Thesis for the Degree of Master of Fisheries Science

Effects of Feed Additive Probiotics on Growth Performance of Blackhead Seabream (*Acanthopagrus Schlegelii*) and Water Quality in Recirculating Aquaculture System (RAS)

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

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사료첨가 프로바이오틱스가 순환여과양식 시스템 내 감성돔의 성장과 수질에 미치는 영향

Advisor: Prof PARK Jeonghwan

by

Dickson Amankwaah

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Abstract

Recirculating aquaculture systems (RAS) for fish culture has been used for decades. The RAS system has advantages of using small land area and minimal water volume for intensive production. Although RAS has proven to be very efficient, it still has some challenges. One of such challenges is poor water quality (high levels of ammonia nitrate and nitrite nitrate) which mainly emanates from accumulated fish waste and uneaten feed. Probiotics have been adopted by many fish farmers as the best alternative to control microbial infections compared to antibiotics, as it improves water quality, boosts fish immune system and helps fish growth. Although many researches have been conducted on the effectiveness of probiotics in ponds and laboratory, little research has been done on its effectives in improving water quality and fish growth in the RAS. To investigate the effectiveness of probiotics in promoting fish growth and water quality, a 60-days experiment was carried out in a RAS on blackhead seabream, *Acanthopagrus schlegelii* using commercial probiotics. The experiment was conducted in four identical RASs and each system consisted of three tanks (70 L each) with a water treatment system. Fifty fingerlings (34 ±0.20 g, 5.95±0.25 cm) were stocked per tank in all the 12 tanks. Probiotics was applied to fish feed in three different concentrations (2.5 g/kg feed T-1, 5 g/kg feed T-2, 10 g/kg feed T-3) and were fed twice daily. Water temperature was controlled using an electric heater with a thermostat and was maintained at 24.5±0.5ºC. Salinity and pH were maintained at 29.5±0.5 ppt and 7.5±1.0 respectively. Specific growth rate (SGR), food conversion ratio (FC), protein efficiency ratio (PER), survival rate (SR) were measured to assess the growth performance and DO, pH, ammonia nitrogen (TAN), nitrite-nitrogen (NO^{-}_{2}-N) and nitrate-nitrogen (NO^{-}_{3}-N), chemical oxygen demand (COD) biological oxygen demand (BOD_5), water color, turbidity were also measured to assess the water quality status during the experimental period. The results showed significant improvement in water quality in probiotics treated tanks as there were reductions in TAN and NO^{-}_{2}-N, COD, BOD_5, turbidity levels (*$P<0.05$*) than in control. The DO and pH, did not show significant differences (*$P>0.05$*) among all the groups although lower levels of pH and DO were recorded in T3. However, there was significant difference in DO (*$P<0.05$*) between T2 and T3 and between control and T2. The growth parameters of the fish did not show

any significant difference (*$P>0.05$*) among all groups as SGR, FCR, PER, SR, average body weight and body length were all statistically not significant (*$P>0.05$*). In this study, probiotics appeared to have effect on improving water quality in the RAS but did not show any clear improvement on fish growth in such experimental period.

Keywords: probiotics, chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), growth rate, water quality

1. Introduction

Aquaculture has been considered a business with very promising future prospects as there has been a consistent decline in marine resources over the years. For the growing population and the increasing world food demand, the best alternative to marine fishing is aquaculture (Martinez et al., 2012). There has been a rise of 14% in the global capture fisheries production whiles aquaculture production has risen to 527% from 1990 to 2018. The aquaculture industry currently employs over 20.5 million people worldwide (FAO, 2020). However, the industry, has some challenges and most of them are related to the destruction of ecosystems mainly through effluents discharge into the environmental and on receiving water bodies (Martinez et al., 2012).

As population increases, it also comes with infrastructural development and land acquisition for farming in urban areas has always been a challenge. For this reason, traditional ponds which requires a very large surface area to operate has to struggle for land and battle with environmental laws and policies due to their effluents discharge.

To be able to produce within a small area with little or no effluents discharged into the environment, recirculating aquaculture system (RAS) is the best alternative to traditional earthen pond or flow through systems, as it has advantages of using minimal water and a small area to produce high yield as well as saving the environment from pollution. RAS is considered a system with very great future prospects with a minimum negative impact on the environment. RAS farming is an excellent usage of technology to manage water quality and promotes fish growth by controlling internal environment. This system also gives farmers easier controls in effluent and waste compared to other systems. RAS has many challenges and one of such challenges is poor water quality which mainly emanates from accumulated fish waste and uneaten feed. This issue often results in disease problems which is associated with pathogenic microorganism biota (Ringo and Birkbeck, 1999). The control of these microorganism has been heavily relied on the use of vaccines for decades. Adverse effects of antibiotics to control diseases have however created other problems. The effects from those measures include its accumulation in tissue and immuno-suppression (El-Haroun et al., 2006; Tukmechi et al., 2007)

Because of these issues, growing concerns about the use of chemical compounds, not only in human medicine also in aquaculture has led to interest in finding other methods of preventing losses in hatcheries (Rollo et al., 2006) and other aquaculture establishments.

The excessive usage of antibiotics as human commensal bacteria led to loss of confidence in the use of antibiotics which lead to its total ban in European Union (EU) countries in 2006 (Angelis et al., 2006). Because of the negative impacts of chemicals and antibiotics on the environment, the usage to control disease and other infections has therefore been discouraged. Probiotics are considered bio-friendly agents that can be administered in aquatic environments to control pathogens and enhance feed utilization, survival, growth rate and water quality without any undesirable side effects on the aquatic organisms and the aquatic ecosystem. (Mohapatra, 2013; Huynh, 2017). Many have reported on the beneficial effects of probiotics on fish and has recommended as a replacement for antimicrobial drugs and growth promoters. Some researchers are of the view that probiotics have advantages for improving fish health in aquaculture and general growth performance, (Allameh, 2017), although others have also shared their dissenting opinions on its effectiveness. Probiotics can be added in to feed to enhance the growth performance such as feed conversion ratio, protein efficiency ratio, digestibility and body composition (Allameh, 2017)

In the RAS, two very important groups of bacteria are present, autotrophs for nitrification and heterotrophs for ammonification (Sugita et al., 2005; Michaud et al., 2009; Gao et al., 2012).

The main objective of this study is to evaluate the effectiveness of commercial probiotics (RHODOMAX®, TIS Bioscience, Chennai, India) composed of *streptococcus*, *Bacillus mesentericus*, *Clostridium butyricum*, red yeast and excipient on growth performance and water quality of blackhead seabream, *Acanthopagrus schlegelii* in the RAS.

2. Literature review

Definition of Probiotics

Probiotics definition has gone through several modifications over the years. The Food and Agricultural Organization (FAO) and World Health Organization (WHO) defines it as live microorganisms that confer a health benefit on the host (David R. Mack, 2005).

Other authors define differently as probiotics as live, dead or constituents of microbial cells that improve the general health of the host organism via improvements in the microbial balance in the environment. It has also been described as viable microorganisms which confer on the host a beneficial effect due to an improvement in the intestinal microbial balance (Giorgio et al., 2010). However irrespective of the differences in its definition, probiotics are considered bio-friendly agents that can be administered in aquatic culture environments to control pathogens enhancing feed utilization, survival, and growth rate of farmed species without any undesirable side effects on treated organisms.

3. Materials and Methods

3.1 Experimental setup

A 60-days experiment was carried out in the Aquaculture Systems Engineering Laboratory (ASEL) in the department of Marine-bio materials and Aquaculture, College of Fisheries, Pukyong National University, Busan, South Korea. The experiment was conducted in four identical RASs and each system consisted of three tanks (70 L each) with a water treatment system. Fifty fingerlings (34±0.20 g, 5.95±0.25 cm) were stocked per tank in all the 12 tanks. Probiotics was applied to fish feed in three different concentrations (2.5 g/kg feed T-1, 5 g/kg feed T-2, 10 g/kg feed T-3) and were fed twice daily. Water temperature was controlled using an electric heater and a cooler with a thermostat and was maintained at 24.5 ±0.5ºC. Salinity and pH were maintained at 29.5±0.5 ppt and 7.5±1.0 respectively.
Each system consisted of three tanks, a solids filter, a water pump, a UV sterilizer, a water cooler and electric pump. Water flow rate was maintained at 300 ml/sec and daily water loss through evaporation and siphoning of uneaten feed and feces was replaced by about 15% of total system water. Water filter was cleaned three times a week to maintain high water quality.

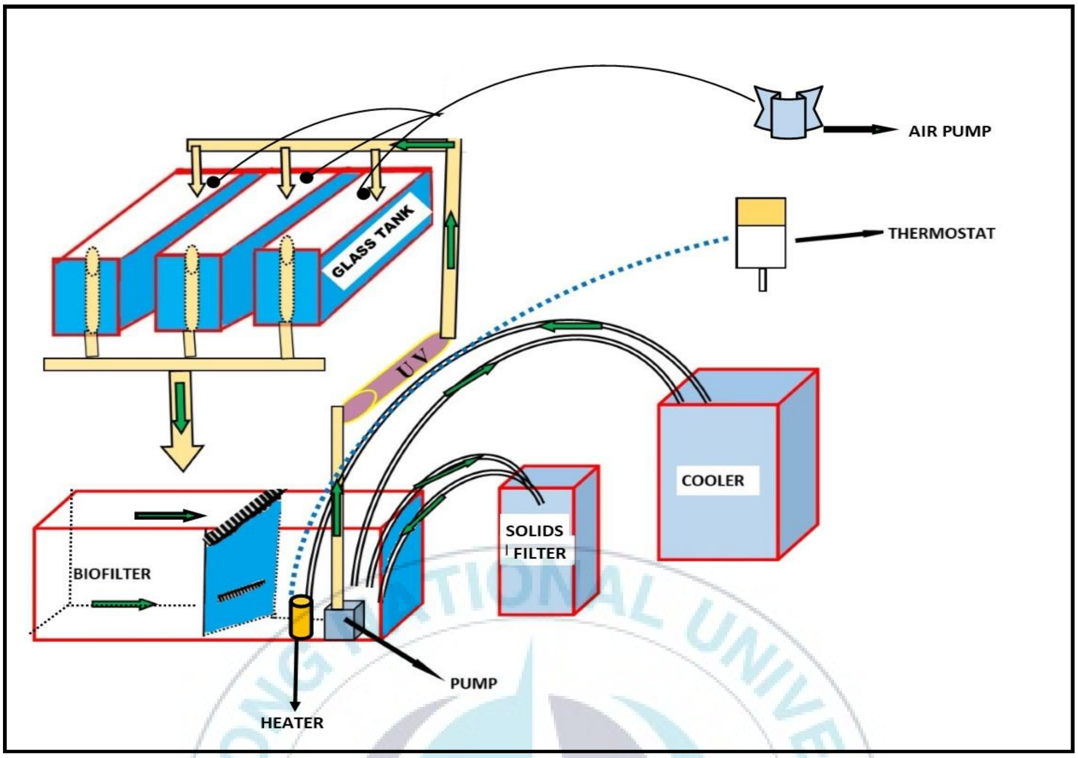

Fig. 1. RAS schematic drawing of the experimental recirculation aquaculture system.

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3.2. Experiment fish and feed

Black Seabream fingerlings with an average weight of 34.2±0.3 g were obtained from Wonheung Susan fish farm in Goeje, South Korea and were moved to AESL and stocked at 50 pieces per tank. Fish were fed on commercial diet according to each treatment. There were three treatments and one control with three replications in each treatment. Treatments were labelled control, T1 T2 and T3.

Probiotics (RHODOMAX®, TIS Bioscience, Chennai, India) was added to the commercial feed (Woon premium Aquafeed, South Korea, 50% crude protein, 10% crude fat, 10%, crude fibre, 18% crude ash, 3% Calcium, 1.2%, Phosphorus 2.7% and 14.0% moisture). Control feed was not added with the probiotics while treatment feeds contained different doses of probiotics (T1; 2.5 g/ kg of feed, T2; 5 g/ kg of feed T3; 10 g/kg of feed) probiotics applied to 1kg of feed).

Feed was mixed the with clean water (20 to 30 ml/kg of feed) and then measured probiotics was mixed with the moisturized feed, dried under room temperature.

3.3. Growth performance parameters

The following growth parameters were measured after 60 days: Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate,

Feed conversion ratio = feed intake/weight gain

Protein efficiency ratio (PER, %P) = Weight gained / protein consumed

Specific growth rate (SGR %/day) = $[(\ln W^f - \ln W^i) / d] \times 100$

where W^f is the final body weight in grams and

W_i is the initial body weight in grams

Survival rates SR (%) = (N^i - N^f x100)/ N^i

where N^i is initial fish number and N^f is final number of fish harvested

3.4. Water sampling and quality analysis

Dissolve oxygen (DO), pH, and water temperature were monitored daily using portable multimeter (HQ 40D, HACH CO, Loveland, Colorado, USA). Salinity was also measured daily with a salinity refractometer and turbidity was checked daily with HACH DR 900 multi-parameter colorimeter (HACH, Co., Loveland, CO, USA). Water samples were taken from each tank of all the four experimental units making a total of twelve samples to analyzed total ammonia nitrogen (TAN), nitrate nitrogen ($NO^-_{3-}N$) and nitrite nitrogen ($NO^-_{2-}N$) twice every with HACH DR 900 multi-parameter colorimeter (HACH, Co., Loveland, CO, USA) using the salicylate method, ferrous sulphate method and cadmium reduction method respectively. Chemical oxygen demand(COD) was determined using titrimetric, mid-level method, whiles BOD was analyzed using dilution method to determine the actual amount of oxygen consumed by bacterial (BOD_5) in oxidizing organic compounds in the water. Nitrogenous biological oxygen demand (N-BOD_5) and carbonaceous oxygen demand (C-BOD_5) were also determined using 2-chrolo-6(trichloromethy) pyridine nitrification inhibitor to know the proportion of dissolve oxygen which was consumed by either heterotrophic and autotrophic bacteria in the oxidation process. All samples and onsite measurements were taken in the morning between 9:00 to 9:30 am before water exchange.

3.5. Total body composition

Proximate body composition was determined at the end of the 60 days' experiment. A total of 9 fish were randomly taken from each system (3 from each tank) and were stored in a freezer at −40° C. Proximate analysis was conducted based on AOAC (1995) methods.

Crude protein content was analyzed using an automatic Kjeldahl distillation method, moisture content was measured at 135º C in a dry oven for 2hours, crude fat was measured using the soxhlet-extraction method and ash content was also analyzed using a muffle furnace at 550°C for 3 hours.

3.6. Statistical analysis

Two-way analysis of variance (ANOVA) was used to analyze the data. Multiple comparisons were performed with LSD test to analyze the differences between treatments. All statistical tests were performed using SPSS software (by SPSS Statistic 20.0 software (IBM, New York USA). Differences were considered statistically significant when $P<0.05$ and the results are expressed as average mean and standard deviation (mean ± SD).

4. Results and Discussion

4.1. Water quality parameters

The results of the experiment showed significant difference (*P<0.05*) among treatments for TAN, NO^-_2-N, NO^-_3-N, COD, BOD_5, C-BOD_5, N-BOD_5, water color and turbidity as shown Table 1. DO and pH on the other hand showed no significant difference (*P<0.05*) among treatments. There was, however, significant difference (*P<0.05*) between control and T2, and between T2 and T3 with respect to DO. Treatment 3 had low level of pH which was statically significant (*P<0.05*) comparable to control, T1 and T2.

The results showed low levels of TAN, NO^-_2-N, COD, BOD_5, C-BOD_5, N-BOD_5, water color and turbidity in the probiotics treated systems were lower than in control while showing high levels of NO^-_3-N in probiotics treated systems. The difference was however very evident in T2 as treatments T1. Multiple comparisons between treatments revealed lower levels of TAN in T2 and T3 which was statistically significant compared to control and T1 but no difference was observed between control and T1.

NO^-_2-N concertation comparison between treatments also showed significantly lower levels in all probiotic treated tanks and compared to control. NO^-_3-N concentration levels also showed high levels of concentration on probiotics treated systems than control. T3 had high level of nitrate but was not statistically different ($P>0.05$) from T2 but was statistically different ($P<0.05$) from control and T1.

The COD_5 levels observed, if compared between treatments also showed significantly higher ($P<0.05$) levels in control and T3 compared to T2 and but no significant difference was observed between control and T3. Treatment one (1) however was comparatively higher than treatment two (2) and the difference was significant ($P<0.05$).

The results on BOD_5 showed same trend as COD as control T1 and T3 recorded high levels compared to T2 just as the C-BOD_5 observed was also significantly higher in control and T1 than T2 and T3 but insignificant difference ($P<0.05$) was observed between T2 and T3. N-BOD_5 was significantly higher ($P<0.05$) in T3 than all other treatments. T2 also recorded higher value than control and T1 but between T1 and control, there was no difference statistically ($P>0.05$)

Table 1. Water quality parameters showing various average mean and standard deviation of DO, TAN, NO^{-2}-N, NO^{-}_{3}-N, COD, BOD_5, C-BOD_5, N-BOD_5, pH, water colour and turbidity.

	Control	T1	T2	T3	*P*-value
DO (mg/L)	6.72±0.71	6.92±0.54	7.41±0.34	6.73+0.81	0.070
TAN (mg/L)	1.13±0.51[a]	0.91±0.53[a]	0.52±0.43[b]	0.47±0.52[b]	0.024
NO^{-}_{2}-N (mg/L)	41.34±1.93[a]	26.72±9.14[a]	14.14±13[b]	8.14±10.04[b]	0.000
NO^{-}_{3}-N (mg/L)	36.53±8.92[b]	55.61±10.82[b]	61.92±20.22[a]	86.8±29.33[a]	0.000
COD (mg/L)	6.51±0.71[a]	5.63±0.82[ab]	3.91±0.73[c]	6.82±0.61[a]	0.000
BOD_5 (mg/L)	3.93±0.54[a]	2.73±0.44[b]	2.14±0.53[b]	3.20±0.34[a]	0.000
C-BOD_5 (mg/L)	3.15±0.62[a]	2.53±0.42[a]	1.60±0.32[b]	1.63±0.24[b]	0.000

N-BOD$_5$ (mg/L)	0.16±0.33^{c}	0.18±0.14^{c}	0.63±0.33^{b}	1.64±0.31^{a}	0.000
pH	7.24±0.53	7.11±0.57	7.04±0.63	6.73±0.71	0.503
Water colour	133.9±23.8^{a}	117.31±27.63^{a}	80.32±8.62^{b}	121.12±42^{a}	0.000
Turbidity	12.12±3.62^{a}	10.72±2.63^{a}	6.62±1.81^{b}	10.42±2.42^{a}	0.002

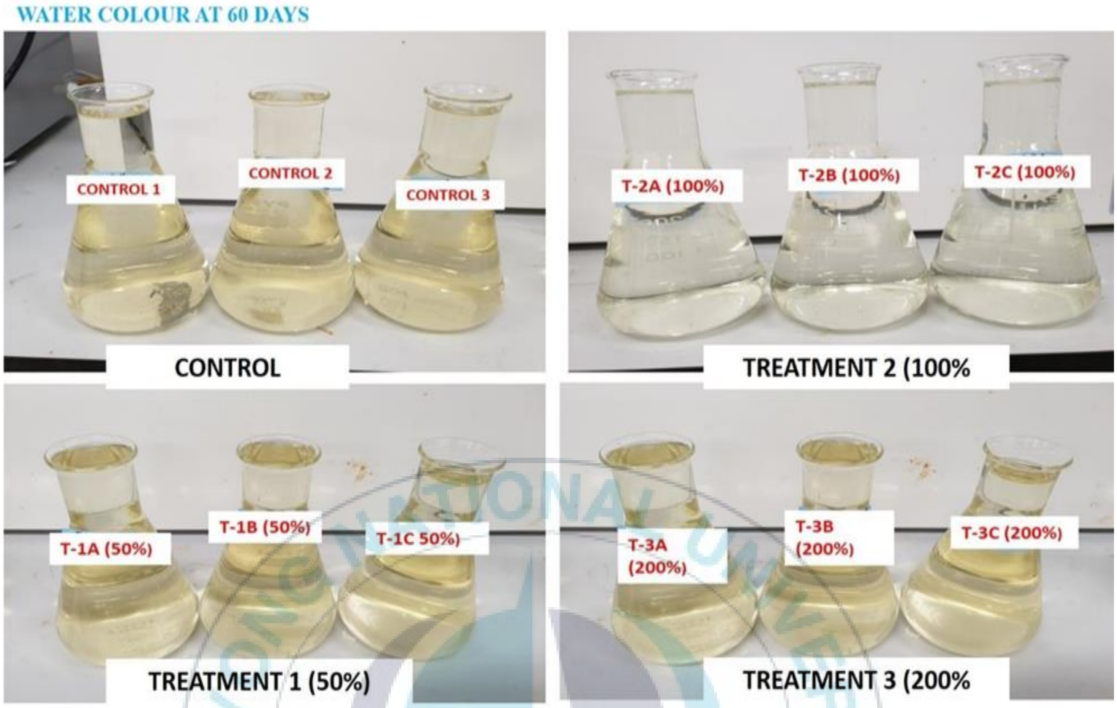

Fig. 2. Water colour of all systems t at the end of at 60 days' experimental period.

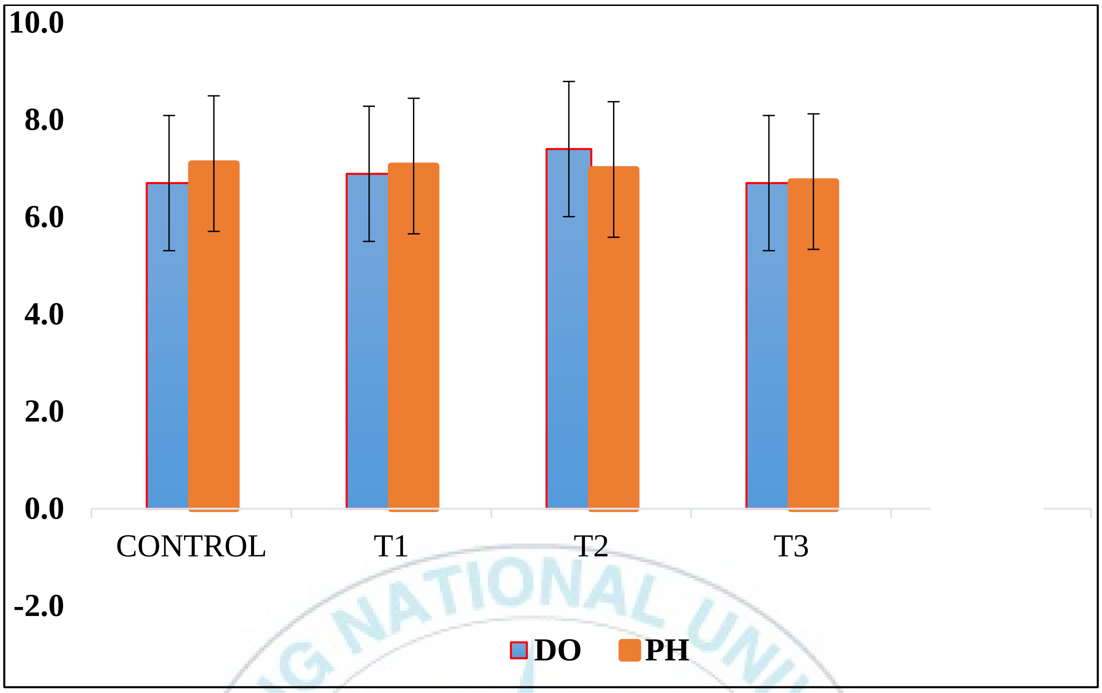

Fig. 3. DO and PH levels of treated systems and control showing the trend between the two parameters in RAS.

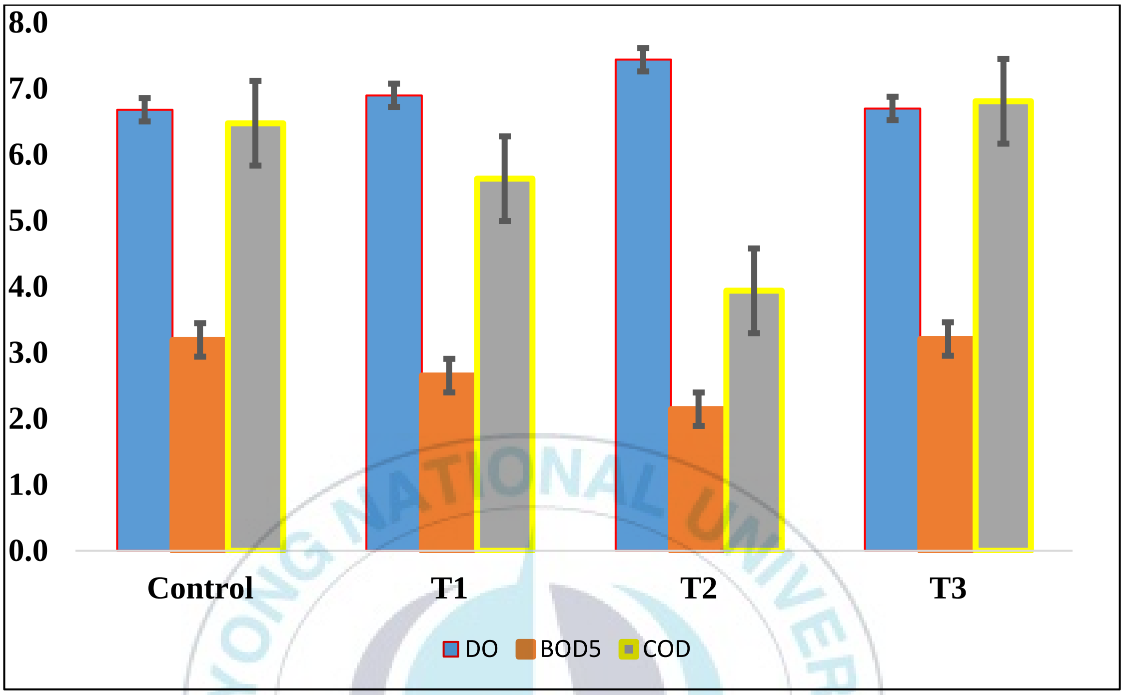

Fig. 4. DO, COD and BOD_5 and levels in treated systems and in control showing total DO consume by bacteria for degradation of organic components and the amount of oxygen which chemically oxidized organic and inorganics components in the systems.

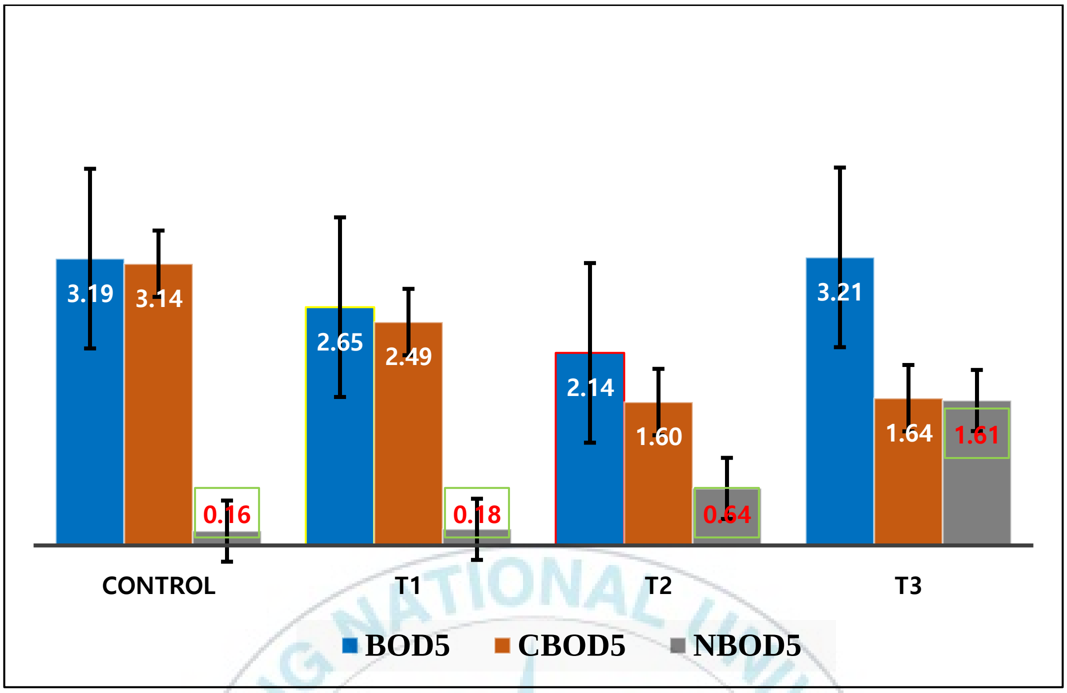

Fig. 5. BOD_5, $CBOD_5$ and $NBOD_5$ levels in treated systems and control showing amount of DO (mg/l) consumed by either autotrophic or heterotrophic bacteria in each system during oxidation

4.2. Growth Performance Parameters

From the results of the experiment, fish growth performance parameters did not show any clear improvement in probiotics treated systems compared to control as it shown on table 2. Generally, SGR, FCR, PER, total weight and survival rate recorded were statistically not different ($P>0.05$). Control however had lower FCR, higher SGR, higher individual average weight and higher total weight gained when compared to T3 and this difference was statistically different *(P<0.05*).

Table 2. Fish growth parameters showing average mean and standard deviation values of SGR, FCR, PER, SR, average weight, total weight and total length after 60 days.

	Control	**T1**	**T2**	**T3**	***P-value***
Av. weight (g)	74.44±0.24	71.12±0.34	73.24±0.51	69.30±0.42	0.07
Total weight (g)	1548.01±1.34	1511.30±1.41	1536.40±1.42	1487.33±1.31	0.08
SGR (%/d)	17.33±0.51	18.04±0.61	17.03±0.24	16.03±0.33	0.06
FCR	1.37±0.13	1.38±0.42	1.39±0.31	1.41±0.44	0.061
Total length (cm)	15.20±0.22	15.2±0.21	15.20±0.31	15.1±0.14	0.130

Survival rate (%)	94.03±1.04	93.02±1.14	94.0±1.02	95.0±1.31	0.120
PER (%)	1.46±0.24	1.42±0.32	1.45±0.41	1.40±0.32	0.062

4.3. Total body composition

Fish body composition analysis in the current research, showed low levels of crude fat compare to the control as indicated in the figure 6. Moisture content was higher in T2 than all other treatments and control. However, levels of crude protein and crude ash were higher in T1 than all other treatments and control.

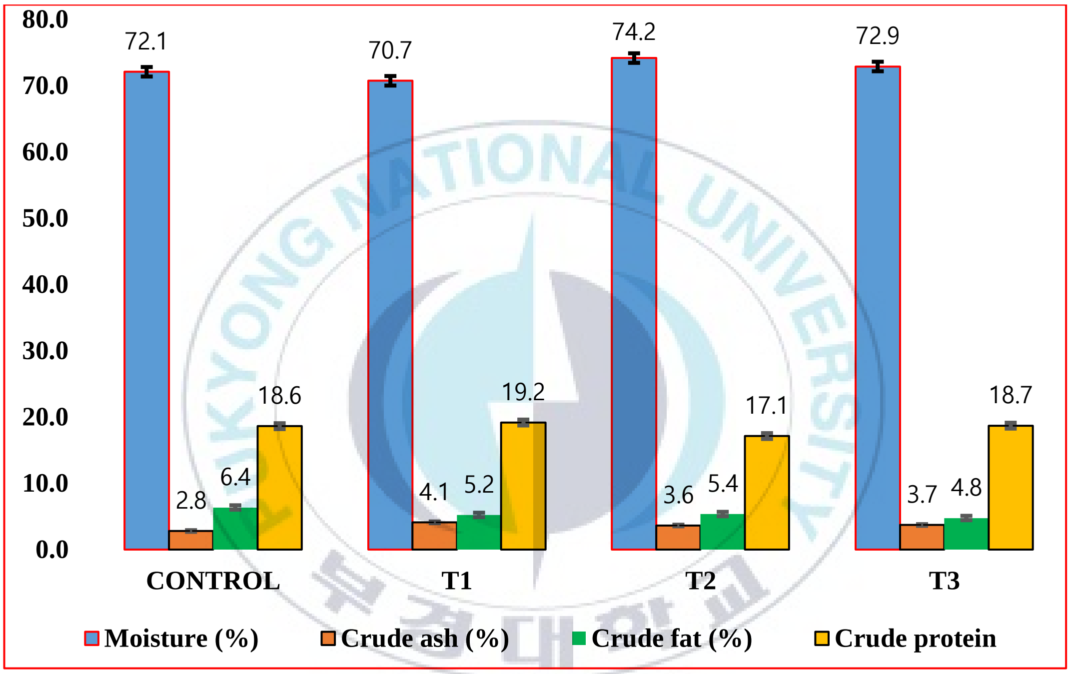

Fig. 6. Body composition of black seabream, *Acanthopagrus schlegelii* after 60 days in a RAS.

5. Discussion

The primary role of heterotrophic bacteria is considered to be the decomposition and mineralization of dissolved and particulate organic nitrogen (Pomeroy, 1974). Probiotic bacteria are known to improve water quality in diverse ways. Heterotrophic bacteria play a significant role in the decomposition of organic matter and production of particulate food materials from dissolved organics (Sunitha, 2016). There are many studies on the relationship between heterotrophic bacteria and water quality (Fang et al., 1989; Liu et al., 1992)

Boyd CE, 1998 reported less effects of probiotics on water or bottom soil quality and attributed it to the low concentrations of probiotics recommended by commercial probiotics manufacturing companies due to the high cost of the product. This research however, is in disagreement with this report as the treatment that was given twice, the manufactures recommended dosage, did not produce any significant results, both in water quality and in growth comparable to the manufactures' prescribed concentration. Even though this research partially supports his findings, as little or no impact was found on some of the parameters investigated, but it was not as a result of low concentration probiotics application as it was concluded in his research.

The research revealed a relatively higher levels of dissolved oxygen in Pond T3 and could be attributed to the beneficial effect of probiotics which favored the mineralization of organic matter (Padmavathi et al., 2012). There was also a significant positive linear correlation between pH of water and the dissolved oxygen as also reported by Jak et al., 1998; Zang et al., 2011 and Mavathi et al., 2012. According to previous research done, bacteriological nitrification is the most practical and easy method of removing ammonia from closed aquaculture systems. Earlier studies on *Bacillus. substilis* (Olmos J. et al., 2014); Gazi et al., 2015; also revealed that, species of *Bacillus* are able to grow under aerobic, facultative aerobic and anaerobic conditions, allowing for switches in nitrogen metabolism which enhances both nitrification and denitrification. (Daims et al., 2015; Narihiro T., 2016) and might have contributed to the comparatively high levels of DO, low turbidly and reduction in TAN in T2. This is because *Bacillus mesentericus* species within genus *Bacillus* are gram-positive, catalase-positive, endosporing, aerobic or facultative anaerobes (Ringo, 2010) *streptococcus faecalis and Clostridium butyricum* that were used for the probiotic, can all decompose organic matter in water under anaerobic condition as they are facultative anaerobic. This can also be the reason of low levels of COD and BOD_5 and turbidity in 100% probiotic treated tanks. From the experiment, it was observed that, BOD_5 which is required to oxide organic matter in the 100% treated pond was significantly lower (2.14 mg/l) with 29.6% (0.6 mg/l) being the actual amount of oxygen consumed by heterotrophic bacteria (N-BOD5) in the oxidation process and 74% (1.6mg/l) carbonaceous oxygen demand. This comparable to control and T2 was significantly lower. T3 recorded BOD level of 3.21 mg/l with 50.3% (1.64 mg/l) being $NBOD_5$ and 49.7% (1.61 mg/l) being

$CBOD_5$ and this shows and exclusive dissolve oxygen competition between autotrophic bacteria and heterotrophic bacteria in the system.

The high levels of BOD_5 and COD in T2 could also be attributed to high concentration of bacterial numbers competing with autotropic bacteria in the water for oxygen which could inhibit the nitrification process.

Matias, et al. (2002) also reported relatively a lower concentration in total TAN, NO^-_3-N, COD and BOD_5, pH in ponds treated with a mixture of *Bacillus sp*. and *Saccharomyces species*, and observed slightly increased in dissolved oxygen and transparency (turbidity which also supports the findings of Boyd CE, et al., (1988) who reported low levels of BOD_5, COD, TAN, NO^-_2-N, NO^-_3-N, in probiotics treated ponds attributed the low levels to the presence of *Bacillu*s species bacteria. When this is compared to previous studies. It suggests that, that probiotics are effective in bioremediation of fish aquaculture. (Narihiro, 2016)

It is therefore very critical to consider the characteristics of probiotics bacteria before is it selected as it is very important because selection of undesirable strains can lead to bad consequences in the host and the culturing environment (YunZhang Sun et al., 2013). Because of this reason, one must therefore consider the viability of the strain, resistance to antibiotics and ability to outcompete other strains (Gomez-Gil et al., 1998). Applying probiotics with the desired bacteria characteristics and appropriate concentration is capable of improving dissolves oxygen in aquaculture as Tucker and Lloyd (1985), reported significantly higher (*P<0.05*) dissolved oxygen concentrations in ponds treated with the bacterial suspension.

pH value is determined by the amount of carbon dioxide, which can react with water as well as carbonate and bicarbonate to form complex but reversible carbonate systems and is also influenced by changes of the ion concentration in the above equilibria. As pH decreases. Hydrogen ions and oxygen react with water, which results in a reduction of the dissolve oxygen (Jin, 1992).

There are several factors that affect heterotrophic bacterial reaction. Most important one is large amount of bacterial biomass produced by this reaction, compared to the autotrophic reaction. Another issue is the modest amount of alkalinity consumed as the carbon source and the resulting high levels of carbon dioxide produced (9.65 g/g TAN (J.M. Ebeling et al., 2006). For this reason, water with high heterotrophic bacteria usually show high CO2 and low pH (decrease in alkalinity and will generally require addition of carbonate, usually in the form of sodium bicarbonate to maintain reasonable alkalinity (100 to 150 mg/L as CaCO3). This implies that low levels of pH observed in treatment 3 was as a result of excessive application of probiotics which increased heterotrophic bacteria numbers in the water.

This explains why systems with relatively low pH also recorded low dissolve oxygen and can there be concluded that pH and dissolve oxygen (DO) has a positive correlation in aquaculture, as also reported by (C. Zang et al., 2010)

From the results of the experiment, it was revealed that there was no significant difference ($P<0.05$) between control and probiotics treated tanks with respect to growth parameters and this infers that probiotics does not have any significant improvement on fish growth which also confirms the findings of Boyd et al., 1984; Tucker and Lloyd (1985); Shariff et al., 2001; H.K. Pal, et al., 2015). Similar trends were reported by Ziaei-Nejad, 2005 and Hidalgo et al., 2006 that *Bacillus* probiotics was able to improved water quality and productivity but not growth.

These findings on the hand is in contradiction with observations made by (Lee S. et al., 2018) on synergistic effects of dietary supplementation of Bacillus subtilis WB60 and manna oligosaccharide (MOS) on growth performance, immunity and disease resistance in Japanese eel, Buruiană et al. (2014); SK, et al. (2017), Muhammad, 2019, also reported similar trend as they also observed improvement in growth parameters in probiotics treated ponds that other ordinary systems. Kristina et al., (2015) indicated that replacing indigenous microbial population with probiotics entirely, would not be prudent in respect to fish growth performance and this might have contributed to the insignificance effect of probiotics on fish growth performance. Considering the water quality parameters and growth performance in the control, it appears that RAS is self-sufficient enough to improve sea water quality (Park et al., 2013) and growth without depending on probiotics but requires regular cleaning of the filtering system, removal of waste feed and sludge and daily monitoring of the water parameters.

6. Conclusion and Recommendations

Probiotics as an alternative to chemicals and antibiotics have proven to be efficient in promoting aquaculture, as they have the potential to improve water quality in the Recirculating Aquaculture system. The usage of probiotics however must not be abused as excessive application of probiotics does not improve fish growth but can have a negative repercussion on water quality as well. Probiotics users must therefore adhere to the recommended dosage prescribed by manufacturing companies on their products. The effectiveness of probiotics in the RAS system is however also depends on waste feed management, filtering system cleansing, proper response feeding and daily water exchange. When the aforementioned parameters are well managed in RAS system, leads to good water quality and growth. It can therefore be concluded that, although probiotics was able to improve some of the water parameters, it could not improve fish growth which is the ultimate goal of every farmer better than the control. The relatively lower levels of TAN, NO^{-}_{2}-N, turbidity, COD, BOD_5 in probiotics treated systems did not show significant improvement in fish growth performance neither did the relatively higher levels of these parameters recorded in the control system also impeded fish growth. Based on this, it can be concluded that RAS is very self-sufficient system which is capable of maintaining good water quality and growth if it is carefully managed well without depending on probiotics.

There has been a lot of controversies and counter research findings surrounding probiotics usage, especially on growth improvement and water quality enhancement. Many commercial probiotics in the market labels on their products many benefits of products which convinces many farmers to patronize their products. Some of the common benefits that are usually written on these products are; “normalizes internal pH, eliminates pathogenic bacteria in the intestines, enhances feed assimilation and promotes better health, increases growth and improves profitability, shortens culture by reaching market fast “etc. But these manufactures do not state the exact culture conditions under which all the aforementioned benefit can be achieved. And this has contributed to the emergence of conflicting research findings on the efficacy of probiotics in Aquaculture. In order to hold probiotics companies accountable for the consequence of their products, they should also state categorically the culture conditions under which all benefits of probiotics mentioned on their products could be achieved.

Farmers must therefore be careful in probiotics usage as one might invest sums of capital and put all his hopes in high expectation on these products and receive very little output in return. From the results of the experiment, even though a clear improvement in growth was not seen in the probiotics treated ponds within the experimental period, I recommend further research to be carried out on this subject for a longer culture period (beyond 60 days) to evaluate the long time effects of probiotics on fish growth and water quality in RAS.

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