



## EFFECTS OF DIFFERENT DOSE OF ZEOLITE (CLINOPTILOLITE) IN IMPROVING WATER QUALITY AND GROWTH PERFORMANCE OF RED HYBRID TILAPIA (*Oreochromis* sp.)

Rabiatul Adawiyah Meor Mohamad Zain, Nur Farrah Izzaty Shaari, Mohamad Faiz Mohd Amin and Musfiroh Jani

Faculty of Earth Science (FSB), Universiti Malaysia Kelantan, Jeli Campus, Jeli, Kelantan, Malaysia

E-Mail: [adawiyahmeor@gmail.com](mailto:adawiyahmeor@gmail.com)

### ABSTRACT

The optimum fish production is totally dependent on the physical, chemical and biological qualities of water. Optimal water quality varies by species and must be monitored to ensure fish growth and survival. The effects of different dose of zeolite to improve the water quality and growth performance of Red Hybrid Tilapia (*Oreochromis* sp.) were investigated in the present study. Twenty tilapia fry were placed in an aquarium with capacity of 30 litre of water. Natural zeolite (clinoptilolite) with different dose of 0 (control), 5, 10, 15, 20 g/L added into the water and the experiment was carried out in 60 days. The obtained result showed that ammonia, nitrite, and nitrate levels were significantly reduced in treated water compared with control. The lowest ammonia levels recorded in 15 g/L treatment of zeolite, consistent with nitrite and nitrate. However for total phosphorus and organic phosphorus (orthophosphates), the lowest concentration obtained was from addition of 10 g/L of zeolite. At the end of study, the growth performance of fish treated with addition of 20 g/L and 15 g/L of zeolite were significantly more compared to other groups. According to the results, it showed that optimum growth of fish can be obtained with a good water quality. In conclusion, the addition of zeolite is significant for the improvement of water quality and growth performance of Red Hybrid Tilapia (*Oreochromis* sp.).

**Keywords:** Zeolite, *Oreochromis* sp., red hybrid tilapia, water quality, growth performance.

### INTRODUCTION

In recent years, production of freshwater fish is increased in Malaysia as the consumption of food fish has increased by 150% since 1961 (FAO, 2013). The commonly species are keli, patin and tilapia which can also be processed into convenience products rather than being sold fresh. Of the commercially cultured freshwater species, red tilapia, *Oreochromis* sp., is one of the popular aquaculture species in Asia including Malaysia. Red tilapia has high demand and value in the market due to its taste and competitive price. The figure set by the Malaysian Department of Fisheries (DoF), state that around 35,400t of tilapia, worth MYR 248 million in production value were recorded in the country last year. In the next four years, DoF aims to increase production to 60,000t; 70% by 2020. On top of that, water quality is the main factor in determining the optimum production of freshwater fish farming (Far Eastern Agriculture, 2016).

The concentration of total ammonia nitrogen (TAN) is the key limiting water quality parameter in aquaculture. In close or recirculating systems for aquaculture such as tank and aquariums, the causes of diseases and fish mortalities come from ammonia that produced by the decomposition of excreta and unfed fish feed (El-Gendy *et al.*, 2015). Ammonia nitrogen is the major pollutant in aquaculture system that is toxic to fish and can give detrimental effect to the growth and health of fish. Therefore, it is important to remove ammonia in water and in a meantime improve the water quality of fish farming systems.

Nitrification and ion exchange are two of the principal methods in removing ammonia. Material such as oyster shell, rock, sand, activated carbon and others are

used to prepare as a substrate for the bacteria involved in nitrification process. Meanwhile, ion exchange is a process in which ions from a synthetic or natural resin are exchanged with specific ions in wastewater. Some natural resin, such as zeolite, is used in removing ammonia from wastewater and clinoptilolite is one of the best zeolites in ammonia removal ion exchange process (Emadi *et al.*, 2001; Farhangi & Rostami-Charati, 2012). Zeolites are microporous crystalline hydrated aluminosilicates, which have been used in various applications due to their unique physicochemical characteristics such as ion exchange and adsorption-desorption properties (Ghasemi *et al.*, 2016).

According to Yildirim *et al.* (2009), there are three main functions of natural zeolite benefits to aquacultures. It acts as an adsorbent for removing ammonia from the pond water that can improve water quality, enriching the ventilating system with oxygen in the aquarium and by using as a feed additive in feed rations that will improve the fish growth and health. In previous study, addition of 12 g/L zeolite could prevent the mortality of beluga sturgeon *Acipenser persicus*. The survival rate of fish also increased with increasing of zeolite concentration (Farhangi & Rostami-Charati, 2012). In addition, clinoptilolite has been used during ornamental fish transportation because of the effectiveness to remove ammonia.

Shrimp culture in Thailand and other Asian countries, had commonly applied zeolite to the ponds to lower the ammonia concentrations by adding 180 to 350 kg per ha of zeolite into the ponds which culture animals are exposed, but there is no research findings to support this practices (Zhou & Boyd, 2014). Even though there were many research conducted on the application of



zeolite in fish culture, there is no specific dosage for specific fish culture. On top of that, it is considered to be an obstacle in application of zeolite for large-scale aquaculture (Ghasemi *et al.*, 2016). The specific dose of zeolite is impossible to be recommended especially in fish rearing system. The dose of zeolite is depending on such factors as the stocking density of fish, protein content in feed, feed stability and definitely the quality of water (Abdel-Rahim, 2017). This study aims to investigate the effect of different dose of zeolite on the growth performance of Red Hybrid Tilapia (*Oreochromis* sp.) and the quality of water.

## MATERIALS AND METHODS

This research was conducted in Aquaculture Laboratory, University Malaysia Kelantan Jeli Campus, Kelantan for 60 days.

### Experimental design

Group of twenty Red Hybrid Tilapia (*Oreochromis* sp.) fry that were obtained from a private hatchery at 2-3 inch average size had been stocked in fifteen aquarium of 30 liter capacity. These aquariums were filled with tap water and anti-chlorine was added to prevent the mortality of the fish and also to purify the water from chlorine. Constant aeration was prepared for each aquarium to supply oxygen for the fish. Each aquarium was assigned a different dose (0, 5, 10, 15, 20 g/L) of zeolite (2-5 mm in size) in triplicate. The zeolite was inserted in a suspended cloth bag before place into the aquarium to prevent coarse particles inside aquarium water from obstructing the pores of zeolite and decreasing ammonium retention (Oz *et al.*, 2016). In this study, Indonesian zeolite, clinoptilolite type was used with chemical compositions as shown in Table-1. The fish was fed with commercial feed two times daily, each time 2 % of average body weight.

**Table-1.** Chemical composition of zeolite.

Elements	Percentage (%)
SiO <sub>2</sub> (Silicon Oxide)	71.10
Al <sub>2</sub> O <sub>3</sub> (Aluminium Oxide)	13.12
Fe <sub>2</sub> O <sub>3</sub> (Iron III Oxide)	0.91
TiO (Titanium Oxide)	0.01
CaO (Calcium Oxide)	1.54
MgO (Magnesium Oxide)	0.99
K <sub>2</sub> O (Potassium Oxide)	2.40
Na <sub>2</sub> O (Sodium Oxide)	0.90
pH	7-8
Loss of ignition	8.72
Cation capacity exchange (CEC)	meq/100g

### Water quality parameter

The physico-chemical water quality parameter in the experimental aquariums was monitored weekly between 09:00 and 10:00 h each sampling day. The physical parameters were monitored for temperature, dissolved oxygen (DO), pH, turbidity, salinity, conductivity and total dissolved solid (TDS). All parameters were measured by using portable multiparameter, YSI model 556 Mps except for turbidity. Portable turbidity meter HACH model 2100Q was used to measure turbidity. Composite column water samples were collected in 500 ml polyethylene bottle, and analyzed for total ammonia nitrogen (salicylate method), nitrite-N (diazotization), nitrate (cadmium reduction) total phosphorus (acid persulfate digestion method) and reactive phosphorus/ orthophosphate (ascorbic acid method), by using HACH DR 6000, UV-Vis Spectrometer following the Standard Methods for the Examination of Water and Wastewater (APHA, 1999).

### Growth performance parameter

Random fish samples from each aquarium were taken biweekly for growth performance analysis throughout the whole experiment. Growth performance parameters were determined by using the following equations:

- Weight gain (WG) = Final weight (g) – Initial weight (g)
- Average daily weight gain (ADG) = Weight gain (g) / times (days)
- Specific growth rate (SGR) = (log of final weight (g) - log of initial weight (g) x 100 / times (days)

### Statistical analysis

Statistical analysis was carried out by using Microsoft EXCEL 2010 and SPSS statistical package program version 20. One-way ANOVA was used for data analysis.

## RESULTS AND DISCUSSIONS

### Water quality parameter

Table-2 shows the average values of the water quality monitored throughout the experimental period. During the experiment, the lowest temperature among the groups was measured at 24.58 °C and the highest was recorded at 26.88 °C. Average temperature for control, 5g/L, 10g/L, 15g/L and 20g/L of zeolite were 25.68±0.75, 25.99±0.87, 25.85±0.83, 25.76±0.80 and 25.79±0.89 respectively and they were within the limits of optimal survival and growth of Tilapia (Popma, 1999; Yıldırım *et al.*, 2009). Throughout the experiment, DO and pH were measured at their lowest levels as 1.67 mg/L and 6.62 and the highest was recorded as 4.16 mg/L and 7.25. In general, tilapia can survive in pH ranging from 5 to 10 but



do best in a pH range of 6 to 9. In addition, pH is an important parameter affecting the sorption process at the water-adsorbent interfaces (Mazloomi & Jalali, 2016). It was reported that optimal pH values for ammonia sorption by natural zeolite were in the range of 5-8 (Huang *et al.*, 2010; Mazloomi & Jalali, 2016; Zhang *et al.*, 2011). This behaviour can be described by the fact that at pH values above 7.0, the  $\text{NH}_4^+$  is converted into unionized-ammonia ( $\text{NH}_3$ ) and the  $\text{NH}_3$  molecules cannot exchange back into the zeolite and thus strips with air. The pH values obtained during this experiment were within the range. Meanwhile the tolerable limit of dissolved oxygen for tilapia is between 3.0-4.0 mg/L (Popma, 1999). But the DO values were below the limits except for treatment with 15 g/L zeolite. This was due to the high concentration of ammonia (Table-2). Mokhtari-Hosseini *et al.* (2016) stated that the existence of ammonium ion in water can cause the depletion of dissolved oxygen levels in water. Besides, the highest and lowest turbidity was recorded at 17.55 NTU and 9.99 NTU in addition of 20g/L and, 15 g/L of zeolite respectively. The other treatments seemed to be constant for turbidity levels.

The average ammonia concentration, in the groups with addition of 5g/L, 10g/L, 15g/L and 20g/L zeolite, was found to be at 0.17 mg/L, 0.11 mg/L, 0.04 mg/L and 0.08 mg/L, respectively, and the highest value recorded (0.18 mg/L) in control group (Table-2). Ammonia concentration in the groups that were added with 15 g/L and 20 g/L zeolite was found to be relatively lower ( $p < 0.05$ ) than control. Thus it was found that addition of natural zeolite had a positive effect in terms of reducing the ammonia concentration levels in water. This result was consistent with (Danabas & Altun, 2011; Ghiasi & Jasour, 2012). In addition, Oz *et al.*, (2016) found that addition of zeolite inside or outside net bag had reduced the concentration of ammonia in aquarium water. However, the use of zeolite in a net bag gave more advantages in terms of high adsorption levels of ammonia. Zeolite exchanges sodium ions for ammonium ions and shifts the ammonia equilibrium away from toxic unionized ammonia, thus preventing an increase in its level (Ghiasi & Jasour, 2012). It was reported that toxicity of unionized ammonia ( $\text{NH}_3$ ) for short-term exposure usually lie between 0.6 and 2.0 mg/L for pond fish, and sub-lethal effects may occur at 0.1 to 0.3 mg/L. It was recommended that the tolerable limits of ammonia should be lower than 0.2 mg/L for pond fishery (Bhatnagar & Devi, 2013).

The lowest concentration of nitrite was recorded at 0.23 mg/L with the addition of 15g/L of zeolite and the highest concentration was obtained from control group at 0.36 mg/L but there is no significant difference among the group ( $p > 0.05$ ). It was mentioned that, the nitrite content should be lower than 0.2 mg/L (AFCD, 2009). According to PHILMINAQ (2008), the nitrite level for hard

freshwater species should be lower than 0.1 mg/L while for freshwater should be lower than 0.5 mg/L. The results of present study were under those values. Meanwhile, for nitrate concentration, it was observed that the application of zeolite decreased obviously the level of nitrate in water (Table-2). Addition of 15 g/L of zeolite resulted as the lowest concentration of nitrate and there was a significant difference ( $p < 0.05$ ) compared to control group which recorded as the highest concentration. Average nitrate concentration in control, 5g/L, 10g/L, 15g/L and 20g/L of zeolite groups were recorded as 7.60 mg/L, 4.99 mg/L, 4.69 mg/L, 4.22 mg/L and 4.98 mg/L respectively. Nitrate is harmless compare to ammonia and nitrite and its levels are normally stabilized in the 50-100 ppm range (Bhatnagar & Devi, 2013).

On the other hand, the content of phosphates (total phosphorus and orthophosphates) in the addition of zeolite has significant difference compared to the control. The lowest values were recorded in the addition of 10g/L zeolite for both total phosphorus and orthophosphate at 4.95 mg/L and 3.83 mg/L. According to Wanchun *et al.* (2011), the removal of zeolite on phosphorus is increased with the decreased of pH. The removal of phosphorus increases in alkaline conditions, because the  $\text{PO}_4^{2-}$  may form precipitate with  $\text{Ca}_2^+$  or  $\text{Ba}_2^+$  and there is a relationship between alkalinity. In a pond with higher alkalinity, the pH shift is reduced (Buttner, 1993). At the end of the experiment, the highest total alkalinity value was recorded in the addition of 10 g/L zeolite. This has proven that, the removal of phosphorus by zeolite increase in alkaline condition.

For total hardness, there was a significant difference between the control group and the group with additional of zeolite ( $p < 0.05$ ). In most waters the concentrations of alkalinity and hardness are similar, but they can be vastly different as alkalinity measures negative ions such as carbonate & bicarbonate and hardness measures positive ions such as calcium & magnesium (Buttner, 1993). At the end of the experiment, the lowest value of hardness was recorded at 21.31 mg/L from control group and the highest value was recorded at 40.03 mg/L with the addition of 10g/L of zeolite. The recommended ideal value of hardness for fish culture is at least 20 mg/L and a range of 30-180 mg/L (Bhatnagar & Devi, 2013). According to Bhatnagar *et al.* (2004) hardness values less than 20 mg/L can causes stress to the fish and 75-150 mg/L is optimum for fish culture.

### Growth performance parameter

The growth performance of the Red Hybrid Tilapia has been determined and data are given in Table-3 for the experimental period of 60 days. At the beginning of the experiments, average weight of fish was  $2.7 \pm 0.02$ g for control and all treatments groups.

**Table-2.** Physico-chemical parameters of aquarium water during the experimental period.

Parameters	Treatments of zeolite				
	Control	5g/L	10g/L	15g/L	20g/L
Temperature (°C)	25.68±0.75 <sup>b</sup>	25.99±0.87 <sup>c</sup>	25.85±0.83 <sup>bc</sup>	25.76±0.80 <sup>bc</sup>	25.79±0.86 <sup>bc</sup>
DO (mg/L)	2.47±0.78 <sup>cd</sup>	2.09±0.33 <sup>bc</sup>	2.47±0.19 <sup>b</sup>	3.05±0.70 <sup>d</sup>	2.90±0.95 <sup>bcd</sup>
pH	6.27±0.83 <sup>bc</sup>	6.49±0.82 <sup>c</sup>	6.42±0.85 <sup>bc</sup>	6.28±0.85 <sup>bc</sup>	6.19±0.91 <sup>b</sup>
Turbidity(NTU)	11.32±4.54 <sup>b</sup>	10.65±3.92 <sup>b</sup>	14.51±8.51 <sup>c</sup>	9.99±7.22 <sup>b</sup>	17.55±7.78 <sup>c</sup>
NH <sub>3</sub> -N (mg/L)	0.18±0.05 <sup>c</sup>	0.17±0.05 <sup>c</sup>	0.11±0.03 <sup>bc</sup>	0.04±0.01 <sup>ab</sup>	0.08±0.02 <sup>ab</sup>
NO <sub>2</sub> (mg/L)	0.36±0.043 <sup>bc</sup>	0.31±0.038 <sup>c</sup>	0.26±0.038 <sup>b</sup>	0.23±0.037 <sup>c</sup>	0.27±0.038 <sup>bc</sup>
NO <sub>3</sub> (mg/L)	7.60±1.38 <sup>c</sup>	4.99±0.91 <sup>bc</sup>	4.69±1.41 <sup>b</sup>	4.22±0.71 <sup>b</sup>	4.98±0.77 <sup>bc</sup>
T. Phosphorus (mg/L)	6.89±0.31 <sup>d</sup>	5.68±0.36 <sup>bc</sup>	4.95±0.30 <sup>b</sup>	5.47±0.31 <sup>b</sup>	6.28±0.29 <sup>cd</sup>
Orthophosphate (mg/L)	5.25±0.23 <sup>d</sup>	4.37±0.33 <sup>bc</sup>	3.83±0.26 <sup>b</sup>	4.67±0.31 <sup>c</sup>	4.60±0.23 <sup>c</sup>
T. Alkalinity (mg/L)	41.00±3.79 <sup>bc</sup>	49.41±5.07 <sup>d</sup>	50.27±4.77 <sup>cd</sup>	32.11±3.60 <sup>b</sup>	37.50±4.48 <sup>b</sup>
T. Hardness (mg/L)	21.31±0.63 <sup>b</sup>	36.25±3.15 <sup>d</sup>	40.03±3.57 <sup>d</sup>	36.08±2.28 <sup>cd</sup>	30.28±0.94 <sup>c</sup>

•Main effect means within same column followed by same letter has no significant difference at  $p > 0.05$  after determined by Tukey's test.

At the end of the experiment, the results showed that, final weights of tilapia kept in aquaria with addition of zeolite are significantly higher than control. As shown in Figure-1, average weight of Red Hybrid Tilapia increased with increasing of zeolite dose as well as days of experimental period. Addition of 20 g/L zeolite resulted as the highest weight gain and the lowest value obtained from control group to be at  $18.2 \pm 4.2$  respectively. In comparison with control, specific growth rate and weight gain in treatments significantly improved. Addition of 20g/L of zeolite also resulted in highest ADW (average daily weight gain) and the difference was significant ( $P < 0.05$ ) compared with other treatments. Previous study from Ghiasi and Jasour (2012) found that, the final weight of Angel fish (*Pterophyllumscalare*) with addition of 10 & 15 g/L zeolite were higher compared to control group (without addition of zeolite). Related to the results of current study, addition of 20 g/L showed the highest weight gain and 15 g/L zeolite resulted in second highest in weight gain of Red Hybrid Tilapia compared to other treatments.

According to these findings it can be concluded that addition of 15 g/L of zeolite in water not just the best for water quality improvement but was also the best dose

for better growth performance of fish. On the other hand, the study performed by Yildirim *et al.* (2009) showed that, the growth performance of Tilapia *zilli* fed with fish feed diets containing 2% of zeolite showed an increase compared to control group. Also, El-Gendy *et al.* (2015) reported that the addition of zeolite at feeding rate 3% of body weight resulted in the highest body weight of Nile Tilapia (*Oreochromisniloticus*). In correlated to these findings, it showed that the addition of zeolite directly in fish diets or addition of zeolite into the water system resulted in better growth performance by the increasing of fish body weight.

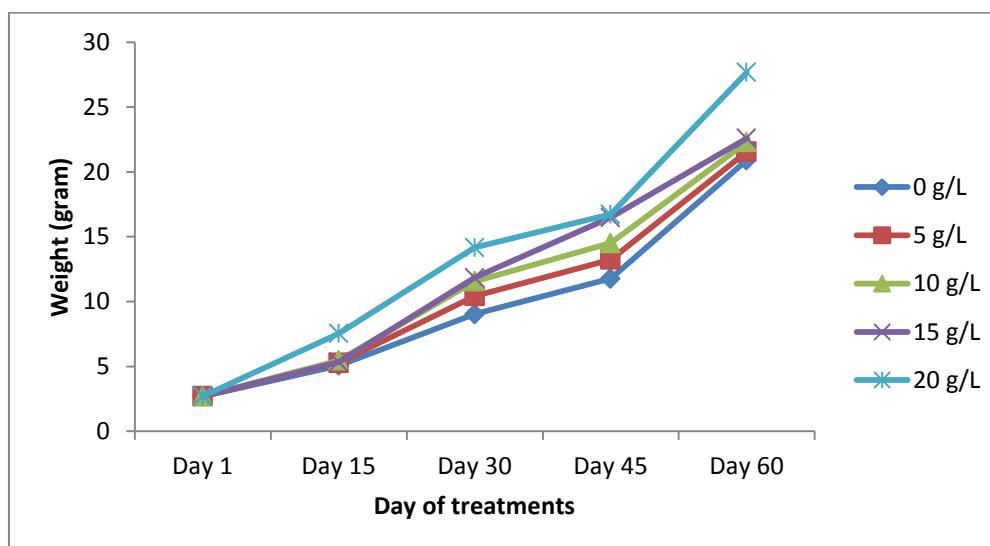
## CONCLUSIONS

Based on the results obtained in this study, it can be concluded that 15 g/L of zeolite was the best dose in reduction of ammonia levels in water. Meanwhile, for the phosphates levels, addition of 10 g/L of zeolite was the best among other treatments. In general, improvement of water quality and growth performance of Red Hybrid Tilapia (*Oreochromis* sp.) was obtained with the addition of (5, 10, 15, 20 g/L) zeolite. However, further research need to be conducted to obtain optimum dosage including the particle size of zeolite.

**Table-3.** Growth performance of red hybrid Tilapia.

Growth parameters	Treatments of zeolite				
	Control	5g/L	10g/L	15g/L	20g/L
Initial weight (g)	2.7±0.01	2.7±0.01	2.7±0.01	2.7±0.01	2.7±0.01
Final weight (g)	20.9±4.2 <sup>b</sup>	21.5±2.6 <sup>b</sup>	22.3±1.7 <sup>b</sup>	22.6±2.1 <sup>b</sup>	27.7±2.8 <sup>c</sup>
Weight gain	18.2±4.2 <sup>b</sup>	18.9±2.6 <sup>b</sup>	19.6±1.7 <sup>b</sup>	19.9±2.1 <sup>b</sup>	24.9±2.8 <sup>c</sup>
Initial length (cm)	2.54±0.01	2.54±0.01	2.54±0.01	2.54±0.01	2.54±0.01
Final length (cm)	9.5±1.76 <sup>b</sup>	9.6±1.77 <sup>b</sup>	9.9±1.94 <sup>bc</sup>	10.0±1.82 <sup>bc</sup>	10.9±1.97 <sup>c</sup>
ADW (g)	0.303±0.216 <sup>b</sup>	0.314±0.182 <sup>b</sup>	0.327±0.211 <sup>b</sup>	0.331±0.176 <sup>b</sup>	0.416±0.218 <sup>c</sup>
SGR (%day <sup>-1</sup> )	1.47±0.15 <sup>b</sup>	1.50±0.09 <sup>b</sup>	1.52±0.05 <sup>b</sup>	1.53±0.07 <sup>b</sup>	1.68±0.07 <sup>c</sup>

•Main effect means within same column followed by same letter has no significant difference at  $p > 0.05$  after determined by Tukey's test.

**Figure-1.** Average weight of red hybrid Tilapia (*Oreochromis sp.*) measured throughout the experimental period.

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