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Growth Performance of African Catfish (Clarias gariepinus, Burchell 1822) Following Direct Injection with Bagrus Bayad Genomic DNA

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Abstract: Study on the growth performance of African catfish (Clarias gariepinus) following direct injection with Bagrus bayad genomic DNA was carried out. The aim was to determine the growth and survival of African catfish injected with Bagrus bayad genomic DNA. Seventy five (75) Clarias gariepinus fingerlings were procured from Maiduguri metropolitan and conveyed to fisheries hatchery complex where the experiment was conducted. The Bagrus bayad samples were obtained from river Gashuwa, Yobe state and conveyed to Biotechnology Department of University of Maiduguri for DNA extraction. After the extraction, the genomic DNA was used to inject the 75 fish which were placed into 5 treatments with each having 15 fish (5 fish per replication). The fish were injected based on the DNA concentration of 0, 5, 10, 15, and 20µl/0.1/fingerlings based on the treatment using 2ml syringe. The injected fingerlings were reared for the period of four (4) months. After the culture period, the data on the growth and survival were subjected to analysis of variance. The result revealed that the growth and survival of the fingerlings were better in fingerlings injected with 10 and 20µl of the Bagrus bayad genomic DNA. This indicates that the growth and survival of Clarias gariepinus fingerlings can be improved through the use of genomic DNA from Bagrus bayad at 10 and 20µl concentrations.

Keywords: Bagrus bayad, effects, genomic, DNA, African catfish, growth, performance.

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1.0 INTRODUCTION

Fish and fisheries product are regarded as best sources of food as they contain higher amount of proteins, vitamins and minerals that can simply utilize by both infants and adults (Abdullahi *et al.*, 2001). In African countries especially Nigeria, fish are eaten at its fresh form or after processing as either smoked or dried and this can be cherished by many people (Adebayo *et al.*, 2008). Fish and fisheries product constitute to 40% of the dietary animal protein consumed by humans in Nigeria. According to Adekoya and Miller (2004), more than 60% of the total animal protein taken by adults especially in rural areas comes from fish and fisheries product. Fish product is higher in nutrient and can easily be digested making it superior over other sources of animal protein (meats or beef, pork and chicken) (Amingheme,

2005). As human population increases, the demand for high quality food especially from aquatic resources is needed. The production of fish through aquaculture is highly needed in order to meet up with the increased human population (Omeji et al., 2013). According to FAO (2007), aquaculture grows quickly than other agriculture sectors as it grows at a rate of 8.8% and above every year since 1970 which is greater than capture fisheries standing at 1.2%. The status of capture fisheries is at decline condition due to over-fishing, habitat destruction, increasing human population and fishing with chemical and other explosive materials (Dunham et al., 2001). In Nigeria, aquaculture is considered as rapidly growing agriculture sub sector, though it was reported that it contribute only 5% to the economy on recent years (Moses et al., 2006). Clarias belongs to the family claridae which is divided into two genera; Clarias and Heterobranchus. They can be distinguished by the presence of rayed dorsal fin followed by large adipose fin, which is present on the Heterobranchus but absent in Clarias. In Clarias, the rayed dorsal fin extends the whole length of the body from its commencement (just behind the head) almost to the tail fin. Three species of Clarias have been described from West Africa. They are Clarias angullaris, C. submarhinatus and C. lazera (Holden and Reed, 1972). They are not easy to identify because they all look similar. The vomerine teeth are the most reliable means for determining the species (Reed, 1967). Bagrus bayad is more or less elongated, the dorsal fin has a smooth spine, and the pectoral fins have spines with serrations on the inside. They have four pairs of barbels. The maxillary barbels may reach to the ventral fin or pelvic fins. This fish is yellow-greenish or blackish with a white belly. The fins are darker, sometimes reddish purple. Juveniles have little black spots on the sides (Froose and Pauly, 2007). Bagrus bayad is a species of fish that belong to the family bagridae. The fish was reported to weigh up to 12.5kg and can attain the length of 112cm. The females grow bigger and larger than the males from the same cohort (Froose and Pauly, 2007). Bagrus bayad are found in most Nigerian territorial water such as lakes, swamps, dams and rivers. Genomic is simply refers to the study of genome. The genome constitutes the genetic made up of an organism including the DNA (deoxyribonucleic acid), genes and the non coding DNA, as well as mitochondrial and chloroplast DNA (Perry and Robert, 1976). The genome of an organism encoded by the genomic DNA is the biological information of heredity which is passed from one generation of organism to the next. Despite the effort of many hatcheries to produce fast growing fingerlings for aquacultural, there are a lot of complain by farmers on the quality of fingerlings supplied for culture. This is because most hatcheries in Nigeria uses fish from the same strain to develop fingerlings for production and the deteriorating performance of the fish declines in growth, milt quality, and disease resistance as well as body deformities. Therefore, this paper tend to produce information on the growth performance of Clarias gariepinus injected with genomic DNA from Bagrus bayad which is easier than other method of improving the fish such as hybridization, uses of hormones, introduction of ploidy, sex reversal and so on.

2.0 MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted at the teaching and research fish farm of Department of Fisheries, University of Maiduguri situated between latitude 11° 51 N and longitude 13° 051 E. Maiduguri is characterized by cold dry climate starting from January to March and on average, the warmest month is April. It has a mean annual rainfall of 800mm. The rainy season usually begins in June and ends in October with the relative humidity of 5-54.5% and atmospheric temperature ranging from 38-40°C during the day which drops to 29-31°C at night (PTN, 2015).

2.2 Experimental Fish

Clarias gariepinus fingerlings used for the experiment were procured from commercial fish farm within Maiduguri metropolitan while *Bagrus bayad* sample was obtained from River Gashuwa Yobe state, both samples were transported to the hatchery unit of the Department of Fisheries, University of Maiduguri separately based on species in 25L Jerican half filled with fresh water. Clarias gariepinus fingerlings samples were acclimatized for 24 hours in 2 x 1m² concrete ponds before were fed 35% crude protein diet at 5% of their body weight twice daily before the commencement of the experiment. *Bagrus bayad* sample was used for the extraction of the genomic DNA which was used in the *Clarias gariepinus* fingerling samples.

2.3 DNA extraction from Bagrus bayad

Bagrus bayad sample was taken to Biotechnology Department, University of Maiduguri for Genomic DNA extraction. The Genomic DNA was extracted from Bagrus bayad tissue sample using Qiuck-DNA universal miniprep kit (catalog No.#D4068 and D4069) ZYMO RESEARCH (South Africa). The genomic DNA was extracted first by adding 400 µl of lysis solution and 20 µl of proteinase K solution to 20µl of tissue inside a sterile DNA free 1.5 µl micro tube. The content of the micro tube was mixed thoroughly by pipetting up and down or using a vortex mixer to obtain uniform suspension. The solution were incubated at 55°C for 20 minutes (using 230 volts VWR Digital Heat Block) until the cells are completely lysed. This was followed by adding one volume of genomic binding buffer to the tube, mixed thoroughly by pipetting up and down or by vortexing to ensure that the samples were homogenized. The mixture were transferred into a Zymo-Spin 11C-XL column in a collection tube and centrifuged at 12,000 x g for one minutes using Eppendorf table top centrifuge 5418. The collection tube and the flow through were discarded. The Zymo-Spin 11C-XL column was transferred to a new 2 ml collection tube, followed by addition of 400 µl DNA pre-wash to the column and centrifuged at 12000 x g for 1 minutes. After the collection tube was emptied 700, µl gDNA wash buffer was added to the Zymo-spin 11c-xl column and centrifuged at 12000 x g for 1 minute. The Zymo-spin 11c xl column was transferred into a clean 1.5 ml micro centrifuge tube and 50 µl DNA elution buffer was added directly to the column matrix. This was incubated at room temperature for 5 min and centrifuged at top speed for 1 minute to elude the DNA, the eluted DNA was used for the directly injection of the Clarias gariepinus fingerlings samples.

2.4 Experimental Design

Seventy (75) five fingerlings of *Clarias gariepinus* (six weeks old) with a total length and weight ranging from 7-10cm and 10-15g respectively were used for the research. The fingerlings were grouped into five treatments and each treatment was replicated (T1, T2, T3, T4 and T5) in a complete randomized design manner (CRD). Fifteen fingerlings were allocated to each treatment (5 fish per replicate). The extracted genomic DNA from the *Bagrus bayad* was used to inject the fingerlings using 0.1xSSC buffer. Four concentrations of 0, 5, 10, 15 and $20\mu l/0.1/fingerling$ were used for each of the treatment. The fingerlings were reared for the period of four months (4 months). During the culture period, the fingerlings were fed with commercial diet constituting 45% crude protein. They were fed twice daily at 5% of their body weight.

2.5 Growth and Survival of *Clarias gariepinus* injected with *Bagrus bayad* Genomic DNA

During the culture period, the following data were recorded; final weight, final length,

survival rate and quantity of feed fed. Later, the below growth indices were calculated and estimated for each of the treatment using the formulae below;

- i) Weight gain (g) = $W_2 W_1$, where W_2 and W_1 are the final and initial weight of fish, respectively (Buacker *et al.*, 1990).
- ii) Mean daily weight gain (MDWG) in gram = $W_2 W_1 / N \times t$, Where = W_2 and W_1 are the final and initial weight of fish, respectively, n = number of fish and t = the culture period (days) (Ahmed *et al.*, 2012).
- iii) Final length (mm) = $L_2 L_1$, where L_2 and L_1 are the final and initial length of fish respectively (Buacker *et al.*, 1990).
- iv) Specific growth rate (SGR % per day) = $\log_e W_i$ $\log_e W_o$ /t×100, where $\log_e W_i$ = $\log_e W_i$ = $\log_e W_o$ = $\log_e W_o$
- v) Feed conversion Ratio (FCR) = Dry weight of feed (g) / Weight gain of fish (g).
- vi) Condition factor (K) = W $\times 100 / L^3$, where W and L are the weight and length of the fish (Ayoola *et al.*, 2012).
- vii) Percentage survival = $n_2 n_1 / t$ 100, where n_2 and n_1 are the final and initial of the fish respectively, t= the culture period (Ayoola *et al.*, 2012).

2.6 Data Analysis

Data obtained from the experiments on the growth performance were subjected to one way analysis of variance for each experiment. The differences between means were determined using Fisher's LSD (p = 0.05) with the aid of Statistix 8.0.

3.0 RESULTS AND DISCUSSION

3.1 Growth and survival of African catfish fingerlings injected with Bagrus bayad genomic DNA.

Table 1 presented the growth performance of Clarias gariepinus fingerlings injected with Bagrus bayad genomic DNA. Higher final weight of 2661.6g was obtained in fingerlings injected with 20µl of genomic DNA from Bagrus bayad, followed by fingerlings injected with 10, 5 and 15ul of genomic DNA as 1789.1, 1302.6 and 956.20g respectively. The least value of the final weight was reported in fingerlings injected with 0µl of genomic DNA as 842.73g. The fingerlings injected with 5, 10, and 20µl did not show any significant variation (p>0.05) between them, fingerlings injected with 0, 5 10 and 15µl show no statistical differences (>0.05). However, fingerlings injected with 0 and 15µl differs (p>0.05) significantly with those fingerlings injected with 20µl of the Bagrus bayad genomic DNA. The final weight presented in this research was lower than the final weight reported by Mohammed et al. (2016) as 5.58kg when worked on the growth of Oreochromis niliticus. Hershberger et al. (1990) also reported lower value of the final weight of 250g on Coho salmon. The differences in the final weight are due to the effect of the experimental materials used (genomic DNA and growth hormone). Higher weight gain of 2591.4g was discovered in fingerlings injected with 20µl of genomic DNA from Bagrus bayad followed by fingerlings injected with 10, 5, and 15µl of genomic DNA as 1765.1, 1271.6 and 930.43g respectively. The least value of the weight gain was observed in fingerlings injected with 0µl of genomic DNA as 823.03g. The fingerlings injected with 5, 10, 15, and 20µl indicated no any statistical differences (p>0.05) with those fingerlings injected with 20µl of the Bagrus bayad genomic DNA. The weight gain of the present work was higher than the value.

Table 1: Growth and survival of African catfish fingerlings injected with Bagrus bayad genomic DNA

Parameters	Concentrations of genomic DNA				
0	5	10	15	20	SEM
Initial weight (g) 3.78*	19.70 ^c	30.976 ^b	24.00 ^{bc}	25.77 ^{bc}	41.20 ^a
Initial length (mm) 20.49*	351.67 ^b	455.00 ^a	391.67 ^b	381.67 ^b	455.00 ^a
Final weight (g) 761.82*	842.73 ^b	1302.6 ^{ab}	1789.1 ^{ab}	956.20 ^b	2661.6 ^a
Final length (mm) 741.66 ^{ns}	1496.7 ^a	1586.7 ^a	2188.7 ^a	987.33 ^a	2116.0 ^a
Weight gain (g) 777.07 ^{ns}	823.03 ^b	271.6 ^{ab}	1765.1 ^{ab}	930.43 ^{ab}	2591.4 ^a
Specific growth rate (a 0.24 ^{ns}	g) 1.33 ^a	1.33 ^a	1.53 ^a	1.30 ^a	1.47 ^a
Feed conversion ratio 0.85^{ns}	2.09 ^a	1.03 ^a	0.64 ^a	1.27 ^a	0.65 ^a
Condition factor 0.05*	0.54 ^d	0.82 ^c	0.82^{c}	0.96 ^b	1.22 ^a
Percentage survival 21.49 ^{ns}	55.33 ^a	46.67 ^a	66.67 ^a	26.67 ^a	55.33 ^a

Means with the same superscript within the same raw are not statistically different (p<0.05)

documented by Iskandar et al. (2018) as 4.7g of mutiara catfish after working on the growth performance of F1 transgenic mutiara catfish. El-Zaeem and Assem (2004) also documented lower weight gain of 13.27g on Tilapia zilli after injecting the fish with shark DNA at different doses. The variation in the weight gain reported from this study might be due to the Bagrus bayad and Shark DNA. Higher final length was revealed in fingerlings administered with 10µl genomic DNA from Bagrus bayad as 2188.7mm followed by fingerlings administered with 20, 5 and 0µl of genomic DNA from Bagrus bayad as 2116.0, 1586.7 and 1496.7mm respectively. The least value of the final length was found in fingerlings administered with 15µl of genomic DNA as 987.33mm. The fingerlings administered with 0, 5, 10, 15, and 20µl did no exhibited any significant differences (p>0.05) to one another. The value of the final length gotten from this research was higher than the value presented by Idowu and Afolayon (2013) as 10.80cm on Clarias gariepinus fed 50% of maggot meal as a supplement to fish meal. The variation in the final length could be as a result of differences in the period of the experiment and feed given. Higher specific growth rate of 1.53 was observed in fingerlings administered with 10µl of Bagrus bayad genomic DNA followed by fingerlings administered with 20, 0, and 5µl of genomic DNA as 1.47, 1.33 and 1.33 respectively. The least value of the specific growth rate was discovered in fingerlings injected with 15µl of genomic DNA from Bagrus bayad as 1.30. The fingerlings administered with 0, 5, 10, 15 and 20µl did not show any significant variation (p>0.05) between the entire treatments. Abdel-Hamid et al. (2000) reported lower value of SGR for carp to be 0.98% per day when fed with maize sativa diets. Variation in the SGR recorded is due to culture season. Higher feed conversion ratio of 2.09 was found in fingerlings injected with 0µ1 of Bagrus

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bayad genomic DNA. Fingerlings injected 15, 5, and 20µl of genomic DNA show the values of feed conversion ratio in the sequences of 1.27, 1.03 and 0.65 respectively. The least value of the feed conversion ratio was obtained in fingerlings injected with 10µl of genomic DNA as 0.64. There was no any significant differences (p>0.05) observed throughout the entire treatment in respect of the feed conversion ratio. The feed conversion ratio observed from this study was lower than the value obtained by Olude et al. (2008) as 2.09 for Clarias gariepinus. Differences in FCR from the two researches could be due to the effects of the experimental materials used. Higher condition factor of 1.22 was discovered in fingerlings administered with 20µl of Bagrus bayad genomic DNA followed by fingerlings administered with 15, 10 and 5ul of genomic DNA as 0.96, 0.82 and 0.82 respectively. Least value of the condition factor was revealed in fingerlings administered with 0µl of genomic DNA from Bagrus bayad as 0.54. The fingerlings injected with 5 and 10µl of the genomic DNA did not indicated any significant variation (p>0.05) between them. However, fingerlings administered with 5 and 10 differs statistical (p<0.05) with those fingerlings administered with 0, 5, 10, 15 and 20µ1 from Bagrus bayad genomic DNA. The condition factor of 1.81 was reported by Opiyo et al. (2014) which was higher than the condition factor gotten from the present research. The variation in the condition factors of the researches is due to the seasons of the research as well as the water quality of the pond management. Higher percentage survival 66.67% was observed in fingerlings injected with 10µl of Bagrus bayad genomic DNA followed by fingerlings injected with 0, 20 and 5µl of genomic DNA as 55.33, 55.33 and 46.67% respectively. The least value of the percentage survival was found in fingerlings injected with 15µl of genomic DNA as 26.67%. The fingerlings injected with 0, 5, 10, 15 and 20µl did not show any significant variation (p>0.05) to one another. Sarka et al. (2004) reported higher value of percentage survival as 90% in Indian carp. Madu and Ita (1990) also reported higher value of the percentage survival of 78.8% in Clarias anguilaris. The variation on the percentage survival might be due the stocking density and proper management of the fish during the experiment.

4.0 CONCLUSION

Based on the result of this study, the growth of *Clarias garepinus* was better in fingerlings injected with 20 and 10µl of genomic DNA from *Bagrus bayad* in term of final weight, weight

gain, final length and percentage survival. This indicates that, the growth and survival of *Clarias gariepinus* fingerlings can be improved by injecting the fish at 10 and 20µl of the genomic DNA from *Bagrus bayad*.

5.0 RECOMMENDATION

It is recommended that, genomic DNA from *Bagrus bayad* should be injected to *Clarias gariepinus* fingerlings in other to harvest their fish in shorter time. Further studies should be carried out on other species of fish to test their genomic DNA on the growth and survival of African catfish fingerlings.

REFERENCES

Abdel-Hamid, M.S.E., Hafiz, M.K., Saneyeldin, M.S., Mohammed, N.B and Ahmed, Z.I.H. (2000). Growth performance of grass carp (*Cternopharyngodon idella*) in earthen ponds as affected by receiving varying feeding inputs. *Egyptian Journal of Applied Science*, 4(8): 125-146.

- Abdullahi S.A, Abolude D.S and Ega R.A. (2001) Nutrient quality for four oven dried freshwater catfish in Nothern Nigeria. *Journal Tropical Bioscience*. 70pp.
- Adebayo-Tayo, B.C, Onilude A.A and Patrick U.G. (2008). Mycofloral of smoked-dried fishes sold in Oyo, Eastern Nigeria. *World Journal of Agricultural Sciences*, 4(3): 346-350.
- Adekoya, B.B. and Miller, J.W. (2004). Fish cage culture potential in Nigeria. *An overview National cultures, Agriculture Focus*, 1(5):10-13.
- Ahmed, M. H., Shalaby, A. M. E, Khattab, Y. A. E and Abdel-Tawwab, M. (2012). Effects of 17a-methyltestosterone on growth performance and some physiological change of Nile tilapia fingerlings (*Oreochromis niloticus*). Egypt, *International Journal of Aquaculture and Biology*, 4(4): 295-311.
- Amiengheme P. (2005). The importance of fish in human nutrition. A paper delivered at fish culture forum, Federal Department of fish Farmers, Abuja. 21pp. Ataguba, G.A, P.A.
- Ayoola, O., Akinwole, O. T. and Fredric, C. A. (2012). Effect of the shape of culture tanks on production of catfish *Clarias gariepinus*. *Journal of Agriculture and Scientific Research*.12(1): 1-8.
- Busacker, G. P., Adelman, I. R. and Goolish, E. M. (1990). Growth method for fish Biology. *American Fisheries Society*, *U.S.A*, 363-387.
- Dunham R. A. Majumdar. K. Hallerman, E. and Main, G. (2001). Review of the status of aquaculture genetics. In K.R.P. Subasinghe, P. Buemo, M.J. Philipa; C. Haugh; S.E. and J.R. Arhur (eds). *Aquaculture in the third millennium*. *Proceedings of the conference on Aquaculture in the third millennium*, Bangkok, Thailand, 20-25 February, NACA, Bangkok and FAO, Rome, 137-186.
- El-Zaeem, S.Y and Assem, S.S. (2004). Application of Biotechnology in fish breeding: production of highly immune genetically modified Nile Tilapia, *Oreochromis niloticus* with accelerated growth by direct injection of shark DNA into skeletal muscles. *Egypt. Journal of Aquatic Biology Fish*eries, 8(3): 67-92.
- FAO (2007). *The State of World Fisheries and Aquaculture*. FAO Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations Rome. 5 22.
- Froese, R. and Pauly, D (2007). "Bagrus bayad" in FishBase. Aug 2007 version.
- Hershberger, W.K., Meyers, J.M., McAuley, W.C. and Saxton, A.M. (1990). Genetic changes in growth of Coho salmon (*Oncorhynchus kisutch*) in marine netpens, produced by ten years of selection. *Aquaculture*, 85: 187-197.
- Holden, M. and Reed, W. (1972). West African Fresh Water Fish London, Longman Group.
- Idowu, R.T. and Afolayon, N. (2013). The physic-chemical parameters of African Ari Zone Man Lake, 1(2): 113-119.
- Iskandar., Buwono. I D.and Mochamad., U. K. A (2018). The growth performance of F1 transgenic mutiara catfish. *Conference Series Earth and Environment Science*.137 (1): 012004
- Madu, C. T. and Ita, E.O. (1990). Comparative Growth and Survival of Hatchlings of *Clarias* sp., *Clarias* hybrid and *Heterobranchus* sp. in the Indoor Hatchery. National Institute for *Fresh water Fisheries Research Annual Report*. New Bussa.pp. 47-50.
- Mohammed, Z. B., Diyaware. M. Y., Raji. O A and Aliyu, M (2016). Evaluation of Camel Testicles for Masculinisation, Growth and Survival of Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1758). Fisheries Society of Nigeria (FISON). 117-123.

- Moses Y. Olufeagba S. O. and Rapheal, A. Z. (2006). Intra-specific hybridization in two strains of *Clarias gariepinus* (Limnaeus, 1758). *Genetics society of Nigeria 30th Annual National Conference*, Nsukka, 5th-8th September, 153-156.
- Olude, O.O., Alegbeleye, W.O.A. and Obasa, S.O. (2008). The use of soaked copra meal as a partial substitute for soybean meal in the diet of Nile tilapia (*Oreochrormis niloticus*) fingerlings. *Livestock Research for Rural Development*, 20 (10): 165-169.
- Omeji, S. Obande, R. A. and Oyaje, J. (2013). Intra-specific hybridization of local and exotic *Clarias gariepinus. International Journal of Modern Biological Research*, 1:34-41.
- Opiyo, M.A., Githukia, C.M., Munguti, J.M. and Charo-karisa, H. (2014). Growth performance carcass composition and profitability of Nile tilapia (*Oreochromis niloticus*) fed commercial and on-farm made fish feed in earthen ponds. *International Journal of Fisheries and Aquatic Studies*, 1(5): 12-17.
- Perry, and Robert P. (1976)."Processing of RNA". Annual Review of Biochemistry, **45**: 605–630. doi:10.1146/annurev.bi.45.070176.003133.
- PTN (2015). Premium time Nigeria.Com retrieved 20-8-2016.
- Reed, W. (1967). Fish and fisheries of Northern Nigeria Ministry of Agriculture, Northern Nigeria.45-49.
- Sarka, G. D., Scilipoti, A., Mazzola, A. and Modica. (2004). Effect of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gult of castellammare, sicily): *a multiple stable isotope study. Aquaculture*, 234:199-213.