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Effects of *Saccharomyces cerevisiae* (Saccharomycetes: Saccharomycetaceae) on *Astronotus ocellatus* as growth promoter and immuno stimulant

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Abstract. Probiotics are live microorganism which can affect the host animal by improving their intestinal flora as supplements to improve growth performance. The aim of this study was evaluation of effects of *Saccharomyces cerevisiae* as supplementation on growth performance, nutritional indexes and some serological parameters in Oscar fish, *Astronotus ocellatus*. One hundred and twenty (120) of Oscar fish fingerlings were divided to 12 aquarium and fed by diets with different levels (0, as control group and 0.5, 1 and 2 per cent of probiotic as treatments) of probiotic, *Saccharomyces cerevisiae*, for 56 days. This experiment was performed in triplicates. At the end of the experiment, biometry was performed for all fish. Furthermore, blood samples were collected from caudal vein by heparinized syringe and then serum was separated. Growth performance was determined and feed utilization was calculated. Lysozyme activity, serum total protein, albumin and globulin were determined. Based on the results, no mortality was seen during the experiment in all groups. Also, no significant differences were seen among treatments and control groups during the experimental period for DWG, BWG, CF, SGR, PER, HSI and VSI ($p > 0.05$). Total serum protein was decreased by increasing of *S. cerevisiae* concentration. But total albumin was not significantly affected by different levels of *S. cerevisiae* ($p > 0.05$). On the other side, total globulin concentration values were significantly different between control group and 2 percent of probiotic. Serum lysozyme activity was increased by increasing in *S. cerevisiae* concentration ($p < 0.05$). Finally, this probiotic can be proposed as an immunostimulant in this species but it may not be proposed as nutritional supplement or growth promoter.

Key Words: probiotic, Oscar fish, growth, immune system, lysozyme.

Introduction. Probiotics are live microorganism which can affect the host animal by improving their intestinal flora as supplements to improve growth performance (Kesarcodi-Watson et al 2008) and they are used as antimicrobial agents (Moriarty 1997). On the other hand, they are effective agents to decrease the effects of stress and resulted in higher production in aquaculture (Ghazalah et al 2010). Mechanism of action of probiotics is very different. Some of these mechanisms are included of inhibition of pathogens via production of antagonistic compounds, competition for attachment to cell, competition for absorption of nutrients, alteration of enzymatic activity of pathogens (Bomba et al 2002). Many studies showed the positive effects of viable microorganisms as probiotic in diets of fish (Brunt & Austin 2005; Pangrahi et al 2005; Barnes et al 2006; Abo-State et al 2009).

Among probiotics, *Saccharomyces cerevisiae* (baker's yeast) is unicellular fungus and it is very cost effective if used in diets of fish (Tewary & Patra 2011). Oral administration or injection of the *S. cerevisiae* has been shown to increase growth performance of tilapia and carp (He et al 2009; Korkmaz & Cakirogullari 2011). Lara-Flores et al (2003) evaluated the effects of probiotics on growth performance in Nile tilapia. They found that the fish fry fed with diet enriched by the yeast, *S. cerevisiae*

revealed greater growth performance than those fed with the same diet without probiotic. The aim of this study was evaluation of effects of *S. cerevisiae* as supplementation on growth performance, nutritional indexes and some serological parameters in Oscar fish, *Astronotus ocellatus*.

Material and Method

Experimental design. The experiment was conducted in 12 glass aquarium in wet laboratory, Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran. This study lasted for 56 days (8 weeks) as from March 2012 to May 2012. Each aquaria was filled with 120 L aerated freshwater and maintained constant throughout the experimental period for 56 days. One hundred and twenty (120) Oscar fish fingerlings (with initial average weight: 8±1 gr and initial average length: 74±1 mm) were transferred to the glass aquarium and acclimatized for two weeks. Aeration was provided continuously except during feeding time to maintain dissolved oxygen level above 6 mg L^{-1}. During this period, the fingerlings were fed based on 3% of body weight twice a day with control diet. Formulation of the basic diet was included of 41.10% crude protein, 12.76% lipid, 7.59% ash, 30.89% carbohydrate and 6.87% moisture. Four diets were prepared by adding 0 (as control group) and 0.5, 1 and 2 per cent of probiotic, *S. cerevisiae* to basic diet (as treatment groups). Diets were stored at -20°C until used. Biometry was performed periodically every 14 days. Water quality (pH, temperature, DO, alkalinity and ammonia) of the experimental aquarium were daily monitored according to methods of APHA (1992) and maintained at normal level. During this period, all fish were exposed to a natural photoperiod (12h/12h). This experiment was performed in triplicates.

Sample collection. At the end of the experiment, biometry was performed for all fish. Furthermore, blood samples were collected from caudal vein by heparinized syringe and transferred into 1.5 ml micro tubes. Blood samples were centrifuged (3000 rpm for 10 min) and serum was separated.

Growth performance and nutritional indexes. Growth performance was determined and feed utilization was calculated by using following equations:
(1) Daily weight gain (DWG, gr) = [mean final body weight (gr) - mean initial body weight (gr)]/number of days;
(2) Body weight gain (BWG, %) = [(final body weight (gr) - initial body weight (gr)) /initial body weight (gr)] ×100;
(3) Specific growth rate (SGR, % day^{-1}) = [(Ln final weight- Ln initial weight) ×100]/ duration in days;
(4) Condition factor (CF) = (fish mass/fish total $length^3$) ×100;
(5) Feed conversion ratio (FCR) = [dry weight of feed (gr)/wet weight gain (gr)];
(6) Protein efficiency ratio (PER) = increase in body weight (gr)/protein intake (gr);
(7) Daily feed intake (DFI, gr/day/fish)= consumed diet × 100/duration in days/fish number per tank;
(8) Hepatosomatic index (HSI) = [weight of liver (gr)/total weight of fish (gr)] ×100;
(9) Viscerosomatic index (VSI) = [weight of viscera (gr)/total weight of fish (gr)] ×100 (Asadi Rad et al 2012).

Serological parameters. Lysozyme activity was measured by turbidimetry method recommended by Ellis et al (1990) with some modification. In summary, 200 µl of bacterial suspension of *Micrococcus lysodeikticus* (0.2 mg mL^{-1} of sodium phosphate buffer, 0.05 molar, pH: 6.2) was mixed with 10 µl of serum samples in 96 well micro plate and optical density was measured after 1 and 6 minutes by ELISA reader at wavelength of 530 nm. PBS was used as blank sample. Each enzyme activity unit is calculated based on enzyme amount which was declined absorbance for 0.001 per min per ml of serum. Lysozyme activity concentrations were compared by using standard curves of egg white lysozyme concentration (sigma) and serum lysozyme activity was revealed in µg mL^{-1}.

Serum total protein was determined by colorimetry method by using spectrophotometer at wave length 495 nm in comparison with standard protein sample by using laboratory diagnostic kit (Pars Azmoon Company) (Thomas 1998).

Serum total albumin was determined by colorimetry method by using spectrophotometer at wave length 495 nm in comparison with standard albumin sample by using laboratory diagnostic kit (Pars Azmoon Company) (Thomas 1998). Serum Total Globulin was calculated by using following equation: Globulin = Protein - Albumin (Kumar et al 2005).

All data were expressed as mean ± standard error. SPSS version 15 and One-way analysis (ANOVA) was used to determination of differences among treatments. Differences were considered significant at level of 0.05 ($p < 0.05$).

Results

Growth performance and mortality rate. No mortality was seen during the experiment in all groups (control and treatment groups) and survival rate was 100% in all treatment groups.

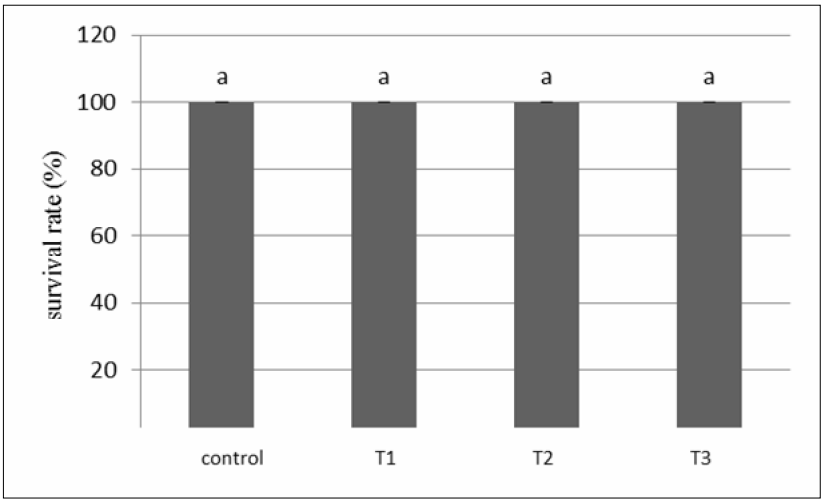

Figure 1. Survival rate of treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

Based on the results obtained from this study, no significant differences were seen among treatments and control groups during the experimental period for DWG, BWG, CF, SGR, PER, FCR, HSI and VSI ($p>0.05$). (Figures 2 to 10)

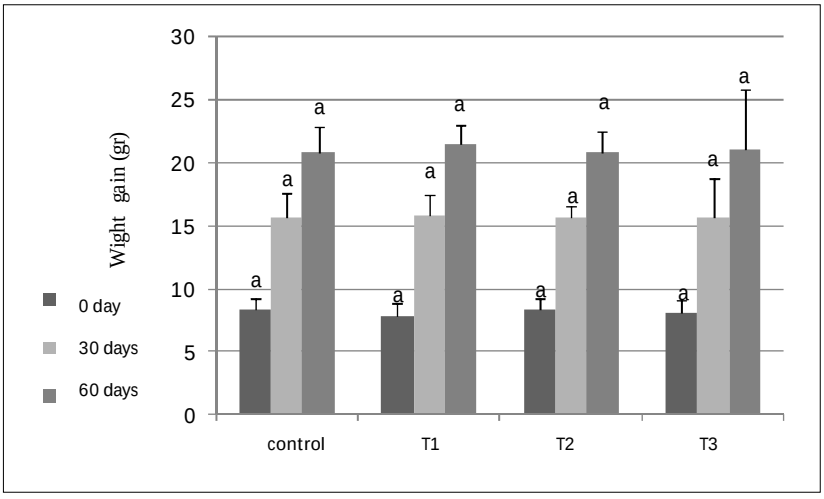

Figure 2. Weight gain of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

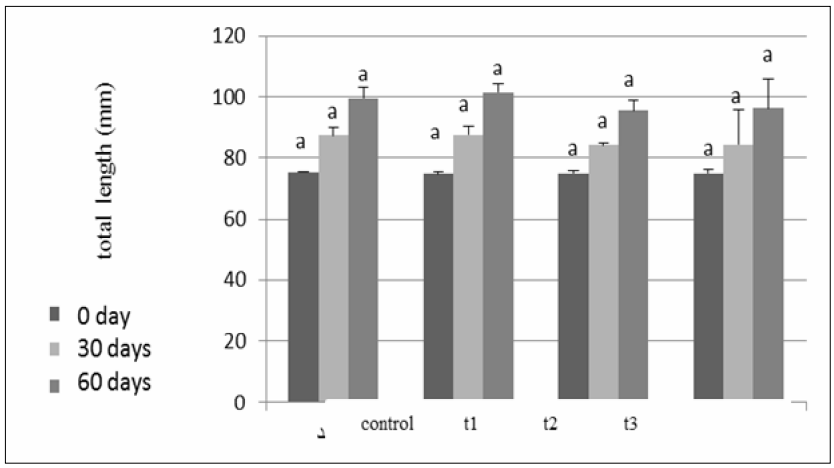

Figure 3. Total length of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

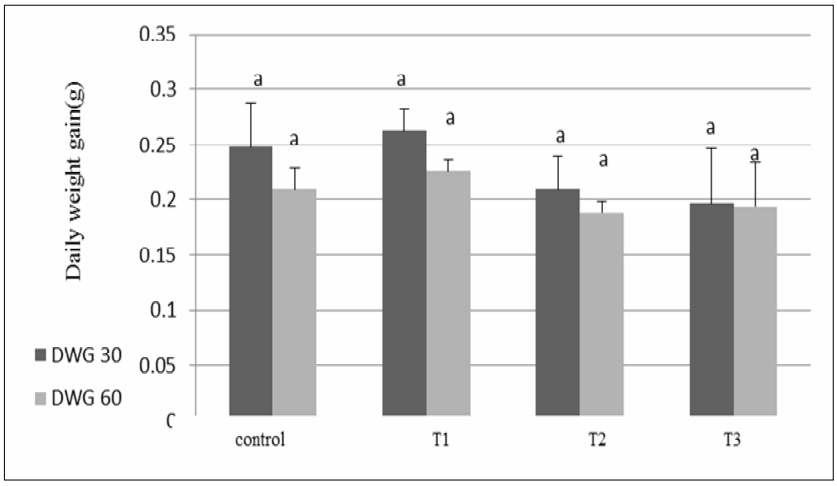

Figure 4. Daily weight gain of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

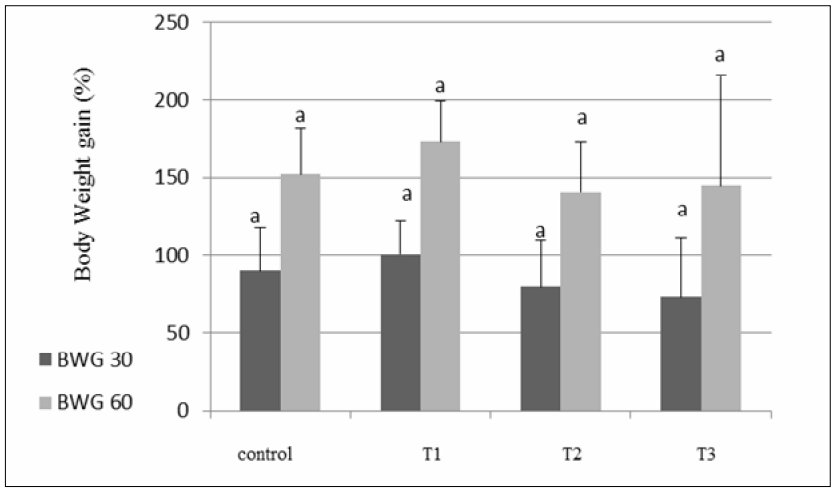

Figure 5. Body weight gain of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

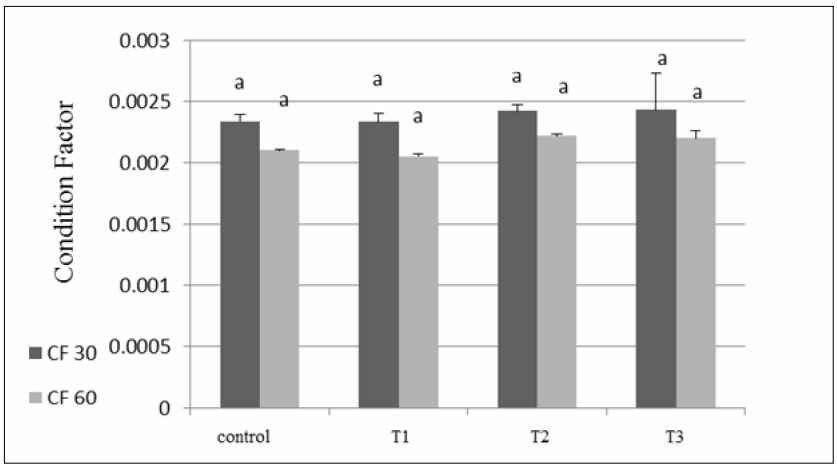

Figure 6. Condition factor of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

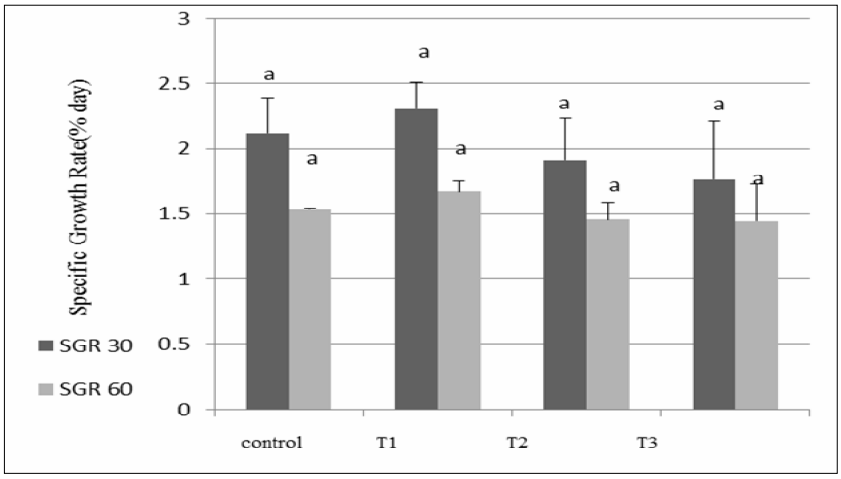

Figure 7. SGR of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

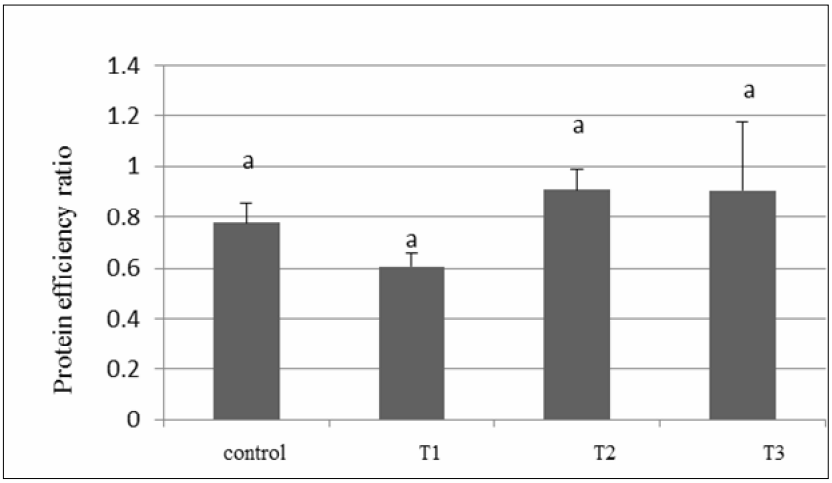

Figure 8. PER of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

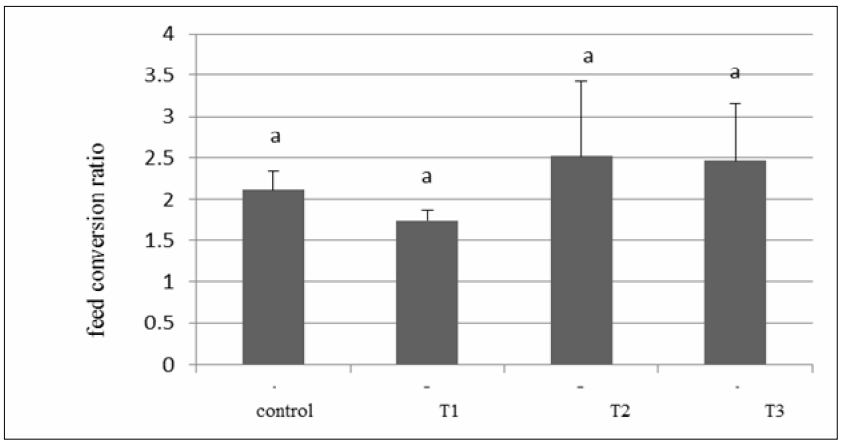

Figure 9. FCR of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

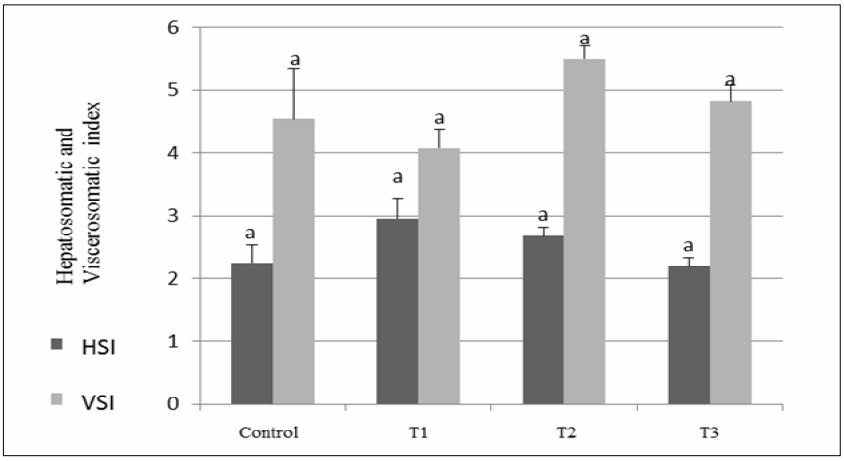

Figure 10. HIS and VSI of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

Serological parameters. There was a significant difference between control group and T2 and T3 and between T1 and T3 ($p < 0.05$) for total protein, but there is not any significant difference between control group and T1 ($p > 0.05$). Also, total serum protein was decreased by increasing of *S. cerevisiae* concentration (Figure 11).

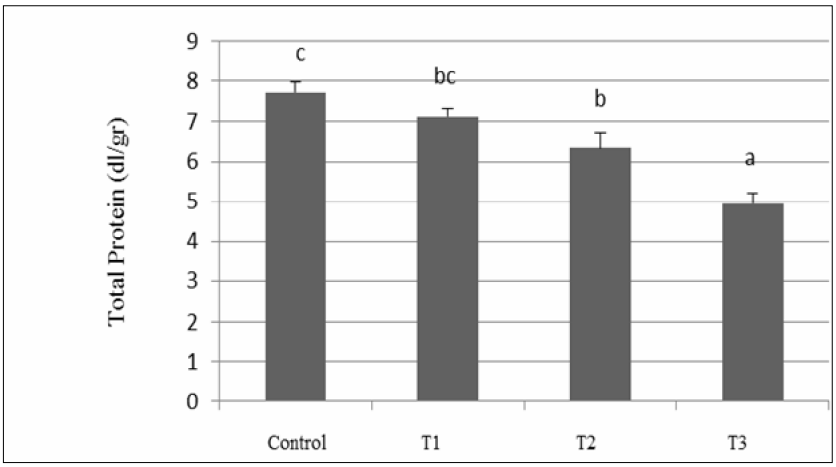

Figure 11. Serum Total Protein concentration of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

Total albumin was not significantly affected by different levels of *S. cerevisiae* ($p > 0.05$) (Figure 12).

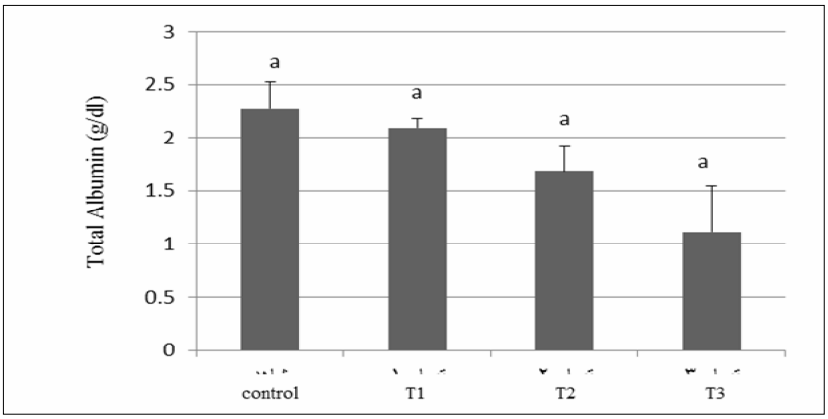

Figure 12. Serum total albumin of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

Total globulin concentration values were significantly different between control group and T3 ($p < 0.05$) and it was decreased by increasing in *S. cerevisiae* concentration (Figure 13).

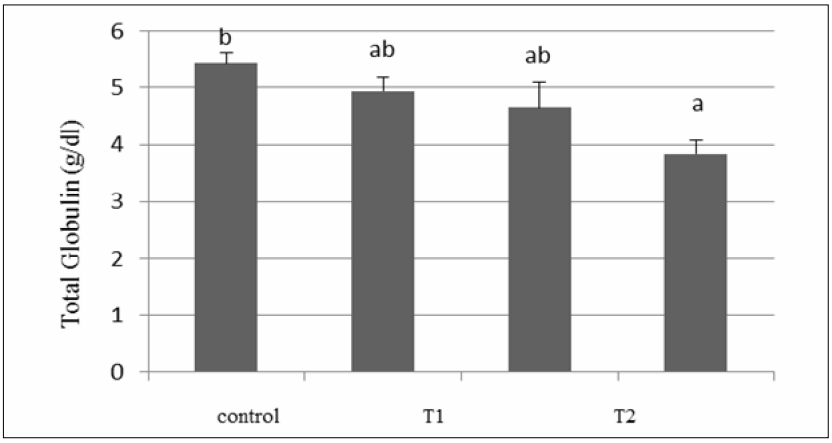

Figure 13. Serum total globulin of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

Serum lysozyme activity was increased by increasing in *S. cerevisiae* concentration and level of lysozyme was dose dependent ($p < 0.05$) (Figure 14).

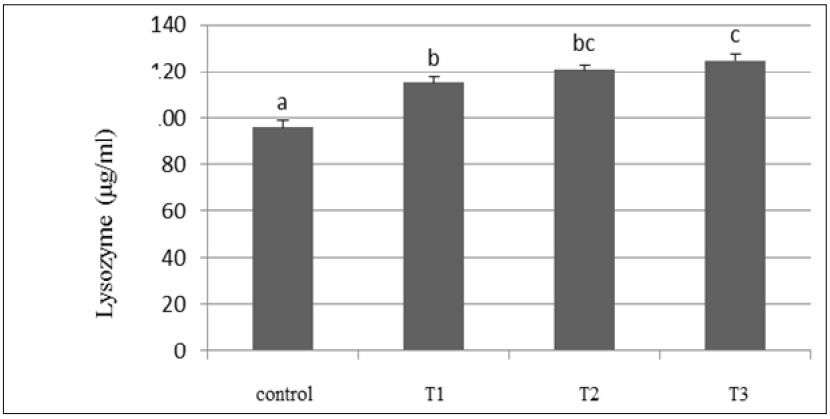

Figure 14. Serum lysozyme activity of different treatments of Oscar fish fed by different levels of *S. cerevisiae*, different letters showed significant differences between groups ($p < 0.05$).

Discussion. Using probiotics in aquaculture as growth promoters and immune stimulants was studied by different researchers in the world (Lara-Flores et al 2003; Carnevali et al 2004; Wang et al 2005; Wang & Xu 2006; Wang 2007; Sakkaravarthi et al 2010; Aftabuddin et al 2013).

S. cerevisiae has been recognized to have the potential effect as a substitute for live food or as a potential replacement for fish meal in aquaculture (Nayar et al 1998; Oliva-Teles & Gonçalves 2001). Researchers have evaluated the nutritional value of *S. cerevisiae* in Nile tilapia (Medri et al 2005; Abdel-Tawwab et al 2008; Korkmaz & Cakirogullari 2011), rohu (Tewary & Patra 2011), lake trout (Rumsey et al 1990), rainbow trout (Rumsey et al 1991) and sea bass (Oliva-Teles & Goncalves 2001) by comparing growth performance and feed utilization. Positive effects of *S. cerevisiae* were seen on survival rate Nile tilapia fry (Abdel Tawab et al 2008), rainbow trout (Siwicki et al 1994) and *Catla catla* (Mohanty et al 1996). According to the results of this study, *S. cerevisiae* has no significant effects on growth performance and nutritional values in Oscar fish. Welker & Lim (2011) declared that probiotics may improve digestion by stimulating production of digestive enzymes or through other alteration in the gut environment, which led to improve growth performance.

The microbial population of gastrointestinal tract is also important for fish nutrition by increasing nutrients uptake and utilization and production of enzyme, amino acids, short chain fatty acids and vitamins and improving digestion process (Merrifield et al 2010; Nayak 2010; Welker & Lim 2011). Lara- Flores et al (2003, 2010) revealed that *S. cerevisiae* supplementation produced significantly higher weight gain and feed utilization efficiency in tilapia fed with diets containing 27% or 40% crude protein compared to the control diet.

Similar pattern that improved growth performance in tilapia fed with *S. cerevisiae* diets have been reported by Marzouk et al (2008), Osman et al (2010) and Ozório et al (2012) which are in despite of results obtained from current study. But, Shelby et al (2006) and He et al (2009) revealed that growth performance of tilapia has not significantly influenced by different levels of dietary supplementation of *S. cerevisiae*. Performance of probiotics which are added to diets of the fish depends on type of the probiotic and its species and their effects on survival rate, growth performance, adaptation with environmental condition in different fish species (Fietto et al 2004). Similar results were obtained by supplementation of diet of rainbow trout with *S. cerevisiae boulardii* on growth indexes (Aubin et al 2005). Different results which are obtained from different studies depended to intra species differences (Lara-Flores et al 2003). Furthermore, type and method of adding *S. cerevisiae* to diet can be effected (Tovar-Ramirez et al 2002).

Ability of probiotic bacteria for enhancement of non specific immune system was studied by some researchers such as Nikoskelain et al (2003), that they said oral administration of probiotics can increase immune response such as phagocytosis, respiratory burst, lymphocyte proliferation and cytokine synthetase. Wang et al (2008), showed that probiotic, *Enterococcus faecium* has significant effect on lysozyme activity, total protein, total albumin, globulin concentration in different experimental groups of Nile tilapia. Merrifield et al (2010) have assessed performance of probiotic on immune system, microbial activity of intestine, growth performance in rainbow trout and they showed better immunological parameters in diets with probiotic in comparison with control group without any probiotic. These results have confirmed the results obtained from this study. In the current years, different types of probiotics were isolated, diagnosed and used as an immune stimulant for prevention from diseases and positive effects on fish health (Brunt & Austin 2005; Wang et al 2008). The yeast can produce beta-glucan and some nucleotides which have affected fish immune system (Sahoo & Mukheri 2001; Li et al 2004). The yeast, *S. cerevisiae*, by producing different metabolites, can increase secretion of gastric enzyme and improve food digestion, stimulate fish immune system, increase survival rate and finally increase growth performance (Ringo & Birkbeck 1999).

Researches showed that extracted glycans from cell wall of *S. cerevisiae*, cause resistance increase against bacterial infection in Atlantic salmon. Probiotics in host

animals established on gastrointestinal tract mucosa and produce inhibitory combinations and stimulated immune system (McCracken & Gaskins 1999). Different researches on positive capability of probiotics in aquaculture have shown that yeast and its derivatives have relative high capabilities for using in aquaculture and are very valuable for increasing of fish resistance against non suitable environmental condition and pathogens and it can be studied as novel biological technology (Ringo & Birkbeck 1999; Sahoo & Mukheri 2001; Li et al 2004). In the current study, lysozyme activity was significant in comparison with control group confirmed that probiotic, increased non-specific immune system. Lysozyme is one of the components of immune system of invertebrate and vertebrate animals (Denev et al 2009). Physiological role of lysozyme has not been defined but it has cooperated in defense of the host against invasive microorganisms. This enzyme hydrolyzes glycoside link between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycan layer of bacterial cell wall. The level of this enzyme is very high in serum, mucosa and skin of fish. This enzyme increased in serum of fish following injection of microbial products and in response to bacterial infection and diets enriched by probiotics (Nikoskelainen et al 2003).

Role of immunoglobulin in protection of human and animals against pathogens is defined. Probiotic bacteria can stimulate immunoglobulin synthesis (Nikoskelainen et al 2003). Other researchers reported effects of probiotics on immune systems of Nile tilapia (Wang et al 2008), Sea bream (Salinas et al 2005), rainbow trout (Raida et al 2003) enhanced immune system in fish.

Growth improvement and resistance against thermal and environmental stress and pathogens is related to important property of probiotic types.

As a result, the establishment, proliferation and function of the probiotics in the digestive tract are largely influenced by various environmental factors, such as water quality, hardness, dissolved oxygen, temperature, pH, osmotic pressure, mechanical friction and the environmental micro flora (Das et al 2008; Mehrim 2009; Ai et al 2011).

Conclusions. Based on the results obtained from this study, the yeast, *S. cerevisiae*, was not significantly effective on growth performance and nutritional indexes and serological parameters, but nonspecific immune system parameters which was examined in this study, has been affected by *S. cerevisiae* in Oscar fish.

It is difficult to draw concrete conclusions and provide specific recommendations on the effects of dietary probiotics on growth performance of Oscar fish given that the studies vary widely with regard to fish age and size, stocking density, diet composition, dietary probiotic concentration, feed allowances, feeding duration and of course, type and source of probiotic. But this probiotic can be proposed as an immunostimulant in this species but it can not be proposed as nutritional supplement or growth promoter.

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