NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 54 Number 1, April 2023 Pg. 275-280
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Growth Performance and Immunity Analysis of *Clarias gariepinus* Juveniles Fed with Moringa leaf, Ginger and Garlic Powder

Ukenye, E. A., Megbowon, I., Oguntade, O. R., Ayo-Olalusi, C. I., Mojekwu, T., Edah, B., Sokenu, B. A., Joseph, J. B., Adeyemi, Y. B., Jubrin, H., Chidume, I., Eze, N. C., Adeleke, R.O. and Igwe, C.

Department of Biotechnology,
Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria
Corresponding Author's email: eekwelem@yahoo.com

Abstract
Natural plants (moringa, ginger and garlic) that have been used as feed supplements in place of antibiotics in aquaculture feeds have shown improved performances. In this study, growth performance, nutrient utilization and immunity of *Clarias gariepinus* juveniles fed with moringa leaf, ginger and garlic powder, their combination and one control (without additive) were observed. Fish samples were equally divided into fifteen transparent tanks of five different experimental groups in three replicates consisting of four diets fed (moringa leaf powder, ginger powder, garlic powder and their combination at 2% inclusion each) and one control(without additive). The feeding trial was done for 8 weeks during which the fish were fed twice daily to satiation. The results revealed that out of the four groups of phytoadditives diet-fed fish, the group fed with garlic supplement diet had the highest and best performance with weight gain (WG) of 381.6 ± 71^{b} and the total weight of fish (TWF) 1010.71; nutrient utilization with feed conversion ratio (FCR) 2.7 ± 0.39^{a}, specific growth rate (SGR) 0.849 ± 1.72^{b} and relative growth rate (RGR) 2.932 ± 0.23^{c}. The group fed with ginger supplement diet has higher performance with WG of 345.4 ± 134^{b} and TWF 1019.88; nutrient utilization with FCR 3 ± 0.84^{a}, SGR 0.798 ± 0.25^{b} and RGR 2.617 ± 1.95^{b}, whereas the moringa fed group has a good performance with WG of 88.61 ± 21.3^{a} and TWF 635.67; nutrient utilization with FCR 7.2 ± 1.54^{b}, SGR 0.315 ± 0.62^{a} and RGR 2.742 ± 1.52^{b} while the group with combined diets has the least performance with weight gain (WG) of $32.8g \pm 26.8^{a}$ and the total weight of fish (TWF) 552.93g; nutrient utilization with feed conversion ratio (FCR) 8.2 ± 0.63^{b}, specific growth rate (SGR) 0.133 ± 0.41^{c} and relative growth rate (RGR) 0.239 ± 1.03^{a}. The agarose gel picture which shows the presence of the TNF gene in all the groups indicated that the immunity gene is expressed in them all. Thus the garlic supplement diet is recommended for better growth, immunity and disease resistance in *Clarias gariepinus.*

Keywords: Growth, Immunity, Clarias gariepinus, Moringa leaf, Ginger, Garlic

Introduction

The aquaculture industry has been globally recognized as the fastest-growing food-producing industry. However, the major challenge with the African catfish (*Clarias gariepinus*) production is the overdependence of aqua feeds on fish meal and antibiotics which are the most expensive ingredients in the aquaculture diet (Raimi and Fatuase, 2021). Traditional use of antibiotics and other chemotherapeutics in aquaculture has been criticized because of the potential development of multiple antibiotics resistant bacteria, accumulation of residues in fish (Monica and Jayray, 2021) and their potential deleterious effect on human health (Agouz *et al*., 2015). The World Health Organization encourages the use of medicinal herbs and plant products to replace or minimize the utilization of chemicals (Mahmoud *et al*., 2019). The use of natural products like medicinal plants as feed additives may be highly beneficial to reinforce the potency of feed utilization and animal productive performance (Levic *et al*., 2008). Medicinal plants have different activities such as growth promotion, increased immunity, disease resistance, antistress, appetite stimulation, aphrodisiac and antimicrobial properties due to the presence of bioactive components such as alkaloids, flavonoids pigments, phenolics, terpenoids, steroids and essential oils (Aklilu *et al*., 2020).

Moringa oleifera commonly called moringa or drumstick tree is a widely available plant with several

important industrial and medicinal uses (Saini *et al*., 2016). The leaves, fruits, barks and roots of moringa have high nutritive value and possess immunostimulant, anti-inflammatory, antiulcer, antibacterial, hypoglycemic, antihypertensive, and hepatoprotective properties (Kazeem *et al*., 2017). It was reported by (Leone *et al*., 2015) that the most used parts of the plant are the leaves, which are rich in vitamins and phytochemicals. In many aquaculture studies, moringa leaves have been used as feed supplements in growth improvement/enhancement and immunity studies (Kazeem *et al*., 2017; Puycha *et al*., 2017; Nyadjeu *et al*., 2020).

Ginger (*Zingiber officinale*) is a generally safe herbal medicine, widely used as a food spice in many types of cuisine. Ginger has been reported to be rich in mineral elements, vitamins, and phytochemicals (Iheanacho *et al*., 2017) which have a high anti-inflammatory and antioxidant effect. Pharmacological studies also reported that ginger has anti-inflammatory, antiplatelet, antibacterial antifungal and antiviral effects on the human body system including the gastrointestinal and cardiovascular systems. It is also known as an antitumor, hypolipidemia and immune modulatory agent in humans as well as animals and fish (Talpur *et al*., 2013; Nyadjeu *et al*., 2020). Hence, supplementing ginger in fish diets has been shown to improve growth, immunity, disease resistance and most importantly survival.

Garlic (*Allium sativum*) have been used to improve the growth and disease resistance of a number of fish and livestock (Shakya and Labh, 2014). Previous studies revealed that garlic (*Allium sativum*) possess antibacterial, antiviral and antifungal activities (Shakya and Labh, 2014), and also play antioxidant, immunostimulant, immunomodulation and significant hepatoprotective role in detoxification (Jegede, 2012). It was reported by (Abayomi *et al*., 2018) that garlic contains a variety of bioactive compounds and phytochemicals such as flavonoid, saponins, and alkaloids allicin (which is responsible for its pungent odor). Many studies have also reported the beneficial effect of garlic on health improvement in man, livestock and fish such as improved growth, feed efficiency and disease resistance (Lee *et al*., 2014; Manoppo *et al*., 2016; Nyadjeu *et al*., 2020). Consequently, this present study was designed to investigate the effect of moringa leaf, ginger, and garlic powder (individually and combined) on the growth and the innate immunity/ disease resistance on *Clarias gariepinus* juvenile. Thus growth performance, nutrient utilization and immunity gene (TNF) of the juveniles fed diets containing the individual and combined phytoadditives were assessed.

Materials and Methods

Processing of Phytoadditives

Freshly harvested moringa leaves (*Moringa oleifera*), ginger roots (*Zingiber officinale*) and garlic bulbs (*Allium sativum*) were washed under running clean tap water and wiped with a clean kitchen towel. Garlic and ginger were individually peeled to remove the foreskin before being diced with a kitchen knife. Additives were sundried for a period of 72hours, then milled to powder using a laboratory milling machine (Model: HK - 860) and sieved with a hand sieve to obtain 2kg each of the powders which were then stored in an airtight cellophane bag and refrigerated at 4°C for further analysis.

Experimental Diets and Acclimatization of experimental fish

Five iso-nitrogenous diets were formulated to contain 35% crude protein with each additive powder added at 2g/100g. Diet "A" (2% moringa inclusion), Diet "B" (2% ginger inclusion), Diet "C" (2% garlic inclusion), Diet "D" (mixture of all three additives: 2% inclusion) and Diet "CT" (0% additives).

Fish Sampling and feeding

A total of 300 live *Clarias gariepinus* juveniles were equally divided into fifteen transparent tanks of five different experimental groups in three replicates consisting of four diets fed (moringa leaf powder, ginger powder, garlic powder and their combination at 2% inclusion each) and one control (without additive). The fish were fed to satiation twice daily with the phytoadditives formulated feed. The feeding trial which involved weekly growth parameters evaluation lasted for eight weeks at the water recirculatory system (WRS) NIOMR, Lagos state. After 8 weeks of the feeding trial (Raimi and Fatuase, 2021), tissue samples (1g each) were collected at the biotechnology laboratory, NIOMR Badore, Lagos state from the ventral region (Cordero *et al*., 2017) of the *Clarias gariepinus* fishes into well labeled sterile 2ml Eppendorf tubes and submerged in RNALater buffer at -20°C for further use.

RNA extraction, cDNA synthesis and PCR amplification

Total RNA was isolated using JENA mini kit (Jena Bioscience, Germany) according to the manufacturer's protocol. The purity and concentration of RNA extract was determined using a Thermofisher 1000 C Nanodrop spectrophotometer. Reverse Transcription Polymerase Chain Reaction (RtPCR) was carried out using Fire script RT cDNA synthesis kits (FIREScript®, Estonia, using Oligo dt primer with the thermal profile: 65° C for 5 mins for denaturation, followed by Primer annealing at 25° C for 10 mins and 85° C for 5 min to inactivate RT enzyme). The housekeeping Gene, Glyceradehyde 3 Phosphate dehydrogenase (GAPDH) and the immunity gene, tumor necrosis factor (TNF-α) were used to quantify the complementary DNA (Primer sequence shown in Table 1), amplification was done with an initial denaturation at 2 min at 94°C followed by 30 cycles of the following; denaturation at 94°C for 30s, annealing at 54°C for 30s and extension at 72°C for 30s.

Statistical analysis

All data were tested for normality before being analyzed in this study. GraphPad Prism software 5.0 was used to plot the graphs and carry out a one-way analysis of variance (ANOVA) to test for significant differences (p

<0.05) among fed treatment groups and control. Duncan's multiple range test was used to compare differences among the treatment means when significant F-values were observed at ($p < 0.05$) level. The data were presented as mean ±SD.

Results and Discussion

Results

Growth Performance and Nutrient Utilization

The addition of the individual and combined moringa leaf powder, ginger powder and garlic powder to the diets fed to *Clarias gariepinus* juveniles for the period of eight weeks had a significant effect on their growth performance (weight gain and total weight of fish) and nutrient utilization (feed conversion ratio, specific growth rate and relative growth rate). the group fed with garlic supplement diet has the highest and best growth performance with WG of 381.6 ± 71^{b} and TWF 1010.71; nutrient utilization with FCR 2.7 ± 0.39^{a}, SGR 0.849 ± 1.72^{b} and 2.932 ± 0.23^{c} followed by the group fed with ginger supplement diet that showed higher growth performance than the moringa and combined diets groups with WG of 345.4 ± 134^{b} and TWF 1019.88; nutrient utilization with FCR 3 ± 0.84^{a}, SGR 0.798 ± 0.25^{b} and RGR 2.617 ± 1.95^{b} while the moringa fed group has a good growth performance with WG of 88.61 ± 21.3^{a} and TWF 635.67; nutrient utilization with FCR 7.2 ± 1.54^{b}, SGR 0.315 ± 0.62^{a} and RGR 2.742 ± 1.52^{b} whereas the group fed with combined diets has the least growth performance with weight gain (WG) of $32.8g \pm 26.8^{a}$ and weight gain (WG) 552.93g; nutrient utilization with feed conversion ratio (FCR) 8.2 ± 0.63^{b}, specific growth rate (SGR) 0.133 ± 0.41^{c} and relative growth rate (RGR) 0.239 ± 1.03^{a}. The growth parameters in terms of weight gain and total weight of fish is shown in Figure 1 while the feed conversion ratio, specific growth rate and relative growth rate which indicates the nutrient utilization is shown in Figure 2.

Qualitative expression of the immunity gene (TNF) on Agarose gel electrophoresis

The amplicons from the polymerase chain reaction process of TNF-α of the *Clarias gariepinus* juvenile fed diets on 2% agarose gel is shown in Figure 3.

Discussion

One of the major problems in aquaculture globally is the inadequate quality of feeds associated with low immunity, reduced growth, and low disease resistance. Although, chemicals and antibiotics are highly used by fish famers to improve fish health and growth, this practice is highly discouraged because of the potential negative effect on the fish produced, the consumer and the environment. In the present study, a general improved performance in growth and nutrient utilization was observed in all the phytoadditives fed groups. The highest and best growth performance and nutrient utilization observed in the garlic powder diet fed group agreed with some previous work on *Clarias gariepinus* and some other fish species (Lee *et al*., 2014; Adeniji *et al*., 2019) that the garlic fed fish had improved weight gain and nutrient utilization value. In addition, it has also been reported by Nyadjeu *et al.* (2020) who suggested that the improved performance shown in the garlic powder-supplemented diet is as a result of the bioactive compounds allin and allicin contained in garlic which has abundant sulphonates responsible for high-performance of intestinal floral and thus improves digestion, nutrient absorption/utilization and growth performance. This is also in agreement with the reports of el- Katcha *et al* (2016) where 50% inclusion of garlic in broilers' diet was observed to significantly increase the level of lymphocytes, neutrophils and heretrophyles count due to the immunostimulatory effect of garlic bulb. The improvement observed in the ginger supplementation diets fed *Clarias gariepinus* was also high, though not as the garlic powder supplementation fed diets group and can be traced to the bioactive compounds in ginger root such as gingerols, flavonoids, phenolic ketone derivatives and volatile oils. These bioactive compounds are known to stimulate appetite and immunity, aid fast-feed digestion and improve the secretion of pancreatic enzymes. Furthermore, the presence of proteolytic and lipolytic enzymes is known to improve the digestion of dietary proteins and lipids (Ibidunni *et al*., 2017; Adegbesan *et al*., 2019). It was also observed that the group fed with ginger has better nutrient utilization and growth performance than the group fed with moringa as reflected in their feed conversion ratio, specific growth rate and relative growth rate as reported by Iheanacho *et al*., 2017; Abdel-Gawad *et al*., 2019 and Nyadjeu, *et al*., 2021, the reason being that ginger is rich in bioactive compounds which increase appetite and improve feed digestion and fish health. However, lower growth performance and nutrient utilization were observed in moringa and combined phytoadditive diets fed *Clarias gariepinus* juvenile than the control group, though the combined group showed the lowest performance. A low growth performance and nutrient utilization of moringa diets-fed fish was also observed in bocourti's catfish (Puycha *et al*., 2017) and in Nile tilapia (Abdel-Gawad *et al*., 2019). Surprisingly, *Moringa oleifera* which is widely known to possess various economic importance and medicinal properties (such as antihypertensive, hepatoprotective, antibacterial, antiulcer and anti-inflammatory) and is expected to improve the growth performance and immunity of fish (Kazeem *et al*., 2017) contrarily has been recorded to show a low growth and nutrient utilization performance. The low performance could be attributed to the antinutrients such as saponins, phytate and tannins present in Moringa leaf. Saponin has an adverse effect on the biological membranes by increasing the permeability of intestinal mucosal cells aiding the free transportation of active nutrients, while phytate inhibits the absorption of nutrients thereby reducing the availability of minerals and protein digestibility (Abdel-Gawad *et al*., 2019). Moreover, the least performance observed in the *Clarias gariepinus* juvenile fed with the combination of the three phytoadditives may be as a result of the effect of the antinutrients present in the moringa leaf which would have inhibited the performance of all other nutrients present in ginger and garlic because the individual and

combined effect of ginger and garlic was reported to improve growth performance, nutrient utilization and innate immunity of fish (Iheanacho *et al*., 2017; Adeniji *et al*., 2019; Nyadjeu *et al*., 2020; Nyadjeu, *et al*., 2021). The gel bands in the agarose gel picture (Figure 3) showed the presence of TNF (immunity) gene in all the groups indicating that the immunity gene is expressed in them all, though their level of expression needs further analysis.

Conclusion

In this study, it is obvious that the inclusion of phytoadditives as supplements in *Clarias gariepinus* juvenile diets is highly beneficial to improve growth, feed utilization and immunity. However, garlic and ginger performed better than moringa and this might have affected the low performance of the combined diets. Thus there might be a need to investigate the effect of the combination of only garlic and ginger on the growth performance, nutrient utilization and immunity of the fish. Moringa leaves can also be subjected to require processing methods to reduce the anti-nutrients in them before including them in fish feed. Also, further study is required to assess the level at which TNF is expressed and regulated in the fish fed with the individual and combination of phytoadditives-supplemented diets.

References

Abayomi, Y., Fagbuaro, S.S. and Fajemilehin, S.O.K. (2018). Chemical composition, phytochemical and mineral profile of garlic (*Allium sativum*). *Journal of Bioscience and Biotechnology Discovery* 3: 105-109.

Abdel-Gawad, E.A., El -Asely, A. M., Soror, E. I., Abbass, A. A. and Austin, B. (2019). Effect of dietary *Moringa oleifera leaf* on the immune response and control of *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*) fry. *Journal of Aquaculture International*. https://doi.org/10.1007/s10499-019-00469-0

Adegbesan, S.I., Obasa, S.O., Akintokun, A.K. and Abdulraheem, I. (2019). Effects of dietary supplementation of *Zingiber officinale*root-powder on growth, nutrient utilization and intestinal microbes of African mud catfish (*Clarias gariepinus*) Fingerlings. *Journal of Aquaculture and Fisheries* 3: 016.

Adeniji, C.A., Wusu,D, and Falana, E.O. (2019). Individual and Combined Effects of Moringa Leaf and Garlic Powder on Growth and Plasma Biochemical Indices of *Clarias gariepinus* Juveniles. *American Journal of Food Science and Technology* 7 (5): 137-145. https://doi.org/10.12691/ajfst-7-5-1

Agouz, H.M., Soltan, M.A. and Meshri, R.N. (2015). Effects of some organic acids and organic salt blends on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Nutrition and Feed 18*(2): 443-450.

Aklilu, E. B., Yasin, H., Wubie, A., Tahir, Z., Fentie, B., Gelaye, M., Tsehay, A., Adem, A., Desalign, M., Ali, Y. and Wubie, G. (2020). Stability Analysis of Seed Yield of Ethiopian Caraway (*Trachyspermum ammi L. Sprague ex Turrill)* Genotypes in Multi-Environment Trials. *Food Science and Quality Management 97,* p-ISSN 2224-6088, e- ISSN 2225-0557. https://doi.org/10.7176/FSQM/97-06

El-katcha, M.I., Soltan, M.A., Sharaf, M.M. and Hasen, A. (2016). Growth Performance, Immune Response, Blood serum parameters, Nutrient Digestibility and Carcass Traits of Broiler Chicken as Affected by Dietary Supplementation of Garlic Extract (Allicin). *Alexandria Journal of Veterinary Sciences 49 (2):* 50- 64. https://doi.org/10.5455/ajvs.21961

Ibidunni, A.S., Olubodun, O.S. and Ikililu, A. (2017) Metabolic activities and health indices of African catfish (*Clarias gariepinus*) fed varying levels of Zingiber officinale root. *Journal of Applied Biology and Biotechnology* 5: 021-028.

Iheanacho, S., Ogunji, J.O., Ogueji, E.O., Nwuba, L.A. and Nnatuanya, I.O. (2017) Comparative assessment of ampicillin antibiotic and ginger (*Zingiber officinale*) effects on growth, haematology and biochemical enzymes of *Clarias gariepinus* Juvenile. *Journal of Pharmacognosy and Phytochemistry 6*: 761- 767.

Jegede, T. (2012). Effect of garlic (*Allium sativum*) on growth, nutrient utilization, resistance and survival of *Tilapia zillii* (Gervais 1852) Fingerlings. *Journal of Agricultural Science 4*: 269. https://doi.org/10.5539/jas.v4n2p269

Kazeem, G.O., Adedayo, F.E. and Thomas, A.O. (2017). Effect of dietary *Moringa oleifera* extract against *Aeromonas hydrophila* infection and transportation-induced stress in African catfish *Clarias gariepinus* (Burchell, 1822) fingerlings. *World Applied Sciences Journal* 35: 88–95.

Lee, D.H., Lim, S.R., Han, J.J., Lee, S.W. and Ra, C.S. (2014). Effects of dietary garlic powder on growth, feed utilization and whole body composition changes in fingerling sterlet sturgeon (*acipenser ruthenus). Asian- Australasian Journal Animal Science 27(9)*: 1303-1310. https://doi.org/10.5713/ajas.2014.14087

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. and Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. *International Journal of Molecular Science* 16: 12791–12835.

Mahmoud, H.K. and El-Hais, A.M.A (2017). Effects of dietary garlic (*Allium sativa*) supplementations on Nile tilapia *Oreochromis niloticus* juveniles' performance under two stocking density. *Egyptian Journal of Nutrition and Feed* 20(1): 115-124.

Mahmoud, R., Aziza, A., Marghani and B., Eltaysh, R. (2019). Influence of ginger and garlic supplementation on growth performance, whole body composition and oxidative stress in the muscles of Nile tilapia (*O. niloticus*). *Advances in animal and veterinary Sciences* 7: 397-404.

Manoppo, H., Magdalena, E.F. and Kolopita, M.R.

(2016) Growth promoter effect of garlic (*Allium sativum*) on carp (*Cyprinus carpio L*). *International Journal of pharmaceutical technology and Research* 9: 283-288.

Monica, K.S. and Jayray, E.G. (2021). Review on probiotics as a functional feed additive in aquaculture. *International Journal of Fisheries and Aquatic Studies* 9(4): 201-207. https://doi.org/10.22271/fish.2021.v9.i4c.2528

Nyadjeu, P., Ekemeni, R. and Tomedi, M. (2020). Growth Performance, Feed Utilization and Survival of *Clarias gariepinus* Post-Larvae Fed with a Dietary Supplementation of *Zingiber officinale-Allium sativum* Mixture. *Journal of Aquaculture and Fisheries* 4: 028. https://doi.org/10.24966/AAF-5523/100028

Nyadjeu, P., Yemdjie, D.D.M., Ndjuissi, N.A.T., Nguenang, G.N., Dedou, N.Y.C. and Tabi-Tomedi, M.E. (2021). Effect of *Zingiber officinale* and *Allium sativum* Powders as Natural Feed Additives Promoting Growth, Feed Utilization and Whole-Body Composition in *Clarias gariepinus* Fry. *Food and Nutrition Sciences* 12: 526-543. https://doi.org/10.4236/fns.2021.126040

Puycha, K., Yuangsoi, B., Charoenwattanasak, S., Wongmaneeprateep, S., Niamphithak, P. and Wiriyapattanasub, P. (2017). Effect of moringa (*Moringa oleifera*) leaf supplementation on growth performance and feed utilization of Bocourti's catfish (*Pangasius bocourti*). *Agriculture and Natural Resources* 51: 286–291.

Raimi, C.O and Fatuase, O.B. (2021). Performance of African Catfish (*Clarias gariepinus*) juvenile fed *Morinda lucida* (Oruwo leaf). *Nigerian Journal of Animal Science* 23(3): 79-89.

Saini, R.K., Sivanesan, I. and Keum, Y.S. (2016) Phytochemicals of *Moringa oleifera*: A review of their nutritional, therapeutic and industrial significance. *Biotechnology* 6: 203–217. https://doi.org/10.1007/s13205-016-0526-3

Shakya, S.R. and Labh, S.N (2014). Medicinal uses of garlic (*Allium sativum*): improves fish health and act as immunostimulant in aquaculture. *European Journal of Biotechnology and Bioscience* 2(4): 44-47.

Talpur, A.D., Ikhwanuddin, M. and Abol Munafi, A.B. (2013). Nutritional effects of ginger (*Zingiber officinale Roscoe*) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. *Aquaculture* 400: 46-52.

Table 1: Primers used for Nucleic acid qualification in this study.

Primer	Forward Sequence	Reverse Sequence	Tm (°C)	Band Size (pb)
GAPDH	GCCCTCTGGTAAAATGTGGA	ATTCCCTTCATGGGTCCTTC	54	450
TNF-α	GGATGGTGGTGTGTGTTGTG	CTGGTACTCTGGTCACGACTC	60	150

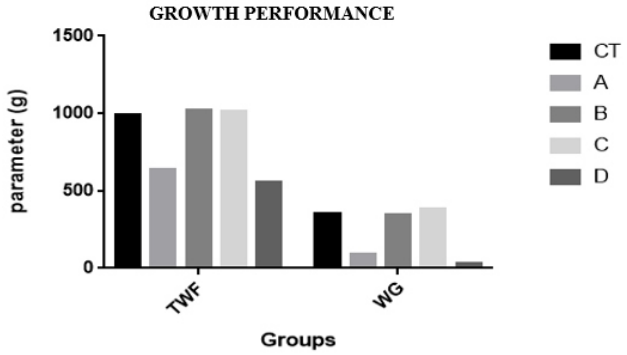

Figure1: Mean weight gain and total weight of fish of *Clarias gariepinus* juvenile-fed diets
CT – control, Treatment A - Fish-fed moringa leaf-based diet, Treatment B - Fish-fed ginger-based diet, Treatment C - Fish-fed garlic-based diet, Treatment D - Fish-fed combination of moringa, ginger, and garlic-based diet, TWF - total weight of fish per tank, WG – weight gain

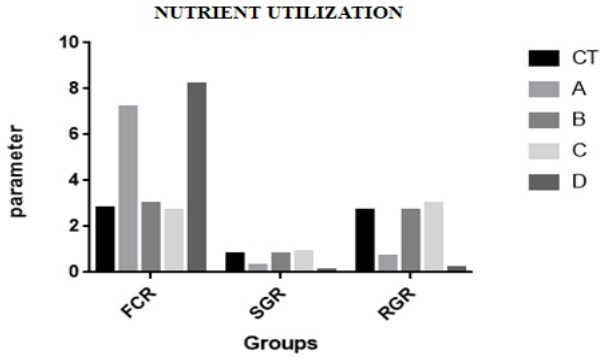

Figure2: Feed conversion ratio, Specific growth rate, and Relative growth rate of *Clarias gariepinus* juvenile fed diets

FCR – feed conversion ratio, SGR – specific growth rate, RGR – Relative growth rate

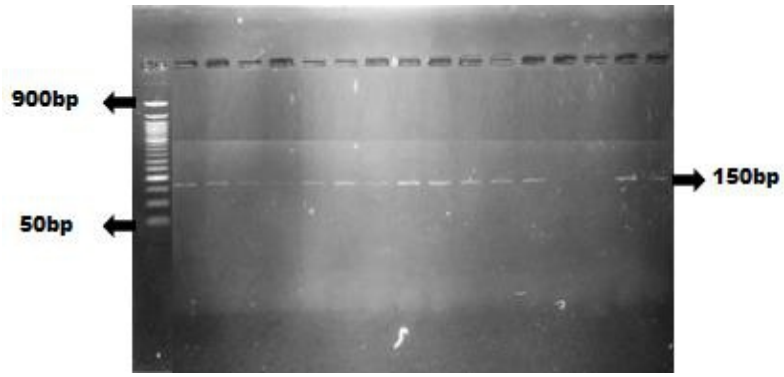

Figure 3: Gel Image capture of TNF-α PCR amplicons ran on 2% Agarose gel electrophoresis, with expected bands size 150bp. Samples were loaded in Triplicate from left to right CT, A, B, C, D.