RESEARCH ARTICLE

Assessment of Probiotic Application on Natural Food, Water Quality and Growth Performance of Saline Tilapia *Oreochromis mossambicus* L. Cultured in Concrete Tanks

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Assessment of Probiotic Application on Natural Food, Water Quality and Growth Performance of Saline Tilapia *Oreochromis mossambicus* L. Cultured in Concrete Tanks

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Abstract
The effects of different levels of commercial probiotics BZT® BIO-AQUA on water quality, phytoplankton populations and total production were evaluated in saline tilapia *Oreochromis mossambicus* production tanks. The experiment consisted of 4 treatments with three replicates utilizing twelve 1 m^3 outdoor concrete tanks. All tanks were stocked with graded, nursed all-male tilapia fingerlings *O. mossambicus* (5.79–6.31 + 0.52 g/fish) for a 60 days culture period. Variations in phytoplankton populations were recorded throughout the experiment among treatments. *O. mossambicus* associated with probiotics to their tanks has a higher significant effects ($p < 0.05$) on final weight, % weight gain, SGR% and FCR than in control treatment, respectively. *O. mossambicus* average body weight (ABW) was higher ($p < 0.05$) and tend to grow with time for overall treatments with better performance in probiotics treated fish. Water quality was better in treatments of *O. mossambicus* associated with probiotics and suggesting providing further control on water quality, growth performance and phytoplankton production, specifically when applied.

Keywords: Probiotics; water quality; phytoplankton; growth and survival; *Oreochromis mossambicus.*

1. Introduction

Addition of artificial feeds in tilapia culture plays an important role especially under conditions of heavy stocking when natural food supply has declined or completely disappeared. The added feeds should be rich in protein, carbohydrate and fats along with vitamins, minerals and growth promoting substances to be physiologically balanced [1]. The high stocking density and introduction of much feeds and chemicals somehow altered the ecology of the culture systems especially ponds. The accumulation of unconsumed feeds and metabolic wastes caused water quality problems and eventually disease outbreaks [2]. For aquaculture to fulfill its potentials in both food and wealth creation, it is inevitable that fish farming will be conducted on an increasingly intensive scale and production methods will become more controlled and efficient [3].

Annual nutrient discharge reported about 5100, 2900 and 29500 kg of nitogen (N), phosphorous (P) and organic matter from a tilapia and catfish farm in USA was due to high level feed remnants, feces and excreta in the water [4]. This increases the level of effluent generated by the system. An excess (P) loading particularly has been associated with algal bloom leading to eutrophication especially in tropical waters. This problem becomes worse with intensive aquaculture because large quantities of feed are introduced to achieve increased fish production. The present knowledge of the ecological consequences of feed wastes is far from exhaustive and recommended for further studies [4]. Managing stable phytoplankton populations is a major challenge in aquaculture systems, especially if the cultured species does not graze phytoplankton directly [5]. Dense phytoplankton blooms with high photosynthetic rates can result in elevated pH levels, causing physical and physiological stress [6] and even mortality might occurs.

However, outbreak of diseases crippled the aquaculture industry in general. In order to enable the industry remaining steadfast, two promising technologies currently practiced are green water technology through tilapia water integration and bioaugmentation through probiotics use. Probiotics are employed in aquaculture to improve water quality by balancing bacterial population in water and reducing pathogenic bacterial load. Probiotics includes bacteria that can improve water quality of aquaculture, and/or exhibit the pathogen in the water thereby increasing production [7]. The Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds, improvement of water quality, enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes [8]. Thus, the use of probiotics in aquaculture has received some attention [9], therefore, mainly the objectives of this study will be to satisfy and to determine the effects of probiotics on water quality particularly nutrients loaded as nitrogen, nitrite and phosphorus, respectively.

Also to determine the effects of probiotics on biological parameters of the culture system in respect to phytoplankton population structures and relate the physical, chemical and biological parameters on the application of probiotics and its effects on physical condition, growth and survival of tilapia (*O. mossambicus*).

2. Methods

The experiment was carried out in 1 m^3 concrete tanks located at the Brackishwater Aquaculture Center, The University of the Philippines Visayas, Leganes, Iloilo, Philippines. Tanks were drained and allowed to dry prior to stocking. All tanks were treated with disinfectant (A) in order to eliminate filamentous algae and parasites. Probiotics was added along with different dosage during seawater culture prior and during the stocking of tilapia *O. mossambicus* as 0.02 g/m^3/week in treatment (B), 0.04 g/m^3/week in treatment (C) and 0.06 g/m^3/week in treatment (D), respectively. Water was added to achieve a depth of 1.00 m and maintained along with well provided aeration throughout the culture period. Apparently, all-male 5.79–6.31 + 0.52 g/fish healthy tilapia *O. mossambicus* fingerlings were held and acclimatized prior stocking in 3 m^3 fiberglass tank at said facility for two weeks time. Twelve concrete tank units were divided into (4) treatments in three replicates each. All treatments were compared with reference to tilapia growth, survival and phytoplankton productivity in the seawater. This comparisons provides basis for determining the growth of tilapia under different levels of a specific probiotics product applied in flow through tank culture system. Further, tilapia at prescribed stocking density was stocked in treatments A, B, C and D at 50 individuals for each tank.

Feeding was done daily and the quantity of food given was computed using the ABW during sampling intervals. Feeding frequency was done twice a day at 5% body weight for four weeks culture period. Samplings were conducted every week to observe the growth in terms of weight increment and survival as well. Upon termination, total biomass, % weight gain, daily weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival rate were calculated. Further, monitoring of physio-chemical and biological parameters was done weekly for dissolved oxygen concentrations (ppm), temperature (°C) and salinity (ppt) in all tanks which were monitored weekly using an YSI Model 57 Dissolved Oxygen Meter (Yellow Springs Instruments Co., Inc., Yellow Springs, Ohio, USA) *in situ*. For ammonia–nitrogen (ppm), nitrite (ppm), phosphorus (ppm) and phytoplankton count (organism/L) determination on the other hand one liter of seawater samples were taken from each tank and immediately transferred to the laboratory for analysis.

2.1. Statistical analysis

Findings of the study were analyzed using one way analysis of variance (ANOVA) utilizing 4*3 complete randomized design (CRD) [10]. Treatments with significant differences were further analyzed using Duncan's multiple range test (DMRT).

3. Results

3.1. Growth parameters

The growth indices of tilapia *O. mossambicus* fingerlings which fed with supplemented diets (Oversea Feed Corporation, crude protein 30%, crude fat 5%, crude fiber 6%, ash 12%, nitrogen free extracts 47% and moisture 12%) and addition of different levels of probiotics BZT® BIO-AQUA to the system were shown in (Table 1). Initial ABW body weight of *O. mossambicus* fingerlings fed the experimental diets at the start did not differ, indicating that groups were homogenous. At the end of the experimental period of 30 days, the groups of fish that had regular application of the probiotics grew well or better than the group of fish without probiotics application as control diet. Upon termination, the ABW of *O. mossambicus* in all treatments shows an increasing trend from the first sampling onwards.

It was observed that, treatment (D) with 0.06 g probiotics obtained a mean final weight of 9.03 g, treatment (C) with 0.04 g probiotics obtained 8.73 g, treatment (B) with 0.02 g probiotics obtained 8.96 g and treatment (A) without probiotics obtained 8.03 g, respectively. The lowest final body weight 8.03 g was achieved by the control, group of fish without probiotics applications. The highest growth was observed in treatment (D) while the poorest was obtained in treatment (A). Statistical analysis showed that treatments with different levels of probiotics application revealed that mean weight of *O. mossambicus* was significantly different from each other.

Total biomass of the fish after culture period was observed significantly higher ($p < 0.05$) in treatments with probiotics compared with free probiotics treatment as higher as treatment (B) with 0.02 g probiotics level as 340.49 g, treatment (C) with 0.04 g probiotic level as 328.78 g, followed by treatment (D) with 0.06 g probiotics level obtained 325.38 g. On the other hand the lowest biomass value achieved by free probiotic treatment as 238.84 g, respectively.

FCR and SGR of fish with BZT® inclusion were superior to those of fish without BZT® inclusion wherein better in treatments with probiotics level 0.02 g which recorded as 1.34, followed by both treatments with 0.04, 0.06 g probiotics levels at 1.56. However, higher value obtained with probiotics free treatment as 1.87. Statistical analysis showed no significant difference ($p < 0.05$) among all treatments with higher FCR. Highest survival rate obtained in treatments (C) and (D) as 98.70%, followed by treatment (B) which has 98%, respectively. On the other hand, low survival rate was achieved by treatment (A) with 97.33%. Generally, treatments with probiotics applications have better survival rates compared to probiotics free treatment. Statistical analysis showed no significant differences in survival among all treatments ($p < 0.05$) (Table 1). Overall, there were no significant differences in growth ($p < 0.05$) among fish offered diets with various inclusion levels of BZT® application to the culture system, respectively.

Table 1: Growth performance of all-male *O. mossambicus* raised in concrete tanks with different levels of probiotics applied to the culture system for 30 days culture period.

Probiotics levels	Initial ABW (g)	Final ABW (g)	Mean weight gain (g)	Total biomass (g/m³)	Weight gain (% Fish–1)	FCR	SGR (%)	Survival (%)
A, Control	5.79 ± 0.02^{a}	8.03 ± 0.01^{a}	2.24 ± 0.00^{a}	238.84 ± 1.12^{a}	38.687 ± 0.31^{a}	1.87^{a}	1.09 ± 0.001^{a}	97.33^{a}
B, 0.02 g	6.16 ± 0.10^{a}	8.96 ± 0.00^{b}	2.81 ± 0.21^{b}	340.49 ± 0.89^{a}	45.617 ± 0.12^{b}	1.34^{a}	1.24 ± 0.060^{b}	98^{a}
C, 0.04 g	5.94 ± 0.01^{a}	8.73 ± 0.00^{b}	2.79 ± 0.12^{b}	328.78 ± 0.90^{b}	46.970 ± 0.41^{b}	1.56^{a}	1.28 ± 0.014^{b}	98.70^{a}
D, 0.06 g	6.31 ± 0.20^{a}	9.03 ± 0.01^{b}	2.72 ± 0.01^{b}	325.38 ± 0.82^{b}	43.106 ± 0.01^{b}	1.56^{a}	1.19 ± 0.002^{b}	98.70^{a}

^{ab}Mean values with different superscript letters in the same column were significantly different among treatments ($p < 0.05$).

3.2. Water quality management

The weekly variations of dissolved oxygen level (ppm) in tanks after 30 days was observed to be higher during the third week with probiotics free treatment (A) as 5.67 ppm, and slightly lower during the fourth week of the culture period in treatment (C) as 3.70 ppm, respectively. Dissolved oxygen readings range from 3.70 to 5.67 ppm. The average temperature of the water in tanks during 30 days ranges from 28 to 29°C. The lowest temperature observed in first sampling in treatment (A), (C) and (D) at 28°C, where as the highest value was observed in treatment (A) during the initial sampling at 29°C and treatment (D) at the final sampling as well. Salinity was maintained at 31 ppt throughout the course of the study.

The average level of ammonia–nitrogen in all tanks ranged from 0.3718 to 0.2709 mg/l, which fall within the limits of *O. mossambicus* tolerance in culture conditions. Highest ammonia–nitrogen level was obtained in treatment (A) without probiotics level at 0.3718 mg/l, where as lowest level obtained in treatment (D) with probiotics 0.06 g at 0.2834 mg/l, while treatments (B) and (C) with probiotics 0.02 and 0.04 g followed the trend as 0.2902 and 0.2709 mg/l accordingly. Significant difference in all treatments compared with treatment (C) was recorded. Table 2 showed the ammonia–nitrogen concentrations during *O. mossambicus* culture period. The average level of nitrite in all tanks ranged from 0.0499 to 0.0323 mg/l, which fall within the limits of *O. mossambicus* tolerance. Highest reactive nitrite level was obtained in treatment (A) without probiotics level as 0.0499 mg/l, where as lowest level obtained in treatment (C) with probiotics 0.04 g as 0.0323 mg/l, while treatment (B) and (D) with probiotics 0.02 and 0.06 g followed the trend as 0.0350 and 0.0364 mg/l accordingly. Statistical analysis showed that treatments (B), (C) and (D) were significantly lower compared with treatments (A).

The average level of reactive phosphorus in all tanks ranged from 0.1673 to 0.1126 mg/l, which fall within the limits of *O. mossambicus* tolerance. Highest phosphorus level was obtained in treatment (A) without probiotics level as 0.1673 mg/l, where as lowest level obtained in treatment (D) with 0.06 g probiotics 0.1126 mg/l, while treatment (B) and (C) with probiotics 0.02 and 0.04 g followed the trend as 0.1526 and 0.1215 mg/l accordingly. Statistical analysis showed that treatments (A) and (B) significantly higher compared with treatments (C) and (D), respectively.

Table 2: Mean values of water quality parameters measured in tanks of all-male *O. mossambicus* with different levels of probiotics.

Probiotics levels	Nitrogen (mg/l)	Nitrite (mg/l)	Phosphorus (mg/l)
A, Control	0.3718 ± 0.027^{a}	0.0499 ± 0.215^{a}	0.1673 ± 0.174^{a}
B, 0.02 g	0.2902 ± 0.034^{a}	0.0350 ± 0.213^{b}	0.1526 ± 0.181^{a}
C, 0.04 g	0.2709 ± 0.078^{b}	0.0323 ± 0.242^{b}	0.1215 ± 0.211^{b}
D, 0.06 g	0.2834 ± 0.063^{a}	0.0364 ± 0.229^{b}	0.1126 ± 0.171^{b}

^{ab}Mean values with different superscript letters in the same column were significantly different among treatments ($p < 0.05$).

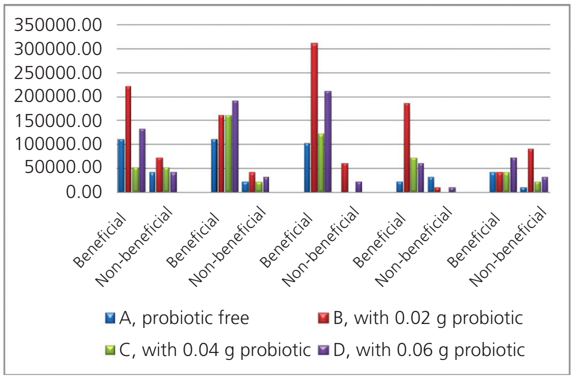
3.3. Biological parameters

Tilapia did not create a consistent long-term shift in the percent contribution of algal groups during the duration of the experiment. Detailed phytoplankton counts were made for sampling dates identified as important time periods regarding changes in phytoplankton population. Plankton analysis showed high values among treatments. Chlorophytes were the dominant group in all treatments throughout the majority of the experiment. *Chlorella*, on the other hand gradually bloomed and maximum density is attained after 6–7 days of culture. Also observed that green algae dominated in terms of algae abundance and number of identified species *chlorella* predominates all the algae presents in all treatments (Figure 1).

4. Discussion

Application of BZT® supplement to fish seawater system appeared to provide beneficial effects on growth of *O. mossambicus* although not on survival. Numerous trials were conducted with microorganisms as probiotics to improve cultivability of food species and to improve human health and welfare. Appropriate probiotics applications were shown to improve intestinal microbial balance, thus leading to improve food absorption [11, 12]

Figure 1: Mean values of dominated beneficial and non-beneficial phytoplankton in tanks of all-male *O. mossambicus* with different levels of probiotics.



and reduce pathogenic problems in the gastrointestinal tract [13, 14]. Results indicated that fish offered BZT® inclusion exhibited greater growth than those without inclusion in control treatment (Table 1).

The final ABW, weight gain and SGR were increased among *O. mossambicus* with application probiotics treatments compared with free probiotic treatment. So we might assume to be considered as a growth promoter/enhancer in fish aquaculture. These results in agreement with [15] and [16] which were reported that the probiotics treated group enhancing growth rate of shrimps and maintaining water quality parameters. Survival of shrimps was significantly greater in treated groups than that of the control group.

Lactic acid bacteria had an effect as growth promoter on the growth rate in juvenile carp but not in seabass [17]. Fluctuations in growth performance occurred during second and fourth week of the culture period due to cyclones and bad weather reasons during the course of the study.

Probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet and by the breakdown of indigestible components [18]. *Streptococcus faecium* improved the growth and feed efficiency of Israeli carp [17]. A several probiotics species were used including *Lactobacillus* sp. [19]. The use of probiotics can improve the nutrition level and immunity of cultured animals to pathogenic microorganisms. In addition, the use of antibiotics can be reduced and frequent outbreaks of diseases can be prevented. Studied the naturally occurring bacteria which are able to promote the growth and survival of *Argopecten purpuratus* larvae by inhibiting the activity of other bacteria that flourish in hatchery cultures [20].

The growth of larvae of seabass fed noticed 1.1% live yeast as a probiotic was increased than control group [21]. Also, survival of larvae was significantly higher than the control. The addition of a grampositive probiotic bacterium increased survival, size uniformity and growth rate of marine fish larvae (snook, red drum, spotted seatrout and stripped mullet) was shown [22]. Much less work has been directed at the immunological enhancement of defense mechanisms of fish by probiotic bacteria or the protective mechanisms of probiotic bacteria in fish [23]. The average of FCR in fish group treatments A followed by group of fish in treatments C, D and B accordingly which were no significantly differed ($p < 0.05$) but improved in comparison with the other groups and better than the control treatment. The FCR was found to be 1.87, 1.56, 1.56 and 1.34, respectively. These results indicated that the best FCR values were obtained for group of fish with different levels of probiotics respectively. The best FCR values observed with probiotics BZT® BIO-AQUA applied to culture water suggested that addition of probiotics improved feed utilization. Similar results have been reported for probiotics use in diets for tilapia fingerlings by [24, 25]. In practical terms, the use of probiotics can decrease the amount of feed necessary for animal growth which could result in reductions of production cost.

A lot of bacterial cultures containing nitrifying bacteria to control the ammonia level in culture water are available commercially and are aimed especially at aquarium hobbyists. Nitrifies are responsible for the oxidation of ammonia to nitrite and subsequently to nitrate. Recent reports demonstrate that many bacterial strains may have a significant algicidal effect on many species of microalgae [26]. In this study, application of commercial probiotics was clearly beneficial for cultured *O. mossambicus* when administered to the water body. It is argued that such probiotics has a role in disease control strategies, growth promotion and improves the physical picture and biochemical parameters among *O. mossambicus* culture in tanks. Results of the experiment showed that *O. mossambicus* stocked in tanks with probiotics gained an increase in body weight compared to that of the tanks without probiotics, and attained a survival rate of 98.70% after 30 days of culture. It is clear from the result that probiotics has immense potential for use in aquaculture. It offers a novel strategy in controlling diseases and improving water quality as well as also helps in disease resistance of tilapia [27]. Moreover, they perform vital roles in rearing water; these prospective probiotics are envisioned to solve the biggest problem facing aquaculture industry 'diseases' in particular. Hopefully, this will then be translated into sustainable production of crustaceans as well as fin fishes and eventually to revival of the industry. Overall commercial probiotics used enhanced feed efficiency results in agreement with the findings of [24, 25].

5. Conclusion

In the present study, there was a significant difference ($p < 0.05$) between treatments in overall means for unionized ammonia–nitrogen, nitrite and phosphorus. Moreover, present study showed further improvement in water quality in terms of ammonia, nitrite and phosphorus in contrast with non-probiotics treatment, respectively. It is reasoned that by maintaining higher levels of these microorganisms in the production systems, which farmers can minimize the buildup of dissolved and particulate organic carbon during the culture cycle while promoting more stable phytoplankton blooms through the increased production of CO_2.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

All authors contributed equally to the production of this manuscript.

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