Effect of dietary probiotic, *Saccharomyces cerevisiae* on growth performance, survival rate and body biochemical composition of three spot cichlid (*Cichlasoma trimaculatum*)

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Abstract. The effects of yeast, *Saccharomyces cerevisiae* on growth performance, survival rate and body biochemical composition was investigated in Three Spot Cichlid, *Cichlasoma trimaculatum* (Gunther, 1867). Eighty four fish (with initial mean weight: 5.01±0.62 gr,) were divided in four groups (in triplicate) including of a formulated diet as a control group and three levels of the probiotic (0.5%, 1% and 2% yeast per food) as experimental diets. All fish were fed twice daily for 8 weeks. Based on the results obtained from this study, Survival rate was greater than 92% in all treatments. Control group without probiotic has exhibited the lowest growth performance among all treatments (P<0.05). The specific growth ratio (SGR), body weight gain (%WG), average daily growth (%ADG), food conversion ratio (FCR) and hepatosomatic index (HSI) were significantly increased in group with 2% of the probiotic compared with the control group (P<0.05). There was no significant difference (P > 0.05) in the effect of the dietary yeast on Survival rate (%SR) and condition factor (CF). A significant increase in the protein content of body was observed by addition of probiotic in the diet. Although no significant differences in lipid, moisture and ash content were observed among treatments. The results of these experiments showed that the using of yeast as additive in the diet of the three spot cichlid is suitable and it is hoped that 2% concentration of yeast in the diet will have the best results on the growth performance and the food efficiency ratio.

Key Words: probiotic, growth performance, biochemical composition, *Cichlasoma trimaculatum*.

Introduction. Nowadays, ornamental fishes are the most popular pets of the world (Velmurugan & Rajagopal 2009) and health management of the fish farms has become an integral part of ornamental fish Quality Assurance programme (Abraham et al 2008). Three spot cichlid (*Cichlasoma trimaculatum*), is extremely strong and very adaptive in natural environment and its breeding in aquarium is easier than other ornamental species (Nico et al 2007).

Control of diseases in the aquatic ecosystems is complicated by the close relationship between pathogens and their host (Olafsen 2001). Antibiotics as the food additives were commonly used in the early 1950s. Although the use of antibiotics for improving health conditions and the food utilization efficiency (Ahilan et al 2004) are confirmed, the abusing of them in aquaculture has resulted in development of many resistant bacteria against antibiotics (Abraham et al 2008). On the other hand, a growing concern about the high consumption of antibiotics has initiated research for alternative methods of disease control (Gildberg et al 1997) and growth promotion (Byun et al 1997).

Probiotics are live microorganisms, which contributed substantially to increase the animal growth and improve health condition by increasing resistance to disease (Fuller 1992); such as *Tetraselmis, Bacillus, Lactobacillus, Streptcoccus, Vibrio, Aeromonas* and *Saccharomyces* (Gastesoupe 1999). Among probiotics, *Saccharomyces cerevisiae* (yeast) is a resistant fungus to antibiotics such as sulfatides and other antibacterial agents.

Several studies have demonstrated certain modes of probiotic action in aquaculture (Kozasa 1986; Uma et al 1999; Macey & Coyne 2005; Lauzon et al 2010), and freshwater ornamental fish culture (Abraham et al 2007a, b; Abraham 2008). Probiotics have been shown to be effective in a wide range of fish species as growth promoter (Burr et al 2005), survival rate increase (Villamil et al 2002), stress resistance (Rollo et al 2006) and immune system enhancement (Gastesoupe 1999).

Onifade et al (1999) reported that activity of yeast is most probably supporting the growth of lactic acid bacteria and a competitive exclusion of pathogenic bacteria and its products, especially the cell wall constituent. Therefore, the present study aimed to determine the effects of probiotic *S. cerevisiae* on growth performance, survival rate and body biochemical composition of *C. trimaculatum*.

Material and Methods. One hundred sixty eight fish *C. trimaculatum* (mean weight, 5.01±0.62 gr and mean length: 6.36±1.22 cm) were obtained from an ornamental fish culture center located in the south of Iran and transferred to fiberglass tanks at the fishery laboratory, Faculty of Marine Natural Resources, Khorramshahr University of Marine Sciences and Technology, Khorramshahr, Iran. Fourteen days before the beginning of the experiment, the fish were distributed randomly among twelve aquariums (with 20 L capacity). During the adaptation period, all fish were fed a commercial diet (Table 1) 3% of their body weight twice daily, at 9:00 and 17:00 for 56 days (from mid March to mid May 2012). The experiment included 4 diet treatments supplemented with different levels of *S. cerevisiae* (including 0, 0.5%, 1%, 2% probiotic concentrations). To prepare experimental diets, probiotic and basic commercial diets together with 400 mL water per kilogram food, were mixed for 45 min and mechanically extruded to obtain pellets. The pellets were dried in a convection oven at 25°C to obtain a moisture level of approximately 100 g kg^{-1} and stored in airtight plastic bags until use. The experiment was carried out in triplicates. Water quality parameters (water temperature, dissolved oxygen concentration, and pH) were recorded daily. Temperature was 27±1°C during the experimental period. Average values for dissolved oxygen and pH were 6±0.5 g L^{-1} and 7±0.3, respectively.

At the end of the experiment, biometry was performed for all fish and survival rates and growth indices were measured. Five fish from each replicate were euthanized for the analysis of proximate body composition. Proximate composition of experimental diets and whole body proximate compositions were analyzed using standard methods (AOAC 1997). Each analysis was conducted in triplicates. Moisture was determined by drying the samples in an oven at 105°C for 24 h to a constant weight. Ash was determined by incinerating the samples in a muffle furnace at 550°C for 12 h. Crude protein (N: 6.25) was measured by Auto kjeldahl units (Buchi, German; model B-414, K-438, K- 371 and K-370). Total lipid was extracted from samples by homogenization in chloroform and methanol (2:1, v/v) (Bligh & Dyer 1959), methylated and transesterified with boron trifluoride in methanol (AOAC 1997). Growth performance and feed utilization were calculated as follows: (1) Body weight gain (BWG, %) = [(final body weight (g) - initial body weight (g))/initial body weight (g)] × 100; (2) Specific growth rate (SGR, % day^{-1}) = [(Ln final weight - Ln initial weight) × 100]/duration in days; (3) Condition factor (CF) = (fish mass/fish total length 3) × 100; (4) Feed conversion ratio (FCR) = [feed dry weight (g)/wet weight gain (g)]; (5) Daily feed intake (FI, g day^{-1} $fish^{-1}$) = diet consumed × 100/duration in days/fish number per tank. The results were analyzed by running a one way ANOVA, using SPSS Software version 11.5. Duncan's procedure was applied for multiple comparisons. Results were considered significant at the level of 0.05.

Four diets with different concentration of *Saccharomyces cerevisiae* (0, 0.5%, 1%, 2%) as probiotic, were prepared and all fish were fed with these diets during the experiment. To providing experimental diets, probiotic and commercial diet were mixed for 45 min and mechanically extruded to obtain pellets of the desired size. The pellets were dried in a convection oven at 25°C to obtain a moisture level of approximately 100 g kg^{-1} and stored in airtight plastic bags until use. In the end of the experiment, survival rate and growth indices were measured.

The results were analyzed by using one way ANOVA. Duncan's procedure was applied for multiple comparisons. Results were considered significant at the 5% level.

Table 1

Composition of commercial diet used for this experiment (Biomar, Incio plus 801)

Food composition	*Rate*
Crude proteins (%)	54
Crude lipids (%)	18
Nitrogen free extract (%)	11
Crude cellulose (%)	1
Ashes (%)	10
Total phosphorus (%)	1.6
Gross energy (MJ Kg^{-1})	22
Digestible energy (MJ Kg^{-1})*	20
Digestible proteins/Digestible energy (g MJ^{-1})	25.4
Vitamin A. added (I.U. Kg^{-1})	7500
Vitamin D3- added (I.U. Kg^{-1})	1500
Vitamin E - added (mg Kg^{-1})	260
Vitamin C - added (mg Kg^{-1})	500
Number of pellets per Kg - indicative	260000

*Biomar digestible energy calculated on proteins, lipids and starch only.

Results and Discussion. The fish were adapted to the experimental condition and no disease or mortality was observed during the adaptation period. Water quality parameters were daily recorded. Temperature ranged 27±1ºC during the experimental period. Average values for dissolved oxygen and pH were 6±0.5 g L^{-1} and 7±0.3, respectively.

There were significantly differences ($p \leq 0.05$) in the different growth parameters among experimental treatments. Maximum weight gain (WG) (86.10%±10.98) was obtained in T3 (2% concentration of probiotic) followed by T2 (1% concentration of probiotic) (57.14%±22.78), T1 (0.5% concentration of probiotic) (41.87%±6.47) and control group without probiotic (20.77%±0.73) respectively (Table 2).

The lowest FCR (Food conversion Rate) (0.47±0.030) was observed in T3 among different concentrations of probiotic in comparison with control group ($p < 0.05$). On the other hand, the fish which were fed by 2% and 1% concentrations had significantly differences with other treatments ($p < 0.05$).

Furthermore, the significantly higher SGR (Specific Growth Rate) (1.47±0.13) was obtained from T3 ($p < 0.05$). ADG (Average Daily Growth rate)(%) and HSI (Hepato-Somatic Index) (%) was significantly reduced in control treatment compared with T3 ($p < 0.05$). Fish in T1 and T2 exhibited intermediate ADG (%) and HSI (%) compared with T3 and control group but it was not statistically significant ($p > 0.05$). CF (Condition Factor) and SR (Survival Rate) (%) were not affected by dietary treatments (Table 2).

Table 2

Initial and final body composition of three spots cichlid fed by experimental diets*

Biochemical parameters (%)	*Initial*	*Experimental diets*			
		(control)	*T1*	*T2*	*T3*
Moisture	78.24±0.43	76.79±1.48^{a}	77.04±2.39^{a}	76.98±0.97^{a}	75.66±1.39^{a}
Crude protein	49.63±0.78	53.00±1.00^{a}	55.42±0.98^{b}	56.07±0.25^{c}	57.44±2.47^{d}
Crude lipid	8.73±0.98	9.02±0.07^{a}	9.21±0.29^{a}	9.88±0.09^{a}	9.63±0.46^{a}
Ash	12.43±0.08	13.18±0.12^{a}	13.34±0.11^{a}	13.54±0.02^{a}	13.51±0.13^{a}

*Values (Mean ± SD) in the same row with different superscripts are significantly different from each other ($p < 0.05$).

The effect of dietary probiotic on body composition of three spots cichlid is presented in Table 3. The results indicated that the yeast, *S. cerevisiae* promoted levels of body crude protein in experimental treatments in comparison with control group ($p < 0.05$); while the crude lipid, moisture content and ash were not significantly increased ($p > 0.05$). The highest body protein level (57.44%±2.47) was recorded in T3 compared to other experimental diets (Table 3) as well as T1 and T2 diets were significantly higher than control group ($p < 0.05$). The highest ash content was found in T2 but it was not statistically significant ($p > 0.05$).

Table 3

Different growth performance of three spot cichlid fed by experimental diets*

Parameters	*Experimental diets*			
	(control)	*T1*	*T2*	*T3*
Initial weight (g)	4.91 ± 0.12^{a}	4.83 ± 0.07^{a}	4.86 ± 0.09^{a}	4.89 ± 0.19^{a}
Final weight (g)	6.05 ± 0.11^{a}	6.85 ± 0.21^{ab}	7.65 ± 1.24^{bc}	9.10 ± 0.52^{c}
WG(%)	20.77 ± 0.73^{a}	41.87 ± 6.47^{ab}	57.14 ± 22.78^{bc}	86.10 ± 10.98^{c}
ADG (%)	1.6 ± 1.1^{a}	2.4 ± 1.1^{ab}	3.01 ± 0.47^{ab}	3.8 ± 0.05^{b}
SGR(%)	0.44 ± 0.01^{a}	0.83 ± 0.11^{ab}	1.05 ± 0.35^{bc}	1.47 ± 0.13^{c}
FCR	1.11 ± 0.14^{a}	1.003 ± 0.125^{a}	0.6867 ± 0.20^{b}	0.47 ± 0.030^{b}
CF(%)	2.52 ± 0.21^{a}	2.48 ± 0.12^{a}	2.56 ± 0.18^{a}	2.32 ± 0.48^{a}
HIS(%)	1.87 ± 0.75^{a}	2.76 ± 0.84^{ab}	3.059 ± 0.74^{ab}	3.21 ± 0.45^{b}
SR (%)	92.59 ± 2.31^{a}	92.85 ± 1.26^{a}	94.327 ± 2.61^{a}	96.48 ± 2.53^{a}

*Values (Mean ± SD) in the same row with different superscripts are significantly different from each other ($P<0.05$); WG%: [final weight (g) - initial weight (g)/ initial weight (g)] ×100; ADG%=[final weight (g) - initial weight (g)/initial weight ×(time (days)]×100; SGR = [In final body weight - In initial body weight/time (days)] ×100; FCR= Feed consumed / Weight gain; CF : [fish weight (g) /(total length) 3 (mm)] × 100; HSI (%) = [weight of liver (g)/ total body weight (g)]×100; SR= [end number of the alive fish/the beginning number of the fish]× 100.

The effects of probiotics have been studied in many aquatic animals. *S. cerevisiae* has been recognized to have potential effects in some fish. Growth improvement has been reported by feeding of *S. cerevisiae* in *Oreochromis niloticus* (Lara-Flores et al 2003; Asadi Rad et al 2012), *Onchorhynchus mykiss* (Pooramini et al 2007), *Epinephelus coioides* (Chiu et al 2010), *Channa striatus* (Dhanaraj & Haniffa 2011) and *Acipenser persicus* (Iranshahi et al 2011). In the current study, the best FCR, SGR, ADG and WG values were observed in T3, suggesting that this yeast improved food utilization. The same results have been reported on *O. mykiss* (Pooramini et al 2009).

Probiotics may improve digestion by stimulating production of digestive enzymes or through other alterations in the gut environment, which could translate to improved growth performance (Welker & Lim 2011). The yeast can be attached to the gut when supplied by food which is led to improve amylase secretion and stimulation of brush border membrane enzyme. Perhaps, enhancement of growth and food utilization by fish may be due to improvement of nutrient digestibility (Lara-Flores et al 2003). Moreover, the study of Wache et al (2006) showed that addition of live yeast can improve diet and protein digestibility which may demonstrate the better growth and food efficiency with yeast supplements. Also the same results were reported by Lashkar Boloki et al (2011) on persian sturgen (*Acipenser persicus*).

Based on the results of the current study, significant differences were revealed in HSI among the experimental treatments. It is indicates that liver contributed significantly to lipid deposition in three spot cichlid. The morphological parameters such as HSI identify possible liver alteration and diseases. Also, CF has been used to assess the general condition of fish (Van der Oost et al 2003). HSI is associated with liver energy stock and nutritional status of the fish (Pyle et al 2005). In this study, CF was not affected by dietary treatments, which is supported by previous studies (Pooramini et al 2009; Kafilzadeh et al 2013).

At the end of the experiment, mortality rate was low. The lowest cumulative mortality of fish was observed in T3 and the highest cumulative mortality was found in control group. Similar effects have been reported for *S. cerevisiae* in diets for common carp, *Cyprinus carpio* (Mazurkiewicz et al 2005; Dehghan et al 2011).

The data obtained from body composition analysis showed that the highest body crude protein was found in fish which were fed with T3 ($P<0.05$). Similar effects have been reported for *S. cerevisiae* in diets for *O. mykiss* fry (Pooramini et al 2009). No significant differences were observed in whole body crude lipid, moisture and ash. The same results were found by Tewary & Patra (2011) on *Labeo rohita* and Kafilzadeh et al (2013) on *Astronotus ocellatus*.

The ability of yeast, *S. cerevisiae* to secrete polyamines such as Putrescine and Spermidine has been linked as essential growth factors (Buts et al 1994). Polyamines play a fundamental role in proliferation, rapid growth and regeneration of tissues (Taoka et al 2006).

Conclusions. In conclusion, under the experimental conditions, the results showed that using of probiotic, S. cerevisiae, make better growth and food utilization in three spots cichlid. Also, dietary supplementation of this probiotic at level of 2% concentration (T3) is an optimal concentration which can be proposed in aquaculture particularly in this species. In practical terms, this means that the using of probiotics can decrease the amount of necessary food for animal growth and it is cost effective.

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