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# Effect of using yeast (*Saccharomyces cerevisiae*) as probiotic on growth parameters, survival and carcass quality in rainbow trout *Oncorhynchus mykiss* fry

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**Abstract**

The effect of *Saccharomyces cerevisia var. elipsoidous* as probiotic in the diet of rainbow trout *Oncorhynchus mykiss* fry was studied during 25 days after the first feeding in ratios of 1%, 5% and 10%. After 25 days there was no significant difference in the fry growth performance ($P > 0.05$). There was also no significant difference ($P > 0.05$) in the effect of the dietary yeast on mortality, condition factor (CF) and food conversion ratio (FCR). Also an experiment with only fish oil (without yeast) was done and all results were compared with the control group. The specific growth ratio (SGR), body weight increase (%BWG) and protein efficiency ratio (PER) were significantly higher in the 5% yeast diet compared to the control diet ($P < 0.05$). A significant increase in the lipid content of the carcass was observed with a probiotic in the diet. With increase of the yeast in the diet, the ash content of the carcass increased and the protein content decreased. The results of these experiments showed that the use of yeast as additive in the diet during the early life stage of the rainbow trout fry is suitable and it is probable that a 5% concentration of yeast in the diet will have the best results on the growth performance and the feed efficiency ratio.

**Keywords**: Probiotic, *Saccharomyces cerevisia*, Rainbow trout, Growth rate, Survival, Carcass quality

## Introduction

Rainbow trout has a promising market potential in Europe, East- and South-Asia and in other regions. It is also an important aquacultural species in Iran, so every factor that can improve the production in trout culture is of economical significance. In many fish species, the larval period is considered critical in the life history. Success of larval rearing depends mainly on the availability of suitable food that is readily consumed, efficiently digested and that provides the required nutrients to support good growth and health (Tovar-Ramirez et al. 2002; Waché et al. 2006). Therefore, the use of probiotics in aquaculture has been introduced.

Probiotic treatment may be particularly useful to secure the settlement of fish intestinal microbiota and it may improve health in fish (Gatesoupe 1999). According to Moriarty (1998), beneficial bacterial cultures, added to water or fish feed, which can subsequently improve the health of the host can be defined as probiotic. The range of probiotics examined for use in aquaculture has encompassed both gram-negative and gram-positive bacteria, bacteriophages, yeasts and unicellular algae (Irianto and Austin 2002).

Several authors have studied the effects of probiotics on the survival rate and the growth of fish larvae and crustacea (Ali 2000), on the digestibility coefficients of nutrients of white shrimp, *Litopenaeus vannamei* (Heizhao et al. 2004), on decreasing the food conversion ratio, growth and survival of the post-larvae of

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*Macrobrachium rosenbergii* (Himabindu et al. 2004) and on survival and growth of the black tiger shrimp, *Penaeus monodon* (Rengpipat et al. 1998). It has been shown that the use of probiotics can decrease the amount of food necessary for fish growth, which could result in reduction of the production rate (Lara-Flores et al. 2003).

Many probiotic bacteria have been proposed for improving the health in rainbow trout (Irianto and Austin 2002). The strains used were generally antagonistic to pathogens (Jöborn et al. 1997; Robertson et al. 2000), and an important feature was the ability to colonize the fish gut (Jöborn et al. 1997; Nikoskelainen et al. 2001). Also the immune system is stimulated in the rainbow trout by several probiotics (Irianto and Austin 2002).

Andlid et al. (1995) suggested that yeast isolated from rainbow trout might also improve health. He paid special attention to their colonization potential. For example, the probiotic yeast *Saccharomyces cerevisae var. boulardii* had some effect on rainbow trout metabolism, since the dietary addition of the yeast increased muscle lipids and red pigmentation, and improved the resistance of trout to *Yersinia ruckeri* (Quentel et al. 2005). According to Waché et al. (2006), polyamine secretion was a possible mediator for the effect of the yeast on rainbow trout fry.

Therefore, in the present study the growth and survival data were used to evaluate the effect of yeast (*Saccharomyces cerevisiae*) on the fry of rainbow trout. Measurements of the carcass chemical composition have been used as a reliable index to estimate nutritional conditions and growth of fish larvae (Rengpipat et al. 1998; Hevroy et al. 2005).

## Materials and methods

### *Larvae rearing*

The experiments were performed at the Reproduction and Culture Complex of Shahid Marjani Sturgeon Fish, Gorgan, Iran. Trout larvae at the first feeding (swim up) stage were obtained from the Trout Reproduction Center, Gorgan, Iran. A batch of 1500 uniformly sized yolk-sac larvae (163 mg±26.27 SD) was randomly divided into 15 groups (five different experiments, three groups of 100 individuals in each) and each group was placed into an identical 35 liter tank with micro-mesh screens on two sides. Water velocity was set at 0.5 L/h; compressed air was used to aerate the tanks using a number of narrow pipes connected to bubblers. The tanks were cleaned daily to avoid pollution by overfeeding or by diet particles.

### *Water quality*

The water quality was regularly monitored. The temperature was maintained at 9.3±1.36 ºC (measured daily, $n$=29) and the oxygen concentration varied between 7.8 and 8.6 mg/L (determined mornings once a week mornings). The total ammonia-nitrogen [($NH_4^+$ + $NH_3$)-N] was always maintained below 0.5 mg/L and the pH values between 8 to 8.2. The residual chlorine was measured weekly whereby the levels stayed below 0.05 mg/L. The photoperiod for this indoor experiment was set at a 12L:12D cycle (light period from 08.00 to 20.00 h) and the light intensity was kept at 40 lx on the tank surface. The physical and chemical variables were maintained as constant as possible by continual renewal of the oxygenated water, and by removing dead larvae and food leftovers (by siphoning) each morning before feeding; dead larvae were removed twice daily and counted.

### *Experimental diets and feeding*

Five different feeding experiments were done with three identical groups of fish larvae. At the end of the first week, the characteristics of one group in each feeding experiment were studied, then at the end of the second week those of the second group and at the end of the third week those of the last group. The five feeding experiments were: (1) artificial food covered with fish oil (without yeast) ($D_0$); (2) artificial food mixed with 1% *Saccharomyces cerevisiae* ($D_1$); (3) artificial food mixed with 5% *Saccharomyces cerevisiae* ($D_5$); (4) artificial food mixed with 10% *Saccharomyces cerevisiae* ($D_{10}$); and (5) only artificial food, the control experiment (C). The artificial food was Biomar-optimal start, in 0.5 mm size (consisting of fish meal, wheat, fish oil, binders, vitamins, minerals and anti oxidants). All groups were fed 5 times per day. The daily ration was about 5% of dry body weight per day (Lovell 1993).

The experimental food mixed with yeast was prepared with probiotic yeast of *Saccharomyces cerevisiae* strain (Institute Daxal, Italy) obtained as commercial preparations. The active dried yeast preparations were powdered by grinding and sifting through 100µm, and then suspended in fish oil. The amount of powder was adjusted in the oily suspensions to obtain a final concentration of $10^6$ Colony Forming Units (CFU) of yeast per gram of experimental food, after which the pellets were coated with the well-shaked suspension (32 ml/kg) (Waché et al. 2006).

***Chemical composition and statistical analysis***

The fish larvae were left unfed for 6 hours before sampling for growth measurements and chemical analysis. Each group of fish was weighed weekly on a balance with a precision of 0.1 mg. The total length of each fish was measured with a sliding caliber with a precision of 0.01 mm.

The amount of food fed per group was recorded weekly and used to calculate the feed efficiency ratios. To determine the composition for moisture, crude protein, crude lipid and ash (AOAC 1990), during the first week 200 fish and after 4 weeks of feeding 50 fish per replicate tank were taken, including samples of the food given. All determinations were done in duplicate. Normal procedures were followed, e.g. moisture by drying the samples at 105ºC overnight, protein by measuring Kjeldahl nitrogen, lipid was analyzed by ether extraction using a Soxhlet system and ash by heating the samples for 5 hours at 550 ºC in a muffle furnace. The results were expressed as percentage of the total body dry weight. The formula for calculation of CF, BWG, SGR, FCR, and PER are as follows:

(Condition Factor) $CF = \frac{W}{L^3} \times 100$ (Lagler et al. 1962)

W= fish weight (wet weight in g); L= fish length (in cm)

(Body Weight Gain percentage) $\%BWG = \frac{BW_f - BW_i}{BW_i} \times 100$ (Ghosh et al. 2003)

$BW_f$ = final weight (g); $BW_i$ = intial weight (g)(Specific Growth Rate)

$$\%SGR = \frac{LnW_f - LnW_i}{t_2 - t_1} \times 100$$ (Helland et al. 1996)

$W_f$ = final weight (g); $W_i$ = initial weight (g); ($t_2 - t_1$) = duration of the experiment in days

(Food Conversion Ratio) $FCR = \frac{F}{W_f - W_i}$ (Helland et al. 1996)

F= feed fed (dry weight in g); $W_f$ = final weight (wet weight in g); $W_i$ = initial weight (wet weight in g.)

(Protein Efficiency Ratio) $PER = \frac{BW_f - BW_i}{AP}$ (Helland et al. 1996)

$BW_f$ = final weight (g); BWi = intial weight (g); AP=Applied Protein

At the end of experiment the number of surviving fish was recorded and used for calculating the mortality. All fish in each tank were pooled for weighing and for growth evaluation. The effects of diet on survival and growth parameters were analyzed using one-way ANOVA. Duncan's procedure was applied for multiple comparisons. Statistical v.9 for Windows was used. Results were considered significant at the 5% level.

## Results

The chemical composition analysis of the carcasses of rainbow trout larvae of each experiment (Table 1) showed that the highest percentage of crude protein was observed for experiment $D_1$ (artificial food coated by 1% yeast) and C (only artificial food: control experiment), the other experiments showed significantly lower figures ($P < 0.05$). The lipid content was the lowest in C group and the highest ash content in carcasses of larvae was found for the experiments $D_{10}$ and $D_0$, which showed significantly higher figures than the other experiments ($P < 0.05$). The highest moisture content was found in the experiments $D_1$ and C; other experiments had significantly lower figures.

The survival rate also showed no significant difference between the experiments ($P > 0.05$). The lowest cumulative mortality of larvae was observed with experimental diet $D_5$ and the highest cumulative mortality was found with diet $D_{10}$ (Fig.1). After 25 days of larvae rearing, the highest average length was observed in fish fed by diet C, with no significant other difference between different treatments, except with $D_1$ fed larvae ($P > 0.05$) (Table 2).

Table1. Proximate composition of trout larvae carcass sampled duration rearing period[1, 2]

| Food experiment | Starting rearing period | Final rearing period | | | | |
|---|---|---|---|---|---|---|
| | | $D_0$ | $D_1$ | $D_5$ | $D_{10}$ | C |
| Moisture(%) | 86.96 | 85.71±0.57$^{ab}$ | 86.52±0.93$^{b}$ | 84.61±0.66$^{a}$ | 84.73±0.68$^{a}$ | 86.52±0.93$^{b}$ |
| Proteins (%) | 70.57 | 73.86±0.36$^{a}$ | 76.77±0.81$^{b}$ | 74.75±0.26$^{a}$ | 74.62±0.46$^{a}$ | 75.87±0.46$^{b}$ |
| Lipids (%) | 11.88 | 12.06±0.12$^{bc}$ | 12.0±0.15$^{b}$ | 12.27±0.14$^{c}$ | 12.11±0.13$^{bc}$ | 11.46±0.09$^{a}$ |
| Ash (%) | 9.38 | 10.33±0.12$^{d}$ | 9.44±0.12$^{a}$ | 10.08±0.11$^{c}$ | 10.43±0.1$^{d}$ | 9.86±0.15$^{b}$ |

Notes:

[1]Values within rows with different superscripts are significantly different ($P < 0.05$).

[2] Mean ± SD.

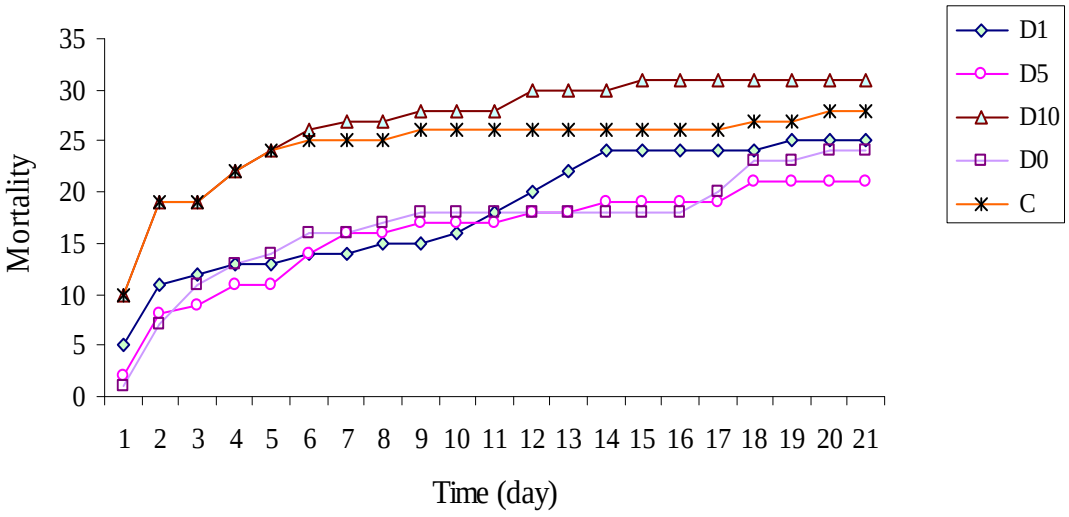


Fig.1. Cumulate mortality of trout (mean, per diet) within 25 days

Strange enough, the previously observed differences were between the fishes with different diets. At the end of the first and second weeks the highest average total length was observed in larvae fed by $D_{10}$ diet which showed significant difference compared to the others fed by other diets ($P < 0.05$). At the end of the experiment, the highest weight was observed with treatment $D_5$, while there were no significant differences with the other diets ($P > 0.05$). At the end of the first and second weeks the highest weight was observed with diet $D_{10}$, but there was no significant difference with diets $D_0$ and C ($P < 0.05$) (Table 2).

Weight increase within the 3 weeks of the experiments showed a significant difference between diet $D_5$ and the other diets ($P < 0.05$). The specific growth rates with diet $D_1$ and $D_5$ showed a significant difference, and with all treatments there were significant differences ($P < 0.05$). At the end of the rearing period, the fish in all experiments had a high condition factor, without any significant difference between any of the diets. ($P > 0.05$) (Table 3).

After 3 weeks of rearing, the lowest FCR was observed in treatment $D_1$ and $D_5$, a significant difference was shown with diet $D_{10}$ ($P < 0.05$). At the end of the 3$^{rd}$ week the highest PER was found with the experimental diet $D_5$ showing significant difference ($P < 0.05$) from all other diets with the exception of diet $D_1$.

## Discussion

During the first week, *Oncorhynchus mykiss* having been fed with different levels of *Saccharomyces cerevisiae*, had a higher growth rate than those fed with a diet of normal artificial food and artificial diet coated by fish oil (C and $D_0$). Research on the effects of the dietary *Saccharomyces cerevisiae* strain and on the rearing conditions in rainbow trout showed that supplementing the trout starter diet with *Saccharomyces cerevisiae* may be

particularly useful for increasing fish growth. Differences in temperature strongly affect fish growth and metabolism (Waché et al. 2006). Noh et al. (1994) and Lara-Flores et al. (2003) showed that fry fed by diets with a probiotic supplement exhibited greater growth than those fed by the control diet without supplement.

Table 2. Results of the biometry of trout fry during experiment

| Diets | | $D_0$ | $D_1$ | $D_5$ | $D_{10}$ | C |
|---|---|---|---|---|---|---|
| First week | Length(mm) | $29.97\pm0.10^{b*}$ | $28.46\pm0.76^{a}$ | $28.94\pm0.49^{a}$ | $30.14\pm0.46^{b}$ | $29.25\pm0.62^{ab}$ |
| | Weight(mg) | $256.83\pm0.29^{a}$ | $242.20\pm11.03^{a}$ | $238.24\pm18.35^{a}$ | $258.72\pm5.91^{a}$ | $239.35\pm13.73^{a}$ |
| Second week | Length(mm) | $34.71\pm0.10^{bc}$ | $33.27\pm0.64^{a}$ | $33.76\pm0.51^{ab}$ | $35.11\pm0.63^{c}$ | $34.65\pm0.61^{bc}$ |
| | Weight(mg) | $378.88\pm14.00^{bc}$ | $339.13\pm19.77^{a}$ | $345.89\pm18.84^{ab}$ | $399.78\pm14.88^{c}$ | $374.31\pm22.39^{bc}$ |
| Third week | Length(mm) | $38.52\pm1.24^{b}$ | $37.04\pm0.63^{a}$ | $38.18\pm0.38^{ab}$ | $38.00\pm0.51^{ab}$ | $37.74\pm0.57^{ab}$ |
| | Weight(mg) | $515.45\pm5.55^{b}$ | $487.03\pm21.4^{a}$ | $523.02\pm17.97^{b}$ | $517.40\pm12.80^{b}$ | $514.22\pm8.91^{b}$ |

Notes:

* Within rows, values with different superscripts are significantly different ($P > 0.05$).

Table 3. Average final weight (g), percent body weight gain (BWG) per day, specific growth ratio (SGR), condition factor (CF), food conversion ratio (FCR) and protein efficiency ratio (PER). Values are mean± standard deviation ($n$=3)

| Treatment Index | $D_0$ | $D_1$ | $D_5$ | $D_{10}$ | C |
|---|---|---|---|---|---|
| Average final weight (g) | $515.45\pm5.55^{b}$ | $487.03\pm21.45^{a}$ | $523.02\pm17.97^{b}$ | $517.40\pm12.80^{b}$ | $514.22\pm8.91^{b}$ |
| BWG% | $36.15\pm4.30^{ab}$ | $43.72\pm4.22^{bc}$ | $51.33\pm3.48^{c}$ | $29.57\pm6.63^{a}$ | $37.62\pm6.02^{ab}$ |
| SGR% | $4.38\pm0.46^{ab}$ | $5.00\pm0.38^{bc}$ | $5.90\pm0.30^{c}$ | $3.67\pm0.72^{a}$ | $4.66\pm0.70^{ab}$ |
| CF% | $0.90\pm0.08^{a}$ | $0.95\pm0.04^{a}$ | $0.92\pm0.03^{a}$ | $0.94\pm0.02^{a}$ | $0.96\pm0.02^{a}$ |
| FCR | $1.18\pm0.15^{ab}$ | $0.97\pm0.10^{a}$ | $0.82\pm0.06^{a}$ | $1.47\pm0.32^{b}$ | $1.14\pm0.18^{ab}$ |
| PER | $1.44\pm0.17^{ab}$ | $1.79\pm0.17^{bc}$ | $2.04\pm0.14^{c}$ | $1.21\pm0.27^{a}$ | $1.60\pm0.25^{ab}$ |

Notes:

* Values within rows with different superscripts are significantly different ($P < 0.05$).

In the present study, the best FCR, SGR and BWG values were observed in diets $D_1$ (artificial food mixed with 1% *Saccharomyces cerevisiae*) and $D_5$ (artificial food mixed with 5% *Saccharomyces cerevisiae*), suggesting that these yeasts improved the utilization of feed. Similar results have been reported for *Saccharomyces cerevisiae* used in diets for carp (Noh et al. 1994), and Nile tilapia (Lara-Flores et al. 2003). In practical terms, this means that the use of probiotics can decrease the amount of food necessary for animal growth, resulting in production cost reductions.

The PER results indicate that supplementing diets with 5% *Saccharomyces cerevisiae* ($D_5$) significantly improves protein utilization in rainbow trout. This contributes to better protein use for growth, a significant quality given that protein is the most expensive nutrient component of feed. Similar results were found by Lara-Flores et al. (2003) who reported that supplementing diets with probiotics significantly improves protein utilization in tilapia.

At the end of the experiment, mortality was low and the survival within groups fed with *Saccharomyces cerevisiae* enriched food did not show any significant difference. Similar effects have been reported for *Saccharomyces cerevisiae* in diets for trout, *Oncorhynchus mykiss (*Waché et al. 2006). The better results found in using supplemented diets suggests that the addition of probiotics improved the digestibility of the diet and protein, which may in turn explain the better growth and feed efficiency seen in using supplemented diets (Lara-Flores et al. 2003). Aubine et al. (2005) hypothesized that the dietary supplement of the yeast increased muscle lipids and red pigmentation.

Growth of the trout fed with yeast-supplemented food was good in the three dietary groups. Pulverization of the yeast suspension in the diet resulted in a lower growth rate. This result is similar to Tover et al. (2002). It can be assumed that the process of incorporation of the pulverized yeast is changing some physical properties of the micro particles, in particular, a decrease of buoyancy was observed and the sprayed particles sank faster. In this case, feed ingestion by larvae could be reduced. Incorporation of the yeast during mixing the raw materials should be considered in further experiments.

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