IJBPAS, December, 2018, 7(12): 2017-2027

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF VARYING DOSAGES OF YAKULT IN THE WATER QUALITY OF RED TILAPIA (*Oreochromis* spp.) UNDER AQUARIA CONDITION

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Received 4th April 2018; Revised 26th April 2018; Accepted 26th Oct. 2018; Available online 1st Dec. 2018

https://doi.org/10.31032/IJBPAS/2018/7.12.4594

ABSTRACT

This study was conducted to evaluate the effect of varying dosages of Yakult containing *L. casei* Shirota strain in the water quality, growth performance, and survival rate of red tilapia (*Oreochromis* spp.) cultured in aquaria. Treatments evaluated were: Treatment 1 (Control), Treatment 2 (20ml of Yakult directly applied in the water), Treatment 3 (40ml of Yakult directly applied in the water), and Treatment 4 (60ml of Yakult directly applied in the water). Results of water quality analyses showed that there were significant differences among the treatments in dissolve oxygen (DO), pH and total ammonia nitrogen (TAN) while temperature and water hardness were comparable ($P<0.05$). For the pH and DO, T1 and T2 are significantly different with T3 and T4, while for TAN, T1 is significantly different among treatments except T2. Regarding the growth performance, there was no significant difference ($P<0.05$) in the gain in weight among the treatments. Likewise, no significant difference among the treatments ($P<0.05$) was observed on survival rate despite, T4 showed the best performance (100%). Results indicate that increasing amount of Yakult decreases the level of DO, increases the acidity of the water and TAN concentration. Thus, there is no effect on the growth performance and survival rate of the fish. Further studies focused on the population of the *L. casei* Shirota strain in water are recommended.

Keywords: red tilapia, Yakult, water quality parameters, probiotics

INTRODUCTION

Aquaculture is one of the world's fastest-growing food sectors [1, 2]. Its intensification was due to the increasing human population which resulted to higher demand for food [3]. However, this also led to environmental degradation due to the waste coming from these facilities and the absence of good management practices [4]. Intensification has caused disease and deterioration of aquatic environment [5]. Depleted dissolved oxygen in the ponds, discharges of concentrated organic waste, gave rise to toxic metabolites such as hydrogen sulfide, methane, ammonia, and nitrites which caused low mortality and low productivity of various crops that led to economic losses [6]. Researchers throughout the world are developing ways to conquer these negative impacts in aquaculture by the application of probiotics [5].

Probiotics are live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host. There are a lot of documented evidences that probiotics can improve the digestibility of nutrients [7], encourage reproduction [8], and increase tolerance to stress [9, 10]. It may also control pathogens and can be viewed as an alternative to antibiotic treatment [11]. In aquaculture, probiotics has been widely used as means of controlling diseases, enhancing immune system response, providing nutritional and enzymatic contributions in the digestion of host, and improving water quality [12]. In the study of El-haroun *et al*. (2006) [13], Nile tilapia (*Oreochromis niloticus* L.) which was supplemented with a commercial probiotic made from *Bacillus licheniformis* and *Bacillus subtilis* attained a significantly higher growth performance and nutrient utilization.

Yakult is a delicious probiotic drink that helps to improve digestion and helps build immunity in human. It is also proven as an important probiotic with many benefits such as the improvement of the balance of intestinal microbiota, volatile fatty acids, antitumor action, stimulation of the immune system, and antimicrobial activity [14]. It contains *Lactobacillus casei* strain Shirota that settle in human intestines alive and restore the balance of the beneficial or friendly bacteria in the gut. In the study of Hernandez *et al.* (2010) [10], results showed that *L. casei* Shirota strain can be used as a probiotics for fish and may help in improving the growth and stress resistance of juvenile Porthole livebearer *Poecilopsis gracili. Lactobacillus* species are commercially prepared probiotic products together with the

Saccharomyces cerevisiae, Bacillus sp., and *Enterococcus* sp., which can give a lot of health benefits [6].

Tilapia is one of the most common freshwater fish species used for aquaculture practices. The Nile tilapia, *(O.niloticus)*, is the most widely cultured tilapia in the world because of its rapid growth, late age of sexual maturity and planktivorous feeding habits. However, the red tilapia, *Oreochromis* spp*.,* is increasing in popularity among producers due to its attractive color, increased marketability and high salinity tolerance in some strains [15]. This species may also pose a high value in the Chinese market someday due to its red color which represents beauty, reunion, good fortune, happiness, success, and harmony [16]. Red tilapia for the Chinese market should, therefore, be well-pigmented; i.e. the entire body should be dominated by red pigments without black spots (blotches) [17].

Since red tilapia is a favorable species to culture and a commercially important food fish worldwide, it is essential to provide a suitable water environment and to increase growth performance that may result to high survival rate. The use of probiotics in aquaculture has just begun because the gastrointestinal microbiota of aquatic organisms has been poorly characterized, and their good effects are not studied extensively [5]. This study will lead to evaluate the existing possibility of Yakult as potential probiotic in aquaculture or water quality enhancer and also as survival promoter for red tilapia (*Oreochromis* spp).

MATERIALS AND METHOD

Experimental fish

A total of 120 Red tilapia (*Oreochromis* spp.) with average weight of 34 g – 44 g were used in this study. The fish samples were obtained from Freshwater Aquaculture Center, Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija and were conditioned for two weeks before the study began. Fish were fed two times a day with fry mash at 8:00 am and 4:00 pm, with a feeding rate of 3% of their body weight.

Experimental units and treatments

Twelve rectangular aquaria with a measurement of 60cm × 30cm × 30cm were set up at the FAC Wet Laboratory. The stocking density was ten Red tilapia per aquarium. Treatments are presented in Table 1. Treatment 1 (no Yakult) is the control while the other three treatments (T2, T3 and T4), were added with Yakult with varying concentrations. Each treatment was replicated thrice and the experimental setup used was Completely Randomized Design (CRD).

Table 1: Experimental treatments in the study

Treatments	Description
1(Control)	No Yakult
2	20ml of Yakult directly applied in the water (every after changing of water)
3	40ml of Yakultdirectly applied in the water (every after changing of water)
4	60ml of Yakultdirectly applied in the water (every after changing of water)

Cleaning of Aquaria

The aquaria were cleaned once every week by wiping the slimes attached on the walls and by siphoning the waste accumulated at the bottom. Water replacement was done 100% by siphoning the water out in aquaria. When the water level reached almost ¼ of aquaria another hose was used to fill the aquaria with clean fresh water. It was done every week during the study prior to the application of Yakult Shirota strain. Aerators were installed in each aquarium to ensure enough source of dissolved oxygen. Once a week, Yakult Shirota strain was added according to their designated dosage. But prior to adding of Yakult Shirota strain, the initial pH, dissolved oxygen, temperature, water hardness and nitrogenous waste of the water were measured.

Water Quality Monitoring

Daily pH, temperature and DO of the water were monitored twice, while water hardness and TAN were done once a week using the appropriate laboratory apparatus or equipment.

Fish Sampling

The experimental fish was sampled every 15 days prior to the addition of Yakult Shirota strain. It was done by weighing the experimental fish in each aquarium with the use of analytical balance. Total sampling was followed.

Data Gathered

Individual weight was determined prior to the start of the study. Gain in weight, survival rate and water parameters such as temperature, pH, DO, total ammonia nitrogen (TAN) and water hardness were determined.

Statistical Analysis

Analysis of Variance (ANOVA) was performed to analyze the data using significance level of $P<0.05$. Comparisons of treatment means of the different treatments were carried out using Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Temperature

Physiology, growth, reproduction and metabolism of tilapia are greatly affected by temperature [18]. Water temperature all

throughout the study ranged from 25.06 °C to 25.19 °C. It is well-suited to the acceptable range of temperature for tilapia which is 25 °C – 32 °C [19]. Furthermore, analysis of variance showed no significant difference among treatments (P<0.05). In the study of Pasala *et. al.,* (2018) [20] water applied with probiotics showed a narrow variation in water temperature which is considered to be characteristic of tropical waters [21, 22, 23, 24]. Optimal environmental condition is necessary to reach the best growth performance of the fish which is essential for the maximization and optimization of its production [25].

pH

The mean value of pH in the study ranged from 7.6 - 8.2. It is still generally within the acceptable level of 6.5 – 9 [26]. Treatments with high dosages of Yakult (T3 and T4) have significantly low levels (P<0.05) of pH compared to T1 (control) and T2. This may be due to the various compounds that can be produced by probiotics, which are inhibitory to the growth of pathogen that may include organic acids (lactic and acetic acids), bacteriocins, and reuterin [27]. *Lactobacillus* species are usually tolerant to high acid environments (pH range between 3 and 8) thereby imparting their benefits [28].

Dissolved Oxygen

DO in aquaculture systems is very vital. Insufficient levels of DO may lead to stress, poor appetite, slow growth, disease susceptibility and mortality in fish [29]. Mean DO levels in the study ranged from 3.24mg/l to 4.15mg/l. It is lower compared to the optimum level for tilapia which is above 5mg/l [30]. However, Nile tilapia has been proven to tolerate DO concentrations as low as 1mg/l; below this level, fish may gasp air from the water surface and utilize atmospheric oxygen [31].

It was observed that treatments with high dosages of Yakult (T3 and T4) obtained significantly low levels (P<0.05) of DO compared to T1 (control) and T2. This might be due to the higher amount of *L. casei* present in the water which also consumes DO in the water thus competing with the fish.

Dissolve oxygen has been found to be positively correlated with pH [32]. In the study, T1 and T2 have higher levels of both pH and DO. It has been proven that carbonates increase the pH of water on hydrolysis and photosynthetic release of oxygen which increases the dissolved oxygen content in the water [20].

Total Hardness

The two most important sources of water hardness are calcium and magnesium.

These are essential in the biological processes of aquatic animals, for example, bone and scale formation in fish [33]. It was observed in the study that highest mean value of water hardness was obtained by T1 (205.01 mg/L) and lowest mean value was attained by T4 (169.84 mg/L). According to Wurts (2002) [33], aquatic animals can tolerate a broad range of calcium hardness concentrations with desirable range between 75 and 200 mg/L $CaCO_3$. Furthermore, analysis of variance revealed no significant difference among treatments ($P<0.05$).

Total Ammonia Nitrogen (TAN)

The mean TAN readings in the study ranged from 1.86 mg/L to 3.06 mg/L. T4 got the highest mean value while T1 got the lowest mean value. ANOVA revealed that there is no significant difference between T1 and T2 ($P<0.05$). On the other hand, both T3 and T4 obtained significantly higher levels of TAN compared to T1 and T2 ($P<0.05$). This connotes that higher levels of TAN is observed in treatments with higher dosages of Yakult. However, results does not conform to the study of Pasala *et al.* (2018) [20] and Hossain *et al.* (2013) [34] wherein treatments with probiotics has lower levels of TAN compared to control. This may be due to the strain and amount of probiotics in Yakult. It is one of the commercially available drinks in the market containing high levels of probiotics with over 30 billion of *L. casei* Shirota strain in each bottle. However, TAN levels are still within the acceptable value which is <4 mg/L, while desirable range is within 0 – 2 mg/L [35].

Gained Weight

ANOVA showed no significant differences on the gain in weight of fish among treatments ($P<0.05$). However, results revealed that T4 which has the highest dosage of Yakult gave the highest mean value of 6.99 g. It has been proven in the study of Aly *et al.* (2008) [36] that probiotics such as *Bacillus subtilis* and *Lactobacillus acidophilus* had positive effect in the growth of *O. niloticus.* Moreover, results of the study of Mohapatra *et al.* (2011) [37] revealed that Rohu (*Labeo rohita*) fingerlings fed with a combination of three probiotics (*Bacillus subtilis, Lactococcus lactis* and *Saccharomyces cerevisiae*) showed higher growth, protein efficiency ratio, nutrient digestibility and lower feed conversion.

Fish Survival

Treatment 4 which has the highest Yakult dosage attained the highest survival rate which is 100%. Results also showed that as the dosage of Yakult increases, survival rate also increases (Table 3). Probiotics may prevent bacterial diseases through a variety

of mechanisms that may increase the survival rate of the fish. However, results showed no significant difference in the fish survival among treatments (P<0.05). This findings corroborate with Sutthi *et al.* (2018) [38] in which the Nile tilapia reared in probiotic-treated water for 90 days showed no significant difference in terms of survival rate.

Table 2: Mean values of water quality parameters: temperature, pH, D.O. Total hardness and TAN for the whole duration of the experiment.

Parameters	Treatments			
	1	2	3	4
Temperature	25.08 ± 1.09[a]	25.14 ± 1.15[a]	25.18 ± 1.08[a]	25.17 ± 1.14[a]
pH	7.89 ± 0.29[a]	7.93 ± 0.29[a]	7.82 ± 0.29[b]	7.83 ± 0.27[b]
D.O.	4.15 ± 0.96[a]	3.97 ± 0.92[a]	3.24 ± 1.109[b]	3.48 ± 1.29[b]
Total Hardness	205.01 ± 66.67[a]	187.32 ± 52.83[a]	179.58 ± 50.35[a]	169.84 ±46.46[a]
TAN	1.86 ±1.26[b]	2.75±1.44[ab]	2.94 ± 0.99[a]	3.06±1.51[a]

Note: Mean and SD in a row with the same letter of superscript are not significantly different at P<0.05.

Table 3: Mean values of survival rate, final weight and gained in weight of fish among treatments for the whole period of experiment.

Parameters	Treatments			
	1	2	3	4
Final Weight (g)	45.16±5.81[a]	48.24 ±2.49[a]	43.83 ±2.95[a]	46.35±6.25[a]
Fish Survival (%)	70 ±26.46[a]	86.67 ± 5.77[a]	93.33 ±5.77[a]	100 ±0.00[a]
Gained in Weight	5.74 ±2.79[a]	5.79 ±2.01[a]	5.37 ±1.84[a]	6.99 ±2.52[a]

Note: Means in rows with the same superscript are not significantly different at P<0.05 level of significance.

CONCLUSION

Yakult containing *L. casei* Shirota strain was tested as probiotic in Nile tilapia reared in aquaria. There were no significant differences on water temperature and hardness. However, increasing dosages of Yakult in the water significantly increases TAN. It was also noted that treatments with Yakult, significantly decreases pH and DO. Higher survival rates were recorded in treatments with Yakult but there was no significant difference in the growth performance of the fish among treatments. Thus, further study is needed focusing on the population of the *L. casei* Shirota strain in water.

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