Water lettuce and water spinach as potential feed ingredients for Nile tilapia *Oreochromis niloticus*

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) is an omnivore and is considered as one of the important aquaculture commodities. Different plant-based ingredients like corn, "ipil-ipil" leaves and even micro and macroalgae were already tested to reduce the cost on the use of animal-based protein source. This preliminary study therefore, is an attempt to assess the potential of water lettuce and water spinach as feed ingredients through average body weight (ABW), specific growth rate (SGR), survival rate and feed conversion ratio (FCR) of juvenile *O. niloticus*. Three treatments [water lettuce (WL), water spinach (WS), water lettuce and water spinach (WL+WS)] were prepared in the form of three different diets [Diet 1 (1:1 water lettuce: fish meal), Diet 2 (1:1 water spinach: fish meal) and Diet 3 (1:0.5:0.5) (fish meal: water lettuce: water spinach)] each replicated thrice and fed to tilapia for 60 days. Tilapia growth showed no significant differences in all diets. The diet with water spinach (*Ipomoea aquatica*) significantly improved the SGR. Based on the result, the *I. aquatica* could be included both in a simple or complex diet together with water lettuce (*Pistia stratiotes*). It took 30 days for *O. niloticus* to adjust to the introduced diets as reflected to their survival rate but the FCR was higher when fed with the test diets compared to the recorded commercially-fed tilapia. Overall, *I. aquatica* has an excellent performance for *O. niloticus* culture.

Keywords: Cichlid*, Pistia stratiotes, Ipomoea aquatica,* ABW, SGR, FCR

INTRODUCTION

There is a rising demand of fish for human consumption worldwide leading to the intensive culture and search for cheaper feed formulas for aquaculture (Bhosale et al. 2010). The commonly used ingredients for tilapia culture include fish meal and soybean meal. These ingredients have higher costs and fish meals add pressure to the marine resources, hence, there is an increasing effort to seek for alternative sources of protein from the local plant resources. At present, various studies are being conducted on the evaluation of protein from plants. It was found that plant sources like leucaena leaves, wheat and corn promote growth performance, feed utilization efficiency and amino acid composition equal or greater than those obtained with fish meal (Tacon 1989; Borgeson 2005; Song et al. 2014).

Tilapia (*Oreochromis niloticus*), an omnivorous fish, is popular and mass cultivated worldwide for having rapid growth, good feed acceptance, adaptation to captivity and white meat with few intramuscular bones (Montoya-Camacho et al. 2018). In the Philippines, tilapia culture had 0.4% contribution to the fisheries sector as of 2019. Furthermore, the total tilapia production reached up to 56,177.85 metric tons in the country last year (Troell et al. 2014; PSA 2019).

The use of less-expensive plant protein sources such as soybean, maize, pea, canola, sunflower seed and "ipil-ipil" as partial or total replacements for fish meal in the diet of tilapia and other cultured organisms is an international research priority at present (Llanes and Toledo 2011; Gonzalez-Salas et al. 2014; Plaipetch and Yakupitiyage 2014; Figueiredo-Silva et al. 2015; Hassaan et al. 2015; Khalifa et al. 2017). Other possible local fish feed plant ingredients with low economic values include water lettuce (*Pistia stratiotes*) and water spinach (*Ipomoea aquatica*). The leaves and stems of water lettuce contain 92.9% moisture, 1.4% protein, 0.3% fat, 2.6% carbohydrate, 0.9% fibers, 1.9% ash, 0.2% calcium and 0.06% phosphorus (Tripathi and Mishra 2007). Another potential plant as tilapia feed ingredient is the water spinach which contains 72.8±0.29% moisture, 11.0±0.50% crude lipid, 17.7±0.35% crude fibers, 54.2±0.68% carbohydrates and a crude protein of 6.30±0.27% (Umar et al. 2007). It also has a good source of minerals like potassium, manganese, iron and magnesium thus, it is being recommended to be used for nutritional purposes both for humans and animals (Umar et al. 2007).

This study therefore aims to find out the potential use of these plants as feed ingredients in the diet of Nile tilapia specifically on its specific growth rate (SGR), survival rate and feed conversion ratio (FCR).

METHODS

Study Site

This study was conducted at the Hatchery of the Fishery Department in Capiz State University-Dayao Satellite College, Dayao, Roxas City, Capiz, Philippines. The area is situated at the middle of the University ponds and the tanks used were shaded and located outside the hatchery infrastructure.

Feed Preparations

For the feed formulation, there are three treatments containing water spinach (WS), water lettuce (WL) and a combination of WL and WS. The ratio of incorporation were adjusted to Diet 1 (1:1 water lettuce:fish meal), Diet 2 (1:1 water spinach:fish meal) and Diet 3 (1:0.5:0.5) (fish meal:water lettuce:water spinach). The WL and WS were sourced from the private fish ponds in Barangay Barra and Barangay Dayao, Roxas City, Capiz respectively (Table 1).

Table 1. Amounts and ingredients of feeds used in the study.

Treatment	Ingredients	Amount (%)
Diet 1	Water lettuce	50
	Fish meal	50
Diet 2	Water spinach	50
	Fish meal	50
Diet 3	Fish meal	50
	Water lettuce	25
	Water spinach	25

All plant ingredients were sun-dried for 3 days, ground into fine particles, sieved or sifted, weighed and mixed by hand for at least 5 min or until well blended before adding the fish meal and cassava flour with water (4:1) as binder. These were further mixed with a wooden ladle and were cooked for 10-15 min under moderate heat. The pre-cooled doughs were then passed through an extruder and the feeds formed were cut into 2-3 mm length prior to one-day sun drying. The pellets were cooled down at room temperature for 30-60 min before storage.

Rearing and Monitoring of *O. niloticus*

Forty-five pieces of juvenile tilapia with an average (±SEM) weight and length of 22.8±1.4 and 9.7±0.6 cm respectively were obtained from the hatchery in Dayao, Roxas City, Capiz. The fish were conditioned for 3 days by feeding them with commercial diet containing 39% crude protein. After conditioning, the fish were stocked in each compartment of a 3-ton-rectangular fiber glass tank (in a shaded area) at 5 fish per 0.3 m^3 with continuous supply of aeration.

The initial feed rate was based on 7% of the fish biomass in all treatments. The daily feeding rate (DFR) was also computed (Table 2).

Table 2. Daily feeding ration (g) of Nile tilapia using diets with water lettuce, water spinach and the combination of water lettuce and water spinach throughout the culture period. WL – water lettuce; WS – water spinach.

Treatment \ Days of Culture	0-15	16-30	31-45	46-60
Diet 1 (WL)	123.9	156.5	158.6	168.0
	72.5	83.0	63.0	68.4
	87.2	110.3	118.7	98.7
Diet 2 (WS)	131.3	149.1	121.8	127.1
	125.0	159.6	127.1	66.2
	105.0	101.9	109.2	112.4
Diet 3 (WL + WS)	131.3	123.9	69.3	70.4
	95.6	158.6	88.2	89.1
	115.5	138.8	99.9	105.0

Statistical Analysis

Average ± standard error of mean (SEM) of the SGR, % survival and feed conversion ratio (FCR) were computed using Microsoft Excel 2013. Statistical Package for Social Sciences (SPSS) version 20 was used to compare the treatments. The growth and feed efficiency data were tested for homogeneity of variance using Levene's tests and then subjected to one-way analysis of variance (ANOVA) at 0.05 level of significance to determine the differences among the three treatments. Furthermore, post-hoc tests using Duncan's Multiple Range Test (DMRT) were carried out to confirm where the differences among groups occurred.

RESULTS

The average body weight (ABW) of tilapia with regard to time apparently increased from day 0 up to day 60 in all treatments. The average body weight of *O. niloticus* fed with diets containing WL, WS and WL + WS had no significant differences except during 30th and 60th day of the culture

(Figure 1). At day 30, the ABW of those fed with WL + WS (36.1±1.9 g) were significantly higher compared to the other treatments. At the end of the culture period, WL + WS (44.2±1.8 g) only differed significantly with those fed with WL (34.2±3.1 g).

The specific growth rates of *O. niloticus* were statistically similar among all treatments throughout the culture period, nevertheless, sharp increase for each treatment was observed at the end of the culture period (Figure 2). On the 15th day, SGR reached up to 1.8±0.3% day^{-1}, 1.3±0.2% day^{-1} and 1.5±0.2% day^{-1} in diets containing WL, WS and WL + WS respectively (Figure 2). Those fed with WL had statistically similar SGR from day 15 to day 30 (0.9±0.3% day^{-1}) but significant increase was apparent at the end of the culture period. Similar trends were observed in the SGR of *O. niloticus* fed with WS and WS + WL in which the significant increases ($p<0.05$) were found after 60-day culture at 2.7±0.1 and 3.0±0.1% day^{-1} respectively.

The survival rate of tilapia decreased after 30-day culture and was maintained up to the end of the culture period (Table 3). No significant differences were found among the survival rates of *O. niloticus* fed with the different diets except at 30-day culture when those fed with WL (86.7±13.3%) were significantly higher than those fed with WL + WS (53.3±6.7%) and WS (80.0±0.0%).

The FCR of the fish also significantly increased from day 30 onwards though the performances of every diet did not vary from each other throughout the culture period. The final FCR of tilapia fed with WL, WS and WL + WS reached up to 7.6±0.8, 7.9±1.0 and 8.1±0.8 respectively.

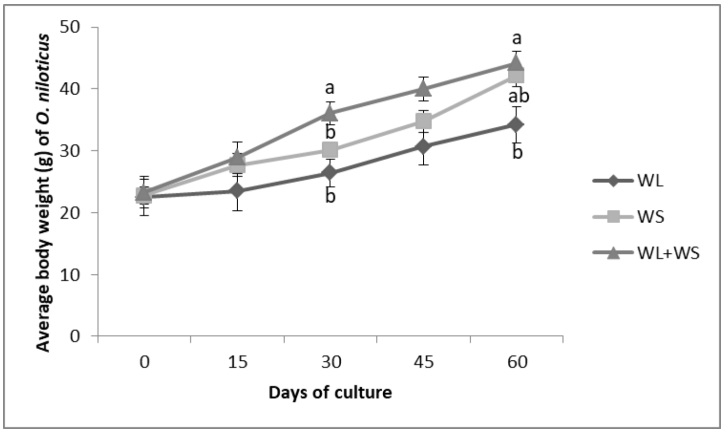

Figure 1. *Oreochromis niloticus* average body weight (ABW) (g) fed diets containing water lettuce (WL), water spinach (WS) and combination of water lettuce and water spinach (WL + WS). Data presented as Mean ± SEM ($p<0.05$), n=3.

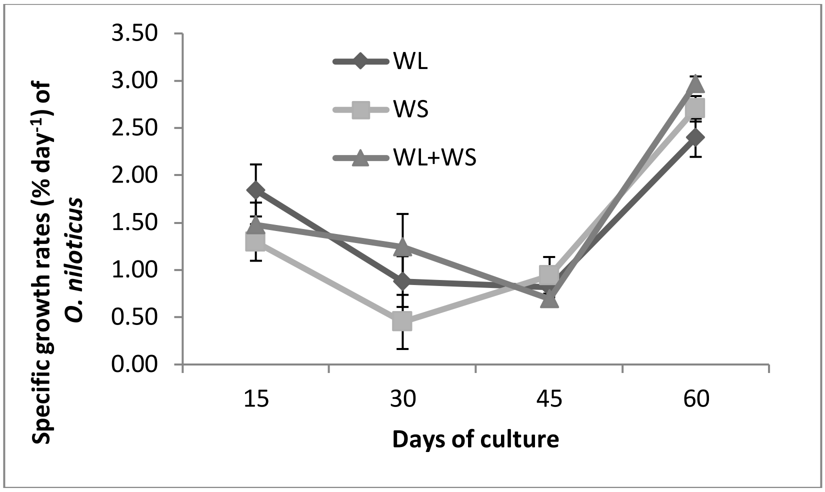

Figure 2. Specific growth rate (SGR) (% day^{-1}) of *O. niloticus* fed diets containing water lettuce (WL), water spinach (WS) and combination of water lettuce and water spinach (WL + WS). Data presented as Mean ± SEM. All treatments showed no significant differences in every period ($p>0.05$; $n=3$) although SGR values on the 60th day were significantly higher than those obtained in other sampling events. ($p<0.05$), $n=4$.

Table 3. Average (± SEM) Survival rate and feed conversion ratio (FCR) of Nile tilapia fed with different organic feeds every 15 days of culture. Values with the same superscripts in every treatment at the same culture period are not significantly different ($p>0.05$).

Days of Culture/ Treatment	**Parameters**	
	Average (± SEM) % Survival	**Average (± SEM) FCR**
Day 15		
Diet 1 (WL)	93.3±6.7	3.7±0.3
Diet 2 (WS)	93.3±6.7	5.6±0.9
Diet 3 (WL + WS)	86.7±6.7	5.0±1.2
Day 30		
Diet 1 (WL)	86.7±13.3^{a}	7.4±0.7
Diet 2 (WS)	80.0±0.0^{b}	3.8±4.5
Diet 3 (WL + WS)	53.3±6.7^{b}	7.6±0.6

Days of Culture/ Treatment	Parameters	
	Average (± SEM) % Survival	Average (± SEM) FCR
Day 45		
Diet 1 (WL)	80.0±11.6	8.0±1.1
Diet 2 (WS)	66.7±13.3	8.6±0.4
Diet 3 (WL + WS)	53.3±6.7	8.3±0.8
Day 60		
Diet 1 (WL)	80.0±11.6	7.6±0.8
Diet 2 (WS)	66.7±13.3	7.9±1.0
Diet 3 (WL + WS)	53.3±6.7	8.1±0.8

DISCUSSION

Tilapia species are generally herbivores with longer coiled intestines compared to carnivores and mouth characteristics necessary for plant shredding (Trewavas 1982). For this reason, several studies had been conducted to replace feed ingredients in the diet of cultured tilapia in consideration to the increasing costs. Studies including protein replacements and additives from different plant sources like duckweed, *Moringa olifera*, jute, maize and soybeans were already tested with promising results (Ritcher et al. 2003; Borgeson et al. 2006; Nnaji et al. 2010; Magouz et al. 2016; Singh et al. 2016; Caipang et al. 2019). Furthermore, macro and microalgae were already evaluated to replace protein in the diets of different cultured aquatic organisms like shrimps, carp and tilapia (Naorbe et al. 2015; Putri et al. 2017; Montoya-Camacho et al. 2018; Puello-Cruz et al. 2018; Sarker et al. 2018; Singh et al. 2018).

In this study, low-valued plants including water lettuce and water spinach were evaluated as potential feed ingredient for *O. niloticus*. The differences in the ABW of *O. niloticus* fed with three different diets were observed at day 30 and day 60 of culture. Those fed with WL alone had significantly lower ABW compared to those with combined WL + WS. This could be attributed by the characteristic of WL (*P. stratiotes*) as an accumulator of heavy metals in its environment (Varun et al. 2017). Aquatic environment, even those that are unpolluted contains toxic heavy metals primarily from discharges of industrial effluents and from atmospheric precipitation (Chavez et al. 2006). These metals in the form of organic and inorganic compounds can undergo methylation through microorganisms in the sediments and the end-product can enter the food chain up to the trophic levels (Chavez et al. 2006). These toxic metals can be taken up by fish via the alimentary tract, gills and skin and these were found to cause damage to some vital tissues and organs affecting the fish growth and survival (Schreckenbach 1982; Svobodova et al. 1993). Examples of heavy metals that were found to accumulate in the tissues of *O. niloticus* include Ni and Pb (Nakkina 2016). Water lettuce, like microalgae, is commonly used as phytoremediation agent in heavy metal-contaminated waters despite its promising nutritional contents (Naorbe and Serrano 2018). It was recorded to contain 8.6-20.5% crude protein, 19.1-21.9% crude fibre, 3.8% crude fat and other trace elements like calcium, magnesium, phosphorus, etc. (Ayoade et al. 1982; Banerjee and Matai 1990; Rodriguez et al. 2000; Jimoh et al. 2016). This species however, has varying nutritional contents depending on its environmental source (Varun et al. 2017).

The plants used in this study were collected from a nearby vacant private fish pond (Brgy. Barra, Roxas City, Capiz) that were once used for aquaculture. The location of this fish pond source was adjacent to the community wastewater outlet, thus it is projected that the water and the plant contain heavy metals from the discharged effluents, previous aquaculture inputs and even from the atmospheric precipitation. Furthermore, the analysis conducted by Rodriguez et al. (2000) showed that *P. stratiotes* had low macro-element like phosphorus (0.26%) which is supposedly beneficial in the digestibility of the dietary fibers and better nutrient utilization (Song et al. 2015). These are the probable cause of the lower ABW of the fish compared to those fed in combination with WS (*I. aquatica*) in this study. The ABW of *O. niloticus* nevertheless, continued to increase regardless of the diet being fed through time. This result was further supported by the SGR of *O. niloticus*. Those fed with WS and WL + WS reached up to 2.7±0.1 and 3.0±0.1% day^{-1} at the end of the culture period respectively. This is higher compared to the SGR of *O. niloticus* fed with diet containing green algae *Caulerpa lentillifera* in the study conducted by Putri et al. (2017) which only reached 2.32±0.07 at 10% inclusion but relatively lower than those fed with commercial diet (40% crude protein) with an SGR of 3.72% (Tavares et al. 2008). The slow growth of *O. niloticus* fed with WS (*I. aquatica*) was probably due to its high carbohydrate and mineral contents and its further combination with the nutrients from *P. stratiotes* (Umar et al. 2007). All treatments decreased in survival rate after 30 days and were maintained up to the end of the culture period. Analysis with respect to time, however, revealed that those fed with WL had higher survival rate than those fed with WL + WS also at 30-day culture. In general, the survival rate of tilapia in this study is lower compared to those conducted by Workagegn et al. (2014) on *O. niloticus* fed different types of formulated diets that attained 100% survival rate and those conducted by Ahmad et al. (2009) on the fish fed *Origanium vulgare* extract which also gave 80-100% survival rate. This could be pointed to the absence of other feed ingredients which led to the lack of other nutrients that could improve the immune system of *O. niloticus*. However, *O. niloticus* fed Diet 1 (WL) had comparable survival rate with those fed fermented and with activator *Lemna minor* and those fed commercial feeds and raised in an earthen pond (Bahnasawy et al. 2003; Pinandoyo et al. 2019). To further improve the survival rate, it is recommended that the protein contents of *P. stratiotes* and *I. aquatic* be concentrated and mixed with other ingredients for definite balanced and complete diet.

Feed conversion ratio (FCR) is a measure of the amount of feeds used to grow each kilogram of a farmed organism. Low values of FCR indicate efficient feeds, low production cost and sustainable process (Martinez-Cordova et al. 2016). In the present study, the FCR of the fish significantly increased from day 30 onwards though the performances of every diet did not vary from each other. Results in this study are higher compared to those fed with commercial feeds at 2.0±0.2 (Bolivar et al. 2011). This is probably due to the high fiber and ash contents of *P. stratiotes* and *I. aquatica*. Fibers are group of complex organic substances mostly found in plants and cannot be digested by enzymes in the intestinal tract of fish and other nonruminant animals, thereby cannot be converted into body mass (Li et al. 2012). The microbial activities in the lower gut of the fish which could aid in the fermentation of this fiber are also limited resulting to the lower dietary nutrient digestibility for growth and metabolism and higher FCR (Glencross 2009). The fibers in the plants used in this study probably reduced nutrient digestibility and absorption and resulted to higher ratio of the converted feeds to flesh (Hilton et al. 1983). The result of FCR in this study is similar to that conducted by Shiau et al. (1989) on Nile tilapia fed a diet containing 10% cellulose that reduced the feed efficiency and glucose absorption of the fish relative to the diet without cellulose. Similarly, high level of ash in the diet could significantly affect the nutrient utilization by fish (Ogunji et al. 2008). Specifically, it could affect the fish dietary apparent digestibility coefficients (ADCs) since these two have inverse relationship or negative correlations (Hajen et al. 1993; Robiana et al. 1997). Apparent digestibility coefficient directs the FCR and another study by Koprucu and Ozdemir (2005) further showed that high content of ash in the diet of tilapia resulted to lower ADCs of dry matter, protein, average amino acid, lipid and energy. This impractical FCR could be addressed by concentrating the protein of both ingredients prior to feed inclusion using either conventional or chemical extraction methods that extract and concentrate the protein from the plants, thereby eliminating other components like fiber and consequently ash (Bleakly and Hayes 2017).

It took 30 days for *O. niloticus* to adjust to the introduced diets as reflected to their survival rate. The experimental diet used in this study had probably poor palatability resulting to feed rejection on the first 30 days of the culture which also reflected on the low weight gain of the fish. Another prospective factor is again, the high amount of fibers that are usually present in the plants used. Usually, fish like tilapia has low cellulose activity (Saha et al. 2006). This also affects the digestibility and gastrointestinal transit rate in the fish although some amount of fiber is required for optimal utilization of diet and overall growth of the fish (Dioundick and Stom 1990; Lanna et al. 2004). *Oreochromis niloticus* used in this study showed similar survival rate with that of *Tilapia rendalli* in the study of Hlophe and Moyo (2011) which adapted to utilizing higher plants by breaking down the fiber, specifically cell wall and later on to the exposure of the cell contents to the digestive processes. In this study, it probably took 30 days for the culture to develop the digestive enzymes and adapt to the new diets. This is in contrast to the study conducted by Bowen et al. (1998) wherein the food intake of the fish has rapid passage and took shorter time for digestion and ingestion resulting to higher survival rate. Overall, the tilapia accepted all the different diets and showed normal behavior and growth.

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