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The effects of different concentrations of probiotic *Saccharomyces cerevisia* on growth performance and survival rate of rainbow trout (*Oncorhynchus mykiss*), fry and resistance against salinity

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In the present study, a yeast strain *Saccharomyces cerevisia* var. *elipsoidous*, acting as probiotic, was administered to rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) fry during a period of 21 days and the effects of the yeast on improvement of growth and resistance against environmental stress were evaluated with respect to fish fed on yeast free feed (control group). The control treatment consisted of a standard commercial diet, and the treatments consisted of the control diet supplemented with 0, 1, 5 and 10% yeast (w/w). The results demonstrate the beneficial effects of probiotics on the characteristics of rainbow trout, as the Specific Growth Ratio (SGR), body weight gain (%BWG) and protein conversion ratio (PER) in 5% yeast-fed fish were significantly (P<0.05) enhanced by probiotic administration. On the contrary, no effect on the fry growth performance, mortality, condition factor (CF), food conversion ratio (FCR) and histological assessment was shown. A significant (P<0.05) increase in lipid content of the carcass was detected in diets with probiotic compareD to 0% and the control treatments. Ash and protein contents of the carcass increased and decreased with an increase in yeast amount, respectively. Challenge with different levels of salinity (10 and 15 ppt) after 24 h revealed 100% survival in treatments containing yeast as probiotic, and difference with control group was significant (p<0.05) indicating that *S. cerevisia* could enhance the resistance against salinity stress. Addition of yeast in concentration of 5% to the diet is recommended during the early period of rainbow trout fry farming to achieve the best results on growth performance and feed efficiency.

Key words: *Saccharomyces cerevisia* var. *elipsoidous*, *Oncorhynchus mykiss*, probiotic, survival and growth rate, carcass quality.

INTRODUCTION

Today, increase in demand for fish products means using intensive methods for more production, accompanied with an increase in infectious agents. This rearing condition can be a source of stress for fish, making them susceptible to disease and triggering high mortality. Due to various adverse effects of antibiotics, their use is

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forbidden in several countries. To date, probiotics are considered a good alternative to the use of antibiotics and in particular, in fish larviculture (Rollo et al., 2006). Probiotics, which are micro-organisms or their products with health benefit to the host, have found use in aquaculture for improving the health of their host and increasing growth rate. The range of probiotics examined for use in aquaculture has encompassed many micro-organisms such as bacteria, bacteriophages, yeasts and unicellular algae (Irianto and Austin, 2002). In several studies, the effects of probiotics on survival and growth rate of fish larvae and crustacea such as digestibility coefficients of nutrients, decreasing food conversion ratio and increase tolerance to stress have been studied (Rengpipat et al., 1998; Ali, 2000; Heizhao et al., 2004; Himabindu et al., 2004; Taoka et al., 2006a). It has been shown that the use of probiotics can increase the amount of given food necessary for animal optimal growth, by which the expense of fish farming might be reduced (Lara-Flores et al., 2003).

Oncorhynchus mykiss has a promising market potential in Asia as well as other areas in the world. Many probiotics have been proposed to improve health quality of rainbow trout (Irianto and Austin, 2002). The stains used were generally antagonistic to pathogens (Jöborn et al., 1997; Robertson et al., 2000), and an important feature was the ability to colonize in fish gut (Jöborn et al., 1997; Nikoskelainen et al., 2001). Moreover, the immune system of rainbow trout is stimulated by several probiotic (Irianto and Austin, 2002).

Andlid et al. (1995) suggested that yeast of *Saccharomyce cerevisae* var, *boulardii* isolated from rainbow trout might also improve the health quality by their colonization potential. This probiotic yeast represented positive effects on rainbow trout metabolism, as it increased muscle lipids and red pigmentation, and also improved the resistance of the fish to *Yersinia ruckeri* (Quentel et al., 2005). The larval rearing period of many fish species, is considered as critical in their life history. To succeed in larval rearing, availability of suitable food that is readily consumed and efficiently digested should be mainly regarded to provide the required nutrients that can support their optimal growth and healthy well (Tovar-Ramirez et al., 2002; Waché et al., 2006).

Although, beneficial effects of probiotics are well known in aquaculture; there is inadequate information about best concentrations of different probiotics used for enhancement of survival, increase in body resistance to stress and infectious diseases and improvement in nutritional parameters such as feed efficiency and feed conversion ratio.

Therefore, more investigation is needed for safe and effective use of probiotic. Therefore, this study was conducted to investigate the effect of *S. cerevisiae* var. *elipsoidous* as a probiotic strain on survival, growth parameters and carcass quality of rainbow trout fry at start feeding.

MATERIALS AND METHODS

Fish fry preparation

A batch of 1500 uniformly sized yolk-sac (*O. mykiss*) alevin (80 ± 26.27 mg) was obtained from the Reproduction Center of *O*ncorhynchus *mykiss,* Gorgan, Iran. In this center, all the eggs were incubated and hatched in spring water (9.34 ± 0.04°C). Alevins were transferred to the Reproduction and Culture Complex of Sturgeon Fish, Gorgan, North of Iran and stored in a Fiberglass tank until yolk-sac absorption. One day before start feeding, the fry (with initial weight and length 127/95 ± 16/73 mg and 24/86 ± 1/38) were randomly divided into 15 groups (five treatments and threereplications of 100 individuals) in a period of 21 days.

Farming condition

Each group was placed into an identical 35 L tank with micro-mesh screens in two sides. Freshwater velocity was set at 0.5 L/min; system aeration was done with compressed air, using a number of narrow pipes, connected to bubbliers. Holding tanks were cleaned daily to avoid pollution caused by overfeeding or aggregation of diet particles. Water quality was regularly monitored. Temperature was measured daily (n = 29) and maintained at 9.3°C during the experiment. Oxygen concentration varied between 7.8 and 8.6 mg/L (determined in the morning once a week). Total ammonia-nitrogen [$(NH_4^+ + NH_3)$-N] was always maintained below 0.5 mg/L and pH value from 8 to 8.2. Residual chlorine level was determined weekly and stayed below 0.05 mg/L. The photoperiod for this indoor experiment was set at a 12 L: 12 D cycle (light period from 08.00 am to 8.00 pm) and light intensity was kept at 40 lux at each tank surface. Physical and chemical variables were maintained as constant as possible by continual renewals of oxygenated water, and removing dead fry and food left-overs by siphoning each morning before feeding; dead fry were removed twice daily and counted.

Food preparation and feeding

The artificial food was Biomar- optimal Start, 0.5 mm in size. All groups were fed 5 times per day. The feeders were operational each time for 5 min. The daily ration was adjusted according to fry weight after 7 to 14 days of rearing (Lovell, 1989). Each ration was about 5% of dry body weights per day. The experimental food, mixed with yeast, was prepared with probiotic yeast *S. cerevisiae* strain (Institut Daxal, Italy) that was obtained as commercial preparations. The active dried yeast preparations were powdered by grinding and sifting through a 100 µm-meshed screen, and then suspended in fish oil. The amounts of powder were adjusted in the oily suspensions to obtain a final concentration of ca.10^6 colony forming units (CFU) of yeast per gram of experimental food, then the pellets were coated with the shaken suspensions (32 ml/kg) (Waché et al., 2006).

Feeding treatments

Five different feeding experiments were conducted on three identical groups of fish fry. In all treatment groups, fish characteristics were determined at a specific time; the characteristics of first, second and third groups of every feeding treatment were studied at the end of the first, second and third weeks, respectively. Five feeding treatments were: (1) artificial food covered with fish oil (without yeast) (D_0); (2) artificial food mixed with 1% *S. cerevisiae* (D_1); (3) artificial food mixed with 5% *S. cerevisiae* (D_5); (4) artificial food mixed with 10% *S. cerevisiae* (D_{10}) and (5) only artificial food, called control experiment (C).

Measurement of growth parameters

Growth measurements and chemical analysis occurred when the fish fry were starved for 6 h. Fish samples of all groups were weighted every week. The weight of 30 fish of every group was measured using a digital scale to the nearest 0.1 mg. Total lengths of all samples were measured using a caliper to the nearest 0.01 mm. The amount of food given per group was recorded weekly and used to calculate feed efficiency ratios. Mortality was calculated according to the number of survived fish at the end of experiment. All fish in each tank were pooled for weighing and growth evaluation as follows:

Condition factor (CF) = $W/L^3 \times 100$ (Lagler et al., 1962)

Where W is the Fish wet weight (g) and L is the fish length (cm).

Body weight gain percentage (%BWG) = $BW_f - BW_i / BW_i \times 100$ (Ghosh et al., 2003)

Where BW_f is the Final weight (g) and BW_i is the initial weight (g).

Specific growth rate (%SGR) (% body weight/day) = $LnW_f - LnW_i / (t_2 - t_1) \times 100$ (Helland et al., 1996)

Where W_f is the Final weight (g), W_i is the initial weight (g), $(t_2 - t_1)$: duration of the experiment in days.

Food concersion ratio (FCR) = $F / W_f - W_i$ (Helland et al., 1996)

Where F is the Feed fed (dry weight in g)

Protein efficiency ratio (PER) = $BW_f - BW_i/AP$ (Helland et al., 1996)

Where AP is the Applied protein.

Proximate composition analysis

To determine proximate composition, 200 fish at first week and 50 fish per each replicate tank after 4 weeks of feeding were taken and water content, crude protein, crude lipid and ash were analyzed using AOAC method (1992). These calculations were conducted in duplicate. Normal procedures have been used, for example, water content was measured by drying samples at 105°C overnight, protein levels were evaluated measuring Kjeldahl nitrogen, lipid was analyzed by ether extraction using a Soxhlet system. Ash content was measured in samples that had been mineralized at 550°C for 5 h in a muffle furnace. Results were expressed as a percentage of the total body dry weight.

Test of salinity stress

20 fish fry of each replicate were sampled and affected by salinity levels of 10 and 15 ppt in air blower-equipped tanks. The mortality and survival rates of fish were recorded after 24 h starving.

Histological assessment

To determine any significant effect of yeast-enriched feeding diets on alimentary tract, rainbow trout fry in each treatment were sampled for histological sections at the end of experimental period. Fish samples were stored in formalin solution (4%) and then eviscerated to remove their digestive tract. Paraffined blocks of fish alimentary tract were prepared and then sliced by microtom to give slices of 4 to 5 µ. Slices were stained by the coloration methods of hematoxiline and eosine and then studied under optical microscope (Mumford, 2004).

Statistical analysis

The results are presented as mean values followed by the SD. The significance of differences was determined using ANOVA, followed by Duncan's test to compare the means of the samples for multi group comparisons, with a statistical software package SPSS 12.0 for windows. Differences were considered significant at $p<0.05$.

RESULTS

Growth parameters

Survival rate showed no significant differences among various treatments ($P>0.05$). The highest and the lowest cumulative mortality of fry were found in feeding diets of D_5 and D_{10}, respectively (Figure 1). The maximum average length was found in fish samples fed with diet C after 3 weeks rearing, indicating significant differences ($P<0.05$) with the feeding diet of D_1 treatment (Table 2). Results indicating the differences between the fish groups with different diets during the experiment period were not always similar to the differences observed after three weeks, at the end of the experiments. D_{10} -fed fish samples indicated the highest significant average length at the end of weeks 1 and 2 than other samples ($P<0.05$). The highest weight was observed in treatment D_5 at the end of weeks 3, not showing significant differences with other diets ($P<0.05$). At the end of weeks 1 and 2, the highest weight was detected in feeding diet of D_{10} while no significant differences was observed in diets of D_0 and C ($P<0.05$) (Table 2). Significant differences ($P<0.05$) were also observed in weight gain of fish samples fed with feeding diet of D_5 and samples in other diets during 3 weeks of the experiment. The specific growth rate of D_5 and D_1 diets showed no significant difference; whereas, fish samples fed with the former diet indicated significant differences with other treatments ($P<0.05$). At the end of the rearing period, no significant difference was observed in condition factor of fish samples in all treatments (Table 3).

After three weeks rearing, the lowest FCR was observed in treatments of D1 and D_5, showing a significant difference with diet D_{10} ($P<0.05$). The highest PER at the end of week 3 was found in feeding diet of D_5, which was significantly different ($P<0.05$) from other treatments with exception of diet D_1.

Carcass proximate composition

The chemical composition analysis of trout fry carcass of each experiment (Table 1) showed that the highest amount of crude protein was observed in samples of D_1 and C treatments than samples in other feeding diets ($P<0.05$). Lipid content was lowest in C treatment

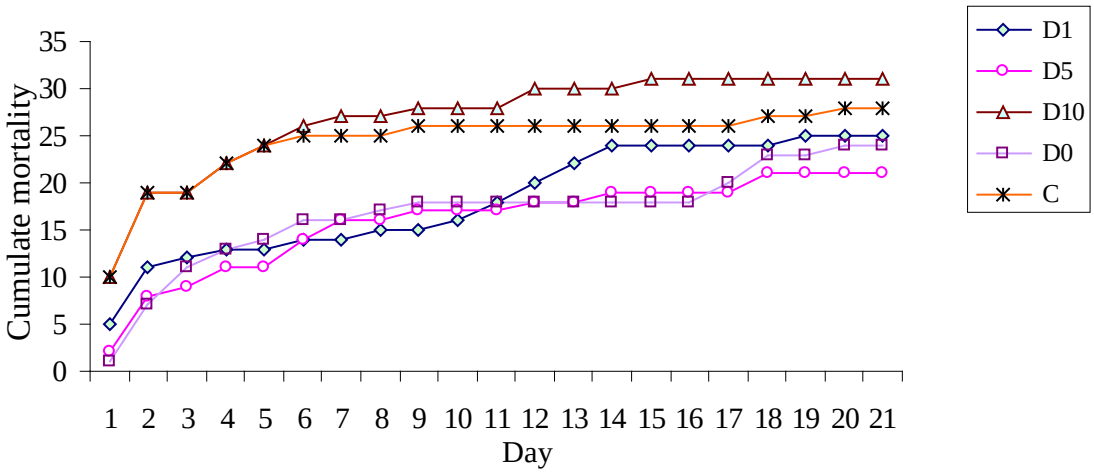

Figure 1. Cumulate mortality of *Oncohynchus mykiss* fry (mean per diet). No significant differences were observed among various treatments.

Table 1. Proximate analysis of trout fry carcass during rearing period, expressed as g kg^{-1} dry weight (mean±SD).

Parameter	Starting rearing period	Final rearing period				
		Feeding diets				
		D_0	D_1	D_5	D_{10}	C
Humidity	869.6	857.1±05.7^{ab}	865.2±9.3^{b}	846.1±6.6^{a}	847.3±6.8^{a}	865.2±9.3^{b}
Proteins	705.7	738.6±3.6^{a}	767.7±8.1^{b}	747.5±2.6^{a}	746.2±4.6^{a}	758.7±4.6^{b}
Lipids	118.8	120.6±1.2^{bc}	120±1.5^{b}	122.7±1.4^{c}	121.1±1.3^{bc}	114.6±0.9^{a}
Ash	93.8	103.3±1.2^{d}	94.4±1.2^{a}	100.8±1.1^{c}	104.3±1^{d}	98.6±1.5^{b}

Values with different superscripts are significantly different in each row (P<0.05).

Table 2. Result of biometry of *Oncohynchus mykiss* fry during rearing period.

Diet	Length / weight	D_0	D_1	D_5	D_{10}	C
End of week 1	Length (mm)	30±0.10^{b}	28.5±0.76^{a}	28.9±0.49^{a}	30.1±0.46^{b}	29.3±0.62^{ab}
	Weight (mg)	256.8±0.29^{a}	242.2±11.0^{a}	238.2±18.4^{a}	258.7±5.9^{a}	239.4±13.7^{a}
End of week 2	Length (mm)	34.7±0.10^{bc}	33.3±0.64^{a}	33.8±0.51^{ab}	35.1±0.63^{c}	34.7±0.61^{bc}
	Weight(mg)	378.9±14.0^{bc}	339.1±19.8^{a}	345.9±18.8^{ab}	399.8±14.8^{c}	374.3±22.4^{bc}
End of week 3	Length (mm)	38.52±1.24^{b}	37.0±0.63^{a}	38.2±0.38^{ab}	38.0±0.51^{ab}	37.7±0.57^{ab}
	Weight (mg)	515.5±5.55^{b}	487.0±21.4^{a}	523.0±18.0^{b}	517.4±12.8^{b}	514.2±8.91^{b}

Values with different superscripts are significantly different in each row (P>0.05).

(11.46%). The highest amount of ash in carcass of fry was found in treatments of D_{10} and D_0, which showed significant differences with other treatments (P<0.05). Fry samples fed with feeding diets of D_1 and C indicated the highest water content (P<0.05).

Histological assessment

Based on our study, no significant difference among various treatments was observed in histological assessment of digestive tract. Histological evaluations in different parts of digestive tract were as follows:

Esophagus

Epithelial with pavement cells was detected in all treatments. As fish fry grow, their esophagus was abundant of phlegm wrinkles (Figure 3).

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 expressed as mean± standard deviation (n = 3).

Treatment index	D_0	D_1	D_5	D_{10}	C
Average final weight (g)	515.4 ± 5.55^{b}	487.0 ± 21.4^{a}	523.0 ± 18^{b}	517.4 ± 12.8^{b}	514.2 ± 8.9^{b}
BWG%	36.1 ± 4.30^{ab}	43.7 ± 4.2^{bc}	51.3 ± 3.5^{c}	29.6 ± 6.6^{a}	37.6 ± 6.0^{ab}
SGR%	4.38 ± 0.46^{ab}	5.00 ± 0.38^{bc}	5.90 ± 0.30^{c}	3.67 ± 0.72^{a}	4.66 ± 0.70^{ab}
CF%	0.90 ± 0.08^{a}	0.95 ± 0.04^{a}	0.92 ± 0.03^{a}	0.94 ± 0.02^{a}	0.96 ± 0.02^{a}
FCR	1.18 ± 0.15^{ab}	0.97 ± 0.10^{a}	0.82 ± 0.06^{a}	1.47 ± 0.32^{b}	1.14 ± 0.18^{ab}
PER	1.44 ± 0.17^{ab}	1.79 ± 0.17^{bc}	2.04 ± 0.14^{c}	1.21 ± 0.27^{a}	1.60 ± 0.25^{ab}

Values with different superscripts are significantly different in each row (P<0.05).

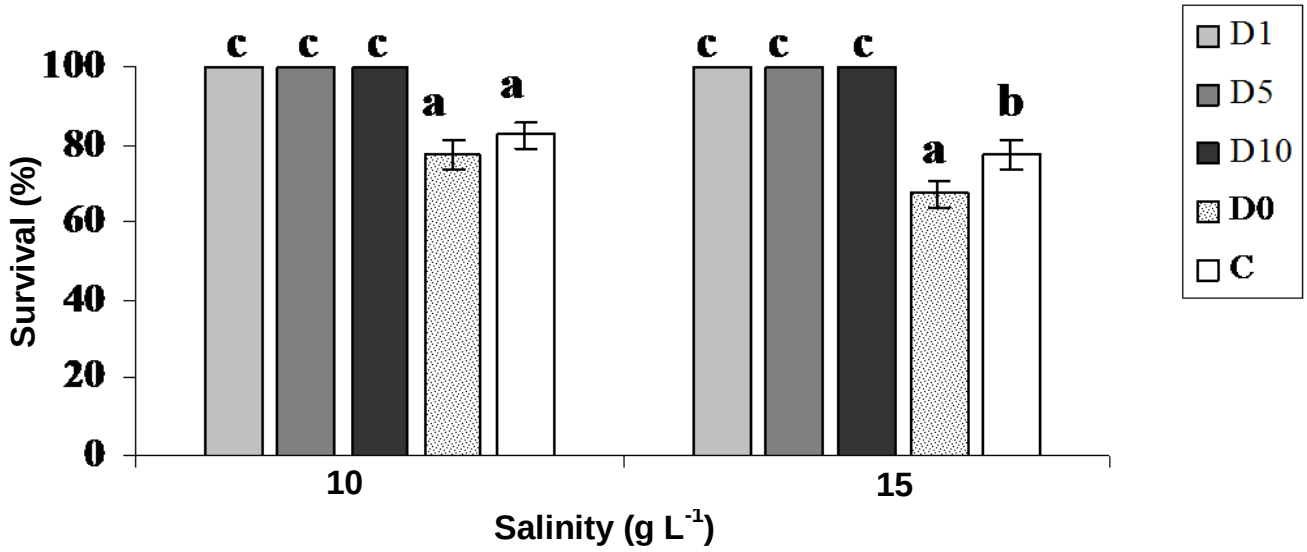

Figure 2. Survival rate in each treatment after challenging with salinity levels of 10 and 15 g L^{-1}.

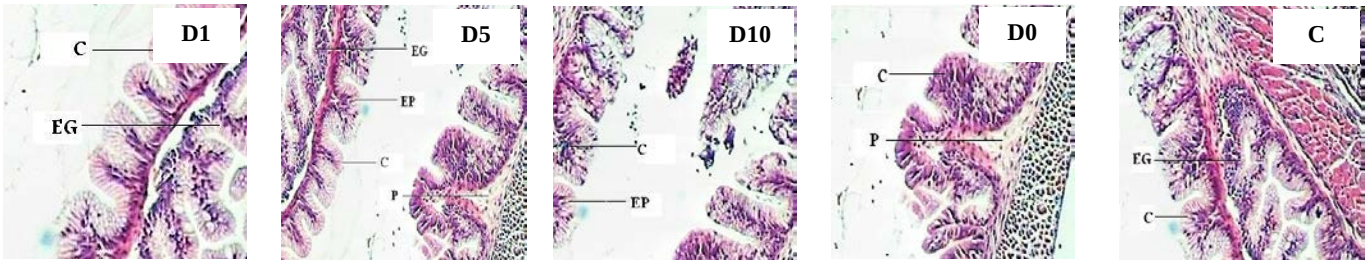

Figure 3. Posterior linear section of esophagus in rainbow trout fry at the end of week 3 (400x). EG, Esophagus glands; EP, epithelial; C, cilia; P, parin. D1, D5, D10, D0 and C implies feeding diets containing 1, 5 and 10% of yeast, fish oil, and the control, respectively.

Stomach

Plenty of wrinkles and secreting glands were observed in phlegm of fry stomach. In all treatments, connective tissue, muscle layers and a number of gland cells were found (Figures 4 and 5).

Salinity challenging

Results of salinity challenged at 10 and 15 ppt revealed that feeding with probiotic yeast *S. cerevisiae* strain had significant effects on survival rate of rainbow trout fry (Figure 2).

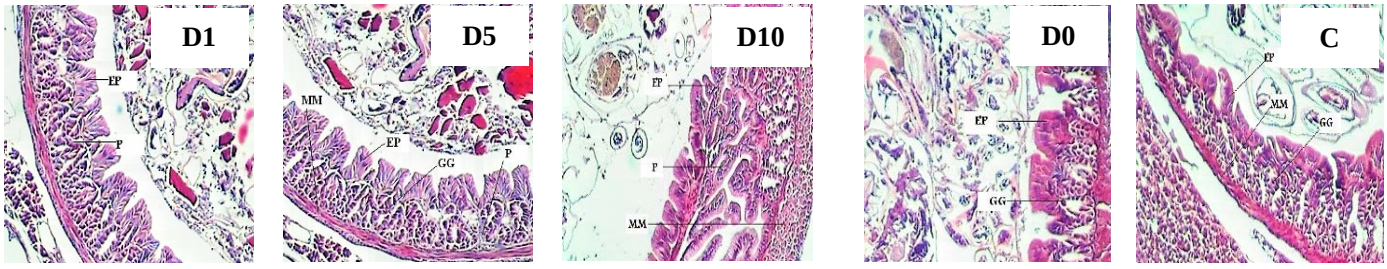

Figure 4. Cross section of stomach in rainbow trout fry at the end of week 3 (400x). EP, Epithelial; GG, gastric glands, P, parin; MM, mucosal muscle. D1, D5, D10, D0, and C implies feeding diets containing 10, 50 and 100 g kg^{-1} of yeast, fish oil, and the control, respectively.

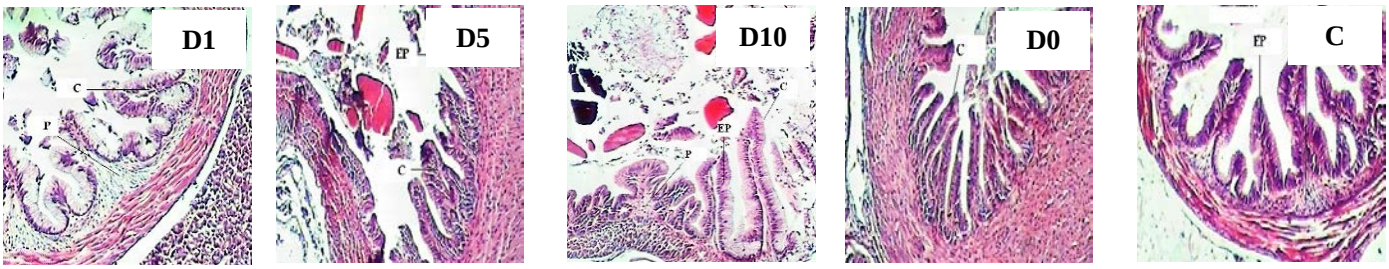

Figure 5. Linear section of intestine in rainbow trout fry at the end of week 3 (400x). C, Cilia; EP, epithelial; P, parin. D_1, D_5, D_{10}, D_0, and C implies feeding diets containing 10, 50 and 100 g kg^{-1} of yeast, fish oil, and the control, respectively.

DISCUSSION

Growth parameters

In the present study, fry of *O. mykiss* fed with different levels of *S. cerevisiae* had a higher growth rate than those fed with normal artificial food and artificial diet covered by fish oil at the first week (C and D_0). Research on the effects of strain of dietary *S. cerevisiae* and rearing conditions in rainbow trout revealed that supplementation of trout starter diet with *S. cerevisiae* may be particularly useful to increase fish growth. Moreover, differences in temperature strongly affect fish growth and metabolism (Waché et al., 2006). Noh et al. (1994) and Lara-Flores et al. (2003) demonstrated greater growth in fry fed with diets containing a probiotic supplement than those fed with the control diet without supplement. The intestinal colonization of early feeding fry with yeast may have some effect on development, for example, by accelerating the maturation of the digestive system. In older fish, dietary yeast may stimulate metabolism and growth (Gatesoupe, 2007). In the present research, the best FCR, SGR and BWG values were observed in diets D_1 [artificial food mixed by 1% *S. cerevisiae* (w/w)] and D_5 [artificial food mixed by 5% *S. cerevisiae* (w/w)] suggesting that these feeding diets improved feed utilization even under stress conditions. Similar results have been reported for *S. cerevisiae* use in diets prepared for Israeil carp (Noh et al., 1994) and Nile tilapia (Lara-Flores et al., 2003). In a similar study, the diet supplemented with yeast produced the best growth performance and feeding efficiency. This was attributed to an increase in the alkaline phosphatase activity, suggesting that yeast is an appropriate growth-stimulating additive in tilapia cultivation (Lara-Flores et al., 2010).

In practical terms, this means that the use of probiotics can decrease the amount of food necessary for optimal growth of the animal by which the expense of fish farming might be reduced. The PER results indicate that supplementing diet with 5% *S. cerevisiae* (D_5) significantly improved protein utilization in *O. mykiss*. This contributes to better protein use for growth, an important quality given that protein is the most expensive feed nutrient component. Similar results were found by Laura-Flores et al. (2003) who reported that supplementing diets with probiotics significantly improved protein utilization in tilapia. In this study, mortality was low and the survival rates within groups fed with *S. cerevisiae* enriched foods did not show any significant differences with other groups at the end of the experiment. Similar effects have been reported for *S. cerevisiae* in diets for *O. mykiss* by Waché et al. (2006). These improvements found when using supplemented diets suggested that the addition of probiotics improved diet and protein digestibility, which may explain the better growth and

feed efficiency seen when using supplemented diets (Lara-Flores et al., 2003).

Proximate composition of carcass

Carcass chemical composition measurements have been used as a reliable index to estimate nutritional conditions and growth of fish larvae (Rengpipat et al., 1998; Hevroy et al., 2005). In the present trial, trout fry in control treatment had the highest and the lowest water and lipid contents, respectively, while yeast-enriched feeding diets revealed no significant difference in lipid content. Moreover, the highest rate of protein was detectable in feeding diets of C and D_1 and fish samples in other treatments showed no significant difference. Aubine et al. (2005) demonstrated that dietary supply of the yeast increased muscle lipids and red pigmentation.

Histological assessment

Based on our study, no significant difference among various treatments was observed in histological assessment of digestive tract. The development of new probiotic strains aims at more active beneficial organisms. In the case of novel microorganisms and modified organisms, the question of their safety and the risk to benefit ratio have to be assessed (Salminen et al., 1998; Ringø et al., 2007a). Today, it is generally accepted that the gastrointestinal (GI) is one of the major routes of infection in fish (Birkbeck and Ringø, 2005). The histological effect of exposing the GI tract of Atlantic salmon to high levels of the *Carnobacterium divergens* was investigated by light and electron microscopy. Results from LM-investigations in that study showed no apparent histopathological changes of the epithelium in the intestine, after exposure of *C. divergens* (Kristiansen et al., 2011). In a similar study, histological investigation of intestine of Atlantic salmon following exposure to one probiotic strain (*C. divergens*), assessed by light and electron microscopy, showed a similar appearance to intact intestinal epithelium (Ringø et al., 2007b). Results of several other studies confirmed lack of histological changes, neither tissue damage nor manifest inflammation provoked by probiotic administration (Picchietti et al., 2007; Harper et al., 2011).

Salinity stress

According to this study, results reveal that feeding with probiotic yeast *S. cerevisiae* strain had significant effects on survival rate of rainbow trout fry in salinity challenging. In feeding studies, useful information on larval requirements to essential components can be achieved by salinity challenging when assessing their physiological condition (Dhert et al., 1992a, 1992b). Challenge tests are proposed as meaningful tools for assessing fish quality in the aquaculture industry, environmental resources management and in research (Wedemyer and McLeay, 1981). The concept is based on the presumptions that stress loading above the acclimation capacity of an organism will weaken it and reduce performance in growth, survival and reproduction, and that the reduction in performance can be quantified by assessing tolerance to reference stressors (Wedemyer et al., 1981). Our results showed 100% survival in treatments containing yeast as probiotic, and there was a significant difference with control group ($p<0.05$). Therefore, probiotic *S. cerevisia* has been shown to enhance the resistance against salinity stress ($P<0.05$). Results of a study investigated the influences of probiotic *Bacilli* sp. on resistance of Persian Sturgeon Larvae against challenge tests including salinity experiment, and showed that the mortality of larvae fed with probiotic was significantly lower than the control group (Faramarzi et al., 2012). Additional of *S. cerevisiae* yeast (0.1%) to feeding diets of Tilapia fry improved their growth and declined the effect of environmental stressors (Lara-Flores et al., 2003). Similar results were obtained by Lara-Flores et al. (2010) who reported that 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.

Recent studies have clearly demonstrated the beneficial effects of these feed additives on immune system modulation, stress tolerance and growth rate of farmed fish (Carnevali et al., 2004; Picchietti et al., 2007; Dimitroglou et al., 2011). One possible explanation is that yeast probiotics provide *β-glucans* and nucleotides that stimulate the immune system of fish (Kulkarni et al., 1986). Results of a study conducted on probiotic benefits of stress tolerance indicated that probiotics supplied in the rearing water and the diet of fish enhanced the stress tolerance and the non-specific immune system of Japanese flounder, providing them a higher resistance against stress conditions and pathogens (Taoka et al., 2006b). In the present experiment, growth rate of trout fry in three groups fed with yeast-enriched feeding diets was observed fine but evidently addition of yeast in concentration of 5% to the diet is recommended during the early period of rainbow trout fry farming to achieve the best results on growth performance and feed efficiency. Pulverization of yeast suspension in the diet resulted in lower growth rate. This result is similar to that of Tovar- Ramirez et al. (2002). It can be assumed that the process of incorporation of the pulverized yeast changed some physical properties of the micro particles; a decrease of buoyancy was observed and the sprayed particles sank faster. In this case, feed ingestion by fry could be reduced. Yeast incorporation during raw

material mixing should be considered in further experiments.

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