Effects of bioencapsulated *Daphnia magna* with *Saccharomyces cerevisiae* on the growth and feeding performance of Persian sturgeon (*Acipenser persicus*) larvae

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Abstract:

BACKGROUNDS: 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 promote the growth and feeding parameters of fish larvae. **OBJECTIVES:** The aim of this study was to determine the growth and feeding performance of Persian sturgeon larvae fed with bioencapsulated *D. magna* with *Saccharomyces cerevisiae*. **METHODS:** *D. magna* were bioencapsulated with *S. cerevisiae* at three concentrations of 5, 5.30 and 5.48 Log CFU ml-1 for 10 hours. P. sturgeon larvae were fed using enriched *D. magna* at 30 percent of their body weight, six times a day. Controlled treatment was fed on unbioencapsulated *D. magna*. **RESULTS:** The results indicated that the *S. cerevisiae* promoted the growth and feeding parameters in P. sturgeon larvae. The final body weight and specific growth rate (SGR) in experimental treatments had significant difference in comparison with control treatment ($p<0.05$). Food conversion ratio (FCR) was decreased significantly in treatment group compared to control one ($p<0.05$). The maximum of lipid productive value (LPV) and protein productive value (PPV) were obtained in the larvae fed on bioencapsulated *D. magna* at 5.30 Log CFU ml-1). **CONCLUSIONS:** This study showed that *S. cerevisiae* had high efficiency in promotion of feeding parameters and growth performance of P. sturgeon larvae.

Introduction

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 (Olafsen and Hansen, 1992). 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 (Hansen and Olafsen, 1999). Successful colonization in digestive system of larvae involves competition with the established microflora for attachment sites and nutrients. 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 pathogenic bacteria in the rearing system (Keskin et al., 1994). 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 (Olsen, 1997).

Probiotics can be defined as live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). 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 (Nogami and Maeda, 1992). 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 (Ringø et al., 1996). Live food e.g. *D. magna* have been used as vectors for delivering compounds of diverse nutritional and/or therapeutic value to larval stages of aquatic animals (Cappellaro et al., 1993), a process known as bioencapsulation. *D. magna* are able to graze bacteria (Michels et al. 1998). The number of bacteria accumulated in live food during bioencapsulation depends on the concentration of the bacterial suspension and the bacterial strain applied (Gomez-Gil et al., 1998; Makridis et al., 2000). *Saccharomyces cerevisiae* has been used as a probiotic and diet additive for various animals. It has been observed to be capable of enhancing feeding efficiency as well as growth (Lara-Flores et al., 2003) 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 carring the beneficial bacteria into digestive tract of fish larvae. There is little information about the bioencapsulation of *D. magna* by probiotic yeast and thus present study was conducted to evaluate the potential effects of different levels of the beneficial probiotic *S. cerevisiae* in bioencapsulation of *D. magna* on the exploitation of nutrient composition of this live food by Persian sturgeon (*Acipenser persicus*) larvae for promotion of feeding parameters.

Materials and Methods

Ten-day old P. sturgeon larvae with initial weight of 50±7 mg and total length of 22±5 mm were obtained from hatchery of Marjanii sturgeon center, Golestan, Iran.

S. cerevisiae (Thepax) containing 1×10^{10} cells mg^{-1} was prepared from Doxal Co.-Italy. The *D. magna* was cultured in earth ponds. *S. cerevisiae* was prepared at three concentrations of 5, 5.30 and 5.48 Log CFU ml^{-1} in broth medium. The newly caught *D. magna* from pond, were collected on a 120 mm-pore-size sieve, washed with fresh water thoroughly and were bioencapsulated with *S. cerevisiae* at the density of 5 g-L at 29±1°C, illumination (2000 Lux), salinity 0.5 ppt and aeration (Michels et al, 1998). After 10h the bioencapsulated *D. magna* was collected on a 120 mm-pore-size sieve, and washed with fresh water. The P. sturgeon larvae were fed with bioencapsulated *D. magna* at 5 (T_1), 5.30 (T_2) and 5.48 (T_3) Log CFU ml^{-1} of *S. cerevisiae*. In control the fish larvae were fed on unbioencapsulated *D. magna*. Each treatment was included in triplicate. The density of fish larvae per was 4-5 fish liter. Sturgeon larvae were fed at 30% body weight six times a day with bioencapsulated *D. magna*.

Each rearing tank was supplied with running fresh water which had been filtered through the special cotton filter (flow rate: 1 L min^{-1}). Water quality parameters consisted of water temperature at 16.8± 0.6 °C, pH 7.6-8.3 and dissolved oxygen above 7.5 mg l^{-1} during the experiment by setting electrical air pump. Fifty fish larvae from each tank were sampled at the termination of the feeding experiment, and the total weight and length of body were measured. The experimental period was 2 weeks. Proximate composition of *D. magna*, fish carcass (initial and final of experiment) were analyzed using the standard procedures described by the Association of Official Analytical Chemists (1990); moisture was determined by oven drying the weight of fresh sample at 100 ^{o}C for 24 h; 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 at 40-60 ^{o}C petroleum ether for 7-8 h in a Soxhlet apparatus and ash was determined by ignition at 550 ^{o}C in a muffle furnace to constant weight. Twenty fish from each tank were sampled at the termination of the feeding experiment, homogenized, and analyzed for moisture, crude protein, crude lipid and ash (on wet weight

basis). Some growth and feeding parameters of the fish were calculated based on the data of carcass analysis and biometry of the larvae. The growth parameters and feeding parameters of the studied fish were calculated on the data of carcass analysis.

Results were analyzed by one-way ANOVA and significant different were determined by Duncan test. The statistics were performed using the software SPSS 15.0 for Windows.

Results

The data of growth parameters are shown 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 (487.22 mg) was obtained in experimental treatment of T2. The *S. cerevisiae* had significant positive effects on the specific growth rate (SGR) and thermal growth coefficient (TGC) in comparison with control treatment ($p<0.05$). The maximum of SGR (11.642% BW day^{-1}) and TGC (1.583 %) were obtained in larvae fed with treatment T2.

Also, the growth parameter of growth coefficient efficiency (GCE %) had the highest level (14.12%) in treatment of T2. Treatment of T2 and T3 had significant difference compared to control treatment ($p<0.05$). Persian sturgeon larvae fed with bioencapsulated *D. magna*, significantly ($p<0.05$) increased the velocity of growth body weight (VW %) and velocity of growth body length (VL %). The best VW (11.405%) and VL (4.594%) were showed in T2 and T3, respectively. The survival rate was significantly ($p<0.05$) increased in T1 (89.00%) and T2 (92.00%), compared to control treatment (88.33%).

Proximate analysis of whole body of Persian sturgeon at the end of the feeding trial and mean values of feeding parameters were shown in table 2 and 3. The best results in this trial obtained in Persian sturgeon larvae fed with bioencapsulated *D. magna*. The maximum of drymatter (12.01%) and minimum of mouisture (88.00%) were seen in treatment T2.

Also, use of bioencapsulated *D. magna* significantly promoted levels of crude lipid, crude energy and carcass dry matter in P. sturgeon larvae compared to the control group ($p<0.05$). However, the crude protein and ash levels were in significant ($p<0.05$). The level of crude lipid had significant difference in experimental treatments compared to control ($p<0.05$). The lowest (5.84%) and the highest (6.77%) of crude lipid were obtained in the control and treatment T3, respectively.

In experimental treatments the food conversion efficiency (FCE) increased while food conversion ratio (FCR) and relative food intake (RFI) decreased compared to control group ($p<0.05$).

The FCR of diets showed an inverse correlation with concentration of yeast ($CFU^{-1}L$) of bioencapsulated suspension of broth. Protein efficiency ratio (PER), lipid efficiency ratio (LER) in treatments T2 and T3 had significant difference compared to the control group ($p<0.05$). The results indicated that the *S. cerevisiae* significantly enhanced levels of protein productive value (PPV), lipid productive value (LPV) and energy productive value (EPV) in experimental treatments compared to the control group ($p<0.05$). The levels of PPV (0.71), LPV (0.102) and EPV (0.367) were significantly higher than control ($p<0.05$). The gastro somatic index (GSI %) in T2 was significantly different from the control group ($p<0.05$).

Discussion

The incorporation of probiotics via live food constitutes a very important potential tool for supplying probionts to the larvae. *D. magna* as one of the most important live foods was used as a vector to carry yeast to digestive system of P. sturgeon larvae, while the most studies have used the probiotics as bioencapsulations by Artemia and rotifer.

The *D. magna* and the *A. urmiana* are the common live food organisms used for the rearing of sturgeon fish larvae. These have been considered as possible vectors for the delivery of different substances, such as probiotics (Jafaryan et al., 2010).

Bioencapsulation of Daphnia with yeast, has not been reported, so far. This study highlights the effects of yeast *S. cerevisiae* on the enhancement of growth of *A. persicus* larvae. The beneficial influence of *S. cerevisiae* on growth parameters of *A. persicus* larvae, were completely observed. The probiotic treatments of T1 and T2 resulted in growth and feeding performances better than control treatment while the treatment T3 had lower effect than the control group. The best performance of fish in terms

Table 1. The growth parameters of Persian Sturgeon larvae in different treatments. Specific growth rate (SGR) = 100 [ln final weight of fish-ln initial weight of fish)]/days of feeding. (Larid and Needham, 1988).Thermal growth coefficient (TGC) = [g final body weight$^{0.333}$- g initial body weight$^{0.333}$]/ [Water temperature days of experiment]. (De Silva and Anderson, 1995).Velocity of growth body weight (VW %) =100 [2(final weight of fish- initial weight of fish)/ days of experiment (final weight of fish+ initial weight of fish)]. (De Silva and Anderson, 1995).Velocity of growth body length (VL %) =100 [2(final length of fish- initial lenght of fish)/ days of experiment (final length of fish+ initial length of fish)]. (De Silva and Anderson, 1995).Food conversion ratio (FCR) = food intake (g) / living weight gain (g). (Hevroy et al, 2005) .Food conversion efficiency (FCE) = [living weight gain (g)/ food intake (g). (Hevroy et al, 2005)Relative food intake (RFI) = 100 [(feed intake)/0.5(final weight of fish- initial weight of fish). days of feeding]. (De Silva and Anderson, 1995).

Treatment / Parameter	Control	T1	T2	T3
FBW(mg)	389.78±54.35 ^{b}	400.89±75.87^{a}	487.22±69.40 ^{a}	427.56±75.48^{a}
SGR (%BW day-1)	10.046±2.342 ^{c}	10.284±2.173 ^{bc}	11.642±2.265^{a}	10.865±1.694 ^{b}
TGC%	1.367±0.294^{c}	1.397±0.281^{bc}	1.583±0.315 ^{a}	1.468±0.227 ^{b}
GCE%	9.43±2.86 ^{c}	9.81±1.09 ^{c}	14.12±2.53 ^{a}	11.09±2.290 ^{b}
VW%	10.768±1.100 ^{b}	10.888±0.955 ^{b}	11.405±0.843 ^{a}	11.160±0.667 ^{a}
VL%	4.037±0.951^{c}	4.293±0.708 ^{b}	4.555±0.674 ^{a}	4.594±0.573 ^{a}
Survival	88.33±1.53^{b}	89.00±1.73ab	92.00±2.00 ^{a}	80.03±1.00 ^{c}

Table 2. Proximate composition of Persian Sturgeon larvae (dry matter base) in feeding treatments.

Treatment / Parameter	Control	T1	T2	T3
Dry matter %	9.67 0.88 ^{b}	9.30 1.51 ^{b}	12.01 1.25 ^{a}	8.75 0.53^{b}
Moisture %	90.33 0.86 ^{a}	90.70 0.86 ^{a}	88.00 1.27 ^{b}	91.30 0.51 ^{a}
Crude protein %	77.08 1.36 ^{a}	77.01 2.52 ^{a}	77.11 0.89 ^{a}	76.97 1.40 ^{a}
Crude lipid %	5.84 0.30^{b}	6.76 0.50 ^{a}	6.74 0.50 ^{a}	6.77 0.40 ^{a}
Crude energy (kcal/g)	4498.47 101.22^{b}	4798.23 99.01 ^{a}	4736.59 102.00 ^{a}	4780.45 180.20 ^{a}
Ash %	10.80 1.00 ^{a}	10.65 1.50 ^{a}	10.42 1.92 ^{a}	10.40 0.95 ^{a}

Table 3. Mean values of some of feeding parameters in *Acipenser persicus* larvae. Protein efficiency ratio (PER) = living weight gain (g) / protein intake (g). (Helland et al, 1996).Lipid efficiency ratio (LER) = living weight gain (g) / lipid intake (g). (Helland et al, 1996). PPV= g retained protein/g protein intake. (Helland et al, 1996).LPV= g retained lipid /g lipid intake. (Helland et al, 1996).EPV= g retained energy/g energy intake. (Helland et al, 1996).GSI (%) = digestive tract weight (g) / living weight gain (g) 100. (Desai, 1970).

Treatment / Parameter	Control	T1	T2	T3
FCR	7.80 ±2.92^{a}	7.52 ±2.49^{a}	6.10 ±2.03^{b}	6.65 ±1.61^{b}
PPV	0.45 ±0.13^{b}	0.46 ±0.12^{b}	0.71±0.22^{a}	0.46 ±0.11^{b}
LPV	0.056 ±0.017^{d}	0.064 ±0.018^{c}	0.102 ±0.032^{a}	0.072 ±0.017^{b}
EPV	0.224 ±0.041^{b}	0.237 ±0.052^{b}	0.367 ±0.075^{a}	0.237 ±0.048^{b}
PER	6.39 ±1.89^{c}	6.52 ±1.88^{bc}	8.92 ±2.80^{a}	7.12 ±1.67^{b}
LER	10.82 ±3.21^{c}	11.04 ±3.21^{bc}	13.72 ±4.31^{a}	12.04 ±2.82^{b}
GSI (%)	18.30 ±3.25^{b}	18.63 ±2.58^{ab}	24.15±3.43^{a}	18.50 ±2.94^{b}
RFI (%)	133.82±30.67 ^{a}	127.10±20.41 ^{a}	99.88±25.99 ^{b}	99.85±27.85 ^{b}
FCE (%)	14.28 ±2.23^{c}	14.57 ±1.24^{bc}	18.11 ±3.69^{a}	15.90 ±2.72^{b}

of growth performance and FCR was recorded for the enrichment *D. magna* with 5.30 Log CFU of yeast-L. Then this concentration of yeast was recognized the best level for process of bioencapsulations of *D. magna* by *S. cerevisiae* in feeding of *A. persicus* larvae. Some reports have shown that yeast *S. cerevisiae* 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 (Oliva-Teles and Goncalves, 2001). In experimental trials, *S. cerevisiae* optimized the feed consumption of *A. persicus* larvae. In the probiotic experimental treatments dry matter, crude lipid and crude energy of *A. persicus* larvae significantly increased while the FCR and RFI significantly decreased. The other feeding parameters as PPV, LPV, EPV, PER, EER and FCE significantly increased ($p<0.05$). Similar effects had been reported for other fishes to increase considerably with the use of probiotic in the diet (Tovar-Ramirez et al., 2004; Lara-Flores et al., 2003). Similar results were reported by Lashkarboloki et al. (2011) who fed *A. persicus* larvae, with bioenriched *D. magna* with extract of *S. cerevisiae* at 150 mg-L. This mixture resulted in the maximum growth and survival rate. Tovar-Ramires et al. (2004), indicated that *S. cerevisiae* increased feeding parameters and better feeding efficiency in sea bass. Also Tovar et al., (2002) reported that a diet supplemented with a suitable amount of the yeast caused faster growth in the sea bass (*Dicentrarchus labrax*) larvae. This suggests that is *S. cerevisiaecan* improve the growth of fish larvae (Andlid et al., 1995).

In this study different results of growth parameters of P. sturgeon were obtained using different levels of *S. cerevisiae* via *D. magna*. The growth parameters of FBW, VW and VL in probiotic trials of T1 and T2 had the highest levels compared to control one. Similar results were also seen where *S. cerevisiae* was used in Nile tilapia, (*Oreochromis niloticus*) larvae (Abdel-Tawwab et al., 2008). The SGR, GCE and TGC significantly increased in experimental treatments similar results were seem by Jafaryan et al. (2010) when, *S. cerevisiae*, and probiotic bacillus were used in *A. persicus*, *Acipenser nudiventris* and *Huso huso* larvae. While He et al. (2009) reported use of *S. cerevisiae* fermentation product (DVAQUA) had in significant effect on growth performance of hybrid tilapia (*Oreochromis niloticus* ×*O. aureus*) cultured in cage system.

Similar results had been reported by Lara-Flores et al. (2003); they showed that *S. cerevisiae* improved feeding efficiency of Nile tilapia juveniles. Noh et al. (1994) and Bogut et al. (1998) studied the effect of supplementing carp feeds with *S. cerevisiae* and bacteria, and found the best growth with the bacteria.

Nevertheless *S. cerevisiae* had negative effects on growth parameters of feeding performance of *A. persicus* when used at 5.48 Log CFU ml-1. Similar to our results, Abdel-Tawwab et al (2008), reported the over dose (5.0 g yeast/kg diet of yeast), decreased the growth and survival rate of Nile tilapia, (*Oreochromis niloticus*).

However P. sturgeon larvae fed with bioencapsulated *D. magna* at 5.30 Log CFU ml-1 showed the best growth and feeding parameters. The results of the present experiment highlighted that use of *S. cerevisiae* via *D. magna* can improve the growth parameters of P. sturgeon larvae resulting in increasing the larvae survival.

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تاثیر مخمر نانوایی پروبیوتیکی (*Saccharomyces cerevisiae*) برای ارتقاء عملکرد رشد و تغذیه لارو تاس ماهی ایرانی (*Acipenser persicus*) در بهره برداری از دافنی ماگنای غنی شده (*Daphnia magna*)

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چکیده

زمینه مطالعه: بهینه سازی ترکیبات میکروبی و ذخیره سازی آن از طریق غذای زنده در فرآیند غنی سازی یکی از مهمترین موارد در آبزی پروری است، بطوریکه آن می تواند معیارهای رشد و تغذیه لاروهای ماهی را افزایش دهد. **هدف:** هدف از این مطالعه تعیین عملکرد رشد و تغذیه لارو تاس ماهی ایرانی در تغذیه از دافنی ماگنای غنی شده با مخمر نانوایی بود. **روش کار:** دافنی ماگنا با سه غلظت ۵، ۵/۳۰ و ۵/۴۸ لگاریتم واحد کلنی در هر میلی لیتر در سوسانسیون غنی سازی برای مدت ۱۰ ساعت غنی شده و توسط لاروهای تاس ماهی ایرانی تغذیه شدند. لاروهای تاس ماهی ایرانی بر پایه ۳۰٪ وزن بدن، ۶ بار در روز تغذیه شدند. تیمار شاهد با دافنی غنی نشده تغذیه گردید. **نتایج:** نتایج نشان داد که ساکارومایسیس سرویزیا معیارهای رشد و تغذیه را در لاروهای تاس ماهی ایرانی ارتقاء داد. وزن نهایی بدن و نرخ رشد ویژه (SGR) در تیمارهای آزمایشی تفاوت معنی داری را در مقایسه با تیمار شاهد داشت ($p<۰/۰۵$). ضریب تبدیل غذای (FCE) بطور معنی داری کاهش یافت ($p<۰/۰۵$). بالاترین ارزش تولید چربی (LPV) و ارزش تولید پروتئین (PPV) در تیمار T2 (لاروهای تاس ماهی ایرانی تغذیه شده با دافنی غنی شده بوسیله ۵/۳۰ لگاریتم واحد کلنی در هر میلی لیتر) بدست آمد. **نتیجه گیری نهایی:** این تحقیق نشان داد که ساکارومایسیس سرویزیا بالاترین کارآیی را در افزایش معیارهای تغذیه ای و عملکرد رشد لارو تاس ماهی ایرانی داشت.

واژه های کلیدی: غنی سازی، دافنی ماگنا، مخمر نانوایی، ارزش تولید چربی.

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