

### The effects of Amax yeast fed to Persian sturgeon (*Acipenser persicus*) larvae via bioenrichment of *Daphnia magna*

Mohammad Lashkar boloki, Hojatollah Jafaryan, Moein Faramarzi, and Hosein Adineh

Gonbad Institute of Higher Education, Golestan, Iran.  
Corresponding author: M. Lashkar boloki, 3772214@gmail.com

**Abstract.** This study was carried out to evaluate the effect of the product of commercial live bakers' yeast (*Saccharomyces cerevisiae*) so called Amax on the growth and survival of Persian sturgeon (*Acipenser persicus*) larvae via enrichment of *Daphnia magna*. The blends of Amax were used in three concentrations of 50, 100, 150 mg L<sup>-1</sup> with *Daphnia* in suspension of broth. Every day *Daphnia* by one of concentrations was bioencapsulated for 10 hours and Persian sturgeon larvae were fed on it. The *Acipenser persicus* larvae were fed from *Daphnia* on the base of the 50 percent of their body weight five times a day. The control group was fed on not enriched *Daphnia*. The gained body weight in experimental treatments of sturgeon larvae had significant difference compared to control treatment ( $p < 0.05$ ). In experimental treatments, Food Conversion Efficiency was increased while the Relative Food Intake significantly decreased ( $p < 0.05$ ). Food efficiency was increased significantly ( $p < 0.05$ ) in experimental treatments. The Amax had significant positive effects on the Specific Growth Rate (SGR), Average Daily Growth (ADG), Relative Gain Rate (RGR), Growth Conversion Efficiency (GCE), Daily Growth Coefficient (DGC) and Thermal Growth Coefficient (TGC) in comparison with control treatment ( $p < 0.05$ ). No significant difference in the condition factor was observed ( $P > 0.05$ ). The Amax had significant positive effects on survival rate in comparison with control treatment ( $p < 0.05$ ). The experiments indicated that the product of *Saccharomyces cerevisiae* has a high ability to increase the growth parameters and feeding efficiency in cultivation system of sturgeon fish.

**Keyword:** *Saccharomyces cerevisiae*, sturgeon, enrichment, *Daphnia magna*.

**چکیده.** در این مطالعه اثرات استفاده از عصاره پودری مخمر نانوائی ساکارومایسیس سرویسیا (تحت عنوان Amax) به عنوان پروبیوتیک در جیره غذایی لارو ماهی تاس ماهی ایرانی (*Acipenser persicus*) از زمان شروع تغذیه فعال به مدت 30 روز مورد بررسی قرار گرفت. لارو های تاس ماهی ایرانی در این مطالعه از دافنی ماگنای غنی شده در سه سوسپانسیون غنی سازی با غلظت های 50، 100 و 150 میلی گرم از محصول مخمري پودري در هر لیتر، مورد تغذیه قرار گرفتند. همچنین از دافنی غنی نشده برای تغذیه لارو های تیمار شاهد برای مقایسه با نتایج سایر تیمار ها استفاده شد. تغذیه لارو های تاس ماهی ایرانی به میزان 50 درصد وزن بدن و روزانه در 5 نوبت از دافنی انجام گردیدند این آزمایش در قالب طرح کاملا تصادفی اجراء گردید. در انتهای دوره بین تیمار ها از نظر رشد اختلاف معنی داری مشاهده نشد ( $p > 0.05$ ). ولی اختلاف معنی داری بین تیمار های مورد تغذیه با دافنی غنی شده در مقایسه با تیمار شاهد مشاهده گردید ( $p < 0.05$ ). مخمر نانوائی بر نرخ رشد ویژه (SGR) ضریب رشد حرارتی (TGC) کارایی تبدیل غذا، نرخ بقا و میزان تولید لارو های تاس ماهی ایرانی در مقایسه با تیمار شاهد، تأثیرات مثبت و معنی دار داشتند ( $p < 0.05$ ). بهترین عملکرد رشد در تیمار چهارم با 150 میلی گرم بر لیتر Amax مشاهده گردید ( $p < 0.05$ ). نتایج این آزمایش نشان داد که استفاده از عصاره پودري مخمر ساکارومایسیس سرویسیا قابلیت بالایی در افزایش معیار های رشد لارو های تاس ماهی ایرانی داشته و در توسعه جیره های کاربردی این ماهی سودمند خواهد بود.

**کلمات کلیدی:** مخمر ساکارومایسیس سرویسیا، پروبیوتیک، غنی سازی، دافنی، تاس ماهی ایرانی

**Introduction.** The demand for animal protein for human consumption is currently on the rise and is largely supplied with terrestrial farm animals. This activity (aquaculture) requires high-quality feeds with high protein content, which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and favor growth. *Acipenser persicus* Borodin, 1879 is an important species for freshwater aquaculture. Improving fish performance and disease resistance of cultured organisms are major challenges facing fish culturists. Therefore, several alternative strategies to the use of antimicrobials have been proposed, such as the use of probiotics as biological control agents. Probiotics, live microbes that may serve as dietary supplements to improve fish growth and immune responses, have received some attention in aquaculture (Gatesoupe 1999; Irianto & Austin 2002; Kesarcodi-Watson et al 2008). The application of probiotics and prebiotics may therefore result in elevated health status improved disease resistance, growth performance, body composition, reduced malformations and

improved gut morphology and microbial balance. Though probiotics are widely used in poultry and swine rearing, little has been done to incorporate them into aquaculture.

The probiotic potential of a wide range of microalgae, yeasts Gram-positive bacteria, and Gram-negative bacteria has previously been evaluated (Irianto & Austin 2002; Gatlin 2002; Siwicki et al 1994). Bakers' yeast, *Saccharomyces cerevisiae*, is used for the bakers industry that contains various immunostimulating compounds such as  $\beta$ -glucans, nucleic acids as well as mannan oligosaccharides, and it has the capability to enhance immune responses (Siwicki et al 1994; Anderson et al 1995; Ortuño et al 2002) as well as growth (Oliva-Teles & Gonçalves 2001; Lara-Flores et al 2003; Li & Gatlin 2003, 2004, 2005). Yeasts are particularly interesting because they provide  $\beta$ -glucans and nucleotides that stimulate the immune system of fish (Sahoo & Mukherjee 2001; Li et al 2004)

There is evidence that the administration of glycans extracted from the cell wall of *S. cerevisiae* induces increased resistance to infection by *Vibrio anguillarum*, *V. salmonicida* and *Yersinia ruckeri* in Atlantic salmon (*Salmo salar*) (Robertsen et al 1990; Raa et al 1992). A previous experiment showed already the doubling of the final mean weight of sea bass larvae fed a yeast-supplemented diet (Tovar-Ramírez et al 2004); also showed an increased of the survival and digestive enzyme activity in *Dicentrarchus labrax* larvae fed diet containing *D. hansenii* (Tovar-Ramírez et al 2004). Most studies dealing with the capacity of yeast or structural polysaccharides to improve disease resistance in fish showed their capacity to reduce mortalities associated with infection by pathogens such as *Aeromonas* (Kumari & Sahoo 2006; Irianto & Austin 2002). Product of dietary *Saccharomyces cerevisiae* (Amax) is a new natural product consisting of yeast cell walls ( $\beta$ -glucans and mannan-oligosaccharides) and cell soluble materials (vitamins, proteins, peptides, amino acids, nucleotides, lipids, organic acids, oligosaccharides, esters, and alcohols), and seldom has living cells in the product (Burgent et al 2004). Thus objective of the present study was to evaluate the effect of product of *Saccharomyces cerevisiae* (Amax) on the growth performance of Persian sturgeon (*Acipenser persicus*) larvae.

## Material and Method

**Enrichment of *Daphnia magna*.** In this study, *Daphnia* were enriched by the use yeast solution at four concentrations (0, 50, 100 and 150 mg L<sup>-1</sup>). *Daphnia* was added into enrichment vase with dose of 2g L<sup>-1</sup>. Enrichment process lasted for 10 h in a 10L-vase with aeration. Light density and temperature was kept at 10 w.m<sup>2</sup> and 19±1°C during the enrichment.

At the end of the process, 80  $\mu$ -enriched daphnia were separated using filtration. The collected daphnia were introduced in bottle of 1L, homogenized and then divided into four equal rations for each replicate.

**Experimental Treatment.** There were totally four treatments with different dose of yeast enrichment. Zero yeast enrichment was considered as a control in this study. The treatments were assigned into the 16 tanks, four replicated for each treatment. Persian sturgeon larvae with initial weight of 80 mg were randomly introduced into the tanks, 100 fish per each tank. Water flow for each tank was approximately 0.7 L min<sup>-1</sup> (see Sealey & Gatlin 2002). The photoperiod of 12 h light and 12 h darkness was performed during the study (according to Jafarian et al 2009).

**Feeding fish larvae.** Fish larvae were fed five times per day during the experiment. Daily feeding ration was estimated based on the 50% larvae body weight for all treatments (Jafarian et al 2009). After feeding, the water flow was stopped in the tanks for 2 h allowing full ingestion of daphnia by the larvae. Larval body measurements were made weekly until the day 28, to record larval growth parameters. At the end of the experiment, all remaining larvae were counted and the growth parameters were measured for all the larvae.

The growth parameters and feeding efficiency were measured by using the following formulas:

Body weight increase (BWI) = BW<sub>t1</sub> - BW<sub>t0</sub> (Tacon 1990);

Food conversion efficiency (FCE) = [living weight gain (g)/ food intake (g)] (Hevroy et al 2005);

Food conversion ratio (FCR) = Total feed consumed (g)/ (n<sub>initial</sub>-n<sub>final</sub>) (De Silva & Anderson 1995);

Specific growth rate (SGR) (%Body weight day<sup>-1</sup>) = [(Ln BW<sub>t1</sub>-Ln BW<sub>t0</sub>) / t<sub>1</sub>- t<sub>0</sub>] × 100;

Thermal growth coefficient (TGC) = [g final body weight<sup>0.333</sup>-g initial body weight<sup>0.333</sup>]/ [Water temperature × days of experiment] (De Silva & Anderson 1995).

Daily growth coefficient (DGC) = 100× (BW<sub>t1</sub><sup>1/3</sup> - BW<sub>t0</sub><sup>1/3</sup>) (Cho 1992);

Relative gain rate (RGR %) = 100× [(BW<sub>t1</sub> - BW<sub>t0</sub>)/ BW<sub>t0</sub>];

Condition factor (CF) = W<sub>final</sub>×100/L<sup>3</sup> (Ai et al 2006);

Average daily growth (ADG) =100× [(final weight of fish- initial weight of fish)/(initial weight of fish) days of feeding] (De Silva & Anderson 1995);

Relative food intake (RFI %) = [F/0.5(BW<sub>t1</sub> - BW<sub>t0</sub>)/(t<sub>1</sub>-t<sub>0</sub>)] × 100 (De Silva & Anderson 1995).

For all equations, BW<sub>t0</sub> and BW<sub>t1</sub> are initial and final body weight of fish larvae and t<sub>1</sub> -t<sub>0</sub> is duration of experiment (De Silva & Anderson 1995). Number of fish is indicated as initial (n<sub>initial</sub>) and final (n<sub>final</sub>). In calculating the specific growth rate, LnBW<sub>t0</sub> and LnBW<sub>t1</sub> are the natural (neperian) logarithm of initial and final body weight of fish larvae (De Silva & Anderson 1995).

**Experimental design and statistical analysis.** The results were presented as means ±SE (Standard error). All the data were subjected to one-way ANOVA to evaluate the effect of yeast enrichment on growth performance of Persian sturgeon larvae. The results were subjected to variance analysis on the means (SPSS 9.0 software) and when F was significant, the Duncan test was performed to compare the means.

**Results.** All the treatments that enriched with yeast product (Amax) showed better results and growth performance than control treatment.

The values of growth parameters of *Acipenser persicus* larvae in different treatments are presented in Table 1. The growth parameters were significantly affected by addition of probiotics to the rearing tanks (p<0.05). The probiotic Amax had positive effect on growth parameters in all of probiotic treatments. The maximum of final body weight (FBW)(600.16±45.68 mg), final body length (FBL)(47.37±1.43 mm), specific growth rate (SGR)(6.89 +0.27% body weight/day), thermal growth coefficient (TGC) (0.07±0.01), daily growth coefficient (DGC)(1.40±0.07), relative gain rate (RGR) (709.78±61.86%) and average daily growth rate (ADG) (23.66±2.05%) were observed in treatment 150mg L<sup>-1</sup> Amax and this was higher than the ones observed in other experimental treatments (p<0.05). The lowest of growth parameters were obtained in control treatment, while the highest condition factor (CF) (0.60±0.04), food conversion ratio (FCR) (7.51±1.27), relative food intake (RFI) (54.21±1.85) were obtained in this treatment where the fish larvae fed on not enriched *daphnia*.

Final fish weight, weight gain increased significantly (p<0.05) with the increase in dose of Amax. The highest survival was recorded in treatment with 150 mg Amax. Results indicated best growth performance in experimental treatment compared to control. The larvae were fed with enriched *daphnia* showed an IBW and SGR significantly higher than the control treatment (p<0.05). In general, the larvae that were fed with the enriched *Daphnia* showed better feeding efficiency than those that were fed with *Daphnia* without enrichment.

**Discussion.** Single cell proteins, including yeast and bacteria, have been viewed as promising substitutes for fishmeal in fish diets. Researchers have evaluated the nutritional value of brewers yeast *S. cerevisiae* in lake trout (Rumsey et al 1990), rainbow trout (Rumsey et al 1991) and sea bass (Oliva-Teles & Goncalves 2001) by comparing growth performance, feed efficiency. Based on these studies, brewers yeast could replace up to 25–50% of fish meal protein without adversely affecting growth of these species. In the present study, yeast product (Amax) was evaluated for its potential as improve of growth performance of *Acipenser persicus*.

Table 1

Feeding efficiency of *Acipenser persicus* in different experimental treatments

Treatment	Control	50 mg L <sup>-1</sup> Amax	100 mg L <sup>-1</sup> Amax	150 mg L <sup>-1</sup> Amax
Initial body weight (IBW) (mg)	74.9±9.71	74.9±9.71	74.9±9.71	74.9±9.71
Final body weight (FBW) (mg)	388.65±10.60 <sup>b</sup>	565.06±50.05 <sup>a</sup>	556.46±47.61 <sup>a</sup>	600.16±45.68 <sup>a</sup>
Body weight increase (BWI) (mg)	0.31±0.01 <sup>b</sup>	0.48±0.05 <sup>a</sup>	0.47±0.04 <sup>a</sup>	0.52±0.04 <sup>a</sup>
Final body length (FBL) (mm)	40±0.75 <sup>b</sup>	46.59±1.59 <sup>a</sup>	46.2±1.65 <sup>a</sup>	47.37±1.43 <sup>a</sup>
Specific growth rate (SGR)	5.73±0.49 <sup>b</sup>	6.66±0.25 <sup>a</sup>	6.67±0.29 <sup>a</sup>	6.89±0.27 <sup>a</sup>
Food conversion ratio (FCR)	7.51±1.27 <sup>a</sup>	5.31±0.56 <sup>b</sup>	5.38±0.51 <sup>b</sup>	4.92±0.39 <sup>b</sup>
Food conversion efficiency (FCE)	13.00±2.64 <sup>b</sup>	18.33±2.08 <sup>a</sup>	18±2.00 <sup>a</sup>	20±1.73 <sup>a</sup>
Condition factor (CF)	0.60±0.04 <sup>a</sup>	0.55±0.01 <sup>a</sup>	0.56±0.01 <sup>a</sup>	0.55±0.01 <sup>a</sup>
Thermal growth coefficient (TGC)	0.05±0.01 <sup>b</sup>	0.06±0.01 <sup>b</sup>	0.06±0.01 <sup>b</sup>	0.07±0.01 <sup>b</sup>
Average daily growth (ADG)	14.07±0.57 <sup>b</sup>	22.08±2.25 <sup>a</sup>	21.70±2.14 <sup>a</sup>	23.66±2.05 <sup>a</sup>
Daily growth coefficient (DGC)	1.02±0.02 <sup>b</sup>	1.34±0.08 <sup>a</sup>	1.33±0.08 <sup>a</sup>	1.40±0.07 <sup>a</sup>
Relative gain rate (RGR)	424.31±14.23 <sup>b</sup>	662.61±67.48 <sup>a</sup>	651.34±64.28 <sup>a</sup>	709.78±61.86 <sup>a</sup>
Relative food intake (RFI)	54.21±1.85 <sup>a</sup>	33.64±5.55 <sup>b</sup>	35.51±3.57 <sup>b</sup>	32.51±2.69 <sup>b</sup>
Growth conversion efficiency (GCE)	10.58±1.02 <sup>b</sup>	20.27±4.20 <sup>a</sup>	18.95±2.70 <sup>a</sup>	21.34±2.73 <sup>a</sup>
Survival rate	79.66±5.50 <sup>b</sup>	96.00±3 <sup>a</sup>	96.33±.57 <sup>a</sup>	97.00±2.64 <sup>a</sup>

Groups with different alphabetic superscripts differ significantly at  $p < 0.05$  (ANOVA).

All the probiotic-supplemented diets resulted in growth higher than that of the control diets, suggesting that the addition of probiotics mitigated the effects of the stress factors. This resulted in better fish performance, with better growth results in the diets supplemented with the yeast. Similar results were observed by Vazquez-Juarez et al (1993) when yeast isolated from the intestines of wild rainbow trout was introduced into the digestive tracts of domestic rainbow trout, producing a significant increase in the growth of the cultured trout. In contrast, the use of Amax in three concentration of enrichment suspension caused growth increases significantly when compared to the control. In accordance with our findings in this study, using probiotic yeast in *Artemia urmiana* nauplii broth, for feeding *Acipenser persicus* larvae had good effects on growth parameters (Jafaryan et al 2008). These results agree with that obtained with catla carp (Mohanty et al 1996), mrigal carp (Swain et al 1996), hybrid striped bass (Li & Gatlin 2003, 2004, 2005), and Japanese flounder (Taoka et al 2006). In accordance with our results Ziaei-Nejad et al (2006) reported that the addition of commercial probiotics in the culture medium of the shrimp *Fenneropenaeus indicus* had a good effect on growth rate. Similar results were obtained when *S. cerevisiae* was added to fish diet for Israeli carp (Noh et al 1994) and Nile tilapia (Lara-Flores et al 2003). These results may be explained by the greater adaptive capacity of yeasts in aquatic environments in contrast to bacteria such as *Lactobacillus* and *Streptococcus*. It is also necessary, however, to consider the possibility of interspecies differences, as suggested by Noh et al (1994), who studied the effect of supplementing common carp feeds with different additives, including antibiotics, yeast (*S. cerevisiae*) and bacteria (*S. faecium*). In contradiction with our results Boyd et al (1984) reported that the adding commercial probiotic did not have any significant effect on growth parameters of channel catfish.

**Conclusion.** The present study indicates that live bakers' yeast positively enhanced growth performance and feed utilization of *Acipenser persicus*. Further research is needed to determine the most appropriate supplement levels for optimum growth results in larger animals at a commercial scale.

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Received: 10 January 2011. Accepted: 01 March 2011. Published online: 24 April 2011.

Authors:

Mohammad Lashkar boloki, Gonbad Higher Education Center, Department of Natural Resources, Iran, Gonbad; Postal code: 4971857765, e-mail: 3772214@gmail.com

Hojatollah Jafaryan, Gonbad Higher Education Center, Department of Natural Resources, Iran, Gonbad; Postal code: 4971857765, e-mail: hojat.jafaryan@gmail.com

Moein Faramarzi, Gonbad Higher Education Center, Department of Natural Resources, Iran, Gonbad; Postal code: 4971857765, e-mail: faramarzimoein@gmail.com

Hosein Adineh, Gonbad Higher Education Center, Department of Natural Resources, Iran, Gonbad; Postal code: 4971857765, e-mail: adineh@gmail.com

Lashkar boloki M., Jafaryan H., Faramarzi M., Adineh H., 2011 The effects of Amax yeast fed to Persian sturgeon (*Acipenser persicus*) larvae via bioenrichment of *Daphnia magna*. *AACL Bioflux* **4**(3):361-367.