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# Impact of yeast (*Saccharomyces cerevisiae*) on the growth performance, hemato-biochemical, physiological parameters and digestive enzymes activity of GIFT Tilapia (*Oreochromis mossambicus*)

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**ABSTRACT**

This study was conducted for 60 days to evaluate the efficiency and influence on different levels of dietary supplementation of *Saccharomyces cerevisiae* (0.15%, 0.30%, 0.45%, 0.60%, 0.75% and 1% named as SC1, SC2, SC3, SC4, SC5 and SC6 as potential probiotic for improving the growth performance, body composition, hemato-biochemical parameters, digestive enzyme activities and stress resistance of GIFT Tilapia (*Oreochromis mossambicus*). The study showed significantly better effects on growth performance ($P<0.05$; 0.01) as compared to control. Moreover, better growth performance was observed in SC4 groups fed with *S. cerevisiae*. Significant differences in crude protein, crude lipids, ash contents, crude fibre and carbohydrates were observed. Hematological parameters indicated that RBC count, HB, HCT and MCHC were significantly higher in SC4 treatments. Total and specific amylase activities, total and specific protease activities as well as total and specific lipase activities were significantly higher in SC6. *Aeromonas hydrophila* and salinity stress challenge tests provided higher survival rates in the treatment SC4 (95%). Hematological parameters indicated highly significant differences ($P<0.01$) in RBC, HB, MCV, MCH, MCHC, leukocyte count, lymphocytes, monocytes, granulocytes, survival after challenge with salinity and *A. hydrophilla*. Differential total leukocyte counts observed maximum in SC5 indicated a significant increase in lymphocytes, monocytes and granulocytes observed higher in SC4 in probiotic treated groups as compared to control which indicated high immune response. It can be concluded that the addition of 0.60% *S. cerevisiae* of the diet enhances growth performance, body composition, hematological parameters, digestive enzyme activities and stress resistance in (*O. mossambicus*).

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## INTRODUCTION

Aquaculture is one of the most important sectors to contribute the nutritional security and one of the fast growing and rapidly expanding industry with appreciable contribution to global animal intake (Wang, 2019; Debnath *et al.*, 2020). Aquaculture is steadily expanding sector of food production in the world and producing animal protein for human consumption (FAO, 2016) and an important source of the diet, revenue for millions of individuals around the globe and affordable protein source in the third world countries (Welker & Lim, 2011; FAO, 2016; 2020). Extensive fish farming alone cannot be able to achieve this high demand and also many wild fish populations are declining due to overharvesting. So, aquaculture gives a solution for all these issues by offering a high protein food that requires less space to farm, a faster harvesting time, lower cost to produce and is less detrimental to the environment (FAO, 2016, 2020). Aquaculture is expanding and applying new technologies on commonly farmed species to promote their survival, growth and stress resistance. New successes of dietitian include symbiotic food supplement, which contains probiotics for the improvement of animal health (Wang *et al.*, 2019). One exciting approach, that emphasis on the use of probiotics bacteria is to develop the well-being of the host by preventing the growth of pathogenic microorganisms and improving the digestion and immune response (Wang & Xu, 2006; Debnath *et al.*, 2020; Dawood, *et al.*, 2020).

Probiotics are environmentally approachable applications to improve fish health and growth (Carnevali *et al.*, 2017; Debnath *et al.*, 2020). According to Ramesh *et al.* (2015), it is a substitute for antibiotics and can suppress pathogens growth without being injurious to the host and its environment. Furthermore, Li *et al.*, (2019b) reported that it has been widely applied in aquaculture and used as a substitute for fish and shrimp antibiotics. Probiotics supplementation in aquaculture results in increasing growth, feed digestibility and increase digestive enzyme activity, increase immunity against pathogens (Zhou *et al.*, 2009) and improve the water quality of culture media (Putra *et al.*, 2021). Probiotics are living microbes or feed additives that exert their effects on the host organism by the production of inhibitory compounds, improving microbial equilibrium modulating and motivating immune function. Probiotics being immune-stimulants and having antimicrobial properties, perform vital role in aquaculture and increasing the stress tolerance, reproduction and in the better digestion of the essential nutrients (Debnath *et al.*, 2020). Probiotics are suitable alternatives to control pathogens to overcome the antagonistic concerns of various antibiotics and other chemotherapeutic agents. They can also be helpful to achieve natural resistance along with high survival rate during larval and post larval stages in fishes (Robertson *et al.*, 2000). The common probiotics in aquaculture industry include various species which belong to *Saccharomyces, Lactobacillus, Bacillus, Clostridium, Enterococcus, Shewanella, Leuconostoc, Lactococcus, Carnobacterium* and *Aeromonas* (De Rodriganez *et al.*, 2009; Kim *et al.*, 2010). Numerous investigations conducted on the dietary application of probiotics have focused on *Bacillus* and *Lactobacillus* species have antimicrobial and immuno-modulatory activities in the host animal (Cutting, 2011). The supplementation of *S. cerevisiae* as probiotics ameliorated the growth, immunity and disease and stress resistance of various fish species and crustaceans (Dawood *et al.*, 2020; Ringo *et al.*, 2012, 2020). In tilapia and carp culture it is effective to enhance the growth rate (Korkmaz & Cakirogullari, 2011; He *et al.*, 2009; Ebrahim & Abou-Seif, 2008).

Tilapia is the most important and second most cultured freshwater fish which is farmed worldwide and indicates 6% of total fish production in farms (FAO & WHO, 2011). The use of new functional ingredients like probiotics and feed additives to increase feed utilization, growth performance and health of fish is also in practice. Tilapia are most abundant species due to enormous adaptability to physical and environmental conditions, captive breeding, resistance to disease and handling stress,

outstanding growth rate, flesh quality, feeding on natural and artificial diets at low trophic level (Welker & Lim, 2011). Tilapia can be cultured in all different types of systems like fresh and salt water and different climates such as subtropical, tropical, and temperate climates (Lim & Webster, 2006). Tilapia is most important cultured fish with low price for mass consumption, good source of protein, and valuable product for export markets (Fitzsimmons, 2006; Welker & Lim, 2011). Tilapias are increasing their acceptability globally and are second to carps by volume of production (FAO, 2016, 2020). The present study was aimed to investigate the effect of probiotics on immunity, stress resistance, growth performance and hematological parameters of GIFT Tilapia (*O. mossambicus*).

## MATERIALS AND METHODS

### Ethics statement

All of the experimental protocols and methods of this study of GIFT Tilapia (*O. mossambicus*) were performed following guidelines and regulations approved by the Animal and Ethics Committee of GC University, Faisalabad. This study did not involve endangered or protected species. No other authorization or ethics board approval was required to conduct the study. Information on animal welfare and methods of sacrifice is not applicable, since the animals were not exposed to any additional stress other than that involved in commercial fishing practices.

### Experimental design and conditions

The experimental fish specimens were fed daily with basal fish feed (5% of their body weight at 9.00 am and 4.00 pm) before the start of the trials. The study involved control and treatment groups with three replicates for each group, and their culture period was 60 days. The trial with *S. cerevisiae* was preceded for 60 days with different doses of *S. cerevisiae*. This 60 days' trial was divided into 6 treatments, i.e., SC1 to SC6 and was fed with basal fish feed plus probiotic (*S. cerevisiae*) @ 0.15% or 0.30% or 0.45% or 0.60% or 0.75% or 1.0 %, respectively and one control (C) group, fed with only basal diet. This trial was also conducted in triplicate.

### Fish procurement and acclimatization

Risk assessment was conducted before starting the experiment and fish husbandry was fixed to maintain fish health by retaining good water quality and overall environment of the stock aquaria. Healthy specimens of experimental fish species (GIFT Tilapia, *O. mossambicus) with similar initial body weight* (8±1 g) were obtained from the Government Fish Seed Hatchery Mianchannu, Pakistan and transported in polythene bags with sufficient amount of oxygen to the Fish Research Laboratory, Department of Zoology, Government College University Faisalabad. Fish was acclimatized in two concrete tanks measuring (400 cm x150 cm x100 cm) for 14 days. During the experiment chlorinated tap water was used and physicochemical parameters of water were determined with temperature, pH and dissolved oxygen were maintained between 25°C, 6.9–7.5 and 6.6–7.5mg/l, respectively. The concentration of ammonia, total dissolved solids and total hardness were maintained at 0.4 to 0.6 ppm, 6.5 to 7.8ppt and 47 to 52ppm, respectively. Fish were fed 2 times a day with basal fish feed throughout the acclimatization period. Water was changed daily and dead fish as well as any fish showing any unusual symptoms were excluded.

### Physicochemical parameters of water

The physicochemical parameters of water were observed daily in fish aquaria. To maintain water quality, water temperature, total dissolved oxygen, pH, and dissolved solids were determined by using multi-parameter apparatus (Hanna Instruments, model HI 9828). The Titration method (AOAC, 2005) as described below was used to evaluate $NH_3$ and water hardness. The water quality helps to meet the specific requirements of the experiments. Water quality parameters (pH, temperature, dissolved oxygen, $NH_3$ and water hardness) were monitored throughout the experiment.

### Determination of ammonia

To determine the ammonia, 50 ml water sample was taken in flask, added two drops of Rochelle salt solution and was mixed. Then Nessler's reagent (2

ml) was mixed. After 10-25 min the Spectrophotometer (Hitachi, U 2800) was used to determine the quantity of ammonia at the absorbance of 425 nm. The anhydrous $NH_4Cl$ was used as a reference standard.

**Total hardness**

The total hardness of water was measured by adding ammonia buffer (1.0 ml) and erichrome Black-T-indicator (5 drops) to water sample (50 ml) in a conical flask. When the color of water turned wine-red, it was titrated against EDTA solution, until a blue color appeared.

To calculate total hardness following equation was used: Total hardness mg/l = ml of EDTA used/Volume of sample (50 ml) X 100

**Determination of nutritional effects (Growth performance)**

The fish was weighed weekly using an electronic weighing machine (Uni Block D450011585 AUW). IBW: Initial body weight, FBW: final body weight, SGR: specific growth rate, FI: feed intake, FCR: feed conversion ratio, PER: protein efficiency ratio, PPV: protein productive value and survival rates were measured by using the following equations:

SGR = ($\ln W_f$ - $\ln W_i$ X 100) / t, where: ln $W_f$ = the natural logarithm of the final weight, ln $W_i$ = the natural logarithm of the initial weight, t = time (days) between $\ln W_f$ and $\ln W_i$,FI = fish weight x feeding level/100, FCR = Feed consumed/Weight gain, WG = FBW (g) - IBW (g), PER = Weight gain (g)/protein fed (g), PPV = (Protein gain (g)/protein fed (g) x 100.

**Proximate analysis (Analytical method)**

The proximate composition for experimental fish was conducted by using the method of analyses adopted by the Association of Official Analytical Chemists (AOAC, 2005). To determine the moisture contents samples were dried at 67-70 °C for 24 hrs. Micro Kjeldahl method was adopted to determine Crude protein (CP) and Kjeldahal distillation unit (UDK 127, Velp Scientifica, Milano, Italy) was used for this purpose. Soxhlet apparatus was used to extract Crude lipid by adding petroleum ether (60-80 °C). Muffle furnace (Hanau, Germany, model M110) was used to detect ash content at 550 °C for 12 hrs.

**Moisture (%)**

Moisture contents were examined by oven drying method (AOAC, 2005). For this purpose, 10g of sample was kept in an oven for 24 hrs at 67-70 °C.

Following formula was used to determine moisture contents: Moisture (%) = Loss in weight (Wt) of sample / weight of sample ×100

**Ash (%)**

Homogenized samples of known weight of organic components were burned with the help of furnace to determine the ash contents. For this purpose, 2g sample was kept in pre-weighed crucibles which were kept in electric furnace (EHRET TK/L 4105) at 450 °C for 12 hrs until the formation of white ash.

Given formula was used for the calculation of ash contents: Ash (%) = weight (Wt) of ash/weight of sample × 100

**Feed preparation**

The basal fish feed was prepared by common ingredients which were purchased from the local market and its proximate chemical analysis was carried out according to AOAC (2000), as shown in Table 1. All these ingredients were mixed with boiled water and converted into paste or into semi moist dough, which was passed through electrical mincer to make pellets and Kenwood Multi-processor was used for this purpose.

These pellets were dried for a few days at the room temperature and then crushed to make fine particles. The proposed doses of Probiotics were added with these crushed fine particles at the time of feeding.

Feed was given twice a day (9.00 am and 4.00 pm each day) @ 5% of body weight for the entire period of experiments.

**Digestive enzyme activities**

Digestive enzymes were extracted from the gut by homogenizing it. For this purpose, hand held homogenizer was used and cooled phosphate buffer (pH 7.5) with the ratio 1/10 (w/v) was poured in it, then it was centrifuged for 5 min at 4 °C and 5000 rpm (Yanbo & Zirong, 2006; Huang *et al.*, 1999). Protein contents, protease enzyme activity, amylase enzyme activity and lipase enzyme activity were determined from the extracts. Method of Lowry *et al.* (1951) was used to assess the protein contents. While protease enzyme activity and amylase enzyme activity were measured by adopting the methods of Anson (1938) and Smith and Roe (1949). Titrimetric method described by (Essa *et al.*, 2010; Teitz & Fiereck, 1966) was used to measure the lipase enzyme activity.

**Carbohydrates (%)**

Carbohydrate percentage was calculated by subtracting the total crude protein, fat and ash contents from 100 (Jabeen & Chaudhry, 2011) and following equation was used:

Carbohydrates (%) = 100 – (Fat + Crude Protein + Ash)

**Crude fiber (%)**

Crude fiber contents were measured by taking 2g of the sample in a conical flask of 250 ml. $H_2SO_4$ (200 ml), 1.25 % was added in the sample and this mixture was boiled for 30 minutes. After boiling, this solution was passed for filtration process through the Whatman filter paper.

The remaining filtrate was shifted into a beaker of 250 ml and 1.25 % NaOH (200 ml) was added in it.

Now it was boiled for 30 minutes in a digestion apparatus then it was again filtered and rinsed repeatedly to make it neutral. Distilled water was used to rinse the filtrate, and its neutralization was checked by pH paper. The residues were shifted into a crucible and kept in an electric oven for drying purpose at 100 °C for some hrs. Then it was allowed to cool by placing in a desiccator and weighed. It was burned again, allowed to cool and weighed. The crude fibre contents were estimated by given equation:

Crude fibre (%) = (wt. of sample + wt. of crucible)/ wt. of sample x 100

**Crude lipid (%)**

A set of Soxhlet system (Soxhlet extractor, thimble, flask, condenser and heating mantle; Behr-lab, D40599) was used to measure the fat contents. Non-polar organic petroleum ether was used as solvent for this purpose. The flask was oven dried (overnight at 60 °C) and sufficient amount of petroleum ether was added in it. The sample (10g) was kept in a thimble and was plugged with cotton wool at the top.

Then an extractor and flask were fitted with each other. It was fitted with the condenser and heating mantle. Now flask was heated, and extraction period was continued for 06 hrs until the solvent was mildly boiled. Lastly, the residual solvent was dried in oven at 60 °C for overnight and retained in desiccators to cool down.

The following equation was used to calculate fat contents: TS: weight of thimble with dried sample (g), T: weight of thimble, S: weight of dried sample (g), S= TS – T, FE: weight of flask with ether extract, F: weight of flask (g), EE: ether extracts (g), E: weight of ether extract (g), E= FE – F; EF (g/kg DM)= E x 100/S.

**Crude protein (%)**

Kjeltec machine (Model Tecator Kjeltec System 8000) was used to calculate % nitrogen. For this determination **s**amples were break down by adding a mixture of $K_2SO_4$: FeSO4: CuSO4 @ 100: 5: 10, respectively until the colour was changed to green. These samples were diluted with distilled water. In the distillation apparatus, NaOH (10 ml) was added with digested samples (10 ml to collect free ammonia in a beaker. Methyl red indicator and 4% boric acid (20 ml) were also added, and the material was titrated against $H_2SO_4$ (0.04 N). Following formula was used to determine protein contents: % of Nitrogen = volume of $H_2SO_4$ used x 0.0014 x volume of dilution/volume of distilate x weight of sample x 100% Crude protein= Nitrogen x factor (6.25).

**Hematological parameters**

After experimental trials (60 days), five fish were taken from all aquariums randomly for hematological analysis of the blood samples was performed according to the protocol (Standen *et al.*, 2013; Casas and Dobrogosz, 2000).

**Collection of blood sample**

During the experimental period blood samples were collected randomly on a weekly basis i.e.; at 0, 15, 30, 45 and 60 days' intervals from both probiotic fed fishes and control fishes from caudal veins. The syringe of 02 ml was flushed with EDTA (Anticoagulant), 150 to 200µl of EDTA was kept in syringe needles and before taking the blood to avoid coagulation. The blood samples were transferred into Eppendorf's (1.5 ml capacity) and stored for analysis.

**Hematological analysis**

After the challenge test blood parameters were also investigated. Blood was taken as described earlier for differential leucocyte examinations by preparing smears. Surplus blood was allowed to clot for 12 hrs. (at 4 $^{o}$C) for serum isolation. For this purpose, clotted blood was centrifuged and then stored at -80 $^{o}$C. Centrifugation was carried out for 5 minutes at 3600 rpm. Then erythrocyte counts (RBC), leucocyte counts (WBC), hemoglobin (HB) and Hematocrit (HCT) values were determined by adopting standard methods suggested by Rawling *et al.*, (2009).

**Immunological parameters**

*Stress resistance and survival rate*

After 60 days' trials, the fish from all treatments were divided into two subgroups to check the survival rate and stress resistance by conducting challenge test on both subgroups. For this purpose, the first subgroup was injected (IP) with pathogenic *A. hydrophila* (0.1 mL of $10^{7}$ cells/mL) whereas, the second one was injected with saline (0.1 mL) as control group. Salinity stress challenge test was also performed to determine stress resistance by using the methods proposed by Soleimani *et al.* (2012). Fish were divided in triplicate from all aquaria and then exposed to 15ppt salinity. All subgroups were monitored daily to determine the survival rate.

**Statistical analysis**

The data from all parameters were analyzed by using two-way ANOVA (analysis of variance). The data were presented as treatment mean ± Standard deviation and the variation of means among different groups were analyzed for the significance at the 95% confidence level. P values < 0.05 was considered to be significant, using Duncan's multiple range test. Software package (SPSS, version 17) was used for statistical analysis.

**RESULTS AND DISCUSSION**

**Growth performance**

Sixty days' trial was conducted to assess the influence of various levels of dietary supplementation of *S. cerevisiae* as a feed supplement on growth performance of *O. mossambicus*. In this trial growth performance was investigated by observing various parameters such as initial body weight (IBW), final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV). Analysis of variance for growth performance after 60 days' trial revealed non-significant differences among treatments and control (C) group for initial body weight ($P>0.05$). In final body weight; weight gain, feed intake, specific growth rate, protein efficiency, feed conversion ratio and protein productive values, highly significant ($P<0.01$) differences were observed in Table 2).

Initial body weight of control (C) and experimental fishes were closer to each other, i.e., 8.82±0.16g in control (C), 8.62±0.06g in SC1, 8.66±0.29g in SC2, 8.90±0.17g in SC3, 8.53±0.17g in SC4, 8.72±0.18g in SC5 and 8.54±0.11g in SC6. Maximum final body weight after 60 days was observed in SC4 which was 38.63±1.22g while minimum in SC1 which was 32.09±0.51g whereas, in control (C) group final body weight was observed 27.53±1.17g. All the treatments exhibited higher FBW compared to control (C) group after 60 days. Maximum Weight gain (WG) was observed 30.1±1.11g in SC4 and minimum 23.47±0.51g in SC1 while in control (C) it was noted 18.71±0.25g in 60 days. Specific growth rate (SGR)

was observed maximum in SC4 (1.09±0.03) and minimum in SC1 (0.95±0.02) while in control (C) it remained 0.82±0.03. Feed intake (FI) was observed maximum 231.78±3.94g in SC4 and minimum 192.54±4.38g in SC1 whereas; in control (C) group it was noted 165.18±8.17 g. Feed conversion ratio (FCR) was observed maximum in SC1 (8.29±0.15) while minimum in SC4 (7.70±0.11) and in control (C) group it was (8.83±0.23). Protein efficiency ratio (PER) was maximum in SC4, SC5 and SC6 (0.43±0.01) and minimum in SC1 (6.51±0.11) while in control (C) group it was observed (0.41±0.01). Protein productive value (PPV) was observed maximum in SC4 (6.94±0.20) and minimum in SC1 (6.51±0.11) whereas, in control (C) it was 6.04±0.10. After 60 days of trial survival rate was observed 100%.

**Table 1.** List of the ingredients and chemical composition (%, DM basis) of the basal fish feed.

| Ingredients | Percentage (%) |
|---|---|
| Fishmeal | 12.0 |
| Soya bean meal | 31.0 |
| Yellow Corn | 20.0 |
| Wheat bran | 25.0 |
| Corn oil | 5.0 |
| Vitamin-mineral premix* | 2.0 |
| Molasses | 5.0 |
| Total | 100 |
| Dry Matter (DM) | 89.19 |
| Crude Protein (CP) | 27.24 |
| Ether Extract (EE) | 6.42 |
| Ash | 10.91 |
| Total Carbohydrates | 55.43 |
| Gross Energy (GE) (Kcal/100g DM)ψ | 439.94 |
| Protein/ Energy ratio (mg CP/Kcal GE)ϕ | 61.91 |

*= Each Kg premix contains: vit. A, 12,000,000 IU; vit. D3, 3000,000 IU; vit. E, 10,000 mg; vit. K3, 3000 mg; vit. B1 200 mg; vit. B2, 5000 mg; vit. B6, 3000 mg; vit. B12, 15 mg; Biotin, 50 mg; Folic acid, 1000 mg; Nicotinic acid, 35,000 mg; Pantothenic acid, 10,000 mg; Mn, 80g; Cu, 8.8g; Zn, 70g; I,1g; Co, 0.15g and Se, 0.3g.ψGE= CP x 5.64 + EE x 9.44 + total carbohydrates x 4.11 calculated according to NRC (1993) ϕP/E ratio= CP/GE x 1000.

Over all outcomes of 60 days' trial indicated that IBW was approximately similar in all treatments and control (C) groups. Final body weight was maximum in SC4 fed with 0.60% dose of *S. cerevisiae*. Weight gain was calculated maximum in SC4 and it was observed that as compared to control (C) group all treatments fed with probiotics have better weight gain. In case of SGR maximum value was found in SC4 and better SGR was noted in all treatments fed with probiotics as compared to the control (C). Maximum feed intake was 231.78±3.94g/fish after 60 days in SC4. FCR was observed Minimum in SC4 (7.70±0.11). PER was observed similar and maximum in three treatments SC4, SC5, SC6 and was significantly increased due to supplementation of probiotic in experimental diets as compared to the control (C) group. PPV was maximum in SC4 which was 6.94±0.20 and gradual increase was observed from control (C) to SC4 (Tables 2). The survival rate in 60 days' experiment was observed 100 % (Tables 3-10).

**Proximate analysis after 60 days**

Analysis of variance for proximate composition analysis after 60 days revealed that significant differences were observed among treatments and control (C) group for moisture and crude protein ($P>0.05$) whereas, highly significant differences were observed in crude lipids, ash contents, crude fiber and carbohydrates ($P<0.01$).

**Table 2.** Comparison of means (±SE) for different parameters of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| SL | Ann. Table No. | Treat. | Days | C | SC1 | SC2 | SC3 | SC4 | SC5 | SC6 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | IBW | 7,15,21,30,37,45,52,60 | 8.82±0.07 | 8.62±0.07 | 8.66±0.05 | 8.90±0.06 | 8.53±0.07 | 8.72±0.05 | 8.54±0.0 |
| 2 | 3 | FBW | 7,15,21,30,37,45,52,60 | 18.42±1.7$^{C}$ | 20.99±1.3$^{B}$ | 24.37±1.67$^{A}$ | 24.29±1.64$^{A}$ | 25.24±1.81$^{A}$ | 24.75±1.70$^{A}$ | 24.99±1.78$^{A}$ |
| 3 | 4 | WG | 7,15,21,30,37,45,52,60 | 9.60±1.16$^{E}$ | 12.37±1.$^{D}$ | 15.71±1.66$^{BC}$ | 15.43±1.64$^{C}$ | 16.71±1.81$^{A}$ | 16.28±1.76$^{AB}$ | 16.45±1.77$^{A}$ |
| 4 | 5 | SGR | 7,15,21,30,37,45,52,60 | 0.96±0.03$^{E}$ | 1.21±0.0$^{D}$ | 1.44±0.07$^{B}$ | 1.40±0.07$^{C}$ | 1.50±0.07$^{A}$ | 1.45±0.07$^{B}$ | 1.42±0.07$^{BC}$ |
| 5 | 6 | FI | 7,15,21,30,37,45,52,60 | 70.83±10.8$^{C}$ | 81.85±12.76$^{B}$ | 95.13±14.65$^{A}$ | 94.59±14.51$^{A}$ | 99.24±15.47$^{A}$ | 97.92±15.19$^{A}$ | 98.07±15.30$^{A}$ |
| 6 | 7 | FCR | 7,15,21,30,37,45,52,60 | 6.37±0.34$^{A}$ | 5.34±0.35$^{B}$ | 5.16±0.37$^{BC}$ | 5.23±0.37$^{BC}$ | 5.04±0.36$^{C}$ | 5.11±0.37$^{C}$ | 5.06±0.37$^{C}$ |
| 7 | 8 | PER | 7,15,21,30,37,45,52,60 | 0.55±0.03$^{D}$ | 0.66±0.05$^{C}$ | 0.74±0.06$^{AB}$ | 0.73±0.06$^{B}$ | 0.76±0.06$^{A}$ | 0.75±0.06$^{AB}$ | 0.76±0.07$^{AB}$ |
| 8 | 9 | PPV | 7,15,21,30,37,45,52,60 | 8.84±0.55$^{D}$ | 10.49±0.83$^{C}$ | 11.92±1.01$^{AB}$ | 11.69±0.97$^{B}$ | 12.22±1.04$^{A}$ | 12.04±1.03$^{AB}$ | 12.18±1.05$^{A}$ |
| 9 | 13 | RBC | 0,15,30,45,60 | 3.54±0.28$^{D}$ | 3.99±0.32$^{C}$ | 4.00±0.21$^{C}$ | 4.27±0.28$^{AB}$ | 4.43±0.44$^{A}$ | 4.14±0.37$^{BC}$ | 3.94±0.25$^{C}$ |
| 10 | 14 | HB | 0,15,30,45,60 | 4.73±0.13$^{D}$ | 5.16±0.21$^{C}$ | 5.18±0.20$^{BC}$ | 5.32±0.25$^{AB}$ | 5.56±0.26$^{A}$ | 5.14±0.25$^{BC}$ | 4.95±0.20$^{CD}$ |
| 11 | 15 | HCT | 0,15,30,45,60 | 23.81±0.31$^{A}$ | 24.03±0.$^{A}$ | 23.99±0.38$^{A}$ | 24.21±0.27$^{A}$ | 24.54±0.40$^{A}$ | 24.38±0.35$^{A}$ | 24.05±0.28$^{A}$ |
| 12 | 16 | MCV | 0,15,30,45,60 | 7.30±0.55$^{A}$ | 6.54±0.49$^{B}$ | 6.20±0.31$^{BC}$ | 6.00±0.39$^{C}$ | 6.49±0.72$^{BC}$ | 6.64±0.63$^{B}$ | 6.41±0.39$^{BC}$ |
| 13 | 17 | MCH | 0,15,30,45,60 | 14.19±0.78$^{A}$ | 13.58±0.$^{ABC}$ | 13.11±0.33$^{BC}$ | 12.73±0.41$^{C}$ | 13.91±1.06$^{AB}$ | 13.31±0.84$^{ABC}$ | 12.87±0.48$^{BC}$ |

The outcomes of proximate analysis after 60 days described that in SC5 the moisture contents were maximum (81.76±0.26 %) while, these were observed minimum in SC3 (80.18±0.70 %). The results presented that moisture contents were recorded 79.67±0.43 % in the control (C) group. It was obvious from the results that protein contents were maximum in SC6 while these were minimum in SC4. Crude lipid was observed maximum in SC2 (22.32±0.41 %) and these were minimum in SC6 (20.62±0.27 %) while in control (C) it was 22.6±0.28%. It was indicated from the results that the maximum value of ash content was observed in SC5 and minimum value was observed in SC3. In this study, after performing experiments on fish the maximum value of crude fiber was observed in SC4 (2.95±0.06 %) and minimum value was observed in SC5 (2.45±0.09) while in control (C) it was 1.59±0.05 %.

After performing analysis on experimental fish, the nutritional value of carbohydrates was found to be maximum in SC6 (4.35±0.07 %) whereas it was minimum in SC2 (3.21±0.06). In control (C) it was observed 3.11±0.10 % (Table 11).

**Table 3.** Comparison of means (±SE) for IBW of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 8.82±0.16 | 8.62±0.06 | 8.66±0.29 | 8.90±0.17 | 8.53±0.17 | 8.72±0.12 | 8.54±0.11 |
| 15 | 8.82±0.24 | 8.62±0.36 | 8.66±0.14 | 8.90±0.26 | 8.53±0.17 | 8.72±0.18 | 8.54±0.16 |
| 21 | 8.82±0.34 | 8.62±0.18 | 8.66±0.12 | 8.90±0.13 | 8.53±0.27 | 8.72±0.26 | 8.54±0.16 |
| 30 | 8.82±0.28 | 8.62±0.21 | 8.66±0.05 | 8.90±0.21 | 8.53±0.26 | 8.72±0.34 | 8.54±0.23 |
| 37 | 8.82±0.25 | 8.62±0.30 | 8.66±0.27 | 8.90±0.25 | 8.53±0.10 | 8.72±0.08 | 8.54±0.18 |
| 45 | 8.82±0.17 | 8.62±0.08 | 8.66±0.12 | 8.90±0.12 | 8.53±0.29 | 8.72±0.10 | 8.54±0.16 |
| 52 | 8.82±0.20 | 8.62±0.23 | 8.66±0.14 | 8.90±0.16 | 8.53±0.28 | 8.72±0.13 | 8.54±0.13 |
| 60 | 8.82±0.28 | 8.62±0.22 | 8.66±0.12 | 8.90±0.23 | 8.53±0.15 | 8.72±0.09 | 8.54±0.16 |
| Mean | 8.82±0.07 | 8.62±0.07 | 8.66±0.05 | 8.90±0.06 | 8.53±0.07 | 8.72±0.05 | 8.54±0.0 |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

The proximate composition analysis of fish muscles indicated that the proximate composition was significantly changed by inclusion of dietary probiotics. The tested diets proved to increase significantly the selected parameters of proximate compositions, including moisture, crude protein, ash contents, crude lipids and carbohydrates as compared to control (C) group. In contrast, it decreases for

crude lipid content, because *S. cerevisiae* fed groups produced low crude lipid in the fish as compared to control (C) group.

**Hematological parameters of 60 days' trial**

Analysis of variance for hematology parameters after 60 days' trail described a non-significant difference among treatments and control (C) group for HCT ($P>0.05$). Highly significant ($P<0.01$) differences were observed in RBC, HB, MCV, MCH and MCHC (Table 2). In the 60 days' trial, initial readings for the hematological parameters of *O. mossambicus* at 0-60 days. The RBC count was observed between the ranges of 2.13±0.08 to 2.91±0.03 among all groups with highest being in SC2 (2.91±0.03) and lowest in SC4 (2.13±0.08). The RBC count was observed as 2.36±0.04 in control. The maximum HB was recorded in SC2 (4.36±0.13) and minimum in SC5 (4.11±0.12).

The HCT of control (C) was observed as 23.15±0.83. The Red Cell Indices like MCV, MCH and MCHC were also calculated, maximum MCV value was in SC4 (11.05±0.21) and minimum value was recorded in SC2 (7.96±0.18). Maximum MCH value was recorded in SC4 (20.38±0.72) and minimum in SC3 (14.46±0.49). Maximum MCHC value was calculated in SC2 (18.81±0.50) and minimum in SC3 (17.58±0.53) while its value was 17.80±0.65 in control.

**Table 4.** Comparison of means (±SE) for FBW of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 10.93±0.32$^{z}$ | 11.49±0.32$^{z}$ | 12.16±0.33$^{yz}$ | 12.30±0.18$^{xyz}$ | 12.14±0.25$^{yz}$ | 12.35±0.23$^{xyz}$ | 12.16±0.39$^{yz}$ |
| 15 | 12.13±0.16$^{yz}$ | 14.19±0.20$^{w-z}$ | 15.86±0.35$^{v-y}$ | 16.00±0.43$^{vwx}$ | 15.93±0.43$^{v-y}$ | 16.02±0.38$^{vwx}$ | 15.84±0.32$^{v-y}$ |
| 21 | 14.43±0.36$^{w-z}$ | 16.99±0.43$^{vw}$ | 19.26±0.36$^{uv}$ | 19.30±0.57$^{uv}$ | 19.53±0.46$^{tuv}$ | 19.52±0.54$^{tuv}$ | 19.44±0.56$^{tuv}$ |
| 30 | 16.83±0.44$^{vw}$ | 16.69±0.53$^{vw}$ | 22.56±0.53$^{r-u}$ | 22.40±0.36$^{r-u}$ | 23.13±0.34$^{q-t}$ | 22.92±0.44$^{q-u}$ | 22.94±0.59$^{q-u}$ |
| 37 | 19.33±0.65$^{tuv}$ | 22.49±0.33$^{r-u}$ | 25.96±0.37$^{n-r}$ | 25.80±0.76$^{n-r}$ | 27.03±0.64l$^{p}$ | 26.42±0.85$^{m-q}$ | 26.74±0.44$^{l-q}$ |
| 45 | 21.73±0.82$^{stu}$ | 25.39±0.82$^{o-s}$ | 29.46±0.51$^{i-n}$ | 29.30±0.89$^{i-n}$ | 30.93±0.65$^{f-k}$ | 29.92±1.14$^{h-m}$ | 30.44±0.54$^{g-l}$ |
| 52 | 24.43±0.16$^{p-s}$ | 28.59±0.96$^{j-o}$ | 32.96±0.64$^{d-i}$ | 32.70±0.64$^{e-i}$ | 34.63±0.46$^{b-f}$ | 33.52±0.42$^{c-h}$ | 34.24±0.92$^{c-g}$ |
| 60 | 27.53±1.17$^{k-p}$ | 32.09±0.51$^{f-j}$ | 36.76±1.46$^{a-d}$ | 36.50±0.70$^{a-e}$ | 38.63±1.22$^{a}$ | 37.32±1.08$^{abc}$ | 38.14±1.47$^{ab}$ |
| Mean | 18.42±1.17$^{C}$ | 20.99±1.43$^{B}$ | 24.37±1.67$^{A}$ | 24.29±1.64$^{A}$ | 25.24±1.81$^{A}$ | 24.75±1.70$^{A}$ | 24.99±1.78$^{A}$ |

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Hematological parameters after 60 days**

The hematological parameters of *O. mossambicus* fed with different doses of *S. cerevisiae* after 60 days' trial, blood samples were collected at 0, 15, 30, 45 and 60 day intervals during the experimental period. The RBC count was significantly higher in SC4 after 60 days (6.23±0.21) as compared to control (C) (4.92±0.06) and other treated groups.

The maximum HB% was recorded in SC4 (7.01±0.20) and minimum in control (C) (5.38±0.10). The HCT was recorded the maximum in SC4 (25.51±0.73) as compared to control (C) group (24.81±1.02).

The Red Cell Indices like MCV, MCH and MCHC were calculated and minimum MCV value was observed in SC4 (4.09±0.24) and maximum values was recorded in control (C) group (5.04±0.22). Maximum MCH value was recorded in SC6 (12.05±0.64) and minimum in SC5 (10.72±0.49). Maximum MCHC value was recorded in SC3 (27.44±0.66) and minimum in control (C) group (21.68±0.47). The results indicated a positive effect represented by significant increase in RBC count, HB, HCT and red cell indices like MCV, MCH and MCHC (Table 12-17).

**Table 5.** Comparison of means (±SE) for WG of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 2.11±0.03$^{z}$ | 2.87±0.05$^{yz}$ | 3.50±0.06$^{xyz}$ | 3.44±0.08$^{xyz}$ | 3.61±0.17$^{xyz}$ | 3.63±0.09$^{xyz}$ | 3.62±0.10$^{xyz}$ |
| 15 | 3.31±0.06$^{xyz}$ | 5.57±0.24$^{wxy}$ | 7.20±0.17$^{vw}$ | 7.10±0.09$^{vw}$ | 7.40±0.08$^{vw}$ | 7.30±0.22$^{vw}$ | 7.30±0.17$^{vw}$ |
| 21 | 5.61±0.11$^{wx}$ | 8.37±0.18$^{tuv}$ | 10.60±0.15$^{stu}$ | 10.40±0.42$^{stu}$ | 11.00±0.48$^{rst}$ | 10.80±0.12$^{rst}$ | 10.90±0.27$^{rst}$ |
| 30 | 8.01±0.13$^{uvw}$ | 8.07±0.09$^{uvw}$ | 13.90±0.43$^{q}$ | 13.50±0.50$^{qr}$ | 14.60±0.51$^{opq}$ | 14.20±0.35$^{pq}$ | 14.40±0.48$^{pq}$ |
| 37 | 10.51±0.17$^{stu}$ | 13.87±0.37$^{q}$ | 17.30±0.51$^{l-o}$ | 16.90±0.13$^{m-p}$ | 18.50±0.51$^{j-m}$ | 17.70±0.17$^{lmn}$ | 18.20±0.40 $^{k-n}$ |
| 45 | 12.91±0.09$^{qrs}$ | 16.77±0.13$^{m-p}$ | 20.80±0.66$^{h-k}$ | 20.70±0.51$^{ijk}$ | 22.40±0.63$^{f-i}$ | 21.20±0.84$^{g-j}$ | 21.90±0.63 $^{f-i}$ |
| 52 | 15.61±0.44$^{n-q}$ | 19.97±0.55 $^{i-l}$ | 24.30±0.37$^{def}$ | 23.80±0.49$^{efg}$ | 26.10±0.49$^{b-e}$ | 26.80±0.85$^{bcd}$ | 25.70±0.34$^{cde}$ |
| 60 | 18.71±0.25$^{j-m}$ | 23.47±0.51$^{e-h}$ | 28.10±0.75$^{abc}$ | 27.60±0.75$^{abc}$ | 30.10±1.11$^{a}$ | 28.60±1.00$^{ab}$ | 29.60±1.15$^{a}$ |
| Mean | 9.60±1.16$^{E}$ | 12.37±1.43$^{D}$ | 15.71±1.66$^{BC}$ | 15.43±1.64$^{C}$ | 16.71±1.81$^{A}$ | 16.28±1.76$^{AB}$ | 16.45±1.77$^{A}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Digestive enzymes activity**

**Digestive enzymes extraction after 60 days (Table 18)**

**Protein content**

According to present study results protein contents were maximum (16.0 ±0.15 mg ml$^{-1}$) in SC6 while they were minimum (14.37±0.38 mg ml$^{-1}$) in SC1 while in control (C) group it was observed 14.07±0.36 mg ml$^{-1}$ (Table 2). Analysis of variance for digestive enzyme activities after 60 days described highly significant difference in protein content of protease and Amylase (P>0.01). Significant differences (P<0.05) in protein contents of lipase were also observed (P<0.05).

Highly significant (P<0.01) differences were observed in total and specific activities in amylase, protease and lipase.

**Table 6.** Comparison of means (±SE) for SGR of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 1.34±0.02 $^{f-j}$ | 1.77±0.02$^{cd}$ | 2.10±0.03$^{ab}$ | 2.01±0.02$^{b}$ | 2.19±0.06$^{a}$ | 2.14±0.07$^{ab}$ | 2.19±0.03$^{a}$ |
| 15 | 0.93±0.01 $^{r-u}$ | 1.44±0.03$^{ef}$ | 1.75±0.04$^{cd}$ | 1.70±0.04$^{cd}$ | 1.81±0.04$^{c}$ | 1.76±0.03$^{cd}$ | 1.26±0.02 $^{g-m}$ |
| 21 | 1.02±0.03 $^{o-t}$ | 1.40±0.01$^{fgh}$ | 1.65±0.03$^{cd}$ | 1.60±0.03$^{de}$ | 1.71±0.02$^{cd}$ | 1.67±0.05$^{cd}$ | 1.70±0.03$^{cd}$ |
| 30 | 0.94±0.02 $^{r-u}$ | 0.95±0.01 $^{q-u}$ | 1.38±0.01$^{fgh}$ | 1.34±0.01 $^{f-j}$ | 1.44±0.04$^{ef}$ | 1.39±0.01$^{fgh}$ | 1.43±0.03$^{efg}$ |
| 37 | 0.92±0.01 $^{r-u}$ | 1.12±0.02 $^{l-q}$ | 1.29±0.03 $^{f-l}$ | 1.25±0.03 $^{h-n}$ | 1.35±0.04 $^{f-i}$ | 1.30±0.03 $^{f-k}$ | 1.34±0.03 $^{f-j}$ |
| 45 | 0.87±0.01$^{stu}$ | 1.04±0.01 $^{o-s}$ | 1.18±0.02 $^{i-o}$ | 1.15±0.03 $^{k-p}$ | 1.24±0.03 $^{h-n}$ | 1.18±0.03 $^{i-o}$ | 1.23±0.02 $^{h-n}$ |
| 52 | 0.85±0.02$^{tu}$ | 1.00±0.02 $^{p-t}$ | 1.12±0.02 $^{l-q}$ | 1.09±0.05 $^{m-r}$ | 1.17±0.03 $^{j-p}$ | 1.12±0.03 $^{l-q}$ | 1.16±0.02 $^{k-p}$ |
| 60 | 0.82±0.03$^{u}$ | 0.95±0.02 $^{q-u}$ | 1.05±0.01 $^{o-r}$ | 1.02±0.01 $^{o-t}$ | 1.09±0.03 $^{m-r}$ | 1.05±0.02 $^{o-r}$ | 1.08±0.02 $^{n-r}$ |
| Mean | 0.96±0.03$^{E}$ | 1.21±0.06$^{D}$ | 1.44±0.07$^{B}$ | 1.40±0.07$^{C}$ | 1.50±0.07$^{A}$ | 1.45±0.07$^{B}$ | 1.42±0.07$^{BC}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Amylase activity**

The total amylase (U ml$^{-1}$) and specific (U mg protein$^{-1}$) amylase activities were significantly higher in those GIFT Tilapia which were fed with different levels of dietary probiotic (*S. cerevisiae*) as compared to those that received the control (C) diet. The maximum total amylase activities were found in SC6 (48.42±1.18 U ml$^{-1}$) and minimum was observed in SC1 while it was recorded 32.37±0.62 U ml$^{-1}$ in the control (C) group fed with control (C) diet. Specific amylase activities

showed the same trend like total amylase. These were observed maximum in SC6 (6.12±0.10 U mg Protein$^{-1}$) and minimum in SC1 (3.44±0.03 U mg Protein$^{-1}$ while in control (C) group it was observed 3.37±0.11 U mg Protein$^{-1}$ .

**Protease activity**

Fish fed with *S. cerevisiae* along with normal diet showed the highest total and specific protease activity when observed after 60 days. While fish fed with only control diet showed minimum value of total and specific protease activity. Maximum total protease activity was recorded in SC6 (6.68±0.06 U ml $^{-1}$) and it was minimum 4.56±0.12 U ml$^{-1}$ in SC1 while 4.24±0.11 U ml$^{-1}$ in control (C) group. The highest Specific activity was observed in SC6 (0.97±0.03 U mg Protein$^{-1}$) and it was recorded 0.49±0.01 U mg Protein$^{-1}$ in SC1 while 0.34±0.01 U mg Protein$^{-1}$ for control (C) group. Results indicated that addition of different probiotic in different doses enhanced the total and specific activity of protease enzyme in GIFT Tilapia as compared to the control diet.

**Table 7.** Comparison of means (±SE) for FI of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 7.65±0.16$^{q}$ | 8.04±0.23$^{q}$ | 8.51±0.10$^{q}$ | 8.61±0.14$^{q}$ | 8.49±0.24$^{q}$ | 8.62±0.04$^{q}$ | 8.50±0.10$^{q}$ |
| 15 | 18.20±0.35$^{pq}$ | 22.35±0.57$^{opq}$ | 23.79±0.43$^{opq}$ | 24.00±0.38$^{opq}$ | 23.90±0.66$^{opq}$ | 24.03±0.35$^{opq}$ | 23.76±1.14$^{opq}$ |
| 21 | 30.30±0.68$^{nop}$ | 35.68±0.46 $^{m-p}$ | 40.45±1.88$^{mno}$ | 40.53±0.46$^{mno}$ | 41.01±0.33$^{mno}$ | 40.99±2.12$^{mno}$ | 40.82±0.94$^{mno}$ |
| 30 | 48.90±1.77$^{lmn}$ | 50.07±1.15$^{klm}$ | 67.68±1.21$^{jkl}$ | 67.20±2.64$^{jkl}$ | 69.39±1.88$^{jk}$ | 68.76±1.62$^{jk}$ | 68.82±1.51$^{jk}$ |
| 37 | 71.52±0.94$^{j}$ | 83.21±0.72$^{ij}$ | 96.10±1.55$^{hi}$ | 95.46±2.98$^{hi}$ | 100.01±2.88$^{hi}$ | 97.68±2.91$^{hi}$ | 98.94±2.01$^{hi}$ |
| 45 | 97.83±1.36$^{hi}$ | 114.21±2.96$^{gh}$ | 132.57±1.76$^{efg}$ | 131.85±2.44$^{efg}$ | 139.23±2.28$^{ef}$ | 134.64±2.46$^{ef}$ | 136.80±2.34$^{ef}$ |
| 52 | 127.04±1.62$^{fg}$ | 148.67±1.96$^{de}$ | 171.39±1.63$^{c}$ | 170.04±4.39$^{c}$ | 180.08±5.77$^{bc}$ | 184.70±3.11$^{bc}$ | 178.05±6.99$^{bc}$ |
| 60 | 165.18±8.17$^{cd}$ | 192.54±4.38$^{b}$ | 220.56±9.82$^{a}$ | 219.00±4.77$^{a}$ | 231.78±3.94$^{a}$ | 223.92±7.43$^{a}$ | 228.84±11.45$^{a}$ |
| Mean | 70.83±10.89$^{C}$ | 81.85±12.76$^{B}$ | 95.13±14.65$^{A}$ | 94.59±14.51$^{A}$ | 99.24±15.47$^{A}$ | 97.92±15.19$^{A}$ | 98.07±15.30$^{A}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Lipase activity**

The total fatty acids liberated of GIFT Tilapia fed with supplementary probiotic along with control (C) diet were significantly high as compared to control (C) group. The highest total fatty acids liberated were observed in SC6 (5.21±0.08 ml) than the total lipase activities of all experimental treatments.

The minimum total fatty acids liberated were observed as 3.67±0.05 (ml) in SC1 while it was 3.19±0.07 (ml) in control (C) group. Like lipase enzyme similar pattern was observed for Lipase specific activity, value recorded for SC6 (0.52±0.02) was higher than that of other treatments and control (C) group. It was recorded minimum as 0.25±0.00 U mg Protein$^{-1}$ in SC1 while 0.19±0.01 U mg Protein$^{-1}$ in control (C) group.

**Immunological parameters**

**Stress resistance and survival rate**

After 60 days' trial, the fish were also divided into two subgroups to evaluate the stress resistance and survival rate of control (C) and treated groups feed with various doses of *S. cerevisiae*. The first subgroup of each treatment and control (C) was inoculated IP (Intra Peritoneal) with 0.2 ml of sterile saline. At the end of the 60 days' trial 20 fish were kept in each tank and subjected to salinity stress challenge. Survival rate was observed daily up to 7 days.

The second subgroup of each treatment and control (C) group was inoculated IP with pathogenic bacteria suspension *A. hydrophila* (0.2 ml of 108x10$^{8}$ CFU ml$^{-1}$). All treatments and control (C) groups of fish were observed for 7 days and the survival rate was recorded.

**Table 8.** Comparison of means (±SE) for FCR of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*Saccharomyces cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 3.63±0.12$^{yz}$ | 2.80±0.08 $^{a-d}$ | 2.43±0.04$^{cd}$ | 2.50±0.05$^{bcd}$ | 2.36±0.06$^{d}$ | 2.39±0.08$^{d}$ | 2.36±0.03$^{d}$ |
| 15 | 5.49±0.15 $^{o-u}$ | 4.01±0.09 $^{w-z}$ | 3.30±0.02$^{zab}$ | 3.38±0.06$^{za}$ | 3.23±0.06 $^{z-c}$ | 3.29±0.03$^{zab}$ | 3.26±0.06$^{zab}$ |
| 21 | 5.40±0.14 $^{q-u}$ | 4.26±0.13 $^{v-y}$ | 3.82±0.06$^{xyz}$ | 3.90±0.15$^{xyz}$ | 3.73±0.06$^{xyz}$ | 3.80±0.09$^{xyz}$ | 3.75±0.14$^{xyz}$ |
| 30 | 6.11±0.17 $^{k-q}$ | 6.20±0.17 $^{j-q}$ | 4.87±0.04 $^{s-v}$ | 4.98±0.14 $^{r-v}$ | 4.75±0.07$^{uvw}$ | 4.84±0.18$^{tuv}$ | 4.78±0.03 $^{t-w}$ |
| 37 | 5.75±0.13 $^{l-r}$ | 5.99±0.03 $^{l-q}$ | 5.56±0.14 $^{m-t}$ | 5.65±0.11 $^{l-s}$ | 5.41±0.09 $^{q-u}$ | 5.52±0.18 $^{n-u}$ | 5.44±0.09 $^{p-u}$ |
| 45 | 7.58±0.06 $^{b-g}$ | 6.81±0.23 $^{g-k}$ | 6.37±0.20 $^{h-l}$ | 6.30±0.14 $^{i-n}$ | 6.22±0.20 $^{j-p}$ | 6.35±0.18 $^{h-m}$ | 6.25±0.13 $^{i-o}$ |
| 52 | 8.14±0.23$^{ab}$ | 4.45±0.05$^{vwx}$ | 7.05±0.12 $^{d-i}$ | 7.15±0.20 $^{c-h}$ | 6.90±0.03 $^{f-k}$ | 6.89±0.09 $^{g-k}$ | 6.93±0.13 $^{e-j}$ |
| 60 | 8.83±0.23$^{a}$ | 8.20±0.15$^{ab}$ | 7.85±0.13$^{bcd}$ | 7.94±0.22$^{bc}$ | 7.70±0.11b-f | 7.83±0.38bcd | 7.73±0.17b-e |
| Mean | 6.37±0.34$^{A}$ | 5.34±0.35$^{B}$ | 5.16±0.37$^{BC}$ | 5.23±0.37$^{BC}$ | 5.04±0.36$^{C}$ | 5.11±0.37$^{C}$ | 5.06±0.37$^{C}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

The results in both subgroups revealed a higher mortality rate in control (C) group than other treatments which were supplemented with different doses of *S. cerevisiae*. Analysis of variance for stress resistance, blood total leucocytic count and leucocytic differential count after 60 days described highly significant differences (P<0.01) in lymphocyte, monocytes and granulocytes. Results of salinity challenge test described that 85 % fish were died in the control (C) group after 7 days of post stress while the dietary probiotic significantly improved the resistance of fish fed with probiotics against salinity stress challenge (P<0.05).

The highest survival rate was observed in the treatment SC4 (95 %) which was supplemented by *S. cerevisiae*, which was significantly higher than all other treatments and control (C) groups (P<0.05). In control (C) group, the survival rate was observed 15 % which was minimum while in SC1, SC2, SC3, SC5 and SC6 it was observed as 70 %, 80 %, 85 %, 85 % and 80 %, respectively (Table 19).

**Table 9.** Comparison of means (±SE) for PER of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 0.92±0.02$^{cde}$ | 1.19±0.03$^{b}$ | 1.37±0.03$^{a}$ | 1.33±0.04$^{a}$ | 1.41±0.02$^{a}$ | 1.39±0.04$^{a}$ | 1.41±0.03$^{a}$ |
| 15 | 0.61±0.02$^{hij}$ | 0.82±0.01$^{ef}$ | 1.01±0.02$^{c}$ | 0.99±0.03$^{cd}$ | 1.03±0.02$^{c}$ | 1.01±0.03$^{c}$ | 1.02±0.04$^{c}$ |
| 21 | 0.62±0.01$^{hij}$ | 0.78±0.01$^{fg}$ | 0.87±0.03$^{ef}$ | 0.86±0.03$^{ef}$ | 0.89±0.03$^{def}$ | 0.88±0.02$^{def}$ | 0.89±0.03$^{def}$ |
| 30 | 0.55±0.01 $^{j-n}$ | 0.54±0.01 $^{j-o}$ | 0.68±0.02$^{gh}$ | 0.67±0.02$^{ghi}$ | 0.70±0.01$^{gh}$ | 0.69±0.01$^{gh}$ | 0.70±0.02$^{gh}$ |
| 37 | 0.49±0.02 $^{k-r}$ | 0.56±0.02 $^{i-m}$ | 0.60±0.02 $^{h-k}$ | 0.59±0.01 $^{h-l}$ | 0.62±0.01$^{hij}$ | 0.60±0.01 $^{h-k}$ | 0.61±0.01$^{hij}$ |
| 45 | 0.44±0.01 $^{n-r}$ | 0.49±0.01 $^{k-r}$ | 0.52±0.01 $^{j-q}$ | 0.52±0.01 $^{j-q}$ | 0.54±0.01 $^{j-o}$ | 0.52±0.01 $^{j-q}$ | 0.53±0.02 $^{j-p}$ |
| 52 | 0.41±0.01$^{qr}$ | 0.45±0.01 $^{m-r}$ | 0.47±0.01 $^{m-r}$ | 0.47±0.01 $^{m-r}$ | 0.48±0.02 $^{l-r}$ | 0.48±0.01 $^{l-r}$ | 0.48±0.01 $^{l-r}$ |
| 60 | 0.38±0.01$^{r}$ | 0.41±0.01$^{qr}$ | 0.42±0.01$^{pqr}$ | 0.42±0.01$^{pqr}$ | 0.43±0.01 $^{o-r}$ | 0.43±0.01 $^{o-r}$ | 0.43±0.01 $^{o-r}$ |
| Mean | 0.55±0.03$^{D}$ | 0.66±0.05$^{C}$ | 0.74±0.06$^{AB}$ | 0.73±0.06$^{B}$ | 0.76±0.06$^{A}$ | 0.75±0.06$^{AB}$ | 0.76±0.07$^{AB}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Results of challenge test with *A. hydrophila* revealed that 80% of experimental fish were died in control (C) group after 7 days of post stress while the other treatment fed with probiotics showed significant resistance against bacteria (P<0.05). The maximum survival rate was detected in the treatment SC4 (95 %) which was supplemented by *S. cerevisiae*, which was significantly higher than all other treatments and control (C) groups (P<0.05). In SC1, SC2, SC3, SC5 and SC6 the survival rate was observed as 75 %, 85 %, 80 %, 90 % and 85 %, respectively.

**Immunity by hematological studies**

Analysis of variance for stress resistance and blood Immunity parameters after 60 days described non-significant differences in HCT (P>0.05). Highly significant (P<0.01) differences were observed in RBC, HB, MCV, MCH, MCHC. Hematological parameters like RBC, HB, HCT, MCV, MCH and MCHC were also observed after challenge test after 60 days' trial. These parameters were decreased slightly as compared to the parameters observed after growth performance trial before challenge test.

This may be due to stress of the implication of bacterial or salinity stress.

**Table 10.** Comparison of means (±SE) for PPV of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 7 | 14.85±0.40 $^{c-f}$ | 19.09±0.53$^{b}$ | 21.97±0.65$^{a}$ | 21.32±0.40$^{a}$ | 22.59±0.61$^{a}$ | 22.40±0.49$^{a}$ | 22.75±0.63$^{a}$ |
| 15 | 9.70±0.31$^{ijk}$ | 13.27±0.36$^{fg}$ | 16.11±0.31$^{cd}$ | 15.84±0.31$^{cde}$ | 16.46±0.27$^{c}$ | 16.23±0.48$^{c}$ | 16.41±0.50$^{c}$ |
| 21 | 9.91±0.24$^{ijk}$ | 12.52±0.24$^{gh}$ | 14.01±0.29$^{fg}$ | 13.66±0.25$^{fg}$ | 14.31±0.39$^{d-g}$ | 14.07±0.47$^{efg}$ | 14.21±0.37$^{efg}$ |
| 30 | 8.73±0.25 $^{k-o}$ | 8.59±0.18 $^{k-p}$ | 10.92±0.17$^{hi}$ | 10.72±0.16$^{hij}$ | 11.24±0.36$^{hi}$ | 11.01±0.38$^{hi}$ | 11.14±0.31$^{hi}$ |
| 37 | 7.83±0.13 $^{l-s}$ | 8.90±0.23 $^{j-n}$ | 9.61±0.23$^{i-l}$ | 9.43±0.28$^{i-m}$ | 9.87±0.16$^{ijk}$ | 9.66±0.26$^{ijk}$ | 9.81±0.13$^{ijk}$ |
| 45 | 7.06±0.17 $^{o-s}$ | 7.83±0.20 $^{l-s}$ | 8.38±0.28$^{k-r}$ | 8.37±0.21$^{k-r}$ | 8.58±0.24$^{k-p}$ | 8.40±0.31$^{k-q}$ | 8.53±0.18$^{k-q}$ |
| 52 | 6.56±0.15 $^{rs}$ | 7.18±0.14 $^{n-s}$ | 7.57±0.23$^{n-s}$ | 7.47±0.09$^{n-s}$ | 7.74±0.15$^{m-s}$ | 7.75±0.17$^{m-s}$ | 7.70±0.20$^{m-s}$ |
| 60 | 6.04±0.10$^{s}$ | 6.51±0.11 $^{s}$ | 6.81±0.23$^{p-s}$ | 6.73±0.14$^{qrs}$ | 6.94±0.20$^{o-s}$ | 6.82±0.27$^{p-s}$ | 6.91±0.14$^{o-s}$ |
| Mean | 8.84±0.55$^{D}$ | 10.49±0.83$^{C}$ | 11.92±1.01$^{AB}$ | 11.69±0.97$^{B}$ | 12.22±1.04$^{A}$ | 12.04±1.03$^{AB}$ | 12.18±1.05$^{A}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

The hematological parameters of *O. mossambicus* after challenge test are shown in Table 2. The RBC count was observed highest in SC4 (6.03±0.14) as compared to the control (C) (4.71±0.14) and other treated groups. The maximum HB was recorded in SC4 (6.22±0.09) and minimum in SC2 (5.15±0.05) while in control (C) group it was observed 4.84±0.16. The HCT was also observed maximum in SC4 (24.99±0.12) and minimum in SC1 (24.05±0.52) while in control (C) group it was 23.18±0.46. The Red cell indices like MCH, MCHC and MCV were also calculated, maximum MCV value was observed in SC2 (4.89±0.09) and minimum value was recorded in SC4 (3.97±0.09) while in control (C) it was observed 4.92±0.18. Maximum MCH values were recorded in SC6 group (12.15±0.27) and minimum in SC4 (10.32±0.40) while in control (C) it was 10.28±0.19. Maximum MCHC value was calculated in SC5 (25.39±0.60) and minimum in SC2 (21.34±0.39) while in control (C) it was 20.88±0.67 (Table 20). Total leucocyte counts after 60 days' experiment showed significant increase in group supplemented with *S. cerevisiae* as compared to control (C) group**.** It was observed maximum in SC5 (44.0±1.53) while minimum in SC1 (35.78±0.89) while in control (C) group it was 32.32±0.69. Differential leucocyte counts also indicated significant increase in lymphocytes, monocytes and granulocytes in treated groups (P<0.05) as compared to control (C) which indicate highly immune response. Results indicated that lymphocytes were recorded higher in SC4 (5.79±0.11) and minimum in SC2 (3.12±0.03) while in control (C) it was 3.58±0.10. Monocytes were also recorded higher in SC4 (2.46±0.04) and minimum in SC2 (2.06±0.04) while in control (C) group it was 2.10±0.06. Granulocytes were observed higher in SC4 (28.26±1.35) and minimum in SC3 group (23.44±0.40) while in control (C) it was 23.45±0.88.

**DISCUSSION**

The study was carried out to evaluate the effects of *S. cerevisiae* on hemato-biochemical, physiological, stress resistance and growth performance of GIFT Tilapia (*O. mossambicus).*

**Table 11.** Comparison of means (±SE) for moisture, Crude Protein, Crude Lipid, Ash Contents, Crude Fiber and carbohydrates (%) of control group and six treatments in the flesh of GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Treatment | Moisture | Crude Protein | Crude Lipid | Ash Contents | Crude Fiber | Carbohydrates |
|---|---|---|---|---|---|---|
| C | 79.67±0.43$^{A}$ | 52.51±0.73$^{B}$ | 22.60±0.28$^{A}$ | 15.68±0.19$^{AB}$ | 1.59±0.05$^{C}$ | 3.11±0.10$^{D}$ |
| SC4 | 80.49±0.40$^{A}$ | 55.89±0.97$^{A}$ | 21.25±0.26$^{AB}$ | 15.36±0.29$^{AB}$ | 2.76±0.06$^{AB}$ | 3.60±0.15$^{BC}$ |
| SC5 | 80.41±0.37$^{A}$ | 55.41±0.49$^{AB}$ | 21.24±0.18$^{AB}$ | 15.81±0.15$^{AB}$ | 2.51±0.09$^{B}$ | 3.21±0.06$^{CD}$ |
| SC6 | 80.18±0.70$^{A}$ | 55.73±0.62$^{AB}$ | 22.32±0.41$^{A}$ | 14.59±0.27$^{B}$ | 2.67±0.07$^{AB}$ | 3.39±0.04$^{CD}$ |
| SC7 | 81.52±0.43$^{A}$ | 54.27±0.73$^{AB}$ | 22.14±0.48$^{AB}$ | 16.05±0.40$^{AB}$ | 2.95±0.06$^{A}$ | 3.59±0.05$^{BC}$ |
| SC8 | 81.76±0.26$^{A}$ | 55.43±0.67$^{AB}$ | 21.17±0.25$^{AB}$ | 16.77±0.33$^{A}$ | 2.45±0.09$^{B}$ | 4.01±0.13$^{AB}$ |
| SC9 | 81.71±0.44$^{A}$ | 56.46±0.55$^{A}$ | 20.62±0.27$^{B}$ | 15.99±0.42$^{AB}$ | 2.73±0.05$^{AB}$ | 4.35±0.07$^{A}$ |

Means sharing similar letter in a column are statistically non-significant (P>0.05).

**Growth performance**

The probiotic supplementation exerted advantageous effects and resulted in higher growth and feed utilization. Since, probiotic bacteria are used by various researchers in fish diets to improve growth performance. In the previous years, efforts were made to discover substitutes to antimicrobials for growth enhancement in the aquaculture. Due to this reason, the use of probiotics is increasing now a day (Agboola *et al.*, 2020; Nhi *et al.*, 2018; Luna-Gonzalez *et al.*, 2013; Zhou *et al.*, 2010; Balcazar *et al.*, 2006). Outcomes of 60 days' trial indicated that the body weight was increased in treatments fed with *S. cerevisae* as compared to control (C) group, all treatments fed with probiotics have better weight gain and SGR as compared to control. Similarly, maximum feed intake and minimum FCR was observed as compared to control (C) group. PER and PPV were gradually increase in treatments as compared to the control (C) group. Therefore, significant differences ($p \leq 0.05$) in growth parameters like weight gain (WG), Specific Growth Rate (SGR) were observed while Survival Rate (SR%) remained constant similar to the results reported by Mohammadi *et al.*, (2016).

**Table 12.** Comparison of means (±SE) for RBC of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 0 | 2.36±0.04$^{no}$ | 2.54±0.07$^{mno}$ | 2.91±0.03$^{k-n}$ | 2.87±0.04l$^{mn}$ | 2.13±0.08$^{o}$ | 2.14±0.04$^{o}$ | 2.76±0.06$^{l-o}$ |
| 15 | 2.51±0.09$^{no}$ | 2.99±0.04$^{j-n}$ | 3.73±0.13$^{ghi}$ | 4.18±0.12$^{fg}$ | 2.93±0.05$^{k-n}$ | 3.16±0.10$^{i-m}$ | 3.31±0.06$^{i-l}$ |
| 30 | 3.34±0.10$^{i-l}$ | 3.67±0.12$^{ghi}$ | 3.61±0.08$^{g-j}$ | 3.52±0.12$^{h-k}$ | 4.98±0.14$^{cde}$ | 4.15±0.06$^{fgh}$ | 3.65±0.03$^{ghi}$ |
| 45 | 4.56±0.16$^{ef}$ | 5.12±0.16$^{cde}$ | 4.63±0.16$^{ef}$ | 4.99±0.19$^{cde}$ | 5.87±0.06$^{ab}$ | 5.32±0.15$^{bcd}$ | 4.87±0.16$^{de}$ |
| 60 | 4.92±0.06$^{de}$ | 5.62±0.16$^{abc}$ | 5.11±0.11$^{cde}$ | 5.81±0.16$^{ab}$ | 6.23±0.21$^{a}$ | 5.91±0.09$^{ab}$ | 5.13±0.13$^{cde}$ |
| Mean | 3.54±0.28$^{D}$ | 3.99±0.32$^{C}$ | 4.00±0.21$^{C}$ | 4.27±0.28$^{AB}$ | 4.43±0.44$^{A}$ | 4.14±0.37$^{BC}$ | 3.94±0.25$^{C}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Survival rate in present experiment was observed 100% due to maintenance of good physio-chemical parameters. Similar results about significant increase in body weight of *Silurus glanis* and *O. niloticus* were observed by addition of *Enterococcus faecium* to fish diet due to the improvement of nutrient absorption and utilization (Abumourad *et al.*, 2014; Lara-Flores *et al.*, 2003; Bogut *et al.*, 2000). Probiotic diet supplementation resulted in better growth performance and feed utilization than in control (C)group and also reduced the culture cost (Bairagi *et al.*, 2004; Yanbo & Zirong, 2006; Adineh *et al.*, 2013), which are also in line with the present study.

**Table 13.** Comparison of means (±SE) for HB of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 0 | $4.12\pm0.07^{n}$ | $4.21\pm0.09^{k-n}$ | $4.36\pm0.13^{j-n}$ | $4.15\pm0.08^{mn}$ | $4.34\pm0.08^{j-n}$ | $4.11\pm0.12^{n}$ | $4.18\pm0.16l^{mn}$ |
| 15 | $4.32\pm0.14^{k-n}$ | $4.68\pm0.19^{i-n}$ | $4.93\pm0.06^{g-m}$ | $5.11\pm0.13^{g-j}$ | $4.84\pm0.12^{h-n}$ | $4.13\pm0.16^{n}$ | $4.74\pm0.14^{h-n}$ |
| 30 | $4.71\pm0.13^{h-n}$ | $4.95\pm0.06^{g-l}$ | $4.51\pm0.14^{j-n}$ | $4.98\pm0.13^{g-k}$ | $5.31\pm0.06^{f-i}$ | $5.12\pm0.12^{g-j}$ | $4.36\pm0.07^{j-n}$ |
| 45 | $5.12\pm0.15^{g-j}$ | $5.63\pm0.17^{c-g}$ | $5.96\pm0.14^{c-f}$ | $5.48\pm0.10^{d-h}$ | $6.31\pm0.14^{abc}$ | $5.98\pm0.16^{c-f}$ | $5.31\pm0.13^{f-i}$ |
| 60 | $5.38\pm0.10^{e-i}$ | $6.31\pm0.21^{abc}$ | $6.13\pm0.10^{b-e}$ | $6.89\pm0.25^{ab}$ | $7.01\pm0.20^{a}$ | $6.34\pm0.14^{abc}$ | $6.18\pm0.22^{bcd}$ |
| Mean | $4.73\pm0.13^{D}$ | $5.16\pm0.21^{BC}$ | $5.18\pm0.20^{BC}$ | $5.32\pm0.25^{AB}$ | $5.56\pm0.26^{A}$ | $5.14\pm0.25^{BC}$ | $4.95\pm0.20^{CD}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

In this study, growth performance results of GIFT Tilapia *(O. mossambicus)* showed agreement with the findings of Lara-Flores *et al.*, (2003), who used *S. cerevisae* for tilapia *O. niloticus*. Ayoola *et al.*, (2013) described that growth performance, specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR) and survival rates were significantly higher (P<0.05) in probiotic supplemented diets which were similar to the outcomes of the present study. Yeasts in our present study also improved the Growth parameters, FBW and SGR of fish as reported by former workers in tilapia and other fishes (Essa *et al.*, 2010; Pooramini *et al.*, 2009; Taoka *et al.*, 2006; Lara-Flores *et al.*, 2003). The positive effects of yeast, *S. boulardii* and *S. cerevisiae* (Tovar-Ramıreza *et al.*, 2002) may be due to polyamine production (Essa *et al.*, 2010). According to Kafilzadeh *et al.*, (2013) *S. cerevisiae* was documented to have the potential effect as a possible replacement of fish meal (Oliva-Teles & Goncalves, 2001) for Nile tilapia (Nhi *et al.*, 2018; Korkmaz & Cakirogullari 2011; Welker & Lim, 2011), Rohu (Tewary & Patra, 2011) and sea bass (Oliva-Teles & Goncalves, 2001). Positive effects were observed in Nile tilapia (Abdel Tawab *et al.*, 2008), while no significant effects were observed on growth performance in Oscar fish (Kafilzadeh *et al.*, 2013).

Different results obtained from various studies depend to intra specific differences (Lara-Flores *et al.*, 2003), type and method of adding *S. cerevisiae* to diet (Tovar-Ramirez *et al.*, 2002; Kafilzadeh *et al.*, 2013; Nhi *et al.*, 2018; Agboola *et al.*, 2020) and similar is true in our current study.

**Table 14.** Comparison of means (±SE) for HCT of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 0 | 23.15±0.83 | 23.42±0.84 | 23.18±0.61 | 23.61±0.47 | 23.53±0.94 | 23.11±0.54 | 23.49±0.48 |
| 15 | 23.46±0.67 | 23.59±0.76 | 23.72±0.55 | 23.58±0.64 | 23.41±0.99 | 23.51±1.00 | 23.91±0.72 |
| 30 | 23.32±0.43 | 23.66±0.61 | 23.98±1.21 | 24.18±0.38 | 24.93±0.90 | 24.72±0.70 | 23.81±0.55 |
| 45 | 24.31±0.19 | 24.62±0.74 | 24.15±0.88 | 24.58±0.62 | 25.32±0.67 | 25.16±0.36 | 24.31±0.74 |
| 60 | 24.81±1.02 | 24.85±0.43 | 24.93±1.17 | 25.11±0.70 | 25.51±0.73 | 25.41±0.62 | 24.74±0.77 |
| Mean | $23.81\pm0.31^{A}$ | $24.03\pm0.30^{A}$ | $23.99\pm0.38^{A}$ | $24.21\pm0.27^{A}$ | $24.54\pm0.40^{A}$ | $24.38\pm0.35^{A}$ | $24.05\pm0.28^{A}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Proximate analysis**

After 60 days' trail, the overall results of proximate analysis indicated that SC6 group showed high levels of crude protein in treatments than control and other treated groups while the crude lipid level was not significantly increased in any treated group, instead it showed the highest value in control (C) group.

The moisture content and ash were recorded highest in SC5. The highest value of crude fibre was recorded

in SC1 compared to other experimental groups and control (C) group but it was not statistically significant. The highest carbohydrate contents were found in SC6 compared to control and other treatments. So, the inclusion of dietary probiotics proved to increase significantly the crude protein and other contents, as compared to control (C) group. In contrast, it was observed that crude lipid contents were decreased due to increasing dose of *S. cerevisiae* in SC6 as compared to control (C) group.

**Table 15.** Comparison of means (±SE) for MCV of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 0 | $9.81\pm0.52^{abc}$ | $9.15\pm0.43^{cd}$ | $7.96\pm0.18^{d-g}$ | $8.23\pm0.40^{c-f}$ | $11.05\pm0.21^{a}$ | $10.80\pm0.17^{ab}$ | $8.51\pm0.50^{cde}$ |
| 15 | $9.35\pm0.44^{bcd}$ | $7.88\pm0.27^{d-h}$ | $6.36\pm0.28^{h-o}$ | $5.65\pm0.18^{j-q}$ | $7.99\pm0.32^{d-g}$ | $7.44\pm0.16^{e-i}$ | $7.22\pm0.46^{e-j}$ |
| 30 | $6.98\pm0.27^{e-j}$ | $6.45\pm0.13^{g-n}$ | $6.59\pm0.41^{g-l}$ | $6.85\pm0.29^{f-k}$ | $5.01\pm0.24^{l-q}$ | $5.96\pm0.28^{i-p}$ | $6.52\pm0.28^{g-m}$ |
| 45 | $5.33\pm0.17^{k-q}$ | $4.80\pm0.19^{opq}$ | $5.22\pm0.19^{l-q}$ | $4.93\pm0.12^{m-q}$ | $4.31\pm0.13^{q}$ | $4.73\pm0.24^{pq}$ | $4.99\pm0.16^{m-q}$ |
| 60 | $5.04\pm0.22^{l-q}$ | $4.42\pm0.23^{pq}$ | $4.87\pm0.25^{n-q}$ | $4.32\pm0.16^{q}$ | $4.09\pm0.24^{q}$ | $4.29\pm0.14^{q}$ | $4.82\pm0.24^{opq}$ |
| Mean | $7.30\pm0.55^{A}$ | $6.54\pm0.49^{B}$ | $6.20\pm0.31^{BC}$ | $6.00\pm0.39^{C}$ | $6.49\pm0.72^{BC}$ | $6.64\pm0.63^{B}$ | $6.41\pm0.39^{BC}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

According to Essa *et al.*, (2010), the moisture content showed no significant differences in the experimental diets. Their results are comparable with our results in all parameters like crude protein, fat and ash contents ranged similar to the chemical composition analysis of the carcass of rainbow trout larvae figured by Pooramini *et al.*, (2009). Silva *et al.*, (2015) describe that Nile tilapia supplemented with probiotics showed no significant difference in proximate analysis between treatment and control (C) groups while many authors have reported enhancements in body composition (Reda & Selim, 2015).

**Table 16.** Comparison of means (±SE) for MCH of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 0 | $17.46\pm0.61^{abc}$ | $16.57\pm0.42^{b-e}$ | $14.98\pm0.73^{c-i}$ | $14.46\pm0.49^{c-j}$ | $20.38\pm0.72^{a}$ | $19.21\pm0.73^{ab}$ | $15.14\pm0.76^{c-h}$ |
| 15 | $17.21\pm0.44^{bcd}$ | $15.65\pm0.73^{c-g}$ | $13.21\pm0.69^{g-m}$ | $12.22\pm0.39^{h-m}$ | $16.51\pm0.59^{b-f}$ | $13.06\pm0.57^{g-m}$ | $14.32\pm0.51^{d-k}$ |
| 30 | $14.10\pm0.74^{e-l}$ | $13.49\pm0.75^{f-m}$ | $12.49\pm0.28^{h-m}$ | $14.15\pm0.76^{d-l}$ | $10.66\pm0.53^{m}$ | $12.33\pm0.32^{h-m}$ | $11.95\pm0.52^{i-m}$ |
| 45 | $11.23\pm0.48^{lm}$ | $10.99\pm0.17^{m}$ | $12.87\pm0.38^{g-m}$ | $10.98\pm0.50^{m}$ | $10.75\pm0.27^{m}$ | $11.24\pm0.31^{lm}$ | $10.90\pm0.33^{m}$ |
| 60 | $10.93\pm0.49^{m}$ | $11.22\pm0.59^{lm}$ | $11.99\pm0.04^{i-m}$ | $11.86\pm0.45^{j-m}$ | $11.25\pm0.58^{klm}$ | $10.72\pm0.49^{m}$ | $12.05\pm0.64^{i-m}$ |
| Mean | $14.19\pm0.78^{A}$ | $13.58\pm0.64^{ABC}$ | $13.11\pm0.33^{BC}$ | $12.73\pm0.41^{C}$ | $13.91\pm1.06^{AB}$ | $13.31\pm0.84^{ABC}$ | $12.87\pm0.48^{BC}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

According to the results described by Noveirian and Nasrollahzadeh (2012), there were no significant differences (P>0.05) in body composition between the treatments which received probiotic. Crude protein and moisture contents are comparable with the present study only but no statistical differences in body composition were observed in probiotic fed groups (P>0.05) however, they improved the body composition. These results are similar with previously described work by Diab *et al.*, (2002); Lara-Flores *et al.*, (2003) and Gafarian *et al.*, (2007). The proximate compositions observed in the current study is in line with the previous study where, significant changes in the crude protein content, the highest was found in

prebiotics and probiotics (live yeast) as compared to the control but low fat and ash contents after 16 weeks' trial in *Channa striata* as freshwater fish contains high protein and low fat. So, inclusion of prebiotics and probiotics led to enhancement of more crude protein and less lipid contents which may be good for food fish (Wee, 1982) but body composition analysis showed no significant differences between dietary groups (Merrifield *et al.*, 2011). According to Ayoola, *et al.*, (2013) chemical composition of African Catfish *Clarias gariepinus* after feeding probiotic diet, the data indicated that moisture contents were found higher as compared to control. Highest crude protein was recorded while lowest lipid contents were obtained in probiotic supplementation, which is in accordance with the present study. Only crude protein and lipids contents were comparable with current study while only moisture and lipid contents are comparable in the present study with Mian and Siddiqui (2014). The similar results were found by Pooramini *et al.*, (2009) on *O. mykiss*; Tewary and Patra (2011) on *Labeo rohita*; Asadi *et al.*, (2012) on *Oreochromis niloticus;* Kafilzadeh *et al.*, (2013) on *Astronotus ocellatus* and Mohammadi *et al.*, (2016) on *Cichlasoma trimaculatum*.

Therefore, it is revealed that the chemical composition analysis described by Essa *et al.*, (2010), Pooramini *et al.*, (2009) and Silva *et al.*, (2015) are in good agreement with the present study.

**Table 17.** Comparison of means (±SE) for MCHC of control group and six treatments in the GIFT Tilapia (*O. mossambicus*) fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Days | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | C | SC4 | SC5 | SC6 | SC7 | SC8 | SC9 |
| 0 | $17.80\pm0.65^{klm}$ | $17.98\pm0.51^{j-m}$ | $18.81\pm0.50^{h-m}$ | $17.58\pm0.53^{lm}$ | $18.44\pm0.23^{i-m}$ | $17.78\pm0.39^{klm}$ | $17.79\pm0.55^{klm}$ |
| 15 | $18.41\pm0.65^{i-m}$ | $19.84\pm0.15^{f-m}$ | $20.78\pm0.60^{e-k}$ | $21.67\pm0.62^{d-h}$ | $20.67\pm0.36^{f-k}$ | $17.57\pm0.61^{m}$ | $19.70\pm0.55^{g-m}$ |
| 30 | $20.20\pm0.63^{f-m}$ | $20.92\pm0.45^{e-j}$ | $18.81\pm0.18^{h-m}$ | $20.65\pm0.52^{f-l}$ | $21.30\pm0.76^{e-i}$ | $20.71\pm0.37^{e-k}$ | $18.31\pm0.67^{i-m}$ |
| 45 | $21.06\pm0.21^{e-i}$ | $22.87\pm0.48^{b-f}$ | $24.68\pm0.71^{a-d}$ | $22.29\pm0.69^{c-g}$ | $24.92\pm0.81^{abc}$ | $23.76\pm0.40^{b-e}$ | $21.84\pm0.64^{d-h}$ |
| 60 | $21.68\pm0.47^{d-h}$ | $25.39\pm0.58^{ab}$ | $24.59\pm0.26^{a-d}$ | $27.44\pm0.66^{a}$ | $27.39\pm0.37^{a}$ | $24.95\pm0.80^{abc}$ | $24.98\pm0.55^{abc}$ |
| Mean | $19.83\pm0.45^{D}$ | $21.40\pm0.70^{BC}$ | $21.53\pm0.73^{ABC}$ | $21.93\pm0.88^{AB}$ | $22.54\pm0.88^{A}$ | $20.95\pm0.83^{BC}$ | $20.52\pm0.74^{CD}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

**Hematological parameters**

Hematological parameters fluctuate due to the size, age, physiological status, environmental conditions and other parameters like quality and quantity of dietary ingredients like protein sources, vitamins and probiotics (Ayoola *et al.*, 2013). The results of the present study showed that RBCs count, Hb %, Hct % were significantly higher in SC4 as compared to the control (C) group. The red cell indices showed maximum RBCs count and minimum MCV value in SC4 after 60 days' trial. Maximum MCH and MCHC values were recorded in SC6 and SC3 which were fed with the probiotics as compared to control. The present study showed significant (P<0.05) results by increasing the blood parameters as compared to the control (C) group which are favorably similar with the previous studies (Rajikkannu *et al.*, 2015; Firouzbakhsh *et al.*, 2012). The hematological analysis of rainbow trout also favored higher values of blood parameters than control group while no significant differences were observed between variants (p>0.05) which confirm improvement of fish health by using probiotic.

The present study is also similar to Marzouk *et al.*, (2008), who found a significant increase in RBCs and Hb values in fish groups fed *S. cerevisae* while a minor decrease was observed when probiotics concentration in diet for rainbow trout were increased. Silva *et al.*, (2015) did not detect variations in red blood cells (RBC), hematocrit (HCT) value and hematological indices but observed higher hemoglobin after using lower concentrations of probiotic like Reda and Selim (2015).

This increase in hemoglobin level was due to enhanced iron absorption in the gut which increased the quantity of iron to yield Hemoglobin in fish (Dahiya *et al.*, 2012; Silva *et al.*, 2015). Therefore, these results indicated a positive effect shown by significant increase in RBCs count, HB %, HCT % and red cell indices which could be credited to the fact that, the probiotics enhanced the blood values because of hemopiotic stimulation (Kamgar & Ghane, 2014) and similar findings are also present in the current study.

**Table 18.** Comparison of means (±SE) for amylase enzyme activity, protease enzyme activity and lipase enzyme activity from GIFT Tilapia (*O. mossambicus*) intestine fed with different doses of probiotic (*S. cerevisiae*) after 60 days.

| Treat. | Protein content - Amylase | Total activit - Amylase | Specific activity - Amylase | Protein content - Proteaase | Total activity - Proteaase | Specific activit - Proteaase | Protein content - Lipase | Total activity - Lipase | Specific activity - Lipase |
|---|---|---|---|---|---|---|---|---|---|
| C | 14.07±0.36$^{B}$ | 32.37±0.62$^{D}$ | 3.37±0.11$^{E}$ | 14.07±0.32$^{C}$ | 4.24±0.11$^{D}$ | 0.34±0.01$^{D}$ | 14.07±0.35$^{B}$ | 3.19±0.07$^{E}$ | 0.19±0.01$^{D}$ |
| SC4 | 14.37±0.38$^{AB}$ | 34.97±0.78$^{CD}$ | 3.44±0.03$^{E}$ | 14.37±0.18$^{C}$ | 4.56±0.12$^{CD}$ | 0.49±0.01$^{C}$ | 14.37±0.27$^{AB}$ | 3.67±0.05D$^{E}$ | 0.25±0.00$^{D}$ |
| SC5 | 15.21±0.39$^{AB}$ | 36.86±0.51$^{C}$ | 4.14±0.08$^{D}$ | 15.21±0.13$^{ABC}$ | 4.90±0.08$^{BC}$ | 0.53±0.01$^{C}$ | 15.21±0.44$^{AB}$ | 3.83±0.15C$^{D}$ | 0.25±0.01$^{D}$ |
| SC6 | 14.75±0.43$^{AB}$ | 43.02±0.39$^{B}$ | 4.81±0.10$^{C}$ | 14.75±0.17$^{BC}$ | 4.81±0.18$^{C}$ | 0.67±0.02$^{B}$ | 14.75±0.35$^{AB}$ | 4.41±0.20$^{B}$ | 0.33±0.01$^{C}$ |
| SC7 | 15.92±0.38$^{A}$ | 40.73±0.76$^{B}$ | 5.25±0.08$^{B}$ | 15.92±0.13$^{AB}$ | 5.45±0.06$^{B}$ | 0.65±0.02$^{B}$ | 15.92±0.44$^{AB}$ | 4.24±0.05B$^{C}$ | 0.43±0.03$^{B}$ |
| SC8 | 15.96±0.21$^{A}$ | 41.75±0.91$^{B}$ | 5.77±0.09$^{A}$ | 15.96±0.23$^{A}$ | 6.21±0.14$^{A}$ | 0.61±0.01$^{B}$ | 15.96±0.46$^{AB}$ | 4.54±0.11$^{B}$ | 0.45±0.02$^{B}$ |
| SC9 | 16.00±0.15$^{A}$ | 48.42±1.18$^{A}$ | 6.12±0.10$^{A}$ | 16.00±0.43$^{A}$ | 6.68±0.06$^{A}$ | 0.97±0.03$^{A}$ | 16.00±0.42$^{A}$ | 5.21±0.08$^{A}$ | 0.52±0.02$^{A}$ |

Means sharing similar letter in a column are statistically non-significant (P>0.05).

**Digestive enzyme activity**

Digestive enzymes like amylase, protease and lipase could be enhanced by adding the probiotics in diet (Ziaei-Nejad *et al.*, 2006; Taoka *et al.*, 2007; Gomez & Balcazar, 2008). This improvement of feed utilization may be due to improvement in intestinal microbial biota which leads to improved nutrient digestibility, better absorption quality and higher enzyme activities (Lara-Flores *et al.*, 2003; Balcazar *et al.*, 2006; Renuka *et al.*, 2013).

The results of the present study presented enhanced levels of amylase, protease and lipase in *O. mossambicus* as compared to control when fed with *L. acidophilus* and *S. cerevisiae* which may be due to the use of probiotics in the gastrointestinal tract. Similar results had been described for other fishes by using different probiotics (Lara-Flores *et al.*, 2003; El-Haroun *et al.*, 2006; Essa *et al.*, 2010). Results of the present study also revealed that different probiotics have different effect on enzyme activities as previously described by Renuka *et al.*, (2013). Same results were also recorded by Yanbo and Zirong (2006) for common carp, *Cyprinus carpio* fed with photosynthetic bacteria and *Bacillus* species. Data on digestive enzyme (protease, amylase and lipase) activity indicated that in trial 2 (60 days' trial) treated with *S. cerevisiae* exhibited better results as compared to trial 1 (30 days' trial) in which *L. acidophilus* was used as probiotic which indicated that *S. cerevisiae* had more significantly increased the digestive ability than that of trial 1. Soleimani *et al.*, (2012) evaluated the digestive enzyme activity in Caspian roach (*Rutilus rutilus*) by using Fructooligosaccharide probiotic in diet for 7 weeks and found the highest digestive enzyme activity which is in line with the present study. A significant reduction in amylase (P<0.05) was recorded in fish fed without probiotics as compared to probiotic treated groups. Amylase and lipase values were higher due to good performance of the probiotic as described by present study and also supported by various authors on different fish species *E. coioides* (Son *et al.*, 2009), *E. bruneus* (Harikrishnan *et al.*, 2010) and *O. niloticus* (Ngamkala *et al.*, 2010). Therefore, this study revealed that the use of probiotics increased digestive enzyme activity and enhanced specific activity of amylase, protease, and lipase.

**Immunity parameters, stress resistance and survival rate**

Salinity stress test is commonly used to estimate fish fitness or quality after feeding probiotics (Dimitroglou *et al.*, 2010; Soleimani *et al.*, 2012; Hoseinifar *et al.*, 2013, 2014).

Salinity challenge test after 60 days' trial indicated that 85 % fish died in the control (C) group after 7 days of post stress while the dietary probiotic significantly improved the resistance of fish fed with probiotics against salinity stress challenge (P<0.05).

The maximum survival rate was noted in the treatment SC4 (95 %) which was supplemented by *Saccharomyces cerevisiae* and was found significantly higher (P<0.05) than the control and other treatments. The previous studies also revealed significant increase against salinity stress resistance in Porthole livebearer (Hernandez *et al.*, 2010); gilthead sea bream (Varela *et al.*, 2010 after feeding *L. acidophilus,* L. *lactis* and probiotic Pdp11 supplemented diet. Similar results were obtained after dietary supplementation of *S. cerevisiae* by Sheikhzadeh *et al.*, (2012).

**Table 19.** Comparison of means (±SE) for the effect of probiotics on total leucocytic count and leucocytic differential count in GIFT Tilapia (*O. mossambicus*) after 60 days trial and survival rate after 7 days of infection challenged with *A. hydrophila*.

| Treatment | Leucocytic count | Lymphocytes | Monocytes | Granulocytes | Survival % (Saline) | Survival % (A. hydrophila) |
|---|---|---|---|---|---|---|
| C | $32.32\pm0.69^{D}$ | $3.58\pm0.10^{C}$ | $2.10\pm0.06^{B}$ | $23.45\pm0.88^{A}$ | $15.00\pm0.58^{C}$ | $20.00\pm0.58^{C}$ |
| SC4 | $35.78\pm0.89^{D}$ | $3.66\pm0.12^{C}$ | $2.14\pm0.05^{B}$ | $24.41\pm1.33^{A}$ | $70.00\pm2.31^{B}$ | $75.00\pm2.31^{B}$ |
| SC5 | $36.56\pm1.11C^{D}$ | $3.12\pm0.03^{C}$ | $2.06\pm0.04^{B}$ | $24.86\pm1.03^{A}$ | $80.00\pm3.46^{AB}$ | $85.00\pm4.04^{AB}$ |
| SC6 | $37.34\pm1.27^{BCD}$ | $3.33\pm0.10^{C}$ | $2.19\pm0.08^{AB}$ | $23.44\pm0.40^{A}$ | $85.00\pm4.04^{AB}$ | $80.00\pm4.04^{AB}$ |
| SC7 | $41.44\pm1.24^{ABC}$ | $5.79\pm0.11^{A}$ | $2.46\pm0.04^{A}$ | $28.26\pm1.35^{B}$ | $95.00\pm4.62^{A}$ | $95.00\pm4.62^{A}$ |
| SC8 | $44.00\pm1.53^{A}$ | $5.43\pm0.13^{AB}$ | $2.14\pm0.08^{B}$ | $27.23\pm0.84^{B}$ | $85.00\pm2.89^{AB}$ | $90.00\pm3.46^{AB}$ |
| SC9 | $42.14\pm0.92^{AB}$ | $5.15\pm0.18^{B}$ | $2.18\pm0.06^{AB}$ | $28.25\pm0.87^{B}$ | $80.00\pm4.62^{AB}$ | $85.00\pm4.62^{AB}$ |

Means sharing similar letter in a column are statistically non-significant (P>0.05).

The results of challenge test with *A. hydrophila* after 60 days' trial 80 % of experimental fish was died in control (C) group after 7 days of post stress while the other treatment fed with probiotics showed significant resistance against bacteria (P<0.05).

The maximum survival rate was detected in the treatment SC4 (95%) which was supplemented by *S. cerevisiae*, which was significantly higher (P<0.05) than other treatments and control (C) groups. Similar results were detected in *L. rohita* fed with different levels of probiotic challenged with *A. hydrophila* and mortality of fish fed with probiotics was reduced significantly compared to fish fed with basal feed. Shoemaker *et al.*, (2006) observed significantly higher reflection of immunity after challenging *O. niloticus* with *Streptococcus iniae*. Previous studies revealed that a challenge test with *A. hydrophila* provided better results in Nile tilapia. Similar results were indicated by Taoka *et al.*, (2006) in Nile tilapia against *Edwardsiella tarda* and *A. hydrophila* (Das *et al.*, 2006; Van-Hai *et al.*, 2009; Putra *et al.*, 2021) while Abd El-Rhman *et al.*, (2009) described that *Pseudomonas* did not offer sufficient defense against *A. hydrophila*. Venkatesan *et al.*, (2012) described that single probiotic can also play effective role against bacterial pathogens like *Bifidobacterium* sp. had higher inhibitory effect against *Salmonella* sp.

All these studies support the current study. Similar results of probiotics were also reported by Balakrishan *et al.*, (2006) and Dahiya *et al.*, (2012) against the pathogenic *Micrococcus* sp., *Bacillus subtilis*, and *Salmonella typhi*. Nayak (2010) described that there is need to determine the dose of individual probiotic used for a specific host because higher doses of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* (Nikoskelainen *et al.*, 2001) were filed against challenge study in *Oncorhynchus mykiss* and high mortality was observed. So, there is need of the effective dose of the probiotic (Souza *et al.*, 2012).

**Table 20.** Comparison of means (±SE) for the effect of probiotics on blood parameters in GIFT Tilapia (*O. mossambicus*) after 60 days trial of stress resistance and survival rate after 7 days of infection challenged with *A. hydrophila*.

| Treatment | RBC (X106/µL) | HB% | HCT (%) | MCV (fl) | MCH (pg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|
| C | 4.71±0.14$^{D}$ | 4.84±0.16$^{B}$ | 23.18±0.46$^{A}$ | 4.92±0.18$^{A}$ | 10.28±0.19$^{B}$ | 20.88±0.67$^{C}$ |
| SC4 | 5.43±0.11$^{ABC}$ | 5.82±0.13$^{A}$ | 24.05±0.52$^{A}$ | 4.43±0.12$^{ABC}$ | 10.72±0.12$^{B}$ | 24.20±0.74$^{AB}$ |
| SC5 | 4.93±0.13$^{CD}$ | 5.15±0.05$^{B}$ | 24.13±0.84$^{A}$ | 4.89±0.09$^{A}$ | 10.45±0.34$^{B}$ | 21.34±0.39$^{BC}$ |
| SC6 | 5.22±0.06$^{BCD}$ | 5.98±0.19$^{A}$ | 24.97±0.81$^{A}$ | 4.78±0.12$^{AB}$ | 11.46±0.25$^{AB}$ | 23.95±0.94$^{AB}$ |
| SC7 | 6.03±0.14$^{A}$ | 6.22±0.09$^{A}$ | 24.99±0.12$^{A}$ | 3.97±0.09$^{C}$ | 10.32±0.40$^{B}$ | 24.89±0.37$^{A}$ |
| SC8 | 5.72±0.15$^{AB}$ | 6.13±0.10$^{A}$ | 24.14±0.30$^{A}$ | 4.22±0.13$^{BC}$ | 10.72±0.24$^{B}$ | 25.39±0.60$^{A}$ |
| SC9 | 5.02±0.13$^{CD}$ | 6.10±0.18$^{A}$ | 24.07±0.23$^{A}$ | 4.79±0.08$^{AB}$ | 12.15±0.27$^{A}$ | 25.34±0.32$^{A}$ |

Means sharing similar letter in a column are statistically non-significant.

**Immunity by hematological studies**

Probiotics are considered as an alternative of the antibiotics in aquaculture generally in fish culture and are helpful to retard mortality and improve growth and survival of fish. A wide range of research has been done on health benefits of probiotics against pathogenic assault (Lategan *et al.*, 2004; Chabrillon *et al.*, 2006; Rimoldi *et al.*, 2020). The resistance against pathogen attacks and enhanced survival was observed as bacterial infection was prevented in *P. pelagicus* (Carnevali *et al.*, 2004, 2006; Ziaei-Nejad *et al.*, 2006; Zhou *et al.*, 2009; Avella *et al.*, 2010; Kesarcodi-Watson *et al.*, 2010; Talpur *et al.*, 2012). The use of probiotics in fish diet improves immune system and pathogenic microorganisms (Irianto & Austin 2002; Balcazar *et al.*, 2006; Nayak, 2010; Mohamed & Refat, 2011). Probiotics are also considered as a substitute to chemotherapy (Kesarcodi-Watson *et al.*, 2008; Abd-El-Rhman *et al.*, 2009; Van-Hai *et al.*, 2009; Giri *et al.*, 2013) as they enhance disease resistance (Irianto & Austin, 2002; Magnadottir, 2006) which is confirm after challenge study with *A. hydrophila* (Das *et al.*, 2006; Putra *et al.*, 2021). Taoka *et al.*, (2006) reported that the probiotics are also effective to enhance fish immunity and resistance against the infection of *Edwardsiella tarda* and other bacteria (Eissa & Abou-ElGheit, 2014). Probiotic treatment is also considered as an effective alternative to improve shrimp health. All these studies support the current study. Hematological parameters can be considered valuable tools to evaluate health after using dietary probiotics as component of fish feed (Irianto & Austin, 2002; Brunt & Austin, 2005). In the present study, total leucocyte counts after 60 days' experiment showed significant increase in group supplemented with *S. cerevisiae* as compared to control (C) group**.** It was observed maximum (44.0) in SC5 while minimum (32.32) in control (C) group. Differential leucocyte counts also indicated significant increase in lymphocytes, monocytes and granulocytes in treated groups ($p< 0.05$) as compared to control. Results indicated that lymphocytes, monocytes and granulocytes were recorded higher in SC4. Similar results about improved immunological parameters were observed in previous studies by various researchers after using different probiotics (Aly *et al.*, 2008; Ferguson *et al.*, 2010). Few studies have described that use of probiotics could stimulate nonspecific immune responses and eliminate the pathogens (Gomez & Balcazar 2008; Ferguson *et al.*, 2010). Several researchers fed *S. cerevisiae* to *Cyprinus carpio* (Mazurkiewicz *et al.*, 2005; Dehghan *et al.*, 2011); *Epinephelus coioides* (Chiu *et al.*, 2010); *Channa striatus* (Dhanaraj & Haniffa, 2011); *Oreochromis niloticus* (Asadi *et al.*, 2012) and *Cichlasoma trimaculatum* (Mohammadi *et al.*, 2016) reported improved immunity with low mortality rates. Yeast *S. cerevisiae* was found effective for better survival in the Zebra fish, *Danio rerio* (Markad & Rane, 2015) which is in good agreement with the present study. The infected groups of fish with *A. hydrophila* maintained on probiotic diets produced better hematological parameters than the control (Chelladurai *et al.*, 2013; Putra *et al.*, 2021) and the same findings are observed in this study.

## CONCLUSIONS

The supplementation of *S. cerevisae* in basal fish feed caused a substantial rise in growth performance indicated by FBW, WG, survival rate, PER and PPV. In case of SGR better value was observed in all treatments fed with *S. cerevisae* as compared to the control. FCR was observed minimum in treatments while PER and PPV were increased due to the use *S. cerevisae* in diets than the control (C) group. The survival rate (%) during growth performance trials remained constant and it was 100 %. Proximate analysis revealed that *S. cerevisiae* promoted the body crude protein, moisture, crude fiber and ash in treatments than control (C) group. The proximate composition analysis indicated that the body composition was significantly changed by the inclusion of *S. cerevisae*. Hematological parameters indicated significantly higher RBC, HB and HCT in SC4 than control group. The Red blood cell indices indicated maximum MCH and MCHC values in SC4 while MCV was observed minimum in SC4. Digestive enzymes affect the efficacy of feed utilization and help the fish to hydrolyze feed ingredients like carbohydrate, protein and lipids. The higher levels of amylase, protease and lipase were observed in *O. mossambicus* fed with *S. cerevisiae* as compared to control. The digestive enzymes (amylase, protease and lipase) activity indicated that *S. cerevisiae* exhibited better results. The salinity challenge test described that after 60 days, 85% fish were died in the control (C) group after 7 days of post stress while, in the treatment groups the dietary probiotic significantly improved the resistance of fish against salinity challenge test ($P<0.05$). The maximum survival rate was noted in the treatment SC4 (95%) which was supplemented with *S. cerevisiae*, which was significantly higher than control (C) groups ($P<0.05$). The challenge test with *A. hydrophila* indicated that after 60 days' trial 80 % of experimental fish died in control (C) group after 7 days of post stress while, the other treatments fed with *S. cerevisae* showed significant resistance against bacteria ($P<0.05$). The maximum survival rate was detected in the treatment SC4 (95%) which was supplemented with *S. cerevisiae*, which was significantly higher than control (C) groups ($P<0.05$). Total leucocyte count showed significant increase in treatments supplemented with *S. cerevisae*as compared to the control (C) group. It was observed maximum in SC5 while minimum in control (C) group. Differential leucocyte counts indicated highly immune response in treated groups ($P<0.05$) as compared to control. The lymphocytes, monocytes and granulocytes were recorded higher in SC4 while minimum in control (C) group. It is recommended that further research on other fish species should be conducted in future.

## AUTHORSHIP

Riffat Yasin and Khizar Samiullah are Co first authors in this work. Also Ahmed Mustafa is Co-senior authors on this work. Inayat Ullah Malik and Shahzad Ahmad are 2nd equal contributors.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the GC University, Faisalabad.

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