International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume: 3 | Issue: 4 | May-Jun 2019 Available Online: www.ijtsrd.com e-ISSN: 2456 - 6470

Oral Administration of Pulverized Wood Charcoal on Growth, Feed Utilization, Survival and Waste Excretion of Red Tilapia (Oreochromis Sp)

Jaypee. S. Samson

Faculty Researcher, College of Fisheries, Freshwater Aquaculture Centre,
Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines

How to cite this paper: Jaypee. S. Samson "Oral Administration of Pulverized Wood Charcoal on Growth, Feed Utilization, Survival and Waste Excretion of Red Tilapia (Oreochromis Sp)" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-3 | Issue-4, June 2019, pp.417-421, URL: https://www.ijtsrd.com/papers/ijtsrd23724.pdf

IJTSRD23724

ABSTRACT

A 35-day feeding experiment was conducted to evaluate the effects of pulverized wood charcoal (PWC) on the growth, feed utilization, survival and waste excretion of red tilapia (Oreochromis sp.). Ninety red tilapias with an initial weight of 7.32 ± 1.31 g were acclimatized and randomly introduced into 9 aquaria in 3 groups with 10 fish per aquarium. Different levels of PWC (0 (T0), 1% (T1), and 2% (T2)) were incorporated in the diets of red tilapia for four weeks. After the experiment, average body weight (ABW), specific growth rate (SGR), relative growth rate (RGR) weight gain (WG), feed conversion ratio (FCR) and survival rate (SR) of the experimental fish were computed. No significant differences were observed on feed utilization, survival, and waste excretion of the fish. Furthermore, ABW and WG of the experimental fish did not show any significant difference, however, significantly higher ($P < 0.05$) SGR and RGR were observed from T1 compared to the other treatments. Consequently, this study showed that supplementation of 1% PWC in the diet is the most suitable in improving the growth performance of red tilapia.

Keywords: *Ammonia, Aquaculture, Feed additive, Growth promotion, Red tilapia*

INTRODUCTION

Tilapia species are known for their ability to tolerate a wide range of environmental factors and stressful conditions. They are considered as one of the most important fish species since the early twentieth century till present (Fitzsimmons 2000). Red tilapia species became popular among culturists due to the similarity in shape with sea bream and excellent growth and feed conversion rates among fresh or brackish water species (Liao & Chen 1983).

FAO (2010) reported that in 2009 the global production of red tilapia was 38 064 ton. Fish require variable protein levels in their feeds to maintain optimal growth and survival that is why the quality of feed is one crucial factor in the aquaculture industry. However, the high levels of protein in fish feeds loads more wastes to the aquatic environment. Common wastes coming from aquaculture facilities are the nitrogenous waste, which is toxic to aquatic organisms and affects their growth and survival. One source of these nitrogenous wastes such as ammonia (Kikuchi et al. 1995) is the undigested commercial feeds given to the cultured species (Quiayum et al. 2014). Aside from that, fish and aquatic organisms contribute to the increase of ammonia level in the water through their waste excretions (Floyd et al. 1990; Yu et al. 2007). As part of the growth and survival of animals, they continuously excrete wastes as a result of their metabolic process in acquiring energy and nutrients from their food. Ammonia is the principal metabolic waste excreted by the fish so accumulates easily in aquatic systems. Because of its negative impact on the health of the fish, ammonia should be regulated before it accumulates and harms the fish. (Floyd et al 1990).

Ammonia is considered a very toxic factor to fish that limits their survival and growth (Person-Le Ruyet et al. 1995). High levels of ammonia may result to gill damage, reduced growth, poor feed conversion, and lower resistance to diseases, increased breathing, cardiac output and oxygen uptake and may result to convulsions and death (Floyd et al. 1990, Buttner et al. 1993, Yu et al. 2007, Sichula et al. 2011, Bhatnagar & Devi, 2013). Ammonia in water is composed of two forms; the ionized (NH_4^+) an un-ionized (NH_3) ammonia (Floyd et al. 1990, Yu et al. 2007). Between these two forms of ammonia, the un-ionized form is more toxic than the ionized form because of its ability to move across cell membranes (El-Sherif & El-Feky 2008; Yu et al. 2007, Bhatnagar & Devi 2013). The predominant form of ammonia present in water is affected by temperature and pH of the aquatic system (Floyd et al. 1990). Both forms are termed as "total ammonia nitrogen (TAN)" (Floyd et al. 1990, Yu et al. 2007).

Modifying the feeds given to the cultured species is one of the recommended ways to reduce the nitrogenous waste from aquaculture. Currently, a natural substance such as charcoal is used in formulating animal feeds because of their ability to absorb nitrogen and ammonia and helps in activating the intestinal function of animals (Quiayum et al. 2014). Charcoal is a form of carbon, which is generally produced as a carbonaceous residue of wood and other industrial wastes after heating the organic matter. It contains 70 to 90% pure carbon and several minerals such as calcium, potassium, iron, sodium, copper, zinc, manganese, magnesium, etc. (Brouwer et al. 1996). Charcoal has a large surface area giving it countless bonding sites which can be used in adsorbing toxins, gases, and drugs without any specific action (Osol, 1975). It has been used as a feed additive for many terrestrial animals because of its ability to absorb gases especially nitrogen and ammonia, activate the intestinal function, and eliminate the poisons and impurities from the gastrointestinal tract of land animals (Banner et al. 2000, Bisson et al. 2001, Kutlu et al. 2001, Samanya and Yamauchi 2001, Mekbungwan et al. 2004). It has also been proven to be a good feed additive in the feeds of aquatic animals such as tiger pufferfish (*Takifugu rubripes*) Japanese flounder (*Paralichthys olivaceus*), *Pangasius hypophthalmus*, *Clarias gariepinus*, Nile tilapia (*Oreochromis niloticus*), red tilapia hybrid (*Oreochromis mossambicus* × *Oreochromis niloticus*) and Gilthead Seabream (*Sparus aurata*) (Thu et al. 2009, 2010, Quaiyum et al. 2014, Jahan et al. 2014, Lawal et al, 2018, Nopadon et al. 2015, Michael et al. 2015, Michael & Helal 2018).

This study aimed to evaluate the effect of pulverized wood charcoal on the on growth, feed utilization, survival and waste excretion of Red tilapia (*Oreochromis* sp.)

MATERIALS AND METHODS

Experimental fish and feeds: Red tilapia fingerlings were obtained from Freshwater Aquaculture Centre, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Experimental fish with an initial weight of 7.32 ± 1.31 g were acclimatized for one week and were starved for 24 hours before the commencement of the experiment. Ten randomly selected red tilapia were placed in each glass aquarium and fed the with their designated feed thrice a day (8:00, 12:00 and 16:00), with an initial feeding rate of 10% of their body weight. Sampling was done once every two weeks to measure their weight and adjust the feeding rate.

This study was designed randomly comprising of 3 treatments with each treatment replicated thrice. Commercially-available diet (35% protein, 4% fat) was used as the control for the experiment. Commercial diet was added with 0% (Control) (T0), 1% (T1) and 2% (T2) PWD and water (200 mL kg^{-1}) (Cho and Lee, 2012). Mixtures were mixed thoroughly and dried at room temperature before storing at -20 °C.

Growth performance and Feed Utilization: To assess the growth performance and feed utilization of the experimental fish, the following data were gathered during the experiment which includes specific growth rate (SGR), relative growth rate (RGR), weight gain (WG), survival rate (SR) and feed conversion ratio (FCR). Parameters were calculated as follows:

$$\text{SGR}\ (\% \cdot \text{day}^{-1}) = \frac{\ln\ [\text{final weight}] - \ln\ [\text{initial weight}])}{(\text{time interval in days})} \times 100$$

$$\text{RGR}\ (\%) = \frac{\text{Final weight of fish - Initial weight of fish}}{\text{Initial weight of fish}} \times 100$$

$$\text{WG (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{SR}\ (\%) = \frac{\text{No. of individuals at the end of the experiment}}{\text{No of individuals at the beginning of the study}} \times 100$$

$$\text{FCR} = \frac{\text{Amount of feed given (g)}}{\text{Weight gain (g)}}$$

Water Quality Monitoring: Water quality parameters such as temperature, dissolved oxygen (DO) and pH were measured using DO meter and pH meter. For the analysis of TAN, water samples were collected from the aquarium tanks and were placed in polyethylene (PE) bottles prior to the analysis in the laboratory. Analysis of TAN was done every week before water change following the phenate method. Briefly, 25 to 50 mL of water sample was filtered through Whatman No. 42, and 10 mL of the filtered sample was transferred into a 50 mL beaker in a magnetic stirrer. While stirring, 1 drop of manganese sulfate ($MnSO_4 \bullet H_2O$) solution, 0.5 mL oxidizing solution and 0.6 mL phenate solution was added. Then, removed from stirrer and allowed 15 minutes for maximum color development before transferring transfer to the 1-cm cuvette. Ammonia-free distilled water (10.00 mL) (reagent blank) and 10.00 mL of the 0.300 mg · L^{-1} solution of total ammonia-nitrogen (standard) were carried through the procedure with each set. With the spectrophotometer at 630 nm, set 0.0 absorbance (100% transmittance) with the reagent blank, the absorbances of the standard and the samples were read. Lastly, the concentrations of the TAN were calculated using the formula:

$$\text{TAN}\ (\text{mg} \cdot \text{L}^{-1}) = \frac{\text{Absorbance of the sample} \times \text{TAN concentration in the standard}}{\text{Absorbance of the standard}}$$

Statistical Analysis: Results for the growth, survival and feed utilization were subjected to one-way analysis of variance (ANOVA) followed by Duncan's test at a significant level of P<0.05.

RESULTS

Table1. Growth performance and feed utilization of Red tilapia fed on diets supplemented with different levels of pulverized wood charcoal (PWC) for 35 days.

Parameters	T0 (0% PWC)	T1 (1% PWC)	T2 (2% PWC)
Initial ABW, g	7.80 ± 0.85^{a}	6.85 ± 2.00^{a}	7.32 ± 1.23^{a}
Final ABW, g	14.71 ± 2.72^{a}	15.18 ± 1.45^{a}	13.84 ± 1.85^{a}
SGR (%)	0.78 ± 0.14^{b}	1.22 ± 0.10^{a}	0.80 ±0.17^{b}
RGR (% · day^{-1})	87.91 ± 21.79^{b}	168.72 ± 21.99^{a}	91.25 ± 24.52^{b}
WG, g	6.91 ± 2.11^{a}	8.33 ± 2.34^{a}	6.52 ± 1.57^{a}
FCR	3.72 ± 0.36^{a}	2.45 ± 0.98^{a}	2.98 ± 0.45^{a}
SR (%)	36.67 ± 5.77^{a}	46.67 ± 15.27^{a}	53.33 ± 37.85^{a}

PWC, pulverized wood charcoal; ABW, average body weight; SGR, specific growth rate; RGR, relative growth rate; WG, weight gain; FCR, feed conversion ratio; SR, survival rate.

Means in column with the same letter are not significantly different at $P < 0.05$

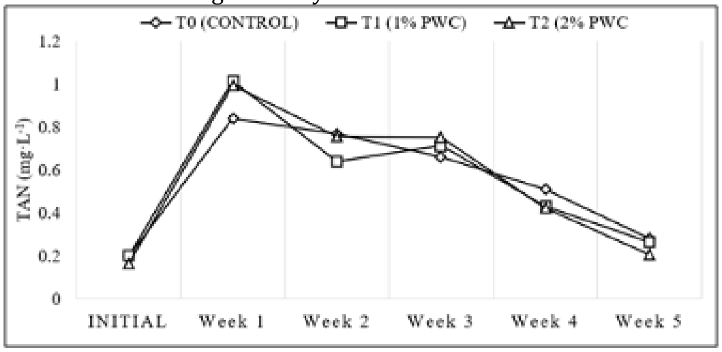

Figure.1. Total ammonia nitrogen (TAN) co+ncentration of water of aquaria stocked by red tilapia fed on diets supplemented with different levels of pulverized wood charcoal (PWC) for 35 days.

Results of growth performance, feed utilization, and survival, at the end of the experiment is shown in Table 1. There were no significant differences observed in the initial and final ABW of the experimental fish. However, PWC inclusion of 1% (T1) significantly ($P < 0.05$) improved the SGR and RGR of the experimental fish when compared to the control group (T0). WG from T1 was also numerically higher compared to the other treatments (T0 and T2), however, the observed increases were not statistically significant based on comparisons with the other groups. Furthermore, although FCR and SR were better on PWC-supplemented diets, no significant differences were observed upon comparing with the control group.

Water quality parameters such as temperature (28.75 ± 0.85 ºC), pH (8.4 ± 0.22), and DO (2.5 ± 0.83 mg · L^{-1}) did not show any significant differences among the treatments. Levels of TAN concentration were found to be varying throughout the culturing period. During the 1st week until the 3rd week of feeding experiment, a high concentration of TAN was recorded in PWC-supplemented diets, with readings of 0.996 to 1.016 mg · L^{-1}, respectively. As the experiment progress, the level of TAN decreases (Fig 1). In the 4th and 5th week of the feeding trial, TAN levels from PWC-supplemented diets were numerically lower, however, the observed decrease was not statistically significant based on comparisons with the control group.

DISCUSSION

The quality of the fish diet is one of the most important factors to consider when it comes to the optimal growth potential of an aquaculture organism. Over the years, numerous efforts were made to formulate a diet that can provide optimal growth, and made of readily available and inexpensive ingredients to reduce the cost of feed production. However, despite the capabilities of the feeds to sustain good fish growth rates, they have a negative environmental impact due to their high nitrogenous wastes particularly ammonia that constitutes almost 80% of these wastes (Kikuchi et al. 1995). With that, the use of feed additives especially charcoals to reduce the nitrogenous waste coming from the aquaculture industry and promoting better growth to the cultured species is gaining popularity. The inclusion of charcoal in the diets of animals has received attention due to its non-nutritive and nondigestible characteristics giving it the ability to adsorb toxins from the gastrointestinal tract thus preventing their absorption into the body. Several researches have demonstrated that charcoal-supplemented diets can improve fish growth, flesh quality, and intestinal function (Boonanuntanasarn et al. 2014, Quiayum et al. 2014, Jahan et al. 2014, Moe *et al*. 2010, Thu et al. 2009, 2010, Lawal et al. 2018, Nopadon et al. 2015, Michael & Helal 2018).

The present study aimed to evaluate the effect of different levels of PWC in the feeds of red tilapia on their growth, feed utilization, survival, and waste excretion. Results showed that 1% inclusion of PWC in the diet significantly improved the SGR and RGR of the fish. The result obtained from this study is in parallel with the report of Nopadon et al. (2015), wherein 1-3% of AC inclusion improved the growth performance of Nile tilapia with optimal inclusion of 2%. Also, Abdel-Tawwab et al. (2017) observed improvement in the growth parameters of Nile tilapia at AC inclusion of 0.2-2%, with optimal inclusion level of 0.7%. Additionally, the inclusion of commercial wood charcoal (CWC) at 3-4% in the diet of red tilapia hybrid (*Oreochromis mossambicus* × *Oreochromis niloticus*) improved fish survival, growth, and feed utilization. In contrast, the present study found no significant improvement in the growth of red tilapia at a 2% level of PWC inclusion. Supplementation of charcoal in the diets have also shown improved growth performance of other aquatic animals. Thu et al. (2009) and Michael & Helal (2018) reported that 4% of charcoal inclusion is the optimal level for tiger puffer fish and gilthead seabream. While 0.5% level of bamboo charcoal (BC) inclusion is reported by Moe et al. (2010) to be the best in juvenile Japanese flounder. In addition, Lawal et al. (2018) found significant improvement in the growth of *Clarias gariepinus* with 0.25% level of AC inclusion. Furthermore, Quiayum et al. (2014) and Jahan et al. (2014) observed significant effects of BC-supplemented diets in pangasiid catfish growth and ammonia loading, however, they reported a dissimilar level of optimum charcoal inclusion of 2% and 1%, respectively. On the other hand, Boonanuntanasarn et al. (2014), reported that growth parameters of Nile tilapia (*O. niloticus*) reared on diets containing 10 g · kg^{-1} body weight AC only showed a numerical improvement with no statistical significance. The same result was observed by Mabe et al. (2017) in juvenile common carp (*Cyprinus carpio* L.) fed with different levels of BC in the diet. These results suggest that the optimal level of charcoal inclusion in the diet of aquatic animals differ among species, animal's life stage, and the nature of charcoal.

The enhanced growth parameters in the present study may be explained due to the capability of PWC to adsorb and eliminate unwanted gases and toxic substances, which can result to improved digestion, metabolism, and better nutrient utilization and absorption (Kutlu et al. 2001, Samanya & Yamauchi 2001, Jahan et al. 2014). Nevertheless, since charcoal is a nonspecific adsorbent, its application in the diets of animals may possibly cause the adsorption of not only toxins but also nutrients from the gastrointestinal tract. Thus, positive and negative impacts on physiological functions, growth and overall health of an organism can be both expected (Boonanuntanasarn et al. 2014, Nopadon et al. 2015).

The SR obtained in all treatments is noticeably low. Mortalities of the experimental fish started during the 3rd and 4th week of the experiment. As an observation, some experimental fish are gliding their bodies along the sides and bottom of the aquarium. It is possible that during the experiment, the fish acquired bacterial disease which is one possible reason why the SR in all treatments is low. Aside from that, the level of oxygen throughout the experiment (2.5 ± 0.83 mg · L^{-1}) is lower than the desired dissolved oxygen level of at least 5 mg · L^{-1}.

In the present study, no significant improvement was observed in the waste excretion of the red tilapia. In contrast, Thu et al. (2009) and (2010) reported that (BC) supplementation reduced the ammonia-N values with tiger puffer fish and Japanese flounder, respectively. Michael et al. (2015) also observed a gradual decrease in ammonia levels in the rearing water area as the CWC levels in red tilapia feeds increases. The same observation was noted by Quiayum et al. (2014), where they found out that increasing level of charcoal incorporation in fish feeds results to lower ammonia level in the water. The possible reason with this is that the duration of the experiment (35 days) is not enough to observe the effect of PWC in the waste excretion of the fish.

CONCLUSION

In conclusion, the result of the present study showed that 1% inclusion of PWC in the diet improved the growth performance of the red tilapia (*Oreochromis* sp.). Therefore, PWC at 1% can be a useful feed additive in the diet of red tilapia as it is cheap and readily available.

ACKNOWLEDGEMENT

The author would like to acknowledge the assistance of the College of Fisheries and Freshwater Aquaculture Centre where the study was conducted. Also, the author would like to extend his appreciation to the Department of Science and Technology–Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP) for financial assistance during the experiment.

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