

Effect of Commercial Probiotics on Growth Performance of Sabaki tilapia, *Oreochromis spilurus*

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Abstract. The use of beneficial bacteria to improve fish health and growth is an innovative method in aquaculture practice. A study was conducted to test the efficiency of two commercial probiotics, such as Sanolife Pro-W and Protexin Balance on the microbial load in culture water and growth performance of Sabaki tilapia, *Oreochromis spilurus* in fiber glass tanks (400 liters) for a period of 75 days. Sanolife was supplied at 3ppm in the rearing water daily, and designated as Treatment 1. Protexin was incorporated at the rate of 1ppm in a supplementary feed, containing 35% protein in diet and considered as treatment 2. A control was also maintained without treating with probiotics in culture water or feed. Healthy and uniform size fish ($3.1 \pm 0.5g$) were stocked at the rate of 25peaces/tank and both treatments and control tanks were set on a randomized design and triplicated. Water quality parameters (physical and chemical), microbial load in water, growth performance and body composition (nutrients) of fish were assessed during the culture period. Significant difference ($p < 0.01$) on growth was observed between control and treatments, the highest growth was recorded in Protexin treated fish. Water quality parameters did not show significant variations. Carcass protein and lipid content showed significant difference between control and treatments. Considerable reduction in bacterial population and improved growth were observed in Sanolife Pro W treated water, when compared to Control. The result shows that Sanolife significantly reduce the bacterial population in culture water and Protexin improves the growth of fish. The outcome of the study has great application in the field of tilapia aquaculture sector in the Kingdom.

Keywords: Probiotics, Growth, Microbial population, Sabaki tilapia, *O. spilurus*.

1. Introduction

Aquaculture is one of the fastest growing business among all sectors of agriculture and marine tilapia culture has gained more attention recently in the Kingdom due to its high market demand (Young *et al.*, 2021). The sabaki Tilapia, *Oreochromis spilurus* is an excellent table fish, has a high salinity tolerance capacity and grows well in sea water salinity up to 45 ppt (FAO, 2015). A major anxiety in intensive culture of tilapia is the increased susceptibility to infectious disease such as Vibriosis and Streptococcosis and various antibiotics and chemicals are being applied in the industry to

treat the diseases (Alyssa *et al.*, 2016). It is well accepted that rather than treating a sick fish, it is a better to control the infectious agents in the rearing water (Aly *et al.*, 2008).

Fish culturists have shown considerable interests to use non-nutrient dietary additives, immune modulators and probiotics to improve health, growth, or to stimulate immune system function against infectious diseases (Ayyat *et al.*, 2014). With increasing demand for environment friendly aquaculture, the use of probiotics is now widely used in aquaculture practice (Vikash Kumar *et al.*, 2016; Kesselring *et al.*, 2019; Ringo, 2020). The fast and healthy

growth of fish culture seriously requires strategies to improve production systems, enhance bio-security and reduce environmental impacts (Avella *et al.*, 2010; Qi *et al.*, 2009).

Zhou *et al.* (2010) reported that *Vibrio* bacteria causes serious diseases in tilapia and affects survival and growth. These opportunistic harmful microorganisms cause disease under unfavorable environmental conditions. The effects include mortality, tissue damage or necrosis and growth retardation. Vibriosis has been also implicated as the cause of high mortalities in juvenile fish worldwide (Nikoskelainen *et al.*, 2003; Kesarcodi-Watson *et al.*, 2008).

Defining alternative strategies to support aquaculture productivity are extremely necessary and the use of probiotics have shown promising results and is now widely accepted as a complementary tool for the management of disease and for improving health and growth of fish (Wang *et al.*, 2008; Avella *et al.*, 2010; Kuebutornye and Abarike, 2019). Extensive work have been done to assess the efficacy of probiotics as feed supplement to enhance the gut bacterial flora and there by improve growth and production of shrimp (Havenaar, *et al.*, 1992; Moriarty, 1998; Skjermo and Vadstein, 1999; Gatesoupe, 1999; Gullian, *et al.*, 2004; Mamdoh *et al.*, 2019; Kesselring *et al.*, 2019; Ringo, 2020).

However, little information is available on the application of probiotics in controlling the harmful bacteria population in tilapia culture (Aly *et al.*, 2008; Zhou *et al.*, 2010). Therefore, this study was conducted to evaluate the effect of two commercial probiotic products such as Sanolife-Pro-W and Protexin Balance on the microbial load in the culture water and growth performance of Sabaki tilapia, *Oreochromis spilurus*, an ideal candidate species for coastal as well as cage culture practice in the Kingdom.

2. Materials and Methods

2.1 Experimental Set Up

Culture experiment was conducted in fiberglass tanks (9 tanks, 400liters) at KAU Fish Farm, Jeddah for a period of 75 days. All culture tanks were cleaned and sundried for one week prior to experiment and arranged in a completely randomized design. There were control (3tanks) and treatments (3 tanks each) for the study and all of them were triplicated. All tanks were filled with filtered seawater (40ppt), provided with adequate aeration, and stocked with healthy and uniform size ($3.1 \pm 0.5g$) fish at the rate of 25 fish per tank.

A commercial probiotic product, Sanolife Pro W (Inve Aquaculture, USA) containing strains of *Bacillus subtilis* and *Bacillus licheniformis* was supplied at 3ppm to the culture water daily. Required quantity (3ppm) of Sanolife was mixed with seawater (1liter) as recommended by the Manufactures and the same was added to the rearing water daily after water exchange (Treatment 1).

Human live probiotic supplement, Protexin Balance (ADM Protexin Ltd, UK) containing strains of *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Bifidobacterium longum* and *Lactobacillus bulgaricus* was given to the fish at the rate of 1ppm through a supplementary feed (Treatment 2) The dose was selected based on the efficiency of Protexin recommended by the Manufactures).

The quantity of Protexin was weighed, dissolved in water and then sprayed it over the feed, 30 mins before feeding for the probiotic have to stuck on feed. A control was maintained without treating with probiotics in culture water or feed.

2.2 Water Quality Analysis

Water quality parameters such as

temperature, salinity, dissolved oxygen and pH in the culture tank were measured daily before water exchange. Temperature and dissolved oxygen were determined with YSI DO Meter (YSI Incorporated, Yellow Springs, USA). pH was measured by a marine portable pH meter (HANNA Instruments, Romania) and salinity was tested by a refractometer (ATAGO, Germany). Chemical parameters *viz.*, Ammonia (unionized), Nitrite (NO₂), Phosphate (PO₄), Magnesium (Mg), Calcium (Ca) and Potassium (K) were measured biweekly using JBL Test kit (GmbH & Co., Germany).

2.3 Feeding and Water Exchange

A standard fishmeal based pellet feed (NAQUA, Jeddah) having 35% protein in diet was supplemented to the fish at the rate of 5% of biomass daily at 8:00am and 5:00pm based on a standard feed table. Water exchange was done at the rate of 5-10 % daily and 50% biweekly from each tank. The unfed and faecal matter in the tanks were siphoned out during water exchange.

2.4 Sampling

For growth assessment, fish sampling was done biweekly. Fishes were collected with a nylon scoop net and anaesthetized using clove oil (4ppm) for measuring length and weight. Individual length and weight of 15 fishes were taken randomly from each tank at every sampling time, and average body weight (ABW) was calculated. Quantity of feed to be fed was calculated according to the weight increment of fish.

2.5 Microbial Analysis

Water samples were collected from each tank biweekly before water exchange for microbial analysis. These samples were used to estimate total colony forming units (CFU), yellow and green *Vibrio*, and luminescent bacteria colonies (Brugger *et al.*, 2012). Bacterial load as colony forming unit per ml

(cfu ml⁻¹) in terms of total bacteria count (TBC), yellow *Vibrio* count (YVC) that forms yellow colonies on thiosulphate citrate bile salts sucrose (TCBS) agar, green *Vibrio* count (GVC) of *Vibrios* that form green colonies on TCBS agar and luminescent bacteria count (LBC) of luminescent bacterial colonies.

2.6 Growth Performance of Fish

On termination of experiment (75th day), growth, survival and biomass of fish in each tank was calculated. Specific growth rate (SGR) was calculated as: $SGR (\%) = \text{Log}_e W_2 - \text{Log}_e W_1 \times 100$. where W1 = Weight of fish (g) at time T1 (Day 1) and W2 = Weight of shrimp at time T2 (Day 75) (Rahman *et al.*, 2022). Feed conversion ratio (FCR) was calculated as: $FCR (\%) = \text{Feed consumption (g)} / \text{Weight gain (g)} \times 100$ (Fry *et al.*, 2018).

2.7 Proximate Composition Analysis

Upon trial completion, fish body was subjected to proximate composition analysis as per AOAC procedures (AOAC, 1990). For this, fish were dried by placing them in a hot air oven for 36 hours at 60°C. The dried and powdered samples were used for the estimation of moisture, dry matter, protein, lipid, glycogen, fibre and ash content.

2.8 Statistical Analysis

One way analysis of variance (ANOVA) was employed to find out the statistical difference on growth, water quality parameters, microbial population between control and treatment tanks using Microsoft Excel software (Excel, 2007).

3. Results

3.1 Water Quality Parameters

Data on physical and chemical water quality parameters recorded during the culture period is depicted in Fig. 1 and 2. The study was initiated in late winter and completed at the beginning of summer. The variation in water

temperature observed was due to the influence of the season. However, the parameters were found to be within the range suitable for fish growth. There was no significant difference ($p>0.05$) in mean values between control and treatments. The enhanced level of ammonia, nitrite, phosphate, and calcium recorded during the second week of the experiment was due to the accumulation of these compounds in water. The values were found to be lower when water exchange was done at 50%, and 50% water exchange was implemented biweekly.

3.2 Microbial Load

Details on microbial population is shown in Fig. 3. Significant difference ($p < 0.01$) in total plate count (TPC) for colony-forming units was found between control and treatment tanks. Total Yellow Vibrio and Green Vibrio colonies were found to be lowered in treated tanks when compare to control. Total luminescent colonies (TLC) were found to be very low when compared to TYC and TGC. The decreased population of harmful bacteria observed in the treatment tanks may be due to the effect of probiotic supplements.

3.3 Growth Performance

Details on growth performance of tilapia in control and treatments are presented in Fig. 4. Fish fed with Protexin incorporated feed (Treatment 2) showed highest growth in length and weight, when compared to the fish grown in Sanolife treated water (Treatment 1) and Control. Significant difference ($p<0.01$) was found between control and treatments. Percentage weight gain in control, Treatment 1 and Treatment 2 were 103.22, 129.03 and 170.96 respectively (Table 1 and 2). Specific growth rate (SGR) was high in Treatment 2, whereas Feed conversion rate (FCR) and survival did not show significant difference between Control and treatments.

3.4 Proximate Composition

Result on proximate composition analysis is presented on Table 3. Crude protein and lipid in the body showed significant difference between control and treatments being the highest in Protexin fed fishes. Moisture, dry matter, fiber and ash content did not vary significantly ($p>0.01$) between control and treatments.

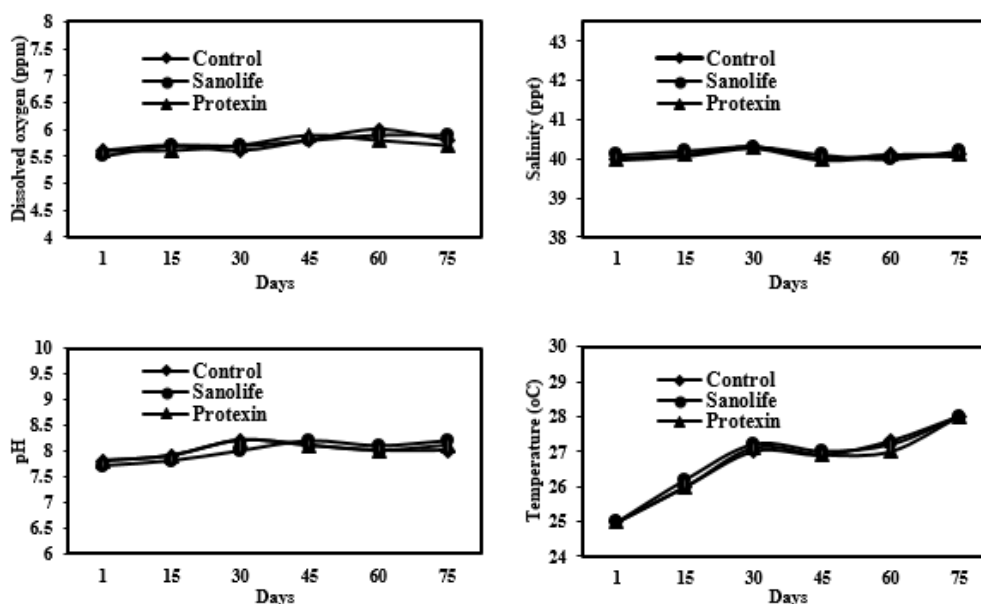


Fig. 1. Water quality (Physical) parameters recorded during the culture period.

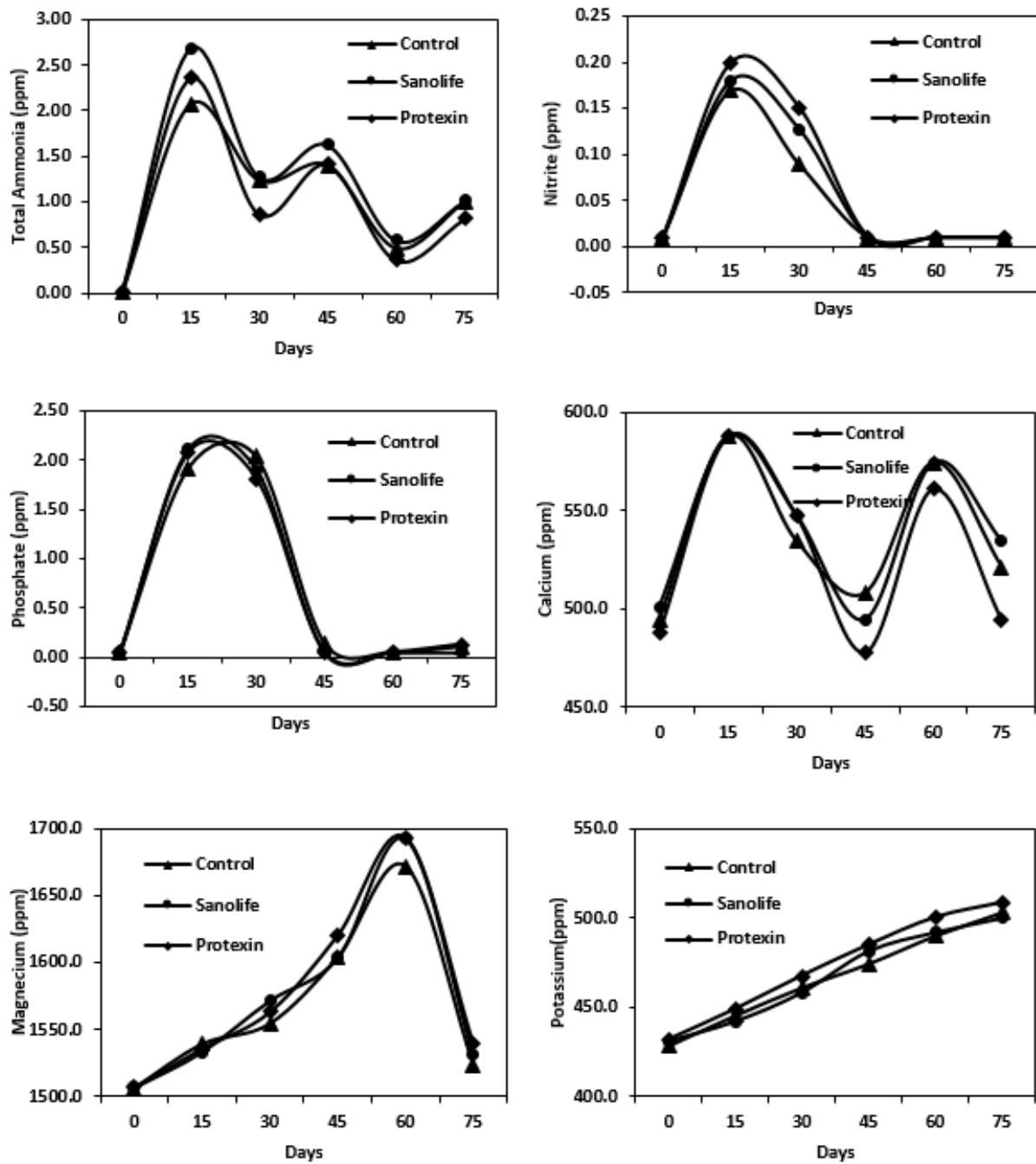


Fig. 2. Water quality (Chemical) parameters recorded during the culture period.

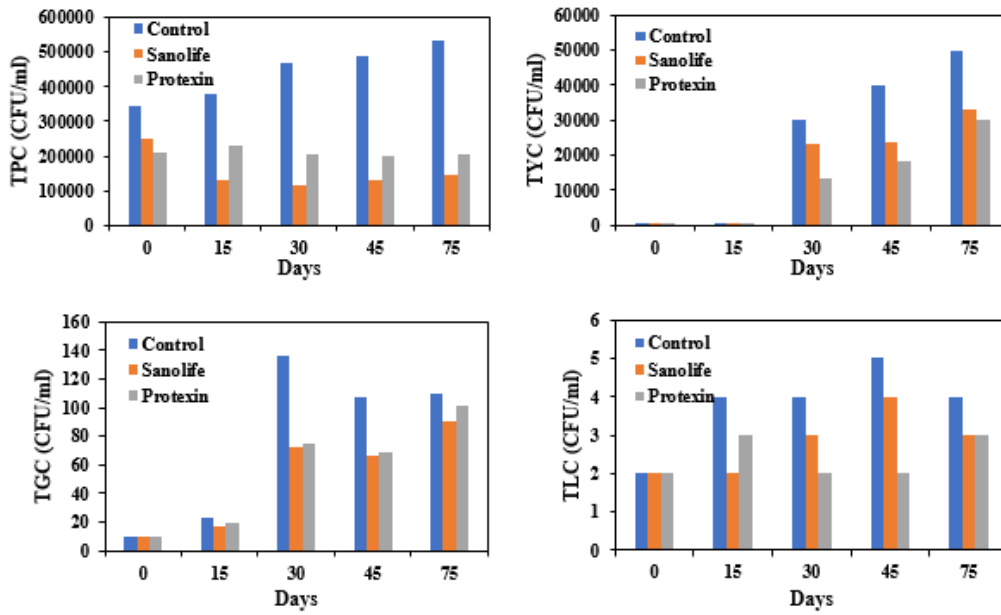


Fig. 3. Microbial population (CFU/ml) recorded during the culture period.
(TPC- Total plate count, TYC- Total Yellow Colony, TGC- Total Green Colony, TLC- Total Luminescent Colony)

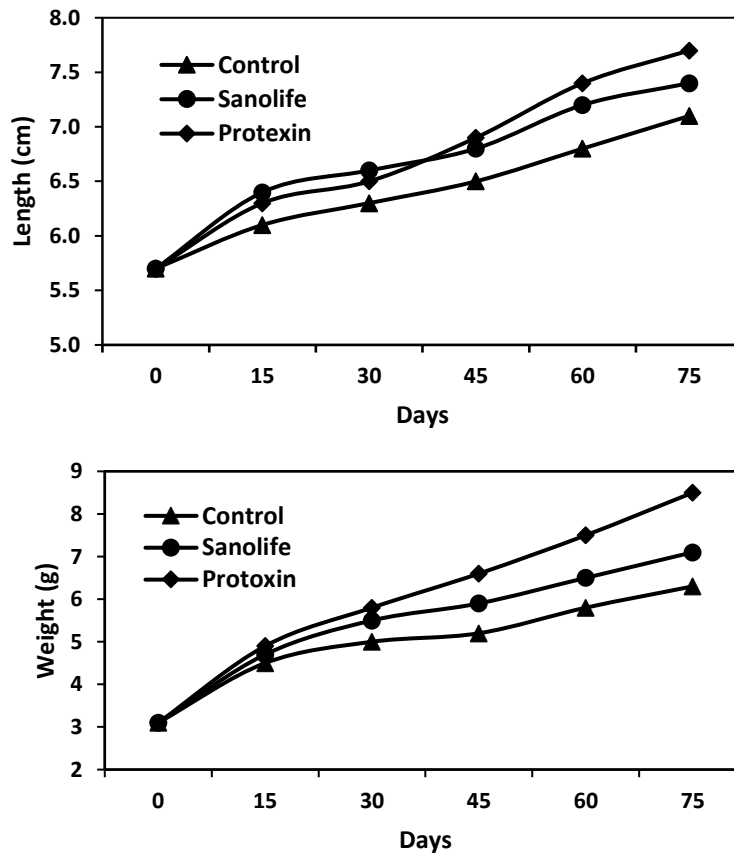


Fig. 4. Growth in length (cm) and weight (g) of tilapia during the culture period.

Table 1. Growth performance of tilapia treated with probiotics.

Parameters	Control	Sanolife	Protexin
	Mean \pm SD	Mean \pm SD	Mean \pm SD
Initial length (cm)	5.7 \pm 0.4	5.7 \pm 0.4	5.7 \pm 0.4
Final length (cm)	7.1 \pm 0.7	7.4 \pm 0.4	7.7 \pm 0.6
Initial weight (g)	3.1 \pm 0.5	3.1 \pm 0.5	3.1 \pm 0.5
Final weight (g)*	6.3 \pm 1.0	7.1 \pm 1.6	8.4 \pm 2.7
Weight gain (g)	3.2	4.0	5.3
Weight gain (%)	103.22	129.03	170.96
SGR %	0.144192	0.196109	0.26913
FCR (%)	2.21	2.12	2.20
Survival (%)	92.0	92.0	94.0

*p<0.01 (ANOVA)

Table 2. Analysis of variance showing significant difference on growth (weight) between control and treatments.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	55.4408	2	27.7204	7.659803	0.000964	3.123907
Within Groups	260.564	72	3.618944			
Total	316.0048	74				

Table 3. Proximate composition analysis of carcass.

Parameters	Control	Sanolife	Protexin
Moisture (%) ^{NS}	73.01 \pm 0.6	73.18 \pm 0.4	73.73 \pm 0.7
Dry matter (%) ^{NS}	26.9 \pm 0.3	26.82 \pm 0.5	26.27 \pm 0.8
Protein (%)*	22.07 \pm 0.9	22.67 \pm 0.5	24.26 \pm 0.4
Total Lipid (%)*	2.82 \pm 0.1	2.01 \pm 0.7	1.29 \pm 0.3
Ash (%) ^{NS}	3.07 \pm 0.2	3.12 \pm 0.3	3.3 \pm 0.5
Crude Fiber (%) ^{NS}	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.01
Carbohydrates (%) ^{NS}	0.1 \pm 0.01	0.1 \pm 0.01	0.1 \pm 0.01

*p<0.01 (ANOVA)

4. Discussion

Sabaki tilapia (*Oreochromis spilurus*) is the most popular and widely farmed marine tilapia species in the Kingdom. Its seed availability to farmers, fast growth rate, tolerance to a variety of cultural situations, appeal with customers, and ease of breeding in captivity are the factors contributing to its

extensive culture (Chithambaran, 2019). Results of the experiment showed that use of probiotics such as Sanolife Pro W and Protexin Balance improved growth and survival of sabaki tilapia, *O. spilurus*. Studies revealed that probiotics are beneficial microbial feed supplement, which can improve the microbial balance of the host digestive tract (Fuller, 1989; Kesselring *et al.*, 2019; Ringo, 2020). Havenaar

et al. (1992) and Moriarty (1997) reported that probiotic consortium of live microorganisms improve the properties of indigenous gut microflora. The Protexin Balance used in the present study was a bacterial consortium containing *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Bifidobacterium longum* and *Lactobacillus bulgaricus*. Manufactures of Protexin Balance claim that the bacterial consortium help to maintain healthy digestive and immune systems in human being (Protexin Pvt. Ltd.). The increased survival of fish fed probiotic-supplemented diets noticed in the present study may be a sign of better health, and this finding is in line with that of Welker and Lim (2011) and Hai (2015), who observed that *Saccharomyces cerevisiae* and *Bacillus subtilis* contain peptide antibiotics, such as subtilin and bacitracin, boosted immunity and increased survival rate in tilapia.

According to studies (Merrifield et al., 2010; Welker and Lim, 2011), probiotics have been shown to modify the gut environment or produce digestive enzymes that increase feed digestion and promote better growth. Opiyo et al. (2019) demonstrated that *Saccharomyces cerevisiae* and *Bacillus subtilis* enhanced growth and survival of fish species such as Nile tilapia, common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) by stimulating gut digestive enzyme activity. Additionally, Zhang et al. (2015) reported that probiotics have the ability to change the morphology of the gut's microvilli and its digestive enzyme production and increases the absorption of nutrients in triangular bream (*Megalobrama terminalis*). Therefore, it is suggested that the enhanced growth observed in the fish fed with Protexin can be correlated with the enhanced feed digestion and nutrient absorption. Protexin might have accelerated

food digestion and speed up the performance of the fish gut flora activities as

In this study, Sanolife Pro W treated water showed a remarkable reduction of harmful bacteria population such as yellow, green and luminescent bacterial colonies. Many studies show that probiotics can control the growth of harmful bacteria population in the rearing medium (Gatesoupe, 1999; Skjermo and Vadstein, 1999; Rengpipat et al., 2000; Hoseinifar et al., 2018). Gullian et al. (2004) reported that the bacterial strain, *Bacillus* P64 showed inhibitory effects against *Vibrio harveyi* and *Bacillus* P64 showed both probiotic and immunostimulatory features when used in shrimp culture. The reduction of *Vibrio* colonies in the present study may be due to the inhibitory effect of *Bacillus subtilis* and *Bacillus licheniformis* present in Sanolife Pro W and Protexin Balance. The favorable environment that Sanolife maintained in the rearing water may have contributed to the improved growth of fish in water treated by Sanolife.

Proximate composition analysis of carcass showed that probiotic supplemented fish has high protein content and low fat content compared to the control. The high protein content in the carcass may be due to the enhanced nutrient deposition in muscle and this is in agreement with the findings of El-Haroun et al. (2006) and Bagheri et al. (2008), who reported an increased level of protein and lipid reduction in *O. niloticus* and *O. mykiss* fed on probiotic supplemented diets. *Saccharomyces cerevisiae* plays a key role in enhancing food intake and resulting increased body composition parameters (Abdel-Tawwab et al. 2008). Hence, the higher carcass protein content observed in this study may be explained by the action of probiotics in the tilapia's stomach making a conducive environment and the efficient conversion of ingested food into

structural protein, which helps to develop more muscle.

The mechanism of probiotic action is complex. However, it acts as a medium to improve water quality, produce inhibitory compounds or antibiotics and compete for chemicals or energy/nutrient. It also acts as adhesion sites, enhance immune responses, supply macro and micronutrients and digestive enzymes and modulate interactions with the environment (Gatesoupe, 1999; Verschuere *et al.*, 2000; Irianto and Austin, 2002; Abidi, 2003; Balcazar *et al.*, 2006; Gomes *et al.*, 2009; Soltani *et al.*, 2019). Good probiotics have characters such as host acceptance, ability of colonization and proliferation within the host, capacity to reach target organs where they can work, and do not contain antibacterial resistance genes (Verschuere *et al.*, 2000; Kesarcodi-Watson *et al.*, 2008). Results of the present study also support this conclusion. The application of probiotics improved feed consumption and digestion, and the number of harmful bacteria was reduced, possibly promoted faster growth of the fish.

4. Conclusions

Results of the study shows that use of probiotics such as Sanolife and Protexin Balance improves growth of Sabaki tilapia and controls the harmful microbial population in the culture water. The data provides base line information for the use of probiotics, Sanolife and Protexin Balance in the culture of *O. spilurus*.

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تأثير البروبيوتيك التجاري على أداء نمو البلطي *Oreochromis spilurus*

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المستخلص. يعد استخدام البكتيريا المفيدة لتحسين صحة الأسماك ونموها من أهم الطرق المبتكرة في استزراع الأحياء المائية. أجريت هذه الدراسة لاختبار كفاءة نوعين من البروبيوتيك التجاريين وهما Sanolife Pro-W و Protexin Balance على الحمل الميكروبي في مياه الاستزراع وأداء نمو البلطي نوع *Oreochromis spilurus* في أحواض مصنوعة من الألياف الزجاجية (٤٠٠ لتر) لمدة ٧٥ يوماً. تم إضافة نوع Sanolife بتركيز ٣ جزء في المليون في مياه الأحواض يومياً، وتم تحديده على أنه معاملة رقم ١. تم دمج نوع Protexin بمعدل ١ جزء في المليون في علف تكميلي، يحتوي على ٣٥٪ بروتين في النظام الغذائي ويعتبر معاملة ٢. كما تم الحفاظ على أحواض مرجعية دون معالجتها باستخدام البروبيوتيك. تم وضع أسماك صحية وموحدة الحجم (٣,١ ± ٠,٥ جرام) بمعدل ٢٥ سمكة / خزان وتم وضع كل من الأحواض المعالجة والمرجعية على تصميم عشوائي وثلاث مكررات. تم تقييم معايير جودة المياه (الفيزيائية والكيميائية)، والحمل الميكروبي في الماء، وأداء النمو وتركيب الجسم للأسماك خلال فترة الاستزراع. لوحظ وجود فرق معنوي ($P < 0.01$) في النمو بين المعاملات، حيث تم تسجيل أعلى نمو في الأسماك المعالجة بالبروتكسين. لم تظهر معايير جودة المياه والتركيب التقريبي للجسم اختلافات كبيرة. كما لوحظ وجود انخفاض كبير في عدد البكتيريا وتحسن النمو في المياه المعالجة بنوع Sanolife Pro W، بالمقارنة مع الأحواض المرجعية. تظهر النتائج أن نوع Sanolife تقلل بشكل كبير من عدد البكتيريا في مياه الاستزراع وأن نوع Protexin يحسن نمو الأسماك. نتائج الدراسة لها تطبيق كبير في مجال الاستزراع المائي للبلطي في المملكة.

الكلمات المفتاحية: البروبيوتيك، النمو، التجمعات الميكروبية، البلطي.