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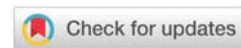
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Research Article

Growth performance of Nile Tilapia (*Oreochromis niloticus*) fingerlings fed with water spinach (*Ipomoea aquatica*) diets

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Abstract

Nile tilapia (*Oreochromis niloticus*) are herbivores with longer coiled intestines compared to carnivores; mouth characteristics necessary for plant shredding. Hence, several studies have been conducted to replace feed ingredients in the diet of Nile tilapia considering the increasing cost. In this study, Water spinach (*Ipomoea aquatica*) was evaluated as a potential feed ingredient for Nile tilapia. A six months feeding trial was conducted to assess the effects of water spinach fish feed composition on the performance of Nile tilapia fingerlings. Five diets were formulated containing 0% (control diet), 5%, 10%, 15% and 20% water spinach composition. Each treatment was carried out in triplicate using 30 Nile tilapia juveniles per replicate with an initial mean weight of 2±1g. The fish were fed at 5% body weight twice per day. Water quality monitoring was done every morning before feeding. There was no significant ($p > 0.05$) variation in water quality parameters between all the treatments. The best growth performance was recorded from a fish-fed 5% diet (180.49±0.83 g), while fish fed with a 20% diet had poor growth performance (128.98± 0.80g). The highest SGR was obtained in fish fed with a 5% diet (1.34±0.05) while the lowest was obtained in fish fed with a 20% diet (1.09±0.05). Except for SGR, WG, FL, and FW, there was no significant difference ($P>0.05$) in other growth parameters of all the treatments. Final weight had a significant difference as determined by One-Way ANOVA ($F(4,316) = 6.363, P=0.00$) between 15% and 20% water spinach composition compared to 5% water spinach composition. Therefore, 5% water spinach composition had the best growth performance.

Introduction

Nile tilapia (*Oreochromis niloticus*) is the most preferred cultured fish species in many tropical and subtropical countries of the world. They are of commercial importance in aquaculture

because they are highly resistant to diseases, exhibit rapid growth, efficient feed conversion, are easy to breed, and have good consumer acceptance [1]. A variety of factors affecting its growth rate include sex, stocking density, decrease in water temperature, and supplemental feeding [2,3].

Fish nutrition plays a significant role in promoting normal growth and sustaining the health of cultured fish. Artificial diets manufactured from various feed ingredients act as the primary source of nutrition in intensive aquaculture systems. Cultured fish are fed with different diets of complete feeds based on species, age, and production purpose in which protein is the limiting factor. Protein, carbohydrates, and lipids are distinct groups of nutrients that the body metabolizes to produce the energy needed for physiological processes and physical activities [4]. However, protein is the most expensive and limiting fish feed ingredient across the globe [5].

This demands alternative sources of fish feed protein away from the traditional animal protein sources already overstretched by the other animal production value chains including dairy and poultry. For this reason, many studies have been conducted on the replacement of animal protein with a lower-cost protein ingredient to reduce the cost of production. Therefore, in order to ensure sustainability in the aquaculture sector, more attention has been devoted to the replacement of fish meal with plant protein sources [6]. Including various leguminous plants such as soybean meal and aquatic macrophytes such as *Lemna minor* and water spinach.

Water spinach (*Ipomoea aquatica*) is a semi-aquatic, floating tropical and sub-tropical plant highly cultivated in the Asian continent. It grows in water or on moist soil and has hollow trailing stems which reach 3 meters. The leaves vary from typically sagittate to lanceolate. The flowers are usually purplish with a mauve center. Propagation is done by either cutting the stem shoots that will root along with nodes or planting the seeds. The origin of water spinach is not quite clear, but it is agreed that it is native to southeastern Asia. In Asia, several sources cite China as the place of domestication [7]. The plant performs best within and above 23.9 °C, with low frost, and high soil moisture. Clay soils and marshy soils rich in organic matter are suitable for water spinach. The ideal pH range for growth is from 5 to 7 [8]. It is a good source of protein and can be used as feed for animals and humans [9]. Water spinach contains significant quantities of amino acids, non-essential amino acids, organic acids, and polyphenols [10]. They are also a source of human food and are cooked or eaten raw [11]. Mainly in southeastern Asia, and also serve in wastewater eutrophication [12].

Fish feeds are expensive and constitute up to 60% to 70% of the total production cost in the aquaculture industry. Therefore, there is a need to replace this with cheaper and easy to culture plant protein sources. In the present study, water spinach was used to replace a fish meal to improve the growth performance of Nile tilapia fingerlings at a lower cost.

Materials and methods

Diet formulation and preparation

Water spinach was harvested from irrigation canals in Ahero, 23 km southeast of Kisumu City and 323 km west of Nairobi, Kenya's capital city. The plants were rinsed of dirt, put on an envelope, and dried. They were sorted, cleaned, air-

dried under the shade then oven-dried at 60° C [13]. Milled into a fine powder, and finally, the proximate composition was determined. Five isonitrogenous diets of 30% crude protein (CP) with different levels of water spinach ranging from 0 % (control), 5 %, 10 %, 15 %, and 20 % were formulated using WinFeed Least Cost Feed Formulation Software version 2.8 with other dietary ingredients incorporated Table 1.

Determination of proximate composition of water spinach

Crude protein was determined using Kjeldal method. 0.2 g of water spinach was weighed in duplicate on a piece of nitrogen-free, ashless Whatman filter paper. The paper was folded and put in a Kjeldal flask into which Kjeldal catalyst tablet and 20 ml of concentrated sulphuric acid were added. This was then heated on a block digester in a fume cupboard until a clear solution was obtained. After cooling, distilled water was added to the Kjeldal flask to about percent full and a drop of phenolphthalein indicator was added. A 400 ml conical flask with 25 ml of 0.1 hydrochloric acid and drops of methyl orange indicator was placed in the outlet of the distillation unit. The Kjeldal flask was then connected to the distillation unit and 40% sodium hydroxide solution was added till the color changed. Distillation was then allowed to take place until the volume in the conical flask rose to 200 ml. the distillate was back titrated with 0.1 N sodium hydroxide until the color changed to yellow.

Crude protein content was determined using the formula below:

$$\text{Crude protein} = \frac{\text{Vol} \times N \times 10^3 \times 14.0067 \times 100}{\text{Total weight of sample}} \times 100$$

Where:

Vol- Volume of NaOH used

N- Normality of NaOH

Lipids were extracted using the modified Folch method [14]. A feed sample weighing 10 g was put in a conical flask. 50 ml

Table 1: Feed formulation (% dry matter).

Ingredients (g/Kg)	Diets(% water spinach composition)				
	0%	5%	10%	15%	20%
Soy Meal	53.8	54.35	54.9	55.45	56
Wheat pollard	9.2	7.65	6.1	4.55	3
Wheat bran	12	10.12	9.25	8.37	7.5
Maize germ	20	16.25	13.5	8.75	5
Water spinach	0	5	10	15	20
Vitamin premix	1	1	1	1	1
MCP	3	3	3	3	3
L-lysine	1	1	1	1	1
Methionine	1	1	1	1	1
Sunflower oil	0	0.63	1.25	1.88	2.5
Total	100	100	100	100	100

of chloroform/methanol (2:1) was then added. The mixture was transferred onto an orbital shaker for 2 hours. This was then followed by filtration through glass wool and transfer of the filtrate into a separating funnel. The residue was recovered and lipids re-extracted with 50 ml of chloroform/methanol (2:1) for one hour on an orbital shaker followed by filtration. The filtrate was washed by addition of 0.2 volumes of 0.5% sodium chloride, mixed well, and left to stand for phase separation. The chloroform phase was recovered and placed in a dry pre-weight volumetric flask. Chloroform was then evaporated in an oven at 55 °C. The weight of the lipids was obtained by subtracting the weight of the empty flask from the weight of the flask with lipids.

The percentage lipid content was determined as follows:

$$\text{Lipid content \%} = \frac{\text{Weight of lipid in grams}}{\text{Weight of sample in grams}} \times 100$$

The moisture content of water spinach was determined by drying (in triplicates) in a Gallenkamp oven at 105 °C until a constant weight is recorded [15]. Ash content was determined by measuring 3 g of sample into a crucible and heated in a Lenton muffle furnace at 550 °C for 24 hours. The residue was weighed and ash content was determined as;

$$\text{Ash content \%} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

The proximate composition of experimental diets (Table 2) shows that the crude protein was highest (29.88%) in the control (0 %) water spinach composition and least (28.50%) in 20 % water spinach inclusion. However, there was no significant difference ($P > 0.05$) in the CP content between the four experimental diets and the control. Total lipids and ash contents were higher in 15 % water spinach composition while total moisture content was higher in 10 % water spinach inclusion.

Experimental design

A total of 450 Nile tilapia fingerlings weighing $2.0 \pm 1\text{g}$ were obtained from the National Aquaculture Research Center Sagana, in Kirinyaga County, Central Kenya. Prior to trial, fish have stocked acclimatization for three days in circular tanks measuring 1m^3 at Kenya Marine and Fisheries Research Institute (KMFRI), Kegati Aquaculture Centre located in South Nyanza part of Kenya. This experiment was conducted for six months from June to November 2019. At the beginning of the experiment, fish were starved for 24 hours,

weighed, and randomly distributed into 15 circular tanks at a stocking density of 30 fish per tank. Water depth in tanks was maintained at 45cm throughout the experimental period, while dissolved oxygen (DO) was maintained at $\geq 2\text{ mg}^{-1}$. Temperature, dissolved oxygen (DO), and pH. were monitored daily using the YSI Multi probe model. NH_4 and NO_2 were analyzed spectrophotometrically every week using standard methods [16].

Growth performance

The entire population in each treatment triplicate was sampled monthly. Water levels were reduced and all fish in each tank were caught using a scoop net and placed in aerated labeled buckets for sampling one at a time to avoid mix-ups. Weight and total length measurements were taken using an electronic balance with an accuracy of 0.01g and a measuring board (60 cm) respectively. The total length was measured from the snout to the tip of the caudal fin. All the measurements were recorded and feeding was adjusted accordingly. Experimental tanks were inspected daily mortalities were recorded if any. The weight measurements were used to determine the specific growth rate (SGR% day^{-1}), mean weight gain (MWG), and average daily growth (ADG). Calculations were done as follows:

$$\text{Specific growth rate (SGR)} = 100 (\text{Ln FBW} - \text{Ln IBW}) / \text{time}$$

$$\text{Average Weight gain (g)} = \frac{\text{Mean weight gain}}{\text{No. of feeding days}}$$

$$\text{Average Daily Growth} = \frac{\text{Mean Weight Gain}}{\text{Number of feeding days}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed fed}}{\text{FBW} - \text{IBW}}$$

Where:

- (Ln = Natural logarithm, IBW= Initial body weight (g), FBW = Final body weight (g))

Statistical analysis

Data collected was keyed in a Ms. Excel spreadsheet on Windows 2013 and presented as mean \pm standard error. The data was then analyzed using One-way analysis of variance (ANOVA) to compare diets with water quality and growth performance. Tukey's HSD multiple comparisons test was done to evaluate specific differences between diets, water quality, and growth performance among the treatments. Values with $P < 0.05$ were considered significant. All analyses were done using Statistical Package for Social Sciences (SPSS) version 20 for windows.

Results

Water quality

Water temperature ranged between 22.52 ± 0.16 °C and 23.26 ± 0.15 °C among the treatment groups (Table 3). Mean pH values ranged between 7.42 ± 0.04 and 7.68 ± 0.03 whereas DO ranged between 2.68 ± 0.1 and 3.06 ± 0.12 mg/l. NH_4 and NO_2 values ranged between 96.38 ± 2.35 , 107.25 ± 2.35 and 10.68 ± 4.76 ,

Table 2: Proximate composition of experimental diets.

	Diets (% water spinach composition)				
	0%	5%	10%	15%	20%
Lipids (%)	4.00	4.35	4.47	4.76	5.09
Moisture (%)	1.92	13.47	13.84	13.55	13.53
Protein (%)	29.88	29.19	28.77	29.14	28.50
Ash (%)	8.34	9.22	10.32	11.16	11.40



38.26±17.02 respectively. Apart from temperature, the mean values of all other water quality parameters monitored did not vary significantly ($p>0.05$) among treatment groups. Temperature shows a significant difference as determined by One-Way ANOVA ($F(4, 670) = 4.019, P=0.003$). Tukey post hoc test shows that there was a statistically significant difference between 20% diet (22.52±0.16, $p=0.015$) and 0% diet (22.59±0.16, $p=0.030$) compared to 5% diet (22.59±0.16).

Fish growth performance

Initial weight and length as well as final length and FCR in all the treatments groups did not vary significantly ($p>0.05$). The initial length and weight ranged between 3.9± 0.09 and 4.32 ±0.11cm, and 1.17±0.06 and 1.35±0.11 g respectively. The final length ranged between 114.84±0.20 and 120.25±0.20 cm and showed significant differences as determined by One-Way ANOVA ($F(4,316) = 5.910, p=0.00$). Tukey post hoc test showed a statistically significant difference between 10% (115.56±0.20, $p=0.05$), 15% (115.23±0.18, $p=0.029$) and 20% (114.84±0.21, $p=0.02$) compared to 5% (120.25±0.20). The final weight ranged from 128.98±0.80 and 180.49±0.83 and showed significant differences as determined by One-Way ANOVA ($F(4,316) = 6.363, P=0.00$). Tukey post hoc test showed a statistically significant difference between 15% (135.98±0.64, $p=0.014$) and 20% (128.98±0.80, $p=0.00$) compared to 5% (180.9 ± 0.75).

Specific growth rate (SGR) ranged between 1.09±0.05 (5% diet) and 1.34 ± 0.04 (0% diet). There was a significant difference in SGR between the treatments as determined by One-Way ANOVA ($F(4,316) = 3.781, p=0.05$). Tukey post hoc test shows

that there was statistically significant difference between 5% (1.0±0.05, $p=0.04$) and 0 (1.34 ± 0.04, $p=0.021$) compared to 20% (1.09±0.05). Weight gain ranged between 28.43±0.75 and 45.43±0.67 and showed significant difference as determined by One-Way ANOVA ($F(4,316) = 6.389, p=0.00$). Tukey post hoc test showed a statistically significant difference between 15% (31.29±0.64, $p=0.016$) and 20% (28.43±0.75, $p=0.00$) compared to 5% (45.43±0.67). Also 0% (42.94±0.64, $p=0.002$) shows significant difference compared to 20% (28.43±0.75) Table 4.

Discussion and conclusion

Generally, Nile tilapia are herbivores with longer coiled intestines compared to carnivores; mouth characteristics necessary for plant shredding. With reference to the increasing cost of feeds, several studies attempt to substitute feed ingredients in the diet of Nile tilapia. In this study, water spinach was evaluated as a potential feed ingredient for Nile tilapia. Water quality parameters were within the acceptable range for the growth and survival of Nile tilapia [17]. Although 5% had the highest final weight compared to 0%, there was no significant difference in growth performance in all experimental fish. Ramzy, et al. (2020) and Manuel, et al. [18]. Also reported no significant difference in Nile tilapia-fed water spinach. Another study by Odulate, et al. [19]. Also found that there was no significant difference in the growth performance of *C. gariepinus* when water spinach was incorporated into the diets. Other studies reported no significant difference in growth performance when other macrophytes were incorporated into fish feeds. These include Nile tilapia-fed *Rubrivivax gelatinosus*,

Table 3: Water quality parameters throughout the experimental period.

Parameters	Diets (% water spinach composition)						F	P
	0%	5%	10%	15%	20%			
Temp	22.59±0.16	23.26±0.15	23.12±0.17	23.11±0.14	22.52±0.16	4.019	0.003	
DO	2.79±0.11	2.80±0.11	2.86±0.10	2.68±0.10	3.06±0.12	1.72	0.14	
Sal	0.09±0.00	0.08±0.00	0.09±0.00	0.09±0.00	0.09±0.00	0.87	0.48	
pH	7.64±0.04	7.42±0.04	7.59±0.03	7.61±0.03	7.68±0.03	8.2	1.83	
NO ₂	18.04±7.42	10.68±4.76	24.17±9.59	38.26±17.02	20.06±8.45	0.98	0.42	
NH ₄	104.72±3.24	106.25±1.25	107.25±2.35	96.38±2.35	115.48±3.45	0.41	0.8	

Temperature (Temp) =°C, Dissolved Oxygen (DO)=mg/l, Salinity (Sal)=g/l, Ammonia (NH₃)= mg/l, Nitrite (NO₂)=mg/l. Values are presented as mean ± standard error (SE).

Table 4: Growth response of *O. niloticus* feed with different compositions of water spinach.

Parameters	Diets (% water spinach composition)						F	P
	0%	5%	10%	15%	20%			
IL	4.32 ± 0.08	4.13 ±0.11	4.06±0.09	3.9+8±0.09	4.04±0.08	0.622	0.647	
FL	117.07 ± 0.19	120.25±0.20	115.56±0.20	115.23±0.18	114.84±0.21	7.29	0.00	
IW	1.20 ± 0.06	1.35±0.11	1.27±0.08	1.17±0.06	1.19±0.07	0.492	0.74	
FW	168.13 ± 0.75	180.49±0.83	139.22±0.75	135.98±0.64	128.98±0.80	5.36	0.00	
SGR%	1.30 ± 0.04	1.34±0.05	1.23±0.05	1.21±0.05	1.09±0.05	3.781	0.005	
DWG	0.26±0.04	0.37±0.04	0.35±0.04	0.34±0.04	0.28±0.04	5.23	0.00	
WG	42.94±0.64	45.43±0.67	38.24±0.75	31.37±0.64	28.43±0.75	4.56	0.00	
FCR	2.24±1.85	1.28±1.27	2.35±4.42	2.69±1.75	3.05±4.63	1.17	0.32	

IW = Initial body weight, FW= Final body weight, IL=Initial body length FL= Final body length FCR = Feed Conversion Ratio, SGR = Specific Growth Rate= ((ln FBW - ln IBW)/180days) x 100. DWG= Daily Weight Gain WG=Weight Gain. Data is expressed as mean ± standard error.



Saccharomyces cerevisiae, and *Spirulina* plants. Grasi, et al. [20]. On *Labeo, fimbriatus* fingerlings fed *Azolla* and Common carp (*Cyprinus capio*) fed *L. minor* diet. [21]. The insignificant difference in growth performance might be attributed to the difference in protein and energy content of the experimental feeds [22].

In the present study, the best growth performance was observed in fish fed a 5% water spinach diet. Ramzy, et al. (2020) reported higher growth in Nile tilapia fed a 25% water spinach diet. Studies on other macrophytes which reported the best growth performance with a 5% diet include Nile tilapia fed *L. minor* diet [23]. Common carp (*Cyprinus carpio*) fed *L. minor* diet [21]. This is due to increased palatability and digestibility of macrophytes at lower inclusion compared to 10%, 15%, and 20% resulting in an increase in feed intake.

Similar to the present study, Ramzy, et al. (2020) reported no significant difference in weight gain on Nile tilapia fed up to 100% water spinach. Odulate, et al. [19]. Reported a decreasing trend in weight gain on *Clarias gariepinus* fed up to 20% water spinach composition. Gangadhar, et al. reported no significant difference in weight gain of *Labeo fimbriatus* fingerlings fed the *Azolla* diet. Bichi and Ahmad [24]. Reported a significant decrease in weight gain when cassava leaf meal was supplemented in the diet of *C. gariepinus*. The difference in weight gain might be attributed to the difference in protein and energy content of the experimental feeds [22].

Similar to weight gain, specific growth rates showed a significant difference in all the treatments. In contrast to the present study, Ramzy, et al. (2020) reported no significant difference in the specific growth rate of Nile tilapia-fed water spinach. Another study on *L. minor* reported no significant difference in the specific growth rate of Nile tilapia fed up to 20% [25]. This could be due to the different palatability of macrophytes when incorporated into the diets of Nile tilapia.

FCR is a measure of the number of feeds used to grow each kilogram of fish. Low values of FCR indicate efficient feeds, low production cost, and sustainable processes [26]. In the present study, the FCR of the Nile tilapia did not vary significantly in all the diets. This is in line with the results presented by Erdal, et al. [21]. This could be attributed to high ash content in the diet which affects nutrient utilization by fish [27]. All experimental feeds were actively fed except a 20% diet. Youssouf, et al. [28]. Reported that Nile tilapia actively fed on 0% to 15% except for the 20% *Azolla* diet. This is probably attributed to high fiber content resulting in the formation of complex inhibitors which affect the palatability and digestibility of the fish [29]. Fibers are organic substances present in plants and cannot be digested by enzymes in the intestinal tract of fish, hence cannot be converted into body biomass [30]. Also, the microbial activities in the lower gut of fish which could help in the fermentation of fiber are limited. This results in lower dietary nutrient digestibility and absorption resulting in lower nutrient digestibility and metabolism [31].

In conclusion, this study demonstrates the acceptance of the water spinach diet by fish indicates that it can be used by fish

farmers to replace a fish meal as a source of protein because they are locally available and easy to culture. Therefore, the current study suggests that 5% water spinach composition results in optimum growth of Nile tilapia and thus recommends future trials in the field for better growth performance. Furthermore, there is a need to determine at what % composition of water spinach meets all the nutritional requirements for best growth performance and the apparent digestibility of water spinach by various growth stages of Nile tilapia [32-34].

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