and feeding parameters better than other treatments and control (Table 2). SGR and biomass increase in T_3 was significantly more than other treatments and control (P<0.05). Final weight in T_3 was higher than other treatments, but had a significant difference with T_1 (P<0.05). Similarly, Seenivasan *et al*. (2012) reported that the enrichment of *Artemia franciscana* naupli with *Lactobacillus sporogenes* cause to higher survival and growth in freshwater prawn *Macrobrachium rosenbergii* and the biomass increase, total weight gain, specific growth rate, condition factor and mean conversion ratio were found to be higher in PL fed with enriched *Artemia* when compared with control**.**

In expressing the positive effects of Probiotics, it can be stated that, probiotics could improve growth performance and feed conversion efficiency of fish by producing short chain fatty acids, digestive enzymes, vitamins and promote fish appetite and the digestion of anti-nutritional factors (Ibrar *et al*., 2017). In this connection, Bagheri *et al*. (2008) reported that, gram-positive bacteria, secret a wide range of exoenzymes and certain essential nutrients to increase growth.

In the present study, FCR in treatments 1, 2 and 3 were significantly lesser than control (P<0.05). In general, it can be stated that, the T_3, in term of effect on growth parameters was in a more favorable condition than others treatments and control. In this regard, Jafarian *et al*. (2007) investigated the effect of probiotic bacillus bioencapsulated with *Artemia urmiana* nauplii in $1.0^9 \times 10^5$, 2.2×10^5 and 3.18×10^5 CFU/mL on growth and survival of *Acipenser persicus* larvae and concluded that, the ability of probiotic *bacillus* to the increase of the growth performance is relatively high and with increasing probiotic concentration, growth parameters were improved. Askarian *et al*. (2011) after feeding Persian sturgeon (*Acipenser persicus*) and Beluga (*Huso huso*) with Chironomidae incorporated with *Lactobacillus curvatus* and *Leuconostoc mesenteroides* for 50 days resulted highest SGR and survival in the higher level of bacteria 2×10^9 CFU per gram food. In the present study, the feed conversion ratio in T_3, was significantly lower than other treatments and control. The reason is that, probiotics improves the feed conversion efficiency by stimulation of digestion due to higher production of digestive enzymes and vitamins (Ibrar *et al.*, 2017). Also Chebanov and Galich (2013) reported that, at feeding of *A. nudiventris* larvae by bioencapsulated *Artemia urmiana* nauplii, enriched for 10 h in bacterial suspension (*Bacillus licheniformis*, *B. subtillis*, *B. polymixa*, *B. laterosporus*, *B. circulans*), the best results were obtained at a higher concentration 3×10^5 CFU/mL. Zare *et al*. (2017) reported that, *Artemia urmiana* nauplii enriched with *Pediococcus acidilactici* as probiotic at a concentration of 10^{10} CFU mL^{-1} had a positive effect on growth and survival of beluga (*Huso huso*) larvae. Along with the mentioned research, this indicates a positive and influential role of probiotic on growth parameters in high concentrations. In the present study, in treatments 1, 2 and 3, survival of larvae was 15.93, 13.63 and 17% respectively higher than control. Like our results, Bagheri *et al.* (2008) reported that in all treatments in the rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic (commercial *Bacillus spp*) larvae survival was more than control. Yanbu and Zirong (2006) reported that addition of the photosynthetic bacteria and *Bacillus* sp. isolated from common carp, to common carp basal diet, showed significantly better results of growth performance and FCR than those with the basal diet. Also another study reported significant increase survival of shrimp larvae reared in water having probiotic Bacillus coagulans (Ibrar *et al*., 2017). In the present study, improve survival of larvae with *Weissiella koreensis* may be associated with the activation of non-specific immune system, effecting T-cell differentiation, decrease penetrability of epithelium for macromolecules and toxins or creating resistance to pathogens (Ibrar *et al*., 2017). This may also be due to, probiotic colonization in the digestive tract and their positive effect on the digestibility of nutrients in feed (Ibrar *et al*., 2017). Due to lower proportion of length to weight of larvae in T_2, condition factor in this treatment was more than other treatment and control, but was significantly more than T_1 and T_3 (P<0.05). The lower condition factor in T_1 and T_3 may indicate that, probiotic may be in some cases, lead to an increase in length over weight. The desired water temperature, dissolved oxygen, salinity and pH to be 20-26^{o}C, over 5.0 mg L^{-1}, 0-0.5 ppt and 6.5-8.5, respectively (Mims *et al*., 2002). In the present study, the parameters were in appropriate range (Table 1). In general, growth parameters resulting from enrichment of *Artemia* nauplii with probiotic in different levels were often better than control group. But it can be stated that, the treatment 3, in term of effect on growth parameters was in a more favorable condition than others treatments and control. Therefore, it is more appropriate for the stellate sturgeon larval stage.

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