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The effects of probiotic bacillus for promotion of growth and feeding parameters in beluga (*Huso huso*) larvae via feeding by bioencapsulated *Artemia*

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Abstract. In this study five species of probiotic bacillus as bacterial blend under the commercial title of Protexin aquatic were used for bioencapsulation of *Artemia urmiana* (Gunther, 1899). This experiment was conducted in a completely random design. *A. urmiana* naupli I was used as a vector to carry probiotic bacillus to digestive tract of *Huso huso (*Linnaeus, 1758*)* larvae. Nauplii with three concentrations of bacteria, 1×10^5, 2×10^5 and 3×10^5 bacteria per milliliter in suspension of broth for 10 hours were bioencapsulated and beluga larvae were fed by them. Beluga larvae were fed 50 percent of their body weight for 6 times a day. The control treatment was fed by unbioencapsulated artemia nauplii. The results indicated that the probiotic bacillus could influence on growth and feeding parameters in beluga larvae. The final body weight and specific growth rate (SGR) for weight in experimental treatments had significant difference in comparison to control treatment ($P<0.05$). In experimental treatments the Food efficiency was increased significantly in comparison with control. The probiotic bacillus had significant positive effects on conversion efficiency ratio (CER), Daily growth coefficient (DGC) and average weight gain (AWG) in comparison to control treatment ($P<0.05$). Also the relative food intake (RFI) significantly decreased ($P<0.05$) while Protein gain (PG) and Energy retained as protein (PD.KJ day^{-1}) significantly increased ($P<0.05$).

Key Words: Probiotic, bioencapsulation, *Artemia* nauplii, specific growth rate, relative food intake.

چکیده: در این مطالعه پنج گونه از باسیلوس های پروبیوتیکی به صورت مخلوط باکتریایی و با نام تجاری پروتکسین آکواتیک برای غنی سازی آرتمیای دریاچه ارومیه مورد استفاده قرار گرفت. این آزمایش در قالب طرح کاملا تصادفی انجام شد. ناپلی آرتمیای ارومیه به عنوان وکتور و برای انتقال باسیلوس های پروبیوتیکی به دستگاه گوارش لارو فیل ماهی *Huso huso (*Linnaeus, 1758) مورد استفاده قرار گرفت. ناپلی ها با سه غلظت از باکتری (1×10^5، 2×10^5 و 3×10^5 باکتری در هر میلیلیتر از سوسپانسین براث) به مدت 10 ساعت غنی سازی شده و لاروهای فیل ماهی توسط آنها تغذیه شدند. لاروهای فیل ماهی بر اساس 50 درصد از وزن بدن خود و 6 بار در روز تغذیه می شدند. گروه کنترل نیز توسط ناپلی آرتمیای غنی نشده تغذیه شدند. نتایج نشان داد که پروبیوتیک های باسیلوسی می توانند بر رشد و پارامترهای تغذیه ای در لارو فیل ماهی موثر باشند. وزن نهایی و نرخ رشد ویژه (SGR) برای وزن در تیمار های آزمایشی تفاوت معنی داری در مقایسه با گروه کنترل داشتند ($P <.005$). در تیمارهای آزمایشی، کارایی غذایی به میزان قابل توجهی در مقایسه با گروه شاهد افزایش یافت. پروبیوتیک های باسیلوسی اثر قابل توجه و مثبتی را بر ضریب تبدیل غذا (CER)، ضریب رشد روزانه (DGC) و میانگین وزن به دست آمده (AWG) در مقایسه با گروه کنترل داشت ($P<0.05$). همچنین میزان نسبی غذای خورده شده (RFI) به صورت معنی داری کاهش یافت ($P<0.05$) در حای که میزان پروتئین به دست آمده (PG) و انرژی ذخیره شده بر مبنای پروتئین (PD.KJ day^{-1}) به طور معنی داری افزایش یافت ($P<0.05$).

کلمات کلیدی: پروبیوتیک، غنی سازی، ناپلی آرتمیا، نرخ رشد ویژه، غذای نسبی خورده شده.

Introduction. The use of probiotics has a long tradition in animal husbendery (Stavric & Kornegay 1995) but has rarely been applied in aquaculture. Probiotics are usually defined as live microbial food supplements, that are administrated in such a way as to enter the gastro-intestinal tract and to be kept alive, this beneficially affects the host animal by improving its intestinal microbial balance and in turn its health (Gatesoupe 1999). Appropriate probiotic applications were shown to intestinal microbial balance, which led to improved food absorption (Fuller 1989). Optimization of zootechnical, nutritional and microbiological factors can reduce the heavy mortalities that often occur during the rearing of marine fish larvae (Olsen 1997). The use of probiotic bacteria has been suggested as an important strategy to accomplish reproductible outputs through biocontrol in cultivation systems for marine fish larvae and crustaceans (Nogami 1992).

The bacterial flora in the larval gut originates from bacteria associated with the eggs, the water in the rearing tanks, and the live food (Ringø & Birkbeck 1999). Intensive rearing of marine fish larvae suffers from heavy mortalities, which may be attributed to bacteria introduced in the rearing system with live food (Keskin et al 1994). Replacement of the opportunistic bacteria with other less-aggressive bacteria may provide a solution. The brine shrimp *Artemia sp.* are 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 (Gatesoupe 1991). This positive effect of probiotics may be attributed to their ability to outcompete other bacteria (Austin et al 1995) or to produce micronutrients important for the development of fish larvae (Ringø et al 1992). Several bacteria have been used as probiotics in the larval culture of aquatic organisms and they can be either delivered directly into the water, or via live carrier such as *Artemia* nauplii and rotifers, or else added to pelleted dry food (Gomez-Gil et al 2000). The aim of this study was to evaluate the effects of probiotic bacillus on the growth and feeding factors of beluga larvae.

Material and Method

Preparing of probiotic bacillus. The probiotic bacillus was prepared from Protexin Co (Iran-Nikotak). The five species of probiotic bacillus as bacterial blend under the commercial title of Protexin aquatic were used for bioencapsulation of *A. urmiana*. The blends of probiotic bacillii (*Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus polymixa, Bacillus laterosporus* and *Bacillus circulans*) from suspension of spores with special media were provided. Three concentrations of bacterial suspension, 1×10^5, 2×10^5 and 3×10^5 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) (Rengpipat et al 1998).

***Artemia* cyst hatching and bioencapsulation**. The cysts of *A. urmiana* from the center of Artemia & Aquatic Animals in Urmia (Iran) were used for this study. The corions of the cysts were removed chemically by using the methodology that proposed by Sorgeloos et al (1977). This process is known as decapsulation. Hatching of the decapsulated cysts was performed in glass cone with 1 liter of seawater (3.0 % salinity) at a density of 5.0 g $liter^{-1}$ and incubated at 30°C with constant illumination and aeration through setting air pump (Gomez-Gil et al 1998). The bioencapsulation of *Artemia* nauplii were accomplished with density of 2 g live nauplii $litter^{-1}$ (Makridis et al 2001) for 10 hours and with three concentration of 1×10^5, 2×10^5 and 3×10^5 bacteries per milliliter in suspension of broth.

Experimental design. Twelve fiberglass tanks (capacity of 50 liters) with three replicates for experimental and control treatments were used. This experiment was conducted in a completely randomized design with four treatments (treatment 1-3 and control). Healthy larvae of beluga (initial weight: 55.30±0.65) were obtained from the fish hatchery of sturgeon center of Marjani (Iran). The density of fish larvae in per tank were 4-5 fish per liter. Beluga larvae in control and experimental treatments were fed 50 percent of their body weight for 6 times a day (3.00, 7.00, 11.00, 15.00, 19.00, and 23.00). The control treatment was fed unbioencapsulated *Artemia* nauplii. Water quality parameters of input water to rearing system were monitored each week throughout the experimental. The water temperature was 18.48 ± 1.44°C, pH was 7.82 ± 0.38, electro conductivity was 1636.75 ± 380.51 and water oxygen level was maintained above 7.62 ± 0.53 mg l^{-1} during the experiment by setting electrical air pump.

Sample collection. The fish were weighted individually at the begining and at the end of the experiment. Before distributing fish to the experimental tanks (in the beginning of exogenous feeding), 50 fish were sampled from the holding tank for biometry and analysis of the initial body composition. In the termination of experiment, 50 larvae from each tank were sampled and the total weight and length of body were measured. The samples of fish were stored in -20°C until analyzing.

Proximate analyses of *Artemia* nauplii and fish. The proximate compositions of samples of fish (initial and final of experiment) and *Artemia* nauplii were analyzed according to the AOAC Procedures (1990) as follows: moisture was determined by oven drying at 105°C for 24 h; crude protein (CP,%N×6.25) by using a micro Kjeltec auto-analyzer; lipid by extracting the residue with 40–60°C petroleum ether for 7–8 h in a Soxhlet apparatus; gross energy (GE) using the automated bomb calorimeter and ash was determined by combustion at 550°C in a muffle furnace to constant weight.

Calculation and statistical analysis. Some growth and feeding parameters of fish were calculated based on the data of carcass analysis and biometry of beluga larvae. Specific growth rate for weight (% BW day^{-1}) and length were calculated by the following formula:

SGR (%Body weight day^{-1}) = $[(\text{Ln } BWt_1 - \text{Ln } BWt_0) / t_1 - t_0] \times 100$

SGR (%Body length day^{-1}) = $[(\text{Ln } BLt_1 - \text{Ln } BLt_0) / t_1 - t_0] \times 100$

where $LnBWt_0$ and $LnBWt_1$ are neperian logarithm of initial and final body weight and also $LnBLt_0$ and $LnBLt_1$ are neperian logarithm of initial and final body length of fish larvae and $t_1 - t_0$ is duration of experiment (De Silva & Anderson 1995). Body weight increase was expressed as: BWI (mg) = $BWt_1 - BWt_0$ (Tacon 1990), where BWt_0 and BWt_1 are initial and final body weight of fish larvae.

Percentage of average weight gain was calculated:

AWG % = $[(BWt_1 - BWt_0)/BWt_0)] \times 100$ (De Silva & Anderson 1995).

Relative food intake (RFI %) = $[F/0.5(BWt_1 - BWt_0) \times (t_1 - t_0)] \times 100$

where BWt_0 and BWt_1 are initial and final body weight of fish larvae and $t_1 - t_0$ is duration of experiment and F is the food intake (De Silva and Anderson 1995).

Conversion efficiency ratio (CER %)= Wet weight instant growth rate×100/Daily food intake rate.

Food efficiency (FE %)= Mean weight gain (g)/food consumed(g) (De Silva & Anderson 1995).

Daily growth coefficient (DGC)= $100 \times (FBW^{1/3} - IBW^{1/3})$ (Cho 1992)

Nitrogen Retention Efficiency (NRE %)= $[(FBW \times N_{final}) - (IBW \times N_{initial})/\text{Gross N intake}] \times 100$

where IBW and FBW are initial and final body weight of fish larvae and $N_{initial}$ and N_{final} is nitrogen content (%) in whole fish body at the initial and end of the trial respectively (Brafield & Liewellyn 1982).

Protein gain (PG)= $[(FBW \times Protein_{final}) - (IBW \times Protein_{initial})/(t_1 - t_0)] \times 100$

where $Protein_{initial}$ and $Protein_{final}$ are protein content (%) in whole fish body at the initial and final of the experiment respectively (Azevedo et al 2004).

Lipid gain (LG)= $[(FBW \times Lipid_{final}) - (IBW \times Lipid_{initial})/(t_1 - t_0)] \times 100$

where $Lipid_{initial}$ and $Lipid_{final}$ are percentage of lipid in fish body at the initial and final of the experiment (Brafield & Llewellyn 1982).

Energy retained as protein (PD.KJ day^{-1})= (Protein gain×23.6 KJ g^{-1}) (Brafield & Llewellyn 1982).

Energy retained as lipid (LD.KJ day^{-1})= (Lipid gain×39.5 KJ g^{-1}) (Brafield & Llewellyn 1982).

One-way ANOVA and Duncan's multiple range test were used to analyze the significance of the difference among the means of treatments by using SPSS program.

Results and Discussion. The proximate composition of beluga and *A. urmiana* nauplii are shown in Table 1 and Table 2. The feeding and growth parameters of beluga larvae are presented in Table 3. Among the three different concentration of probiotic bacillus in both of bioencapsulation of *Artemia* nauplii, which was fed by Belug larvae, the highest results obtained in T1 (bioencapsulated *Artemia* with 1×10^5 CFU/ ml). Final body weight and specific growth rate of beluga larvae, were significantly ($p<0.05$) affected by probiotic bacillus. In the experimental treatments, growth parameters were significantly ($p<0.05$)

higher than control treatment. The highest average of weight gain was obtained in the experimental treatment of T1.

Table 1

Proximate composition of *A. urmiana* nauplii

Crude protein (%)	Crude lipid (%)	Crude energy (Cal./g)	Dry matter (%)	Moisture (%)	Ash (%)
56.83	21.2	4727.49	9.09	90.91	3.75

Table 2

Proximate composition of Beluga (*Huso huso*) larvae in the first of exogenous feeding

Crude protein (%)	Crude lipid (%)	Crude energy (Cal./g)	Dry matter (%)	Moisture (%)	Ash (%)
64.88	11.2	4199.88	11.11	88.89	6.35

The results indicated that the probiotic bacillus could influence on growth parameters in beluga larvae. The gained body weight in experimental treatments of larvae had significant difference in comparison to control treatment ($P<0.05$).

Probiotic bacillus had significant positive effects on the specific growth rate (SGR), daily growth coefficient (DGC) and conversion efficiency ratio (CER) in comparison to control treatment ($P<0.05$). The maximum of SGR for body weight of beluga larvae obtained in treatment of T1 while SGR for body length was shown in T4 (fed on by bioencapsulated *Artemia* with 3×10^5 CFU ml^{-1}). Significant different in experimental treatments (T1, T2 and T3) were not obtained regarding of FBW, SGR, AWG and DGC. The feeding parameters significantly increased in experimental treatments in comparison to control treatment. The relative food intake (RFI) in experimental treatment in comparison to control (60.83 %) decreased while nitrogen retained efficiency (%), Protein gain (g day^{-1}) and energy retained as protein (PD.KJ day^{-1}) significantly were higher ($P<0.05$) than control. The protein retained was significantly increased ($P<0.05$). In comparison to control treatment (0.0169 g day^{-1}), the maximum of Protein gain obtained in treatments of T1 was (0.0191 g day^{-1}). No significant difference was shown between the experimental treatments of T1, T2 and T3 ($P>0.05$). Also the energy retained as protein (PD.KJ day^{-1}) in beluga larvae which was fed by bioencapsulated *Artemia* nauplii, was significantly increased ($P<0.05$). While probiotic bacillus significantly decreased the lipid gain (g day^{-1}) in T1 and T2, but in treatment of T3 in comparison to control, it was increased. No significant difference was observed between the control and T4 ($P>0.05$). The same pattern was obtained about the energy retained as lipid (LD.KJ day^{-1}).

Table3

Growth and feeding parameters of Beluga (*Huso huso*) larvae in experimental treatments (trial 1-3) and control

Treatment / Parameter	Control Unbioencapsulated *Artemia* nauplii	T1 Bioencapsulated *Artemia* nauplii with 1×10^5 CFU/ ml	T2 Bioencapsulated *Artemia* nauplii with 2×10^5 CFU/ ml	T3 Bioencapsulated *Artemia* nauplii with 3×10^5 CFU/ ml
Initial weight (mg)	55.30 ± 0.65	55.30 ± 0.65	55.30 ± 0.65	55.30 ± 0.65
Final body weight (mg)	217.71±32^{b}	244.28±2.87^{a}	235.44±21^{a}	234.94±30^{a}
Body weight increased (mg)	163.48±31.54^{b}	185.76± 33.89^{a}	180.45±30.44^{a}	182.69± 38.29^{a}
Average weight gain (%)	293.67±41.33^{b}	341.74±43.58^{a}	325.75±46.79^{a}	324.85±48.36^{a}
Specific growth rate for weight (% BW day^{-1})	11.764±2.45^{b}	12.957±2.29^{a}	12.678±1.84^{a}	12.642±2.12^{a}
Specific growth rate for length (% BL day^{-1})	4.6115± 0.8338^{b}	4.8349± 0.9132^{ab}	4.7218± 0.6795^{b}	5.0109± 0.7929^{a}
Daily growth coefficient (%)	2.2866± 0.4825^{b}	2.4525± 0.4652^{a}	2.4619± 0.3691^{a}	2.4582± 0.4301^{a}
Relative food intake (%)	60.83±12.73^{a}	51.425±7.63^{b}	52.33±5.195^{b}	53.08±6.495^{b}
Food efficiency (%)	33.30±5.37^{b}	39.10±3.79^{a}	38.69±4.38^{a}	37.28±5.80^{a}
Conversion efficiency ratio (%)	56.177± 10.450^{b}	72.467± 11.253^{a}	67.323± 15.461^{a}	66.561± 16.312^{a}
Nitrogen retained efficiency (%)	54.7952± 7.5922^{b}	63.1441± 8.1570^{a}	60.4854± 6.4235^{a}	59.7658± 7.7017^{a}
Protein gain (gday^{-1})	0.0169± 0.0020^{b}	0.0191± 0.0032^{a}	0.0184± 0.0036^{a}	0.0182± 0.0038^{a}
Lipid gain (gday^{-1})	0.00158±0.00037^{a}	0.00128±0.00028^{b}	0.00115±0.0002^{c}	0.00160±0.00034^{a}
Energy retained as protein (PD.KJ day^{-1})	0.4004±0.0757^{b}	0.4517±0.0892^{a}	0.4354±0.0663^{a}	0.4309±0.0803^{a}
Energy retained as lipid(LD.KJ day^{-1})	0.0624±0.0149^{a}	0.0508±0.0115^{b}	0.0453±0.0794^{c}	0.0634±0.0132^{a}

Discussion . In the present study, nauplii of *A. urmiana* were used as a vector to carry the probiotic bacillus to digestive tract of beluga larvae. The probiotics in this experiment promoted the feeding and growth parameters in beluga larvae in experimental treatments in comparison to control treatment.

All the probiotic treatments resulted in better growth performance and some of feeding parameters than the control. The beneficial influence of probiotc bacillus (blend of bacillus) on the feeding efficiency of *Huso huso* larvae, was completely observed. The results indicated that the probiotic bacillus had significantly effects on the growth and feeding parameters in experimental treatments. The better body weight and SGR for weight and length were obtained in experimental treatments (T1). Similar results were observed by Gatesoupe (1991) in using *Bacillus toyoi* on turbot (*Scophthalmus maximus* Linnaeus, 1758), Swain et al (1996) in Indian carps that improved the growth factors and feeding efficiency and Ghosh et al (2003) on the Rohu.

Noh et al (1994) and Bogut et al (1998) also proved that the commercial probiotics of *Streptococcus faecium* improved the growth factors and feeding efficiency of carp. However, in trial T1, beluga larvae were fed by bioencapsulated *Artemia* nauplii in suspension of 1×10^5 bacteria per milliliter, obtained the best body weight and food Efficiency (FE). Results of this study also showed that different concentration of probiotic had different effects on growth parameters. However growth parameters decreased with the increasing level of probiotic bacillus. Same results were obtained by Bairagi et al (2004) and Ghosh et al (2003) on the Rohu (*Labeo rohita* Hamilton, 1758). They used different concentration of *Bacillus circulans* (isolated from intestine of *Labeo rohita*) as bacterial supplementation in diet of this fish. The best growth and feed utilization efficiency of Rohu obtained in concentration of 1.5×10^5 CFU $100gr^{-1}$ of diet. While the using of *B. circulans*, reduced the lipid digestibility and carcass crude lipid. The level of carcass lipid decreased from 89.45% (in control) to 77.12% in experimental treatments. In the present study the lipid gain decreased in experimental treatment in comparision to control. Ghosh et al (2003) indicated that the supplementation of bacteria cells induced a reduction of lipid deposition, probably by reducing lipid digestibility, and increase in body protein in fish. Same results were obtained by Bairagi et al (2002) and Ghosh et al (2002) on the Rohu fingerling (*Labeo rohita*). Ghosh et al (2002) indicated that 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. The photosynthetic bacteria and *Bacillus sp*. (isolated from the pond of common carp) was used in diet of common carp (*Cyprinus carpio* Linnaeus, 1758) by Yanbo and Zirong (2006). The results indicated that this probiotics increased growth parameters and digestive enzyme activities. The results of this studies showed that bacterial probiotics can increase the growth and feeding efficiency in fish. The different bacterial strains used in the present study as probiotics were effective in stimulating fish performance that the blends of bacteria had the best results.

Conclusions. This experiment indicated that the probiotic bacillus have the highest ability to promote the growth parameters in *Huso huso* larvae. Different concentrations of probiotic bacilluse had different effects on the growth and feeding parameters in Beluga larvae. In general, the findings can be useful in the performance of larviculture of this species.

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