NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 54 Number 1, April 2023 Pg. 593-600
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Inclusion of *Annona Muricata* to Diets of *Clarias gariepinus* Parentss and its Efficacy on the Growth of Subsequent Generations

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Abstract
The objective of the extant study was to evaluate the weight, length, and survival of *Clarias gariepinus* first and second filial generation. The knowledge on the weight, length, and survival of *Clarias gariepinus* first and second filial generation is curbed, however, such information is precise for the culturing of the species, to discover the health condition of the fish and it will be beneficial in biological and genetic study, hence, aid to source for various ways to reduce inbreeding, enhance the growth and survival of the species. The growth in weight was ascertained using a sensitive weighing digital balance, while the growth in length was determined using the meter rule. The hatchlings were from the previous breeding exercise whose parents and F_1 broodstock were fed with different inclusion levels of *Annona muricata*. 750 hatchlings of F_1 and F_2 with mean body weight and length of 0.03g and 0.5cm; 0.05g and 0.7cm were stocked in an indoor flow-through fibre pond (1.0 x 1.0 x 1.0 m^3) in triplicate in a completely randomized design. The 3-day-old larvae of both F_1 and F_2 generations were fed *ad libitum* with 0.2mm coppens as a starter feed twice daily for 7 days. While 0.3 to 3.0mm were further used based on the weight and length of the fish for 49 days. Every week the weight and length were measured and mortality was recorded. **Results:** The results revealed continuous and exponential growth and a significant difference between the hatchlings across the various treatments and across the generations. The weight, length and survival rate were higher in hatchlings whose parents consumed *A. muricata,* the diet 5 (4% inclusion) of the F_1 generations had 40.22g, 21.6cm and 94% hatchlings survived after 56 days and F_2 generations had 63.9g, 22.9cm and 92% of the hatchlings survived while the least was recorded in the 0% inclusion designated as D1 the F_1 recorded weight 30.1g, length 12.9cm and the survival was 53% while the F_2 was weight 16.1g, 13.6cm and 38% survived. The temperature of the F_1 during breeding and rearing ranged between 27 to 29°C while that of the F_2 generations was between 18 to 24°C. The research revealed that both F_1 and F_2 generations that the parents consumed *A. muricata* performed significantly better than the generation that did not consume the additive, in addition, temperature and stocking density play an essential role in the growth and survival of the fish.
Keywords: Weight, Length, Survival, First and second Filial Generations and Annona muricata

Introduction

The *Clariids* constitute an excellent food protein of high commercial value. It is very essential for aquaculture promotion and sustainability in Nigeria (Onyia *et al*., 2015[b]). Catfish (*Clarias gariepinus*) is one of the most important groups of farmed fishes in the world; it commands a very good commercial value in Nigerian markets (Onyia *et al*., 2016). They are characterized by their ability to grow and develop on a wide range of both artisanal and natural feed. It has a very high yield potential, and tolerance to low oxygen, and can also be reared using available ingredients, and additives. It can grow fast in both intensive and extensive culture systems (Ochokwu *et al.,* 2019). Catfish produce thousands of eggs in a breeding period; and withstand both handling and environmental stress (Ochokwu *et al.,* 2021). It can also withstand disease and many other adverse conditions that can kill some fish species. Fish growth either weight (g) or length (cm) refers to various metrics used to assess the increase in size and development of fish populations or individual fish within the populations (Jisr *et al*., 2018). Data *et al*. (2013) reported that measuring the length and weight of fish provides basic information about their size and body condition. Growth in length and weight indicates how well the fish are developing, and the ability to assimilate the feed and convert it to flesh (Feed conversion ratio) (Ayisi *et al*., 2017). Growth rate measures the speed at

which fish grow over a certain period. It can be calculated by comparing size measurements at different times (Ali and Bhaskar, 2016). Dinh *et al*. (2022) stated that some of the parameters in growth determinations are the condition factor, which indicates the overall health and well-being of the fish. A higher condition factor usually suggests good health and growth potential Dinh *et al*. (2022). The growth of fish is essential in population Monitoring, it aids in tracking growth parameters, and scientists can monitor the health and dynamics of fish populations (Rahman *et al*., 2023). Changes in growth rates or condition factors can indicate environmental stressors, changes in food availability, or overfeeding (Jobling, 2008), while Fogarty and Collie (2019) argue that the growth rates and condition of fish are crucial for sustainable fisheries management. Fisheries managers use growth data to set harvest quotas, determine fishing seasons, and implement size limits to ensure the long-term viability of fish stocks (Fogarty and Collie, 2019). It also helps to optimize feeding regimes, stocking densities, and other management practices to maximize growth rates and production efficiency (Singh and Srivastava, 2021). It provides valuable information about the role of fish within aquatic ecosystems. Changes in growth rates may reflect alterations in habitat quality (Griffen and Drake, 2008). Growth parameters are essential for scientific research on fish biology, physiology, and ecology (Santos *et al*., 2022), while Berg *et al*. (2018) expressed that they provide baseline data for studying environmental factors, genetics, and other variables on fish growth and development.

Annona muricata (soursop) also known as Graviola, guanabana, or Brazilian pawpaw is a valuable, medicinal, and edible indigenous fruit tree. It is a lowland tropical, fruit-bearing tree of the family Annonaceae found in the rainforests of Africa, South America, and Southeast Asia (Rady *et al*., 2018). *A. muricata* has the largest fruits in the genus. It is astringent and cholagogic in nature (Uno *et al*., 2017). It aids indigestion. In Nigeria, *A. muricata* is found in the South, however, it is dominant in the South East and generally found in every house. Minh (2018) reported that *A. muricata* has large, glossy, dark green leaves (Moghadamtousi *et al*., 2015), which have edible, green heart-shaped fruits (Coria-Téllez *et al*., 2016). The leaves (Rady *et al*., 2018), pericarp (Kuete *et al*., 2016), fruits (Sun *et al*., 2016), seeds and roots (Pieme *et al*., 2014) of *A. muricata* have been used in traditional medicine. It also has several medicinal uses such as in the management of diabetes and its complications, as an antioxidant and anti-mutagenic agent. Rady *et al*. (2018) reported that the leaf extract is used in the treatment of cancer and skin infections such as eczema. It is rich in protein, vitamins, minerals, and phytochemicals. The research aimed to expose the growth of the F_1 and F_2 generations whose parents consumed *A. muricata* as a feed additive.

Materials and Methods

Fish and experimental design

The research took place in the hatchery complex, Department of Fisheries, Modibbo Adama University, Yola, with a latitude of 9°13'48"N and a longitude of 12°27'36"E, from March 21st to May 19th, 2022 and February 12 to April 10 2023. The F_1 and F_2 of *C. gariepinus* used for the growth assessment were procured from previous breeding exercises, according to Ochokwu *et al*. (2016). In brief, the *C. gariepinus* Parents and F_1 broodstock were fed with diets containing different inclusion levels of *Annona muricata* (D1 0%, D2 1%, D3 2%, D4 3%, and D5 4% inclusion levels) for 56 days (19 January to 21st March 2022) and F_1 broodstock (12th December 2022 to 11th February 2024). The mean body weight of the parents for both sexes was 550–710 g and the length 41–46.7 cm while the mean body weight of the F_1 was 1400 to 1850g and the length 48.2 to 60.1cm. At the end of the feeding trial, the female fish were induced with ovulin at 0.5 ml/kg. The latency period lasted for 9 to 12 hours at a temperature of 29°C and 26°C. The eggs were stripped while the males were euthanized with clove oil before incising the abdomen. A 0.2-ml syringe was used to extract out 0.3 ml of the milt to fertilize 1g of the eggs, while the abdomen was stitched back to heal (7 days) before returning to the culture pond. The mixture was activated with 5 ml of saline solution for perfect fertilization and the production of the developed embryo.

Growth parameters

In the first phase (21st March to 19th May 2022), 750 hatchlings (F_1) from the parents-fed *Annona muricata* with a mean body weight of 0.03g and a length of 0.5cm were stocked in an indoor flow-through fiber pond (1.0 x 1.0 x 1.0 m^3) in triplicate in a completely randomized design while in the second phase from February 12 to April 10 2023, 750 hatchlings (F_2) from the F_1 fed *Annona muricata* with a mean body weight of 0.05g and a length of 0.7cm were stocked in an indoor flow-through fiber pond (1.0 x 1.0 x 1.0 m^3) in triplicate in a completely randomized design. The 3-day-old larvae from both F_1 and F_2 generations were fed *ad libitum* with 0.2mm coppens as a starter feed twice daily, at 7 a.m. and 5 p.m. for 7 days. While 0.3 to 1.0mm were further used based on the weight and length of the fish for 49 days. Every week the weight and length are measured using a sensitive weighing balance and meter rule. The management practices carried out include general cleanliness of the hatchery, maintaining flow-through operation, siphoning of uneaten feeds and debris from the pond water, monitoring the quantity of the feed given and avoidance of overfeeding. It was observed that the uneaten feeds, unhatched eggs, or debris became a medium for fungi growth. The fungi development and growth began 10 to 14 hours after they remained in the culture pond at a water temperature of 27°C. It is essential to convey that 0.2 mm of Coppens feed was used because of its effectiveness, availability, cheapness, and acclaim among the aqua-culturists in Nigeria. The growth in weight and length was monitored weekly.

Statistical analysis

Data collected for growths in weight, length and survival of First and second filial generations were presented in a line graph and histogram using Excel 2013.

Results and Discussion

Results

Growth in weight (g) and length (cm) of Clarias gariepinus f_1 generation

The growth in weight (figure 1) of the *Clarias gariepinus* hatchlings (F_1 generation) shows continuous and exponential growth among the treatments. The initial weight (g) ranged from 0.03 in 0% to 0.07g in 4% inclusions. While the final weight (at 8 weeks) ranged from 30.1g in 0%, to 40.2g in 4% inclusion. The increase in length (figure 2) ranged from 0.3 in 0% to 0.6cm in 4% inclusions, while in the final length, it increased to 12.9 in 0% and 21.6cm in 4% inclusions. The hatchling survival (figure 3) was highest in the 4% inclusions; 94% survived, followed by the 2% inclusions (88%), and the least in the 0% inclusion 53% survived respectively.

Growth in weight (g) and length (cm) of Clarias gariepinus f_2 generation

The growth in weight (g) of *C. gariepinus* second filial generation (F_2) fed for 8 weeks is presented in Figure 4. The initial weight of the hatchlings ranged from 0.04g in Diet 1 to 0.09g in Diet 5 designated as week 1, and in the final weight, the least was 32.6g in Diet 1 and the highest was 49.6g in Diet 5. Figure 5 shows the differences in the length (cm) parameters of the *C. gariepinus* second filial generation (F_2) fed for 8 weeks. The initial length of 0.06cm was the least recorded in Diet 1 the highest was in Diet 5 (0.9cm) and the final length ranged from 13.6cm in Diet 1 and 22.9 in Diet 5 respectively. The values of the survival rate of the F_2 generation are presented in Figure 6. The highest percentage of survival is in Diet 5 (92%), followed by Diet 4 (82%), and the lowest was in Diet 1 (38%).

Water quality parameters of Clarrias gariepinus f_1 and f_2 fed A. muricata leaf diet

The water quality parameters are presented in Table 1. Temperature ranged from 16 to 29°C across the weeks, water pH ranged from 6.7 to 7.2, dissolved oxygen 5.0 to 5.5mg/l, and ammonia 0.01 to 0.2mg/l. the differences in the temperature range across the weeks were because of climate change.

Discussion

There was an increase in the growth in weight (g) and length (cm) of *C. gariepinus* f_1 and f_2 generation. The 4% inclusion level (Diet 5) performed significantly better than the other fish followed by Diet 4 and the least was in 0% inclusion (Diet 1). The enhanced growth and survival recorded can be inferred from the diet acceptability and environmental balance this is agreed with Afe and Omosowone (2019) who reported an increase in weight and survival of fish when the diet was supplemented with *Acacia auriculiformis* leaf. Subsequently, previous research reported the essential of plant leaves in improving growth, nutrient utilization and survival of the fish. The same trend was seen in Ochokwu *et al*. (2020) who observed an increase in the growth and survival of *C. gariepinus* that was fed with a *Telfairia occidentalis* leaf diet. In addition, Satimehin and Tiamiyu (2019) observed that the fish that were fed different hydrothermally processed rubber leaves-based diets did better than the fish that consumed the control diet. Sanusi and Bakar (2018) reported that *Anona muricata* is known for its rich nutrient content, such as vitamins, minerals, and bioactive compounds, some compounds in *Anona muricata* have been studied for their potential immuno-modulatory effects, with improved immune system of f_2 gives them a better resistance to diseases and stress (Kim *et al*., 2016), ultimately leading to increased growth and survival. Another factor is digestibility, *Anona muricata* leaf meal contains some amount of soluble fibre which aids in digestibility leading to better nutrient absorption and utilization (Afzaal *et al*., 2022). Some compounds in *Anona muricata* are linked to metabolic effects, including potential impacts on lipid metabolism. Hence changes in their metabolic activities, could trigger growth (Zubaidi *et al*., 2023). Furthermore, Turner (2009) expatiates epigenetic (correlation between behaviour and environment which trigger a change in the operation of the gene) effect, hence the f_1 generation's exposure to *Anona muricata* induced epigenetic changes that were passed on to the F_2 generation. Epigenetic modifications can affect gene expression and potentially influence growth (Handy *et al*., 2011). Also, interactions with Microbiota and the consumption of some plant leaves can influence the gut microbiota of fish. A balanced and beneficial gut microbiota can enhance nutrient absorption and overall health, potentially leading to increased growth (Wang *et al*., 2021). If the f_1 generation consist of fish with naturally higher growth potential and was selectively bred, the improved growth in the f_2 generation might be due to genetic factors (Afzaal *et al*., 2022). Subsequently, the f_2 generation from the previous f_1 parents that were fed with *A. muricata* demonstrated a high survival rate when contrasted with the control (0% inclusion). It could be inferred that at 8 months the fish that did not consume *a. muricata* were not fully matured for breeding. These are some of the major factors that influence the growth and survival of the hatchlings. The reduced growth recorded in the f_2 is a result of the low temperature of 18°C during the breeding and rearing period another factor is the stocking density, this concurs with Okunsebor *et al.* (2015) who established that temperature can affect the hatchability and growth of *C. gariepinus* hatchlings, furthermore, he reported that the fish under 28°C temperature performed better than the lower temperature.

Conclusion

Including *A. muricata* leaf in the diet of *C. gariepinus* parentss and F_1 significantly improved the growth and survival of the F_1 and F_2 hatchlings. The parentss that consumed 4% inclusion of *A. muricata* significantly produced hatchlings with higher growth rates and survival. It can be inferred that the inclusion of *A.*

muricata in the diet of *C. gariepinus* stimulates the growth of the subsequent generations. In addition, growth parameters are critical indicators of the health, productivity, feed acceptability, utilization and sustainability of fish populations, influencing various aspects of fisheries management, aquaculture, and ecological research. Tracking these parameters helps ensure the responsible stewardship of aquatic resources for future generations. It also revealed the significance of plant additives, inclusion in the diet of fish and its potency in aquaculture survival and sustainability.

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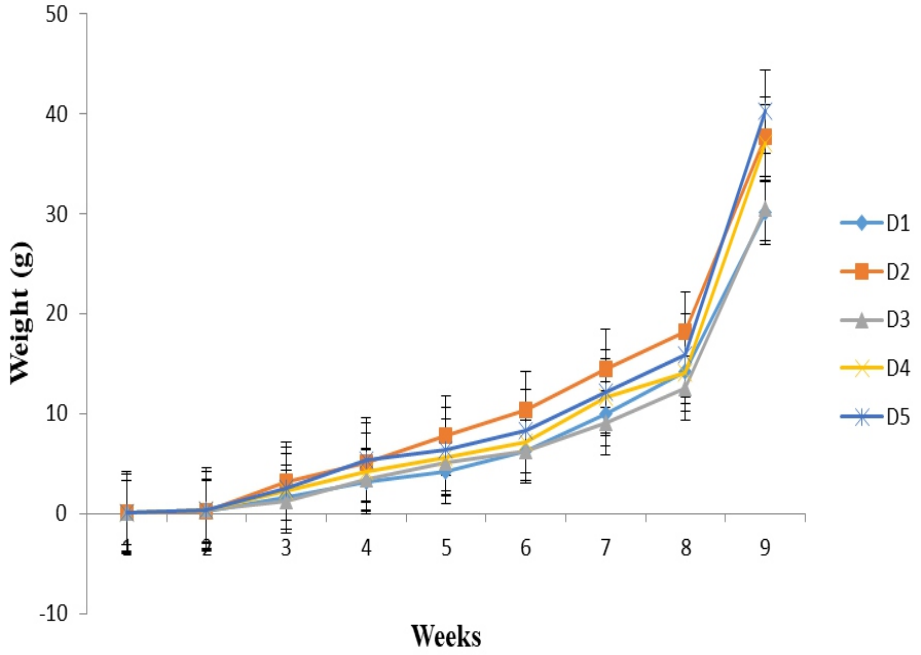

Figure 1: Growth in weight (g) of the f_1 generations

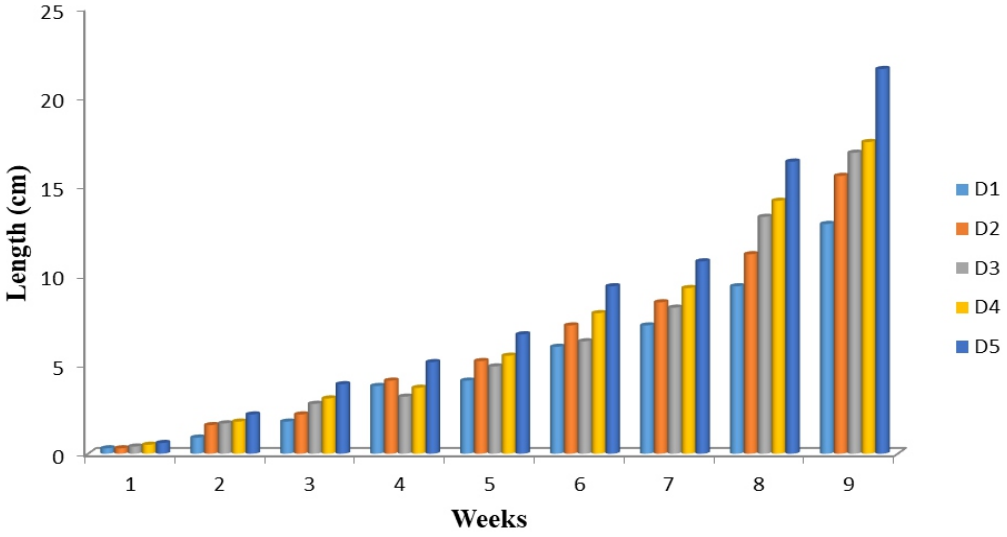

Figure 2: Growth in length (cm) of the f_1 generations

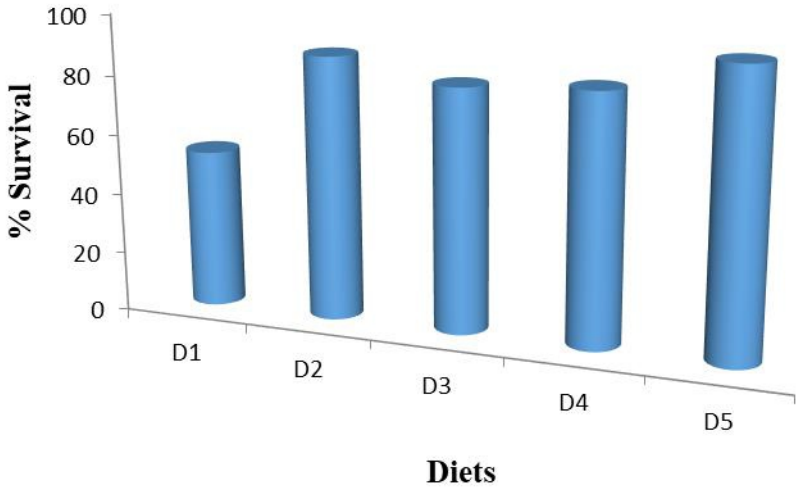

Figure 3: Percentage survival of the f_1 generations

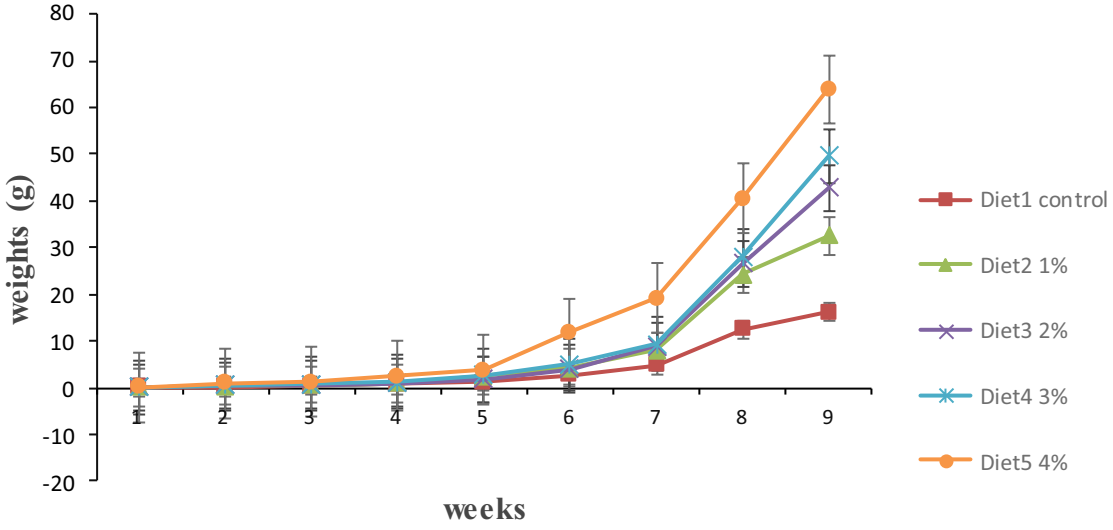

Figure 4: Growth in weight (g) of Clarias gariepinus f_2 generations

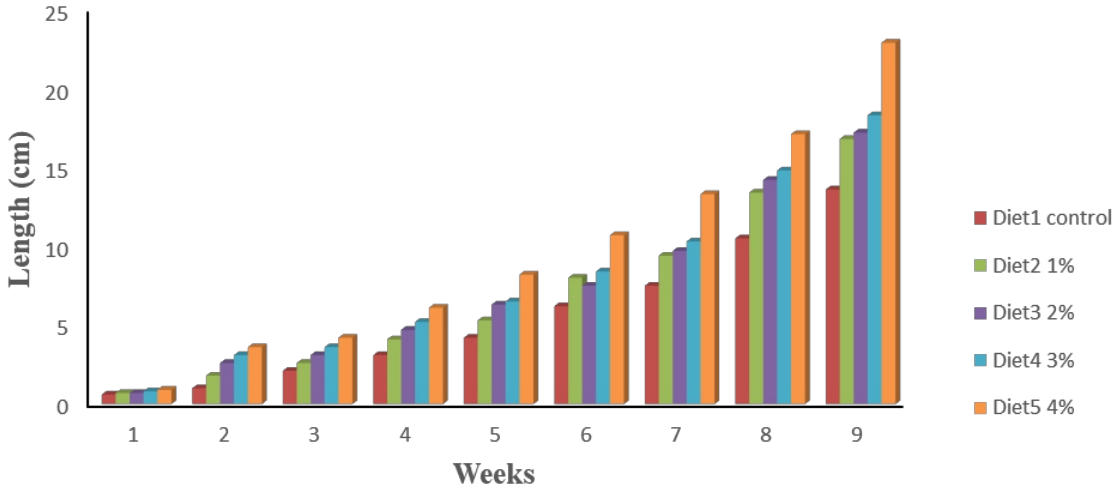

Figure 5: Growth in length (cm) of Clarias gariepinus f_2 generations

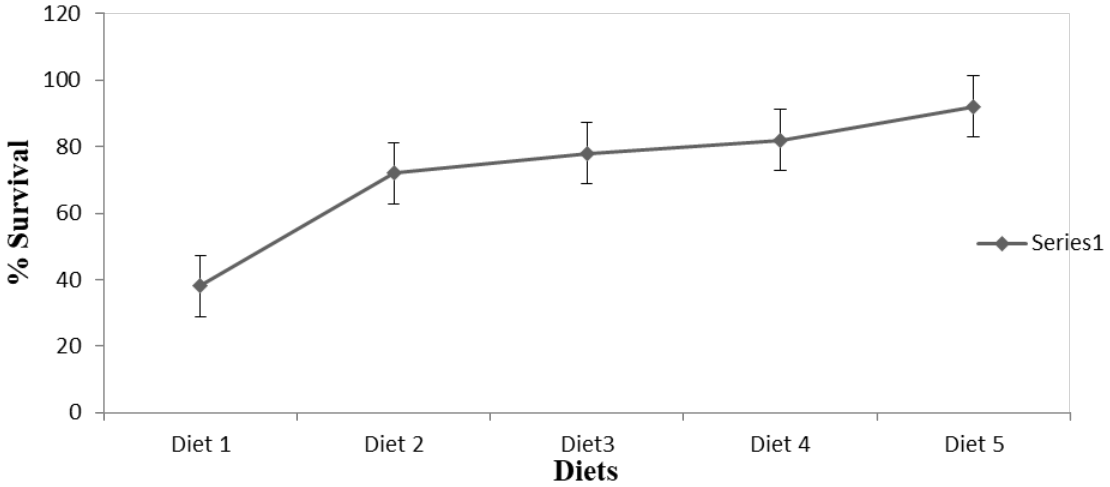

Figure 6: Survival rate of Clarias gariepinus f_2 reared for 8 weeks

Table 1: Water Quality Parameters of *Clarias gariepinus* Broodstock Fed *A. muricata* Leaf Meal

Parameters	Minimum	Maximum	Range
Temperature ^{0}C	16.00	29.00	22.50
pH	6.70	7.20	6.95
Dissolved Oxygen mg/L	5.00	5.50	5.25
Ammonia mg/L	0.01	0.2	0.11