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The impact of ozonated water treatment on growth rate of ‘Srikandi’ tilapia (*Oreochromis Aureus X Niloticus*)

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Abstract. The impact of ozonized water treatment on ‘Srikandi’ tilapia was assessed using ozone reactor with an airflow velocity of 1.5 L / min at a voltage of 10 kV, which leads to that the dissolved oxygen (DO) content increases from 0.99 to 11.11 mg / L. The ozonized water treatment was divided into five groups based on the length of treatment period: 5 minutes as group I, 10 minutes as group II, 15 minutes as group III, 20 minutes as group IV and 0 minute (Reference case). The fish growth rate was measured in terms of length and weight per seven days for 30 days. The result indicated that the fastest growth rate of ‘Srikandi’ tilapia occurred at the group III (length growth: 7.82 cm; weight growth: 7.72 g in 30 days). The fastest Specific Growth Rate (SGR) of the fish occurred at the group II (1.281%), and the fastest Relative Growth Rate (RGR) of the fish occurs at the group III (4.538%). The oxygen content, temperature, salinity to match the growth of Tilapia ‘Srikandi’ are vital elements in Tilapia farming management. These results are considered to be useful to increase the production rate of ‘Srikandi’ tilapia farming.

1. Introduction

Salt water pond-fish farming in coastal areas has following advantages than the corresponding fresh water pond-fish farming i.e. high tolerance of salinity up to 30 ppt with survival rate >80%, rapid growth (can reach 200 grams in three months with salinity pressure), high protein contain as a food source of animal protein, high content of omega 3(reach >105 mg/100 g meat) and omega 6 (reach >230 mg/100g of meats) fatty acids, has a better meat taste and chewy meat texture, and can grow up in polyculture system [1]. Aquaculture can be defined as human efforts to increase the water productivity through aquatic farming of aquatic biotas. Aquaculture is a breeding activity to gain benefits via reproduction, growth, and aquatic organism quality increase In line with the increase demand of seafood production around the world, a productive aquaculture is urgently needed to produce organisms in controlled environment and subsequently to gain profit [2].

Tilapia is cultivated in fresh water commodity in hatchery and enlargement because of it benefits that can be compared with some fresh water fishes, especially in rapid growth, easy to breed, easy in maintenance process, and high adaptation in environment changes [3]. Tilapia habitat originates in fresh water of rivers, lakes, stanks, and swamps, but can tolerate in large salinity (eury haline) so that it can live in brackish water and salt water of ocean. The fish tolerant availability of salinity is 0-35 ppt



(part per thousands), but the optimal salinity for its growth is 0-30 ppt. Tilapia still alive in salinity range of 31-35 ppt, but the growth is slow [4]. Tilapia can be farmed in coastal ponds because of their tolerance to salinity. Along with the development of technology, tilapia has been developed with a new genetic strain that has a high tolerance to salinity, namely 'Srikandi' (*Oreochromis aereus x niloticus*). This fish is the result of hybridization between black arwana tilapia (*Oreochromis niloticus*) with blue tilapia (*Oreochromis aureus*) done by Research Institute for Fish Breeding (BPPI) Sukamandi, West Java in 2012. The main problem in the development of saltwater tilapia is the availability of tilapia juveniles that can be maintained in salt water. The saltwater tilapia is produced by the result of gradual adaptation of fresh water. Another obstacle is the low survival rate of the salted tilapia juveniles as a result of saltwater adaptation when compared to tilapia farmed in freshwater [5]. 'Srikandi' classification based on Decree of Minister of Maritime Affairs and Fisheries No. KEP.09/MEN/2012 as follows:

Phylum	: Chordata
Sub-Phylum	: Vertebrata
Class	: Pisces
Sub-Class	: Teleostei
Ordo	: Percomorphi
Sub ordo	: Perchoidea
Family	: Cichlidae
Genus	: <i>Oreochromis</i>
Species	: <i>Oreochromis aureus x niloticus</i>



Fig. 1. A. Hybrid species 'Srikandi' tilapia (*Oreochromis aureus x niloticus*); B. *Oreochromis aureus* (Steindachner, 1864) [6]; C. *Oreochromis niloticus* (Linnaeus, 1758) [7].

Efforts had been done to improve fish health and growth for commercial purposes, one of them is water ozonation treatment. Ozone is a molecule that consists of three atoms of oxygen. It is an unstable oxygen if it is compared by O_2 [8]. Ozone (O_3) can functionate as a cleaner, deodorizing, and disinfectant that can clear all microorganisms as bacteria, viruses, fungus, seeds, etc. Ozone is the strongest oxidizing agent after fluorine. If it is compared to chlorine, ozone has 3250 times disinfectant power faster and 50% oxidative reaction stronger. Before or after reaction process, ozone always produce oxygen (O_2), so ozone technology is very friendly to environment or it can be called as the future green chemistry [9]. Ref [10] emphasized that, ozonation of water has been found to effectively reduce biochemical oxygen demand, chemical oxygen demand, dissolved organic carbon, color, nitrite, turbidity, total organic carbon, and/or total suspended solids. However, potential hazard related to ozone may also has to be taken into account. Excess ozone residual remaining in the culture water may cause significant harm to cultured species. Therefore, its various concentrations of treatment to specific cultured biota may urgently needed to adjust the certain level for their optimum growth.

2. Methods

2.1. Measuring Optimum Ozone Concentration from Generator

The study used a free air source that was introduced into the reactor with a flow velocity of 1.5 liters/minute at a voltage of 1.38 kV, 10 kV, and 12.5 kV. The graph on Fig. 2 shows the relationship between the stresses used and the resulting concentrations. Based on the graph, the ozone concentration increased in line with increasing voltage applied, owing to increase of electric

field, resulting increased ionization production. As shown on the graph, the voltage of 10 kV generated optimum ozone concentration, thus it was used for 'Srikandi' tilapia treatment to increase their growth.

2.2. Fish Acclimatization

This research used 5 aquariums with 50 x 50 x 50 cm in volume. Twenty five fishes were treated in the aquarium. Fish acclimatization was applied for 7 days to adapt new environment, allowing juveniles adapt before ozone treatment.

2.3. Ozone Treatment

The ozone was produced by ozone reactor with an airflow velocity of 1.5 liters / minutes at a voltage of 10 kV, which impact on the DO content increases from 0.99 to 11.11 mg / L. Complete randomized design with ozone as single factor was applied, consisting 4 tested group of treatments and a Reference treatment, i.e. 5 (treatment I), 10 (treatment II), 15 (treatment III), 20 (treatment IV) minutes of ozonation and e result of this without ozonation as a reference case.

Fish feeding was applied three times a day with the amount of feed as much as 0.5 grams. The water quality was observed daily to maintain pH and temperature in the aquarium.

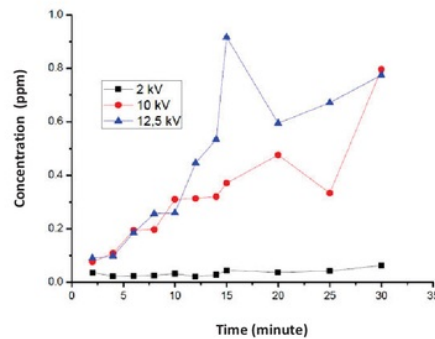


Fig. 2. The concentration of ozone as a function of time at a voltage of 2 kV, 10 kV, and 12.5 kV using airflow velocity of 1.5 L / min.

2.4. The weight and length measurement

The weight and length of tilapia 'Srikandi' were measured every 7 days for 30 days period of study. The observed growth was calculated as length and weight. The growth in length was calculated as follows:

$$L = L_t - L_0 \quad \dots\dots\dots(1)$$

where :

- L** = Length growth (cm)
- L_t** = Length after period of *t* time (cm)
- L₀** = Initial length (cm) [11].

Specific Growth Rate (SGR) in length was counted using formula as follows:

$$SGR_{(Length)} = \frac{(\ln L_t - \ln L_0)}{t} \times 100\% \quad \dots\dots\dots(2)$$

where :

SGR = Specific Growth Rate of length (%)

- L_t = The last average body length (cm)
 L_o = The initial average body length (cm)
 t = Time (days)

Absolute weight growth was calculated as follows:

$$G = W_t - W_o \quad \dots\dots\dots(3)$$

where :

- G = Absolute Weight Growth (g)
 W_t = The last weight growth (g)
 W_o = The Initial weight growth (g) [12].

Specific Growth Rate (SGR) in weight was counted similar to formula (2)

Relative Growth Rate (RGR) was counted using formula as follows :

$$RGR = \frac{W_t - W_o}{W_o \times t} \times 100\% \quad \dots\dots\dots(4)$$

where :

- RGR = Relative Growth Rate (% per days)
 W_t = The last weight (g)
 W_o = The initial weight (g)
 t = Time (days) [13]

2.5. Data Analysis

All datas of tilapian growth in length and weight of the five groups of treatments were analyzed using *Analysis of Variance* (ANOVA) to access the differences in their variances. *Post Hoc* test was performed if significant differences occurred within the treatments.

3. Result and Discussion

Based on **Fig.3**, it can be explained that the length growth of tilapia increased for 28 days of research. Based on the treatment time, it can be shown that the highest length value in ozonation process for 15 minutes in 7,82 cm and the lowest length value in ozonation process for 5 minutes in 7,1 cm.

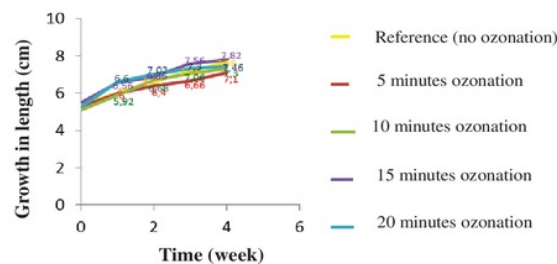


Fig. 3. Graph of 'Srikandi' Tilapia growth based on length (cm) and time duration of ozonation (per weeks)

Meanwhile, the growth in weight of 'Srikandi' tilapia has increased during 4 weeks of measurement, as shown on the Fig. 4. Based on the duration of ozone treatment, it was shown that the highest weight value was found in 15 minutes ozonation treatment, i.e. 7.72 g. The lowest weight value was in 5 minutes ozonation treatment, i.e. 6.56 g.

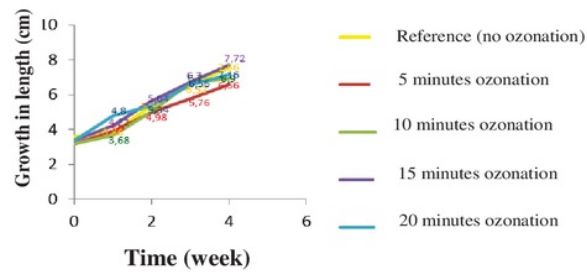


Fig. 4. Graph of 'Srikandi' tilapia growth based on weight (g) and time of ozonation (per weeks)

3.1 Ozonation Influences through 'Srikandi' Tilapia Length Growth

Based on the graph on **Fig. 5**, The average growth in length of 'Srikandi' tilapia at all treatments were Treatment III (2.32 ± 0.915), Reference (2.26 ± 0.900), Treatment II (2.2 ± 0.901), Treatment IV (2.16 ± 0.867), and Treatment I (1.8 ± 0.684), all in centimeters (cm). In general, the long-term growth of 'Srikandi' tilapia showed an increase during the 28-days treatment. The value of the daily specific growth rate (SGR in length) was obtained from the experiments conducted during the 28-day trial. Daily growth serves to calculate the growth percentage of fish length per days, as shown in **Table 1**.

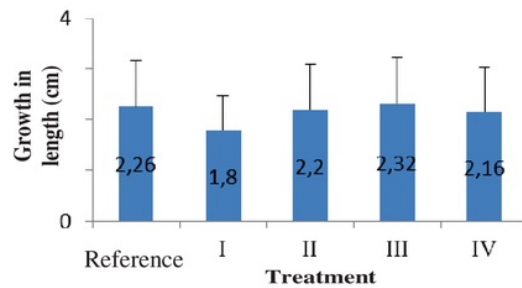


Fig. 5. 'Srikandi' tilapia growth in length for every treatment (\pm SD).

Table 1. 'Srikandi' Tilapia SGR_{length} for all treatments.

Treatments	SGR_{length} (% per days)
Reference	1,249 %
I	1,044 %
II	1,281 %
III	1,257 %
IV	1,221 %

The highest value of SGR obtained in Treatment II (1.281%), while the lowest value obtained at Treatment I (1.044%); however, the value was not higher than those at the reference. Statistical analysis using ANOVA showed that there was no statically significant difference in the ozonation treatment on long growth of fish.

3.2 Impact of Ozonation on Growth of 'Srikandi' Tilapia

The average growths of 'Srikandi' tilapia for each treatment were Treatment III (4.32 ± 1.763), Reference (3.96 ± 1.698), Treatment IV (3.76 ± 1.483), Treatment II (3.7 ± 1.691) and Treatment I (3.36 ± 1.359), all in grams (gr).

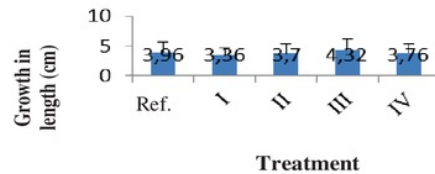


Fig. 6. Graph of growth in weight of tilapia on each treatment (\pm SD).

The weight of 'Srikandi' tilapia for 28 days tes shows the difference in weight growth of each treatment. Specific weight rate (SGR weight) was obtained from the experiments conducted during the 28-day trial. Daily growth serves to calculate the growth percentage of fish length per day. The growth of 'Srikandi' Tilapia is shown on **Fig. 6**.

The highest value of SGR weight was obtained at Treatment III (2.929%), while the lowest value was obtained at Treatment I (2.564%) where the value was not higher than Reference (2.560 %), as shown in **Table 2**. Statistical analysis was conducted to determine the effect of ozonation treatment on the growth in weight of 'Srikandi' tilapia. However, statistical analysis using ANOVA showed that there was no statistically significant difference of ozonation treatment to fish weight growth.

Table 2. SGR weight value od 'Srikandi' Tilapia in evey treatments

Treatments	SGR in weight (%per days)
Reference	2,650 %
I	2,564 %
II	2,744 %
III	2,929 %
IV	2,660 %

3.3 Relative Growth Rate of 'Srikandi' Tilapia

The relative growth rate of 'Srikandi' tilapia in each treatment within 28 days of trial was obtained as shown in **Table 3**.

Table 3. 'Srikandi' Tilapia RGR value

Treatments	RGR (%)
Reference	3,929 %
Treatment I	3,750 %
Treatment II	4,129 %
Treatment III	4,538 %
Treatment IV	3,950 %
Treatment V	3,929 %

Ref.[14] stated that the rate growth was the absolute growth difference measured by time sequence. Growth can be divided into two, which are absolute growth and relative growth. Absolute growth is the average of total size of each age, whereas relative growth was the percentage of growth at each time interval. Based on **Table 3**, the highest relative growth rate of

'Srikandi' tilapia was Treatment III (4,538%). It was found that aqueous ozone was very effective in significantly reducing microbial contamination on live catfish, especially in significant reductions in total plate counts [15]. Furthermore, it has been demonstrated that the use of ozonized seawater reduced and eliminated bacterial pathogens in mariculture facilities and extended marine food products storage. After treatment, *Vibrio* counts and shrimp disease were eliminated, ozonized seawater decreased the time required for normal molting of shrimp and the total growth cycle was reduced [16]. Despite the benefits of ozone for its effectiveness in oxidizing agent, higher ozone concentrations are reported a risk to cultured fish stocks causing gross tissue damage and stock mortalities, and also are a risk to bacterial films on the biofilter [17].

4. Conclusion

Efforts to increase the production rate of 'Srikandi' tilapia (*Oreochromis aureus niloticus*) farming had been done using ozone reactor with an airflow velocity of 1.5 L/min at a voltage of 10 kV, which led to that the dissolved oxygen (DO) content increased from 0.99 to 11.11 mg / L in the water column. Treatments to assess *Srikandi* tilapia growth using aquarium size 50x50x50 cm and sea water with salinity ranging from 25-30 ppm as growing medium for the fish has been done. The result showed that the fastest growth rate of tilapia occurred at the group III (Its length growth was 7.82 cm and its weight growth was 7.72 g in 30 days.). The fastest Specific Growth Rate (SGR) occurred at the group II (1.281%), and the fastest Relative Growth Rate (RGR) occurred at the group III (4.538%). These results are considerably useful as an alternative solution to increase the production rate of 'Srikandi' tilapia farming. The oxygen content, temperature, salinity to match the growth of Tilapia 'Srikandi' are vital elements in tilapia farming management. Further studies can be done on impact of ozonation dosage variation on fish growth rate.

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