Original Research Article

Frequency of water change on growth performance, nutrient utilization and liver histology of Nile tilapia (*Oreochromis niloticus,* Linnaeus, 1758)

Abstract

The study was conducted to investigate the frequency of water change on the growth performance, nutrient utilization and body indices of *Oreochromis niloticus* juveniles. *O. niloticus* of average body weight of 15 g were stocked and fed with 42% crude protein diet twice daily. The experiment had three treatments with 50% changing of water, every 2days, every 4days and every 7days. The water quality parameters were not significantly different among the treatments except for pH. Weight gain, percentage weight gain and specific growth rate were slightly higher in the 4days interval of changing water with values; 57.65±8.95 g, 33.93±5.16 % and 0.52±0.07 % day^{-1} respectively. Higher but not significant yield in relation to the volume of water used was obtained in 7days interval (0.46±0.09 Kg m^{-3}). Slightly higher values of condition factor, viscerosomatic index and hepatosomatic index were recorded in the 4days water changing interval. Higher glycogen content was observed in water changes every 4days. All the livers were healthy with the mildest vacuolations in the treatment with water changes once in a week. The results showed that *O. niloticus* juveniles can be reared with water changes every 7days with no negative consequences. Water changes every 4days tended towards the best.

Keywords: Body indices; glycogen; growth performance ; Nile tilapia; water change; water quality.

Introduction

Fish is an important source of human protein, it is considered as a relatively cheap source and it is readily available in most part of the world. Although the consumption varies across the world, the global per capita average stood at 20.5 Kg as of 2018 (FAO 2020). Overall global fish production is increasing with percentage contribution from aquaculture growing faster than that of capture (Dauda et al. 2018). Aquaculture has grown from less than 15 % contribution of total global fish production in 1990 to over 45 % in 2018, and for Africa, it has grown from less than 2 % to over 18 % within the same period (FAO 2020). Cichlids are regarded as the second most cultured finfish in the world, only after carps, and the most cultured in Africa, with Egypt producing the highest amount in Africa (Kpundeh et al. 2013, Dauda et al., 2018). Although Nigeria is the second highest producer of fish from Aquaculture in Africa, the bulk of fish from Nigeria is African catfish, with a very little amount of Cichlids. According to El-Kadi and El-Morsy (2016), Cichlids are considered as the best aquaculture candidates because of their ability to tolerate, adverse environmental conditions, wide salinity range, relatively poor water quality, as well as ability to grow and reproduce in captivity and resistance to diseases. Among the most cultured Cichlids is *Oreochromis niloticus*, otherwise known as Nile tilapia. Nonetheless, Cichlids like other cultured fish species is faced with certain problems in Nigeria, amongst which are high cost of feed, water supply both in quality and quantity, inadequate capital (Dauda et al. 2015). Water supply is a major requirement for fish culture and it must be available in adequate quantity and quality for successful fish culture (Ajani et al. 2011). Unfortunately, water availability is a global problem, hence, the needs for effective and efficient management of limited available water for optimal fish production (Akinwole et al. 2016) Also, wastewater from fish culture could constitute a nuisance to the environment, so, even when water is available in abundance, it should still be well managed to limit negative impacts of wastewater on the environment (Piedrahita 2003, Dauda et al. 2019). Over time, several means of managing water in aquaculture has evolved through various fish culture systems, such as recirculating aquaculture systems, aquaponics, flow-through systems, stagnant renewal systems and biofloc technology (Timmons et al. 2002, Tidwell 2012). Stagnant renewal system involves culturing of fish in a facility with water changes with time especially as the water is perceived inadequate for fish culture. However, most fish culturists do this arbitrarily with no reference standards. While some do it too frequently, some left the water in the system longer than necessary without the proper understanding of what could be the effect of changing water frequency on the fish culture. Although a too frequent change of water may always make clean water available to the fish, this may have consequences of increasing cost of production (Crab et al. 2012), through increase the cost of water used, as well as an increase in releasing of wastes to the environment, this may also deny the fish access to natural microbiota that may be of advantage to the fish (Dauda et al. 2019, Dauda 2020). On the other hand, delaying wastewater for a long time in the culture facility may lead to accumulation of toxic metabolites beyond what the fish can tolerate. Hence, resulted in reduced feed intake, poor growth, susceptibility to diseases as well as extreme lethargy and death (Timmons et al. 2002, Boyd 2003, Martins et al. 2010). Hence, the needs to seek for water changing frequency that can support optimum growth of fish with minimum negative impacts on the environment. An earlier study by Okomoda et al. (2015) noted a difference in the performance of African Catfish cultured at different water changing frequency and opined water changes between four and eight days is adequate for fingerlings of African catfish. However, the water chnaging frequency may be species and age dependent. This study, therefore, examined the performance of Nile tilapia fingerlings reared in stagnant renewal systems with different water changing frequency.

Materials and methods

Study area and material source

The research was carried out in the wet laboratory of the Department of Fisheries and Aquaculture, Federal University Dutsin-Ma, Dutsin-Ma, Katsina State, Nigeria. The rearing of the fish was done using rectangular shape plastic tanks of 44 cm x 29 cm x 25 cm with a capacity of 30 l of water. The tanks were gotten from Kano State. Borehole water from the school reservoir was used for all the treatments. Ninety pieces of *Tilapia* juveniles of average 15 g body weight were obtained from a private farm in Kano town and transported down to Dutsin-Ma in plastic kegs with the top opened. The fish were acclimatized for 7 days in a 500 l plastic tank and fed commercial diet of 2 mm with 42 % crude protein, twice a day at 5 % body weight per day.

Experimental design and procedure

The design used was a complete randomized design (CRD) comprising of three treatments which include: 50 % of the tank water was changed every 2days, every 4days and once in a week as treatment 1, 2 and 3 respectively, with each of the treatment in triplicates. The experiment was carried out for eight weeks, each tank was filled with 20 Liters of water and stocked randomly with ten fish each. The tanks were left to normal weather vagaries with no control of the lighting period. All the fish stocked were fed a commercial extruded pellets of 2 mm size and 42 % crude protein that was purchased in Kano metropolis. They were fed to apparent satiation every day (morning and evening) and their weight was recorded every week for eight weeks duration of the experiment.

Performance evaluation

The examined water quality parameters, temperature, pH, total dissolved solids, electrical conductivity, total ammonia-nitrogen and nitrate were measured using Lamotte instruments following the description of Dauda and Akinwole (2014). The daily feed intake, and length and weight of the fish from each tank at the end of the eight weeks experiment were used in the evaluation of growth performance. The body indices were examined by dissecting three fish from each tank, and the viscera and liver were harvested and measured. The livers from each treatment were fixed in 10 % phosphate-buffered formalin for histological analysis. All the calculations were done following the description of Jimoh et al. (2019)

Weight gain = Final weight – initial weight

Percentage weight gain = Weight gain/initial weight x100

Specific growth rate = ((Ln W2 – Ln Wi)/ number of days for fish culture) X 100

Feed intake = summation of daily feed intake for the number of culture days

Food conversion ratio = Feed intake/ wet weight gain

Protein effciency ratio = Weight gain/protein intake

Lipid efficiency ratio = Weight gain/lipid intake

Yield (per water used) = Weight of fish/volume of water used for fish culture

Viscero-somatic index = (weight of viscera/weight of fish) x100

Hepatosomatic index = (weight of liver/weight of fish) x 100

Condition factor = (W/L^3) x100

Liver histology

The fixed livers were transferred from formalin to 70 % ethanol after 24 hours, from where they were processed using the methods described by Dauda *et al*. (2017) for both periodic acid Schiff (PAS) and hematoxylin and eosin. The slides were examined after staining under a light microscope (Olympus BX51, Olympus, Tokyo, Japan) attached to a charge-coupled device camera (Olympus U-CMAD-2: Olympus, Tokyo, Japan) with Leica image analyser software. The images were analysed following the description of Romano *et al*. (2018).

Statistical Analysis

The data obtained were presented using descriptive statistics mean±standard error. The differences among the treatments for each parameter was tested using analysis of variance (ANOVA), after the test of homogeneity. Where a significant difference was observed, Duncan multiple range test was used as a follow-up. IBM SPSS version 23 was used for the analysis.

Results and discussion

Water quality parameters

As shown in Table 1, the temperature was not different significantly ($P > 0.05$) among the treatments though it was slightly higher in water changes of 4days interval (26.47±0.41 ^{0}C). The temperature observed during the study were within the range of 24 to 28 ^{0}C in all the three treatments. The temperature was suitable for culturing tropical fish as it was within the recommended range of 20-30 ^{0}C (Dauda et al. 2014). pH is an important water quality parameter because optimum health of the fish is related to the level of pH (Al-Hafedh et al. 2003). In this study, pH value was similar in 2days and 4days water changing intervals. The highest pH was observed in 7days interval changing (7.12±0.07) and it was significantly higher ($P < 0.05$) than that of 4days interval. However, all the pH values were within the recommended range (6.5-8.5) for warm water fish culture (Akinwole 2005). A higher value of (528.42±149.24 mg l^{-1}) was recorded in 7days water changing interval for total dissolved solid while the least value was recorded in the 2days water changing interval. The difference between the treatments was not found significant ($P > 0.05$). Also, there was no significant difference among the treatments for electrical conductivity ($P > 0.05$) where the highest (0.77±0.22 µs cm^{-3}) value was recorded in the 7days water changing interval. Slightly higher values of total dissolved solids and electrical conductivity may be due to the accumulation of solid waste due to longer time of changing the water. However, the values were lower than that observed by Dauda et al. (2017) in the biofloc culture of African catfish and the values within the range for this study were not reported harmful to the fish. Total ammonia-nitrogen (TAN) is the most critical water quality parameter in culturing fish (Al-Hafedh et al. 2003, Dauda et al. 2014). It consists of two fractions, un-ionized ammonia (NH_3) and ionized ammonia of which the first one is extremely toxic to fish (Romano and Zeng 2013; Dauda 2020). Ajani et al. (2011) noted that fish continuously exposed to more than 0.2 mg l^{-1} of the unionized form of ammonia may exhibit reduced growth and susceptible to disease. Total ammonia-nitrogen was recorded to be similar in 4days and 7days water changing with a higher value of (0.38±0.07 mg l^{-1}) compared to 0.31±0.06 mg l^{-1} recorded in the 2days interval of changing water, the difference among the treatments was not significant ($P > 0.05$). All the TAN reported were still within the recommended level (< 8.8 mg l^{-1}) for warm water fish culture (Akinwole 2005). Nitrate is one of the products of ammonia oxidation, though it is not generally considered to be of serious concern to fish culture (Verdegem et al. 2006) except at very high concentration of above 200 mg l^{-1}. It can cause a nuisance to the environment even as low as 20 mg l^{-1} (Dauda et al. 2014). Nitrate was not significantly different among the treatments ($P > 0.05$) in this study and the values were both within a healthy range for fish culture and discharge into the environment.

TABLE 1: Water quality in the rearing of *Oreochromis niloticus* at different frequencies of changing water

Parameter	2days interval	4days interval	7days interval

Temperature (^{0}C)	26.07±0.54	26.47±0.41	26.20±0.49
pH	6.95±0.07^{ab}	6.91±0.04^{b}	7.12±0.07^{a}
Total dissolved solids (mg l^{-1})	450.67±148.42	494.75±152.43	528.42±149.24
Electrical conductivity (µs cm^{-3})	0.68±0.23	0.72±0.23	0.77±0.22
Total ammonia nitrogen (mg l^{-1})	0.31±0.06	0.38±0.07	0.38±0.07
Nitrate (mg l^{-1})	0.20±0.03	0.17±0.04	0.18±0.04

Different letters as superscript in each row indicate significant differences ($P < 0.05$)

Growth performance and nutrient utilization

Overall growth performance of fish is affected by water quality parameters (Ajani et al. 2011). The similarity in water quality parameters among the treatments is an indication that the fish reared at different frequencies of changing water have the same influence of water quality. This may lead to similar growth performance, especially if other culture conditions are similar. Mean values of selected growth and feed utilization parameters obtained in the experiment are as shown in the (Table 2). As for the growth parameters, weight gain, and specific growth rate were relatively higher in the 4days interval of water changing with values; 57.65±8.95 g and 0.52±0.07 % day^{-1} respectively but the difference was not significant ($P > 0.05$) among the treatments. Percentage weight gain having almost similar value although it was slightly higher in 4days interval (33.93±5.16 %). The slightly lower growth performance observed in 7days water changing interval may be due to longer days of changing the water which led to the highest total dissolved solids. It might already be stressing the fish, and if prolonged may cause significant growth reduction (Moyle and Ceck 2000, Poon et al. 2002, Okomoda et al. 2016). Total dissolved solids might also cause toxicity through an increase in salinity, changes in ionic composition of the water and toxicity of individual ions (Timmons and Lorsodo 1994, Dauda et al. 2019). In terms of feed consumed, a slightly higher amount of feed was consumed in 4days and 7days treatments respectively, compared to the 2days treatment. However, the difference was not significant ($P > 0.05$) among the treatments. The FCR obtained in 7days and 4days were very similar and slightly higher but not significantly different from that of 2days interval. There was no mortality in any of the treatment throughout the experiment. Higher yield in related to the volume of water used was obtained from 7days interval (0.46±0.09 Kg m^{-3}) and the least in 2days interval (0.20±0.02 Kg m^{-3}) but the difference was not significant among the treatments ($P > 0.05$). This put 7days water changing interval at an advantage over other treatments, as a lower amount of water is used and this may lead to a reduced cost of production. Okomoda et al. (2016) also reported similar growth and nutrient utilization performance between African catfish juveniles with water changing every day and ever four days, although water changing frequencies showed varying differences.

TABLE 2: Growth and nutrient utilization of *Oreochromis niloticus* reared at different frequencies of changing water

Parameters	2 days interval	4 days interval	7 days interval
Initial weight (g)	159.60±3.20	169.85±0.55	170.53±15.14

Final weight (g)	212.50±7.50	227.50±9.50	217.0±15.82
Weight gain (g)	52.90±4.30	57.65±8.95	46.47±9.09
Percentage weight gain (%)	33.11±2.03	33.93±5.16	28.01±6.67
SGR (% day^{-1})	0.51±0.03	0.52±0.07	0.44±0.09
Feed intake (g)	124.02±3.26	144.06±2.77	134.39±12.74
Feed conversion ratio	2.34±0.08	2.50±0.40	2.89±0.99
Survival (%)	100.0±0.00	100.0±0.00	100.0±0.00
Yield (Kg m^{-3})	0.20±0.02	0.32±0.05	0.46±0.09

Different letters as superscript in each row indicate significant differences (P < 0.05)

Body indices

Table 3 showed that all the body indices observed during the experiment were not significantly different (P > 0.05) among the treatments. Although, higher values of condition factor, viscerosomatic index and hepatosomatic index (1.70±0.03, 4.72±0.31 % and 1.72±0.20 %) were recorded in the 4days water changing interval respectively. The condition factor gives the information on the physiological state of fish to its welfare. It was greater than one among the treatments which indicate a suitable response of fish to the experimental condition. This is in line with the findings of Jimoh et al. (2019) on the assessment of prebiotic potentials in selected leaf meals of high dietary fibre on growth performance, body composition, nutrient utilization and amylase activities of a tropical commercial carp fingerlings. The similar viscerosomatic index and hepatosomatic index of the Nile tilapia among the treatments indicated similar well-being status of the fish reared at different water changing frequency, and the values obtained are comparable with that of Dauda et al. (2017) and Romano et al. (2018).

TABLE 3: Body indices of *O. niloticus* reared at different frequencies of changing water

Parameter	2 days interval	4 days interval	7 days interval
Condition factor	1.65±0.50	1.70±0.03	1.68±0.03
Viscerosomatic index (%)	4.54±0.38	4.72±0.31	5.62±0.38
Hepatosomatic index (%)	1.48±0.15	1.72±0.20	1.61±0.06

Different letters as superscript in each row indicate significant differences (P < 0.05)

Liver histology

The livers sections from the three treatments as shown in (Figure 1) showed that there was glycogen accumulation in the cytoplasm of the hepatocyte, as indicated by the pink areas of positive PAS. The glycogen is densely accumulated in the cytoplasm hepatocyte of T2 (4day interval). It was mild in T1(2days interval) and T3(7days interval). This shows a relatively higher energy deposit in T2 than T1 and T3, however, all the treatments have considerable high energy deposit which indicates that the livers are not stressed and healthy (Dauda et al. 2017). The liver sections (Figure 2) for Hematoxylin & Eosin showed a few vacuolizations in all the treatments, with treatment 3(7days interval) having the mildest observable lesions. The observation are not indicators of bad liver function, a similar report was earlier documeted by Ebrahimi et al. (2017).

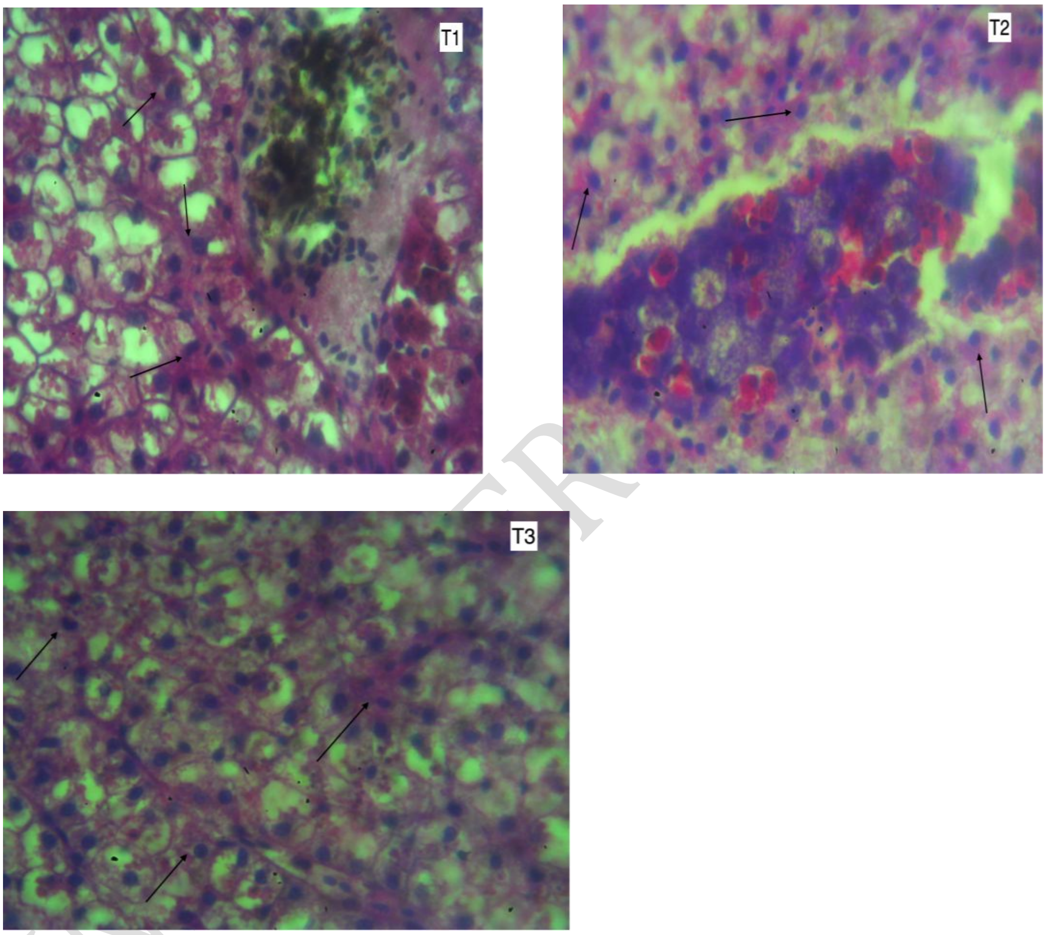

Figure 1: Sections of the liver (PAS staining) of the *O. niloticus* reared at different frequencies of changing water (PAS x 400). The glycogen deposits are seen as pink areas of PAS-positive (arrows) .T1: Glycogen accumulations are seen in the cytoplasm of the hepatocyte and around the sinusoids. T2: densely glycogen deposits in the cytoplasm of the hepatocytes. T3: mild sinusoidal glycogen accumulations.

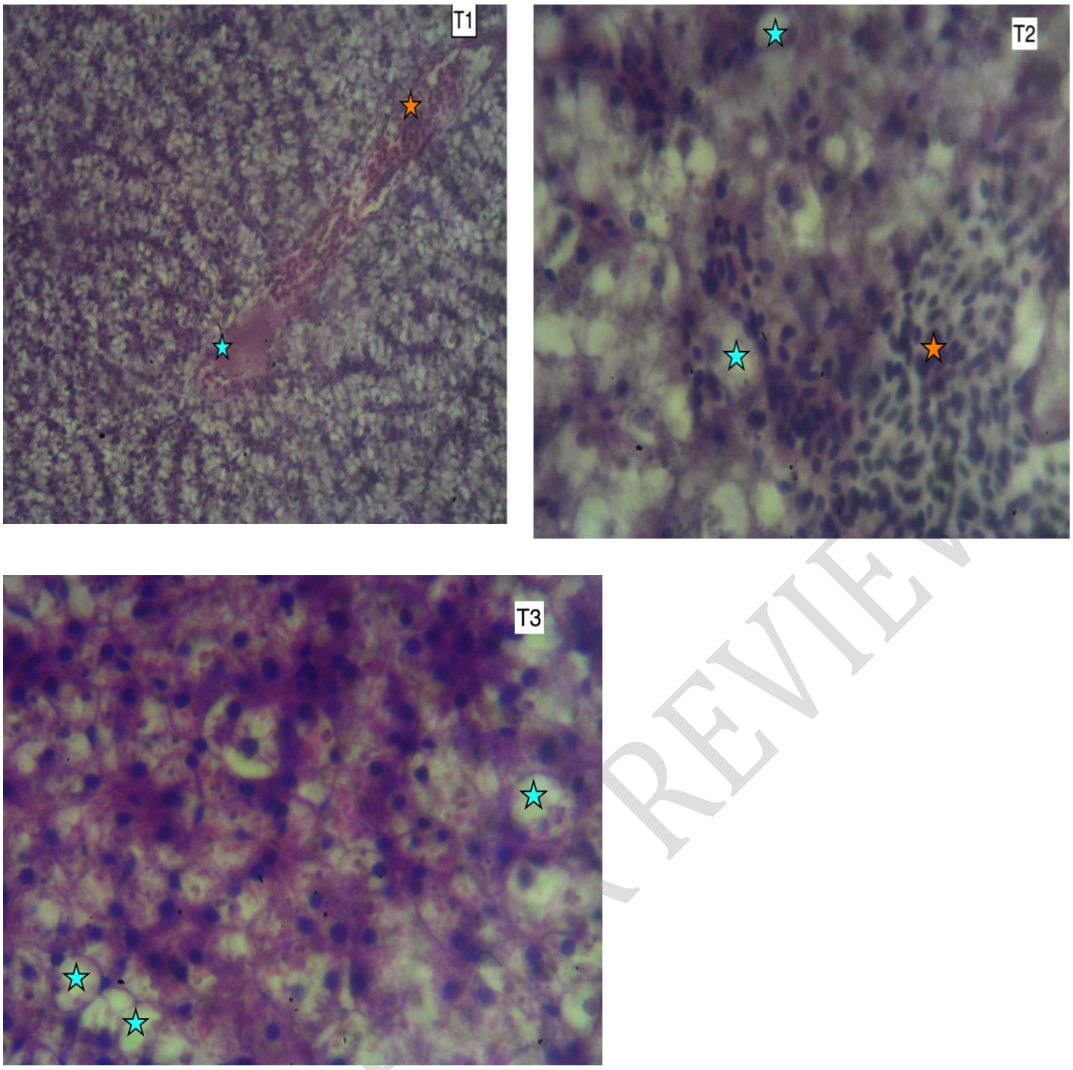

Figure 2: Sections of the liver (H&E staining) of the *O. niloticus* reared at different frequencies of changing water (H&E x 400). The blue stars indicate congestion areas of vacuolation and red star shows the sinusoidal infiltration of RBC. T1: hepatocytes vacuolations (H&E x 100). T2: diffuse areas of vacuolations. T3: mild vacuolations are observed.

Conclusion

The findings of this research revealed similar water quality in the three treatments despite the differences in the frequency of changing water. The growth performance, nutrient utilization and body indices were also similar among the treatments. Therefore it can be concluded that *O. niloticus* juveniles can be reared up to stocking density of 11.5 Kg m^{-3} at water changing interval of 7days interval without any negative effect on the growth performance and well-being of the fish.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

Ajani, E.K., Akinwole, A.O and Ayodele I.A. (2011) Fundamentals of Fish Farming in Nigeria. Walecrown publishers Ibadan.

Akinwole, A.O. (2005). Effect of media size and depth on performance of sand filter for fish farm wastewater treatment. *Ibadan Journal of Agricultural Research* 1(1): 19-25.

Akinwole, A.O., Dauda, A.B. and Oyewole, E.B. (2016), Influence of partial and total static culture water withdrawal on water quality, growth and feed utilization of *Clarias gariepinus* juveniles. *International Journal of Fisheries and Aquatic Studies (IJFAS) 4(6)*: 175 – 178. www.fisheriesjournal.com

Al-Hafedh, Y.S., Alam, A. and Alam, M.A. (2003), Performance of plastic biofilter media with different configuration in a water recirculation system for the culture of Nile tilapia *(Oreochromis niloticus). Aquacultural Engineering* 29: 139-154.

Boyd, C.E. (2003), Guidelines for aquaculture effluent management at the farm-level. *Aquaculture* 226: 101-112.

Crab, R., Defoirdt, T., Bossier, P., and Verstraete, W. (2012), Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* 356: 351-356.

Dauda, A.B. (2020), Biofloc technology: a review on the microbial interactions, operational parameters and implications to disease and health management of cultured aquatic animals. *Reviews in Aquaculture* 12, 1193-1210 https://doi:10.1111/raq.12379

Dauda, A.B. and Akinwole, A.O. (2014), Interrelationships among water quality parameters in recirculating aquaculture system. *Nigerian Journal of Rural Extension and Development* 8(4): 20-25.

Dauda, A.B., Akinwole, A.O. and Olatinwo, L.K. (2014), Biodenitrification of aquaculture wastewater at different drying times in water reuse system. *Journal of Agriculture and Food Technology* 4(2): 6-12.

Dauda, A.B., Dasuki, A. and Bichi, A.H. (2015), Analysis of constraint to Aquaculture development in Sudano-Sahelian region of Nigeria. *Tropical and Subtropical Agroecosystem* 18; 189-193

Dauda, A.B., Romano, N., Ebrahimi, M., Karim, M., Ikhsan, N., Kamarudin, M.S, and Ekasari, J. (2017), Different carbon sources affects biofloc volume, water quality and the survival and physiology of African catfish *Clarias gariepinus* fingerlings reared in an intensive biofloc technology system. *Fisheries Science* 83: 1037-1048.

Dauda, A. B., Romano, N., Ebrahimi, M., Teh, J. C., Ajadi, A., Chong, C. M., Karim, M., Ikhsan, N. and Kamarudin, M. S. (2018). Influence of carbon/nitrogen ratios on biofloc production and biochemical composition and subsequent effects on the growth, physiological status and disease resistance of African catfish (*Clarias gariepinus*) cultured in glycerol- based biofloc system. *Aquaculture*, 483, 120–130. https://doi.org/10.1016/j.aquaculture.2017.10.016

Dauda, A.B., Ajadi, A., Tola-Fabunmi, A.S. and Akinwole, A.O. (2019), Waste Production in aquaculture: sources, components and managements in different culture systems**.** *Aquaculture and Fisheries*, 4, 81-88. https://doi.org/10.1016/j.aaf.2018.10.002

Ebrahimi, M., Daeman, N. H., Chong, C. M., Karami, A., Kumar, V., Hoseinfar, S. H. and Romano, N. (2017). Comparing the effects of different dietary organic acids on the growth, intestinal short-chain fatty acids, and liver histopathology of red hybrid tilapia (Oreochromis sp.) and potential use of these as preservatives. *Fish Physiology and Biochemistry* 43: 1195–1207. https://doi.org/10.1007/s10695-017-0365-0

EL-Kadi, S.M and EL-Morsy, A.M. (2016), The effect of water aquaria change on Nutrient utilization and microbial activity of Nile tilapia *(Oreochromis niloticus). International Journal of Fisheries and Aquatic Studies* 4(4): 196-205.

FAO. (2020), The State of World Fisheries and Aquaculture (2020). *Sustainability in action*. Rome. https://doi.org/10.4060/ca9229en

Jimoh, W.A, Kamarudin, M.S, Sulaiman, M.A. and Dauda, A.B. (2019). Assessment of prebiotic potentials in selected leaf meals of high dietary fibre on growth performance, body composition, nutrient utilization and amylase activities of a tropical commercial carp fingerlings. *Aquaculture Research* 50: 3401-3411.

Kpundeh M. D., Xu P., Yang H., Qiang J.and He J. (2013), Stocking Densities and Chronic Zero Culture Water Exchange Stress' Effects on Biological Performances, Haematological and Serum Biochemical Indices of GIFT Tilapia Juveniles *(Oreochromis niloticus). Journal of Aquatic Research and Development* 4, 189.

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d'Orbcastel, E. and Verreth, J.A.J. (2010), New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering* 43(3): 83–93.

Moyle, B. P. and Cech, J. J. Jr. (2000), *Fishes: An Introduction to Ichthyology*. Fourth edition. Upper Saddle River NJ.

Okomoda, V. T., Tiamiyu, L. O. and Iortim, M. (2016), The effect of water renewal on the growth of *Clarias gariepinus* fingerlings. *Croatian Journal of Fisheries* 74: 25-29. DOI: 10.1515/cjf-2016-0005

Piedrahita, R.H. (2003), Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture* 226: 35–44.

Poon, W. L, Hung, C. Y. and Randall, D. J. (2002), The effect of aquatic hypoxia on fish. Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong, SAR, China. EPA/600/R-02/097.

Romano, N. and Zeng, C. (2013), Toxic effects of ammonia, nitrite, and nitrate to decapod crustaceans: a review on factors influencing their toxicity, physiological consequences, and coping mechanisms. *Reviewes in Fisheries Science* 21(1): 1-21.

Romano, N., Dauda, A.B., Natrah, I., Karim, M., and Kamarudin, M.S. (2018), Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies and biochemical composition of African catfish *Clarias gariepinus* juvenile**s**. *Aquaculture Research* 49 (12): 3691-3701. https://doi.org/10.1111/are.13837.

Tidwell, J.H. (2012), *Aquaculture production systems*. World Aquaculture Society published by Wiley-Blackwell, USA 434pgs.

Timmons, M. B. & Lorsodo, T. M. (1994), *Aquaculture water reuse systems, engineering design and management*. New York, USA: Elsevier Science B.V.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. and Vinci, B.J. (2002), *Recirculating Aquaculture Systems*, 2nd Edition. Cayuga Aqua Ventures, New York.

Verdegem, M.C.J., Bosma, R.H. and Verreth, J.A.J. (2006). Reducing water use for animal production through aquaculture. *International Journal of Water Resources Development* 22: 101-113.