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**EFFECT OF FERMENTED TARO CORM ON THE WATER QUALITY OF RED
TILAPIA (*Oreochromis* sp.) CULTURED IN OUTDOOR CONCRETE TANKS**

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ABSTRACT

This study was conducted to evaluate the effect of fermented taro corm (FTC) on water quality of Red tilapia (*Oreochromis* spp.) reared in outdoor concrete tanks. The treatments evaluated were: Treatment 1 (Control), Treatment 2 (0.005% of total water volume, 230 grams of FTC), Treatment 3 (0.010% of the water volume, 460 grams of FTC), and Treatment 4 (0.015% of the total water volume, 690 grams of FTC). Water quality parameters monitored are within the desirable level except for dissolved oxygen (DO). Application of fermented taro corm detected significant differences ($P < 0.05$) among treatments in terms of pH in which T2, T3, and T4 were significantly higher than the T1 ($P < 0.05$). T4 was significantly higher ($P < 0.05$) on DO readings. Comparison of treatment means in terms of weekly alkalinity showed that T1 was significantly lower than the rest of the treatments on the following week of the study ($P < 0.05$). Comparison among treatments on the gain weight of red tilapia revealed that T2, T3, T4 were significantly lower to T1. Results indicate that FTC decreases dissolved oxygen concentration in water; regulates the acidity of the water, increases alkalinity of the water and increasing amount of fermented taro corm applied in water causes slower growth of the species cultured in concrete tanks.

Keywords: red tilapia, fermented taro corm, water quality parameters, bioremediator

INTRODUCTION

Tilapia has been a prominent freshwater aquaculture species worldwide and mostly, Nile tilapia (*Oreochromis niloticus* L.) is the preferred cultured species. However, nowadays, red tilapia (*Oreochromis* spp.), is gaining popularity due to its market acceptability and for relative tolerance to a wide range of water temperature, dissolved oxygen (DO), salinity, pH, light intensity and photoperiods [1]. It can also pose a higher price in the market since well-pigmented red tilapia resembles some high valued-marine species such as sea bream (*Chrysophrys major*) and red snapper (*Lutjanus campechanus*) [2].

The growing popularity of red tilapia among consumers and the ever-increasing need to improve food production imposes the need to seek other production alternatives to culture this species [1]. Unfortunately, water pollution, diseases and natural hazards have become serious limiting factors reducing the production of fishes [4]. Sources of mortality in aquaculture systems include degraded water quality, nutritional imbalance, toxicants in the water as well as in improperly stored feed, poaching, pollutants and diseases [5]. The main water quality parameters that are essential for cultured organisms are transparency, temperature,

dissolved oxygen, pH, ammonia, etc. [4]. A consistent water source and good quality of water is vital in aquaculture.

In view of all potential environmental impacts as well as economic impacts through disease transmission and environment deterioration, it is suggested that the improvement in aquaculture waste management is a highly desirable objective [6]. In aquaculture, the use of living organisms primarily microorganisms (probiotics) is emerging as one of the most useful alternative technology for improving water quality, survival and growth rates and increased the health status of some aquatic animals as a means of bioremediation [3]. It has a very important role to play in the degradation of organic matter thereby significantly reducing the sludge and slime formation in tanks [7]. In addition, the application of probiotics as a biotechnological tool for bioremediation has been suggested to be effective in controlling and reducing wastes, pathogenic diseases, and toxic gases. Probiotics can be applied in the pond water or can be directly applied to the host or on the ambient environment of the cultured species [8]. It can also be added to its aquatic environment in several ways: addition via live food [9]; bathing [10];

addition to culture water [11], or as addition to artificial diet [12].

Fermented taro corm (FTC), or poi is a Hawaiian word for the primary Polynesian staple food made from the corm of the taro plant (*Colocasia esculenta* L.) [13]. Originating in Asia, this root crop is now found primarily in tropical and subtropical regions and was a major dietary staple in the Pacific islands. It can also be used as a nutritional supplement for weight gain in patients with conditions such as failure-to-thrive, cancer cachexia, aids, pancreatitis (cystic fibrosis), and some of the induced weight loss conditions of the gastrointestinal tract, such as inflammatory bowel disease [13].

The fermentation process of FTC is reported on the study of Huang *et al.* (1994). Acid production in FPC changes pH from 6.3 to 4.5 within 24 hours, and reaches its lowest pH on the fourth or fifth day of fermentation, when the FTC is usually discarded [14]. The predominant bacteria in FTC are *Lactococcus lactis* and *Lactobacilli*, both of which are lactic acid-producing bacteria and that the FTC contains significantly more of these bacteria per gram than yogurt [14]. It has been hypothesized that FTC can be a potential probiotics. The concept of probiotics in aquaculture is a biological

control agent by means of bioremediation. It can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms [15]. In addition, it is emerging as one of the most useful alternative technologies for removing contaminants from the environment, restoring contaminated sites, and preventing further pollution [16]. In this study, the efficacy of FTC was assessed as a bioremediator on the water quality specifically on Dissolved Oxygen (DO), pH, Total Ammonia Nitrogen (TAN), temperature and alkalinity of red tilapia cultured in outdoor tanks.

MATERIALS AND METHODS

Experimental units and treatments

Eight (8) 0.46 m³ outdoor circular concrete tanks were used in the experiment. Each tank was stocked with ten one month old mixed sex Red tilapia (*Oreochromis* spp.) fingerlings.

The study was done for one month and had four treatments which were replicated twice (Table 1). T1 (no FTC), while the other three treatments (T2, T3 and T4), was added with FTC with varying concentrations (%) of the total water volume. The experimental setup used was Completely Randomized Design (CRD).

Table 1: Experimental treatments used in the study with the description

Treatments	Descriptions
T1 (control)	no FTC added
T2	0.005% of the total water volume (230 g of FTC)
T3	0.01% of the total water volume (460 g of FTC)
T4	0.015% of the total water volume (690 g of FTC)

Preparation and application of fermented taro corm (FTC)

The FTC was made by following the ratio of taro corm to water (100 g taro corm : 10 ml water). The taro corms were obtained in the local market. It was steamed first, and then peeled, mashed and mixed until it formed a smooth and pasty starch. Then the pasty starch of the taro corm was put in sterilized jars, and stored in a dark and warm place for 2 to 4 days to allow fermentation. FTC was directly applied in the designated tanks one week after the stocking of red tilapia fingerlings. This was done in the succeeding weeks until the end of the study.

Sampling

Sampling of weight of the stocked red tilapia fingerlings was done weekly after before cleaning of tanks to determine the daily feeding ration. Five (50%) fingerlings

in each tank were weighed individually using a digital weighing scale.

Feeding management and cleaning of tanks

Commercial fry mash feeds was fed on the experimental fish twice a day, 8:00-9:00 AM and 3:00-4:00 PM. The feeding rates used for determining the appropriate amount of feed requirement was the 10% the average body weight (ABW) of the experimental fish.

The water in each tank was partially drained once a week throughout the study. It was replaced with new and fresh water after siphoning out waste from the bottom and cleaning.

Monitoring of water quality parameters

Water quality parameters such as dissolved oxygen (mg/L), pH, and temperature (°C) was recorded daily during the entire duration of the study. The pH was

measured using the pH meter instrument while the dissolved oxygen and temperature were measured by a DO meter device with temperature reading. These water quality parameters were measured at 10:00-11:00 in the morning and 3:00-4:00 in the afternoon

Chemical water quality parameters such as alkalinity (mg/L CaCO₃) total ammonia-nitrogen (mg/L) were recorded weekly throughout the duration of the study. Water samples were collected at 7:00-8:00 in the morning and analyzed in the laboratory. Chemical water quality parameters were analyzed after 6 days of the FTC application in the tanks.

Data gathered

The data gathered were initial weight and final weight of the fish and water quality parameters such as DO, pH, temperature, alkalinity and total ammonia-nitrogen (TAN).

Statistical Design and Data analysis

The data were subjected to Analysis of Variance (ANOVA) for Complete Randomized Design (CRD) using statistical program for social studies (SPSS) version 16.0. The Least Significant Difference was used to determine the differences among treatments.

RESULTS AND DISCUSSION

Temperature (°C)

Temperature is very important physical water quality parameter. This can affect the metabolic rate of the fish in the water and phytoplankton abundance. Across time mean weekly readings were within the acceptable temperature requirement for tilapia culture of 25°C to 32°C [17].

Analysis of variance revealed no significant difference among treatments ($P < 0.05$) except for week 4 of the morning in which T4 was significantly higher than the rest of treatments. Noted numerical differences in temperature among the mean of the treatments might be due to the turbidity of the water on treated tanks caused by the taro particles that remained after the application of FTC. During these periods, temperature relatively increases due to the turbidity of the water caused by the residue of FTC after the application that increases heat absorption ability of the water. During midday, water temperature increased with increasing turbidity [18].

pH (power of Hydrogen ion)

Power of hydrogen (pH) is defined as the negative logarithm of the hydrogen ion activity [17]. In fish culture, pH of the pond water plays a vital role for expressing acidity or alkalinity of the water on which 7 is neutral, lower values are more acid, higher values are more alkaline.

The pH weekly readings during the study ranged from 7.19 to 8.74 which are within the permissible range (6.5 to 9.0) for tilapia culture [19]. All treatments applied with FTC have significant differences with control. The numerical differences of the mean pH readings among treatments can be caused by the amount of microbes present in FTC. The carbon dioxide produced by the aquatic organisms could be converted to bicarbonate ions which have buffering action may have contributed. The assumption of Huang (1994) that FTC may contain certain microorganisms same with probiotics could be correct, which significantly regulated pH of the water.

Dissolved Oxygen (mg/L)

All living microorganisms like fish need dissolve oxygen (DO) for survival and growth, therefore it is important that DO level should always be maintained at the acceptable level. All treatments treated with FTC have lower level of DO recorded throughout the study. This might be the reason why the aquatic organisms reared in the tanks except for T1 begin to gasp at the surface of the water and rapid breathing was observed. This could have been indicative that the level of oxygen they received is not enough for them to survive. The ideal level

of DO should be 5 mg/L and above for the survival of most species [20].

Analysis of variance for morning and afternoon DO concentrations showed significant differences among treatments throughout the study. Treatment 1 was significantly higher than all of the treatments. It was observed that increased level of FTC decreased the DO level in the morning and afternoon. The lower DO levels in tanks that received FTC could be possibly due to more number of bacteria that consume DO to support their metabolic activities [21]. Other reason could be related to the turbidity of the water in the treated tanks. Suspended taro corm particles caused turbidity in which higher temperature can be recorded that also leads to decrease of DO.

Total Ammonia Nitrogen (mg/L)

Ammonia is one of the most important limiting factors for fish cultured in tanks and recirculating systems. The primary source of ammonia is the protein metabolism of the fish, feces and urine, uneaten feeds and decomposing organic matters. Obtained mean values ranged from 0.24 mg/L to 1.03 mg/L. Under tilapia culture, the ammonia should be less than 1 mg/L [22]. At the beginning, TAN level among all treatments are high but slightly decreased on the following week of the study. However, noted

numerical differences among treatments on the TAN readings shows no significant difference on the analysis of variance.

Alkalinity (mg/L CaCO₃)

Alkalinity is the total concentration of bases in water expressed in milligrams per liter of calcium carbonate (CaCO₃) [17]. The values obtained were within acceptable concentration which must be 20-400 mg/L [23]. Analysis of variance on the weekly alkalinity readings shows significant differences among treatments. In week 3, T1 was significantly lower among all of the treatments. T2 was comparable to T3 but significantly lower to T4, and T3 was significantly lower to T4. Alkaline forming elements present in the composition of FTC might have increased the concentration of alkalinity in the water. The results coincides with the findings of Brown and Valiere (2004) that despite of acidic pH, poi and taro are considered alkaline foods because their alkaline forming elements (Na, K, Ca, Mg) exceed their acid-forming elements (S, P, Cl).

Gain in weight (g)

Gain in weight (g) is another indicator of growth performance of an organism. Table 3 shows the mean results of gain weight per treatment of the study. T1 obtained the highest gain weight mean value while T4 obtained the lowest. Comparison among treatments revealed that T2, T3, T4 were significantly lower to T1 while T4 was significantly to T2. The increase in the amount of FTC resulted in decrease of gain weight of cultured red tilapia. Gain weight of red tilapia could be influenced by the turbidity of the water and the low concentration of dissolved oxygen caused by the suspended solids from the FTC. During the study, turbidity on the treated tanks was observed, consequently, the cultured red tilapia spotted at the surface of the water and gasping for air. Decreased oxygen availability is also considered a major factor in determining food intake of fish. The present study agrees with the study of Tsadik and Kutty (1987) on the influence of ambient oxygen on feeding and growth in *O. niloticus* that the fastest rate of gain in weight was at high DO or vice versa [24].

Table 2: Mean summary of the water quality parameters observed in Red tilapia (*Oreochromis* spp.) reared in outdoor concrete tanks

WATER QUALITY PARAMETERS	WEEKS	TREATMENTS			
		1	2	3	4
Temperature (°C) (morning)	1	25.07±1.62 ^a	24.67±1.61 ^a	24.81±1.26 ^a	24.95±1.25 ^a
	2	24.56±1.74 ^a	26.26±1.74 ^a	25.65±2.38 ^a	25.84±0.3 ^a
	3	24.92±1.33 ^a	24.50±1.27 ^a	24.48±1.23 ^a	24.80±1.18 ^a
	4	26.53±0.68 ^a	26.21±0.90 ^a	26.25±0.92 ^a	26.54±0.94 ^b
Temperature (°C) (afternoon)	1	28.54±2.88 ^a	28.05±2.17 ^a	27.17±1.31 ^a	27.56±0.97 ^a
	2	28.12±0.77 ^b	28.09±0.93 ^{ab}	27.74±0.43 ^a	27.81±0.34 ^a
	3	29.65±3.80 ^a	28.17±3.86 ^a	28.62±1.97 ^a	29.00±1.84 ^a
	4	30.18±3.30 ^b	29.98±3.36 ^{ab}	30.58±1.7 ^a	30.56±1.40 ^a
pH (°C) (morning)	1	7.79±0.32 ^b	7.56±0.28 ^a	7.56±0.23 ^a	7.48±0.22 ^a
	2	7.99±0.27 ^c	7.80±0.17 ^{ab}	7.65±0.13 ^{abd}	7.58±0.07 ^{ad}
	3	7.93±0.32 ^a	7.78±0.21 ^a	7.67±0.20 ^a	7.72±0.12 ^a
	4	8.31±0.20 ^b	8.08 ±0.26 ^b	7.91 ±0.26 ^b	7.83±0.24 ^a
pH (afternoon)	1	8.15±0.30 ^b	7.90±0.17 ^a	7.78±0.14 ^a	7.75±0.16 ^a
	2	8.45±0.37 ^b	8.05±0.43 ^{ab}	7.74±0.43 ^a	7.81±0.34 ^a
	3	8.50±0.26 ^b	8.33±0.21 ^{bc}	8.17±0.19 ^{ac}	8.26±0.27 ^a
	4	8.74±0.35 ^b	8.50±0.31 ^{ab}	8.34±0.28 ^a	8.26 ±0.26 ^a
Dissolve Oxygen (mg/L) (morning)	1	4.16±2.14 ^b	0.55±0.51 ^a	0.33±0.28 ^a	0.30±0.32 ^a
	2	3.24±0.87 ^d	1.23±1.26 ^c	0.11±0.06 ^{ab}	0.17±0.20 ^{ab}
	3	6.19±1.52 ^c	2.51±0.87 ^a	1.20±0.64 ^{ab}	0.90±1.09 ^{ab}
	4	8.31±0.20 ^c	3.96±2.30 ^b	1.36±1.82 ^a	0.74±0.85 ^a
Dissolve Oxygen (mg/L) (afternoon)	1	8.23±3.64 ^b	0.48±0.77 ^a	0.26±0.32 ^a	0.21±0.97 ^a
	2	11.72±3.59 ^b	1.37±1.01 ^a	0.09±0.04 ^a	0.11±0.11 ^a
	3	10.52±3.96 ^c	6.58±2.52 ^b	3.19±0.53 ^a	2.86±1.72 ^a
	4	12.57±2.30 ^c	7.85±2.96 ^b	2.54±2.03 ^a	8.26±0.26 ^a
Alkalinity (mg/L CaCO ₃)	1	384.00±8.91 ^a	305.00±2.82 ^a	166.00±15.56 ^b	296.50±92.72 ^a
	2	91.00±14.14 ^a	166.00±5.66 ^b	200.00±26.9 ^b	195.00±2.83 ^b
	3	190.00±0.00 ^b	310.00±14.14 ^c	280.00±71.71 ^{bc}	555.12±7.07 ^a
	4	220.00±14.14 ^b	320.00±15.14 ^a	305.20±21.07 ^a	282.26±53.68 ^{ab}
Total Ammonia Nitrogen	1	0.76±0.14 ^a	0.90±0.25 ^a	1.03±0.01 ^a	1.06±0.15 ^a
	2	0.50±0.028 ^a	0.47±0.03 ^a	0.52±0.02 ^a	0.28±0.02 ^a
	3	0.24±0.07 ^b	0.28±0.01 ^{ab}	0.32±0.04 ^a	0.41±0.03 ^{ab}
	4	0.33±0.025 ^b	0.39±0.06 ^{ab}	0.41±0.03 ^{ab}	0.51±0.09 ^a

Table 3: Mean values of gain in weight mean±standard deviation of cultured red tilapia per treatment for 1 month

Treatments	Gain in weight (g)
1	8.63±3.86 ^c
2	4.71±2.42 ^{bd}
3	3.17±2.09 ^{ab}
4	2.09±1.42 ^a

Note: Means with different superscript letters are significant at 0.05 level

CONCLUSION

Fermented taro corm decreases dissolved oxygen concentration in water, which is lethal to the red tilapia. It might regulate the acidity of the water, thus red tilapia experienced lesser stress in treated water. It could also be an effective agent in increasing the alkalinity of the water, thus it may buffer the water against rapid pH changes. Increasing amount applied in water may cause slower growth of the species cultured in concrete tanks.

REFERENCES

- [1] Balcazar, J., Aguirre, A., Gómez, G. and Paredes, W. 2006. Culture of hybrid red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) in marine cages: effects of stocking density on survival and growth. Retrieved from https://cals.arizona.edu/oip/ista6/6abstracts/Balcazar_Rojas/Culture_of_hybrid_red_tilapia_text.doc on November 17, 2017.
- [2] Thodesen, J., Rye, M., Wang, Y.X., Li, Y.X., Bentsen, H.B., Yazdi, M.H. and Gjedrem, T. 2013. Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth, survival and external color traits of red tilapia (*Oreochromis spp.*) after four generations of multi-trait selection. *Aquaculture*, 416(417): 354-366.
- [3] Balcázar, J.L., Blas, I.D., Ruiz-Z, I., Cunningham, D., Vendrell, D. and Múzquiz, J.L. 2006. The role of probiotics in aquaculture. *Veterinary Microbiology*. 114(3-4):173–186.
- [4] Patil, P.N., Sawant, D.V., and Deshmukh, R.N. 2012. Physico-chemical parameters for testing of water – A review. *International Journal of Environmental Sciences*, 3(3): 1194-1207.
- [5] Stickney, R.R. 2016. *Aquaculture: An Introductory Text*. 3rd ed. CABI. Wallingford, Oxfordshire, UK. P. 351 p.
- [6] Crooker, P.C. and Contreras, J.O. 2010. Bioremediation of aquaculture wastes. *Current Opinion in Biotechnology*, 21(3): 313–17.
- [7] Santos, G. 2014. *Probiotics: An essential tool in intensive shrimp aquaculture*. Science and Solutions, A magazine of BIOMIN Holding GmbH Industriestrasse, Herzogenburg, Austria, 8 p.
- [8] SEAFDEC. 1999. Promoting appropriate aquaculture technology for more fish in Southeast Asia.

- SEAFDEC Aquaculture Department. Tigbauan, Iloilo, Philippines. 24 p.
- [9] Gomez-Gil, B., Herrera-Vega, M., Abreu-Grobois, F. and Roque, A. 1998. Bioencapsulation of two different *Vibrio* species in nauplii of the brine shrimp (*Artemia franciscana*). *Appl. Environ. Microbiol.*, 64:2318–2322.
- [10] Austin, B., Stuckey, L.F., Robertson, P.A.W., Effendi, I. and Griffith, D.R.W. 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii*. *J Fish Dis* 18: 93–96.
- [11] Spanggaard, B., Huber, I., Nielsen, J., Sick, E.B., Pippert, C.B., Martinussen, T., Slierendrecht, W.J. and Gram, L. 2001. The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. *Environ. Microbiol.* 3: 755–765.
- [12] Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S. and Menasaveta, P. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiotic bacterium (*Bacillus S11*). *Aquaculture* 191: 271–288.
- [13] Brown, A.C. and Valiere, A. 2004. The medicinal uses of poi. *Nutr. Clin. Care*, 7(2): 69–74.
- [14] Huang, A.S., Lam, S.Y., Nakayama, T.M. and Lin, H. 1994. Microbiological and chemical changes in poi stored at 20°C. *Journal of Agricultural and Food Chemistry*, 42: 45–48.
- [15] Cruz, P.M., A.L. Ibáñez, O.A., Hermosillo, and H.C. Saad. 2012. Use of probiotics in Aquaculture. Retrieved from <http://dx.doi.org/10.5402/2012/916845> on November 17, 2017.
- [16] Balcazar, J., I. De Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell and J. Muzquiz. 2006. The role of probiotics in aquaculture. *Veterinary Microbiology*, 114: 173–86.
- [17] Boyd, C.E. 1990. *Water Quality in Ponds for Aquaculture*. Department of Fisheries and Allied Aquaculture. Alabama. Agricultural Experimental Station. Auburn University. Alabama. Birmingham Publishing CO. Birmingham, Alabama. P. 318.

- [18] Paajimans K.P., W.Takken, A.K. Githeko, A.F.G. Jacobs. 2008. The effect of water turbidity on the near-surface water temperature of larval habitats of the malaria mosquito *Anopheles gambiae*. International Journal of Biometeorology, 52 (8): 747-753.
- [19] Dewalle, D.R., Swistock, B.R. and Sharpe, W.E. 2011. Episodic flow – duration analysis: assessing toxic exposure of brook trout (*Salvenius fontinalis*) to episodic increases in aluminium. Canadian Journal of Fisheries and Aquatic Sciences, 52(4): 816-827.
- [20] Boyd, C.E. 2001. Water Quality Standards, Dissolved Oxygen. Global Aquaculture Advocate, 92: 70-71.
- [21] Knud-Hansen, C.F. 1998. Pond Fertilization: Ecological Approach and Practical Application. Pond Dynamics/Aquaculture Collaborative Research Support Program, Oregon State University, Corvallis, Oregon.
- [22] Lakshman, R. and P. Soundarapandian. 2008. Effect of commercial probiotics on large scale culture of black tiger shrimp *Penaeus monodon* (Fabricus). Research Journal Microbiology, 3:198-203.
- [23] Swann, L. 2017. A Fish Farmer's Guide to Understanding Water Quality. Retrieved from <https://extension.purdue.edu/extmedia/AS/AS-503.html> on May 24, 2018.
- [24] Tsadik, G.G., and M. Kutty .1987. Influence of Ambient Oxygen on Feeding and Growth of the Tilapia, *Oreochromis niloticus* (Linnaeus). <http://www.fao.org/docrep/field/003/AC168E/AC168E00.htm> on September 17, 2018.