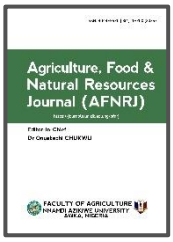
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Original Article

Genetic improvement of African catfish (*Clarias gariepinus*) using Nile perch (*Lates niloticus*) deoxyribonucleic acid (DNA)

OPEN ACCESS

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ABSTRACT

This study investigates the potential of genetic improvement in African catfish (Clarias gariepinus) through direct intramuscular injection of Nile perch (Lates niloticus) deoxyribonucleic acid (DNA). A total of 225 African catfish fingerlings were divided into five DNA concentrations of 0 µg (T1), 1 µg (T2), 2 µg (T3), 3 µg (T4), and 4 µg (T5) per fish, with each group replicated three times in a completely randomized design. The experiment was conducted in 15 plastic tanks, each holding 15 fish, and the fish were fed a commercial diet for 20 weeks. Data on growth performance were subjected to one-way analysis of variance. The length-weight relationship was analyzed using regression and correlation analysis. Results on growth performance indicated that fish injected with 4 µg of Nile perch DNA showed significantly better ($p \leq 0.05$) growth performance, including final body weight (114.59 g), mean weight gain (108.55g), percent weight gain (1802.84%), average daily weight gain (0.78 g/day), and survival rate (84.45%), compared to other groups. The indices of the length-weight relationship "b" values were 0.3564 for T1 (control), 1.2776 for T2 (1 µg), 0.2170 for T3 (2 µg), 0.3686 for T4 (3 µg), and 0.0434 for T5 (4 µg), exhibiting a negative allometric growth pattern ($b < 3$) across all treatment groups. The correlation coefficients (r) for T1, T2, T3, T4, and T5 were 0.630, 0.597, 0.818, 0.669 and 0.839, respectively. The findings suggest that Nile perch DNA injection can enhance growth parameters in African catfish. The study advocates for further research on optimal DNA dosing and long-term effects in aquaculture.

K E Y W O R D S : *Aquaculture, Direct intramuscular injection, DNA concentration, Growth performance, Gene*

INTRODUCTION

Fish is a crucial source of nutrition, providing essential animal protein, fatty acids, vitamins, and amino acids, especially for vulnerable populations like children, pregnant women, and those with cardiovascular conditions (FAO, 2016). However, challenges such as overfishing and habitat destruction threaten traditional capture fisheries (Barde *et al*., 2020), while aquaculture emerges as a sustainable solution to meet rising global demand. Aquaculture, the fastest-growing food production sector, now accounts for about half of global fish production (FAO, 2020).

The African catfish (*Clarias gariepinus*) is a key aquaculture species, with Nigeria being the largest global producer (FAO, 2016). Nile perch (*Lates niloticus*), native to Africa, is a

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significant freshwater species known for its large size and reproductive capacity (Asnake, 2018).

The development of genetically improved fish has been ongoing since the creation of the first genetically modified mammals. The first transgenic fish in aquaculture were goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*), and as of 2003, more than 35 species had been genetically engineered in research laboratories worldwide (Cebec *et al.*, 2020). Various fish species have been genetically engineered for academic and practical purposes, including enhancing traits related to growth, maturation, flesh quality, freezing tolerance, and disease resistance (El-Zaeem, 2012).

A foreign gene can be transferred into fish *in vivo* by introducing DNA either into embryos or directly into somatic tissues of adults. A commonly used method to introduce foreign DNA into embryos include microinjection, electroporation, sperm-mediated gene transfer and gonad-mediated gene transfer (El-Zaeem, 2012). Direct transfer of DNA into fish tissues is a simple method that produces quick results and eliminates the requirement for transgenic individual screening and germline carrier selection (El-Zaeem *et al.*, 2012a). Gene transfer and expression following intramuscular direct injection of foreign DNA into skeletal muscles of fish has been achieved by several studies and indicates a possible easy and rapid way for improving fish characteristics (El-Zaeem *et al.*, 2012b). Despite the significance of the African catfish as a commercial species in many countries, its production faces challenges due to long production cycles and increasing demand, impacting industry efficiency and sustainability (Awe, 2017). The Nile perch, the biggest and largest freshwater fish, known for its fast growth, diseases resistance, adaptability, meat quality and market acceptability, could serve as a valuable source of genetic material for enhancing the traits of African catfish through direct intramuscular DNA injection (Asnake, 2018). This study is therefore designed to produce a genetically improved African catfish with enhanced growth performance through direct intramuscular injection of Nile perch DNA.

MATERIALS AND METHOD

Experimental Fish

The experiment was conducted at the Fish Hatchery Complex of the Department of Fisheries and Aquaculture, Federal University Dutse, Jigawa State, Nigeria. Three hundred (300) African catfish fingerlings with an average total length of 9.51 ± 0.20 cm and weight of 6.18 ± 0.32 g were procured from a A4 global fisheries, Kano and conveyed to the study site in a 50-litre plastic container half filled with fresh water. The fish were acclimatized to the experimental condition in a plastic tank of 1,500 litre capacity for two weeks and fed with a commercial diet (Blue crown, 2mm) containing 45% crude protein at 5% body weight twice daily in two equal rations at 9.00 and 17.00 hrs.

Experimental Design

Two hundred and twenty-five (225) African catfish were randomly divided into five treatments, namely: T1 (0 µg, control), T2 (1 µg), T3 (2 µg), T4 (3 µg) and T5 (4 µg), and each treatment was replicated three times in a completely randomized design. Fifteen (15) plastic bowls measuring 100 liters capacity were used for the feeding experiment in a flow through system, with 15 fish per experimental unit.

Source of Foreign DNA

Two Nile perch with an average total length of 25.45 ± 2.19 cm and weight of 244.51 ± 62.21 g were obtained from the local fisherfolk at Dingare landing site of the River Hadejia. Liver tissues were promptly collected and preserved in 99% ethanol in a 100 ml sample collection tube to prevent DNA degradation and stored at -20°C before being process for DNA extraction. Following preservation, the tissue samples were transported to the molecular biology laboratory of the North East Zonal Biotechnology Centre of Excellence, University of Maiduguri, Borno State, for genomic DNA extraction.

DNA Extraction

High molecular weight DNA was extracted from the liver (El-Zaeem *et al.*, 2012a; El-Zaeem *et al.*, 2012b) of Nile perch according to the protocols of Promega (2023). The quantity and purity of the extracted DNA were measured using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, USA) at absorbance of 260/280 nm, while the quality was evaluated using 1% agarose gel electrophoresis (Invitrogen, USA). The DNA was stored at –20°C until ready for further analysis. The extracted DNA was further cleaved using EcoR1 type II restriction enzyme (New England Biolabs® Inc. R0101S) to obtain DNA fragments of different sizes, facilitating their integration into the host genome following the manufacturer's instructions. EcoRI recognizes and cuts DNA at the sequence 5'-GAATTC-3', producing sticky ends with overhanging single-stranded DNA. Agarose gel electrophoresis was conducted following the protocol outlined by Williams *et al.* (1993) to confirm the restriction enzyme digestion of the genomic DNA. Digested DNA were evaluated on a 2% agarose gel (Invitrogen, USA) and stained with 10 µl of ethidium bromide. DNA fragment lengths were compared with a 100 bp DNA marker. The amplified pattern was visualized under UV transilluminator and photographed by gel documentation system (InGenius 3 GENE *Syc*).

Injection of Foreign DNA *in vivo*

The digested DNA obtained from Nile perch was diluted using 0.1x saline-sodium citrate (SSC) buffer into five different concentrations (0 µg, 1 µg, 2 µg, 3 µg and 4 µg/0.1ml/fingerlings) and injected into the target host *in vivo* using a hypodermic needle (disposable insulin syringe, 1 ml). The injection was administered at an angle of 45° intramuscularly at the inception of the fin, just above the lateral line (Woynarovich and Horvath, 1980).

Experimental Management

The fish were fed commercial diet (Blue crown, 2 mm) containing 45% crude protein at 5% body weight daily twice daily in two equal rations at 9.00 and 17.00 hrs for a total culture period of twenty (20) weeks (140 days). The quantity of feed was adjusted biweekly based on the new weight of fish. Remains of feed were siphoned out of the culture medium and same quantity of water removed during siphoning was replaced immediately on a daily basis. Total renewal of whole water was done every three days, and the bowls bottoms were scrubbed to remove dirt from the medium. Dissolved oxygen, pH and temperature were recorded weekly using dissolved oxygen meter (Hanna H19146) and pH/temperature pen type (Comboly Hanna H198130), respectively throughout the study period.

Measurement of Growth Parameters

The length and weight of each fish from each experimental unit were measured at the commencement and end of the experiment using top loading sensitive digital scale (Model: SF - 400) and fish measuring board, respectively. The following growth parameters were estimated for each of the treatment as follows:

i) Mean Weight Gain (MGW) was determined as described by Busacker *et al*. (1990) as:

$$(MWG)\ (g) = W_2 - W_1 \qquad (1)$$

Where W_2 = final mean weight (g), W_1 = initial mean weight (g)

ii) Percent Weight Gain (%WG) was calculated according to Wannigamma *et al*. (1985) as:

$$(\%WG) = (W_2 - W_1)/W_1 \times 100 \qquad (2)$$

Where W_2 = final mean weight (g), W_1 = initial mean weight (g)

iii) Average Daily Weight Gain (ADWG) was carried out in accordance with the procedure of Bagenal (1978) as:

$$(ADWG)(g/day) = MWG/T \qquad (3)$$

Where T= culture period (days)

iv) Specific Growth Rate (SGR) was carried out according to Brown (1957) as:

$$(SGR)(\%/day) = [(logW_2 - logW_1) \times 100]/T \qquad (4)$$

Where $LogW_2$ = logarithm of final mean weight, LogW1 = logarithm of initial mean weight, T= culture period (days)

v) Condition Factor (K) was determined according to Brown (1957) as:

$$(K) = (W_2 \times 100)/{L_2}^3 \qquad (5)$$

Where W_2 = final mean weight (g), L_2 = Average total length (cm)

vi) Percentage Survival (%S) was calculated as described by Bagenal (1978) as:

$$(\%S) = (S_2/S_1) \times 100 \qquad (6)$$

Where S_2 = Final number of fish, S_1 = Initial number of fish

Length-Weight Relationship

The length-weight relationship LWR was estimated by using the equation provided by Le Cren (1951) as:

$$W = aLb \qquad (7)$$

where W = Weight of fish (g), L= Total length of fish (cm), a = Regression constant or intercept, b = slope, or an exponent indicating isometric growth when equal to 3.0; negative allometry at < 3, and positive allometry at > 3.

The parameters "a" and "b" were estimated by linear regression on the log transformed equation according to Ricker (2011):

$$\log W = a + b \log L. \qquad (8)$$

Statistical Analysis

Data obtained on growth parameters were subjected to one - way analysis of variance (ANOVA) and the mean significant differences among the different groups were compared using Duncan multiple range test ($p < 0.05$). Length-weight relationship was analyzed using regression analysis. ANOVA and length-weight relationship were performed using IBM SPSS Statistics version 27.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) and Microsoft excel, respectively.

RESULTS

Growth Performance of African Catfish injected with Varying Concentrations of Nile Perch DNA

The growth performance of African catfish injected with varying concentrations of Nile perch DNA is presented in Figure 1-2. At the onset of the experiment, there were no significant differences ($p > 0.05$) in the initial body weight (IBW) and initial body length (IBL) of African catfish among the treatment groups. Fish injected with 4 µg (T5) of DNA exhibited the highest mean final body weight of 114.59 g, mean weight gain of 108.55 g, percent weight gain of 1802.84%, average daily weight gain of 0.78 g/day, and a percentage survival rate of 84.45%, indicating significant improvement ($p \leq 0.05$) compared to the other injected groups and the control. Additionally, the highest specific growth rate of 1.03%/day was recorded in the fish injected with 4 µg of DNA, although this did not significantly differ ($p > 0.05$) from the results of fish injected with other doses or the control group. The control and 1 µg (T1) group exhibited the highest condition factors of 1.04, which were significantly ($p \leq 0.05$) greater than those of the other injected groups. Furthermore, the final body length of 23.57 cm was significantly ($p \leq 0.05$) increased in fish injected

with 4 µg of DNA, showing a higher mean compared to the other injected and control groups.

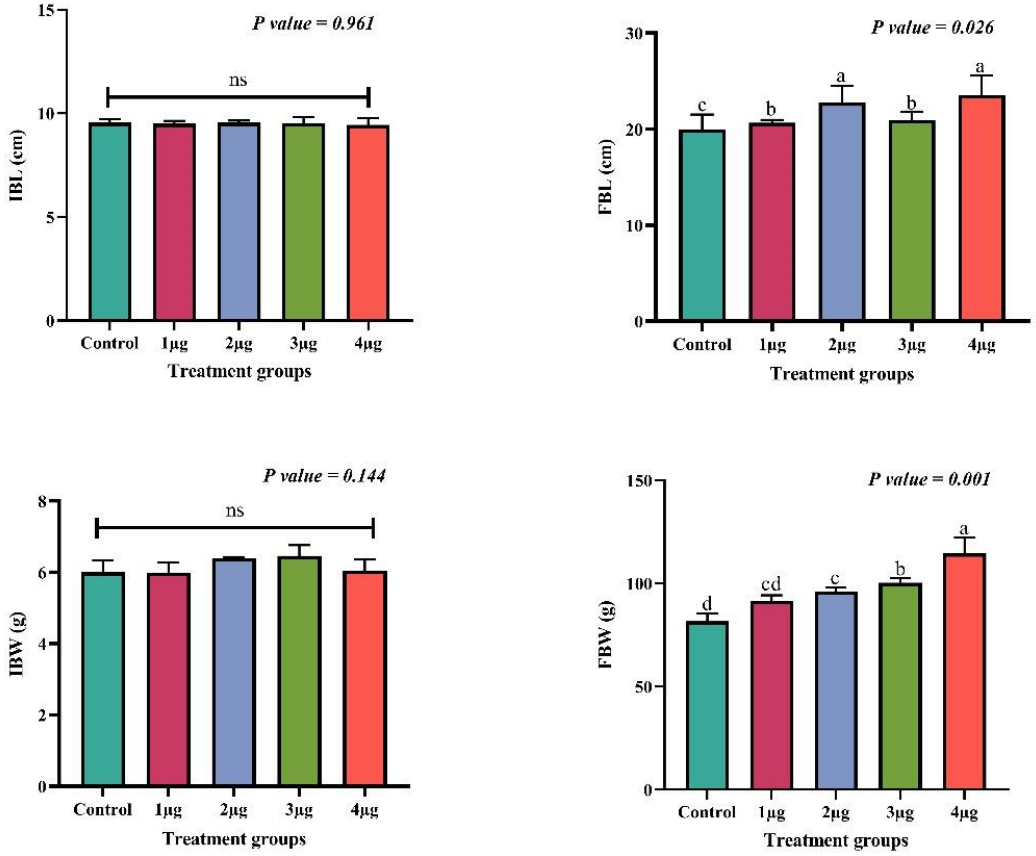


Figure 1: The growth performance of African catfish injected with varying concentrations of Nile perch DNA
IBL= Initial body length, IBW= Initial body weight, FBL= Final body length, FBW= Final body weight.

Length-weight Relationship of African Catfish injected with Varying Concentrations of Nile Perch DNA

The length-weight relationship of African catfish injected with varying concentrations of Nile perch DNA is shown in Table 1. The "b" values were 0.3564 for T1 (control), 1.2776 for T2 (1 µg), 0.2170 for T3 (2 µg), 0.3686 for T4 (3 µg), and 0.0434 for T5 (4 µg). The results indicated that African catfish exhibited a negative allometric growth pattern ($b < 3$) across all treatment groups. The indices of the length-weight relationship (LWR) in the treatments revealed 'a' value of 1.4483 in T1, 0.2810 in T2, 1.1688 in T3, 2.4884 in T4, and 2.1180 in T5. The regression coefficients (R^2) for T1, T2, T3, T4, and T5 were 0.3625, 0.3539, 0.6862, 0.4532, and 0.0029, respectively. Consequently, African catfish follow the cube law, demonstrated a positive correlation between total length and body weight, with "r" values of 0.630 for T1, 0.597 for T2, 0.818 for T3, 0.669 for T4 and 0.839 for T5.

Table 1: Length-weight Relationship of African Catfish Injected with Varying Concentrations of Nile Perch DNA

Treatment	b	a	R^2	Growth Pattern	r
T1 (Control)	0.3564	1.4483	0.3625	NA	0.630
T2 (1 µg)	1.2776	0.2810	0.3539	NA	0.597
T3 (2 µg)	0.2170	1.1688	0.6862	NA	0.818
T4 (3 µg)	0.3686	2.4884	0.4532	NA	0.669
T5 (4 µg)	0.0434	2.1180	0.0029	NA	0.839

Key: b=slope, a=intercept, R^2=Regression coefficient, NA=Negative allometric, and r=correlation coefficient

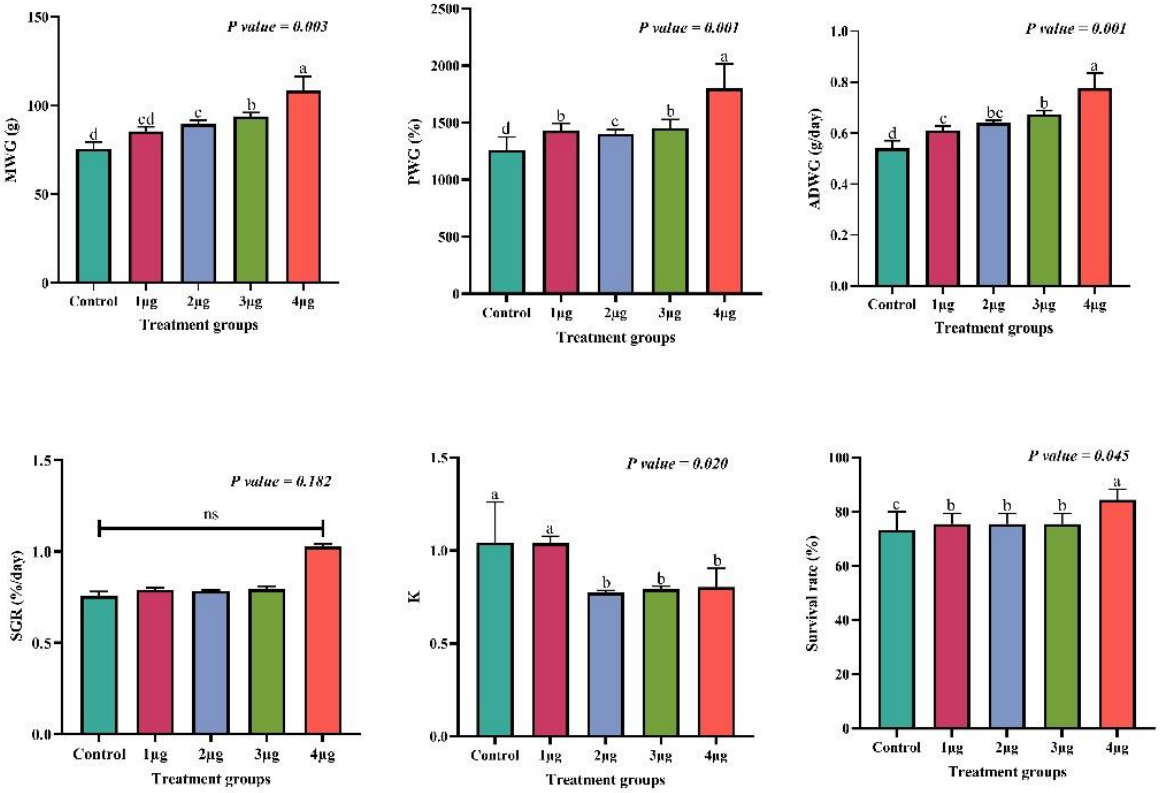


Figure 2: The growth performance of African catfish injected with varying concentrations of Nile perch DNA

MWG= Mean weight gain, %WG= Percent weight gain, ADWG= Average daily weight gain, SGR, Specific growth rate, K= Condition factor, %S= Percentage survival

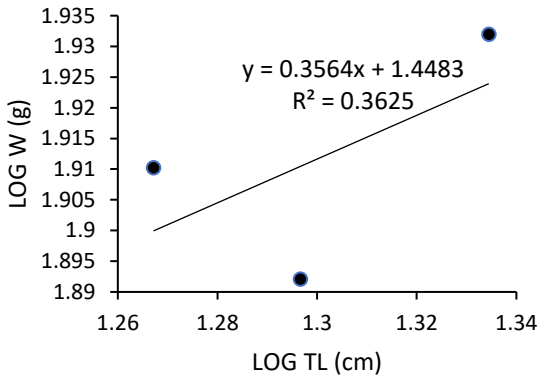


Figure 3: Linear Regression of Total Length and Weight of African Catfish Injected with 0 µg (control) of Nile Perch DNA

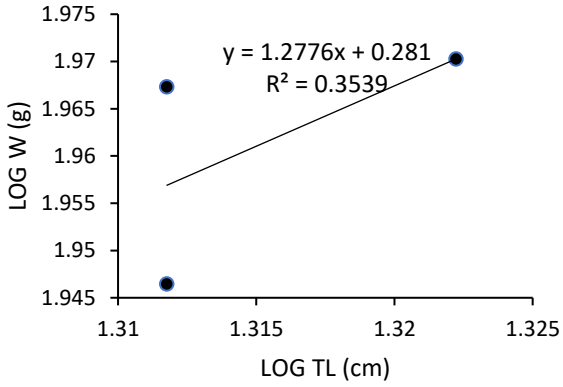


Figure 4: Linear Regression of Total Length and Weight of African Catfish Injected with 1 µg (T2) of Nile Perch DNA

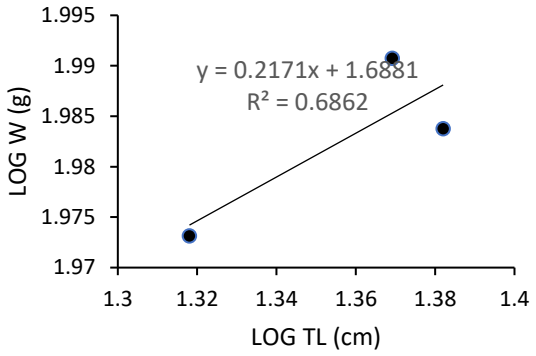


Figure 5: Linear Regression of Total Length and Weight of African Catfish Injected with 2 µg (T3) of Nile Perch DNA

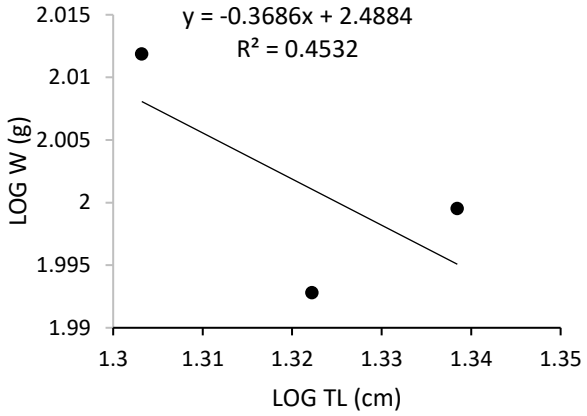


Figure 6: Linear Regression of Total Length and Weight of African Catfish Injected with 3 µg (T4) of Nile Perch DNA

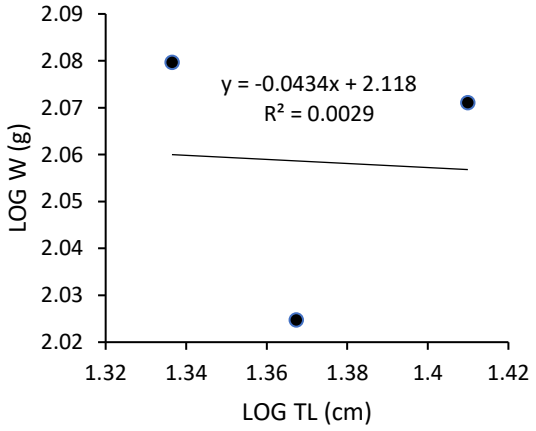


Figure 7: Linear Regression of Total Length and Weight of African Catfish Injected with 4 µg (T5) of Nile Perch DNA

DISCUSSION

Growth Performance of African Catfish injected with Varying Concentrations of Nile Perch DNA

The results revealed that DNA injection significantly affected the growth parameters and overall performance of the fish. Fish injected with 4 µg of Nile perch DNA exhibited the highest Mean Final Body Weight (FBW), Mean Weight Gain (MWG), Percent Weight Gain (%WG), Average Daily Weight Gain (ADWG), and Percentage Survival (%S), showing significant improvement ($p \leq 0.05$) compared to other injected groups and the control. This aligns with previous studies indicating that DNA injection can enhance growth performance. Assem & El-Zaeem (2005) observed significant weight gains in *Tilapia zilli* injected with shark DNA, and Barde *et al.* (2020) reported substantial final weights in African catfish fingerlings administered with genomic DNA from *Bagrus bayad*.

The highest Specific Growth Rate (SGR, %/day) was recorded in fish injected with 4 µg of DNA, although this did not significantly differ from fish injected with other doses or the control group. This finding is consistent with that of Abdel-Hamid *et al.* (2000), who reported an SGR of 0.98% per day for carp fed maize sativa diets, and El-Zaeem (2012), who documented an SGR of 1.32% per day in grey mullet injected with catfish DNA.

The Final Body Length (FBL) was also significantly ($P \leq 0.05$) increased in fish injected with 4 µg of DNA, showing a higher mean compared to other injected and control groups. This result supports the findings of Barde *et al.* (2020), who observed a significant increase in the final length of catfish fingerlings injected with genomic DNA from *Bagrus bayad*.

The control group had the highest Condition Factor (K) (1.04), which was significantly ($p \leq 0.05$) greater than that of the injected groups. This could suggest that while DNA injection promotes growth, it might also impact the overall condition of the fish. Assem & El-Zaeem (2005) reported a condition factor of 2.00 in *Tilapia zilli* injected with 40 µg of shark DNA, highlighting that different DNA sources and dosages might yield varying impacts on fish condition factors.

The Percentage Survival (%S) was significantly higher in fish injected with 4 µg of DNA, indicating that DNA injection not only promotes growth but also enhances survival rates. This finding is corroborated by Assem & El-Zaeem (2005) who reported 100% survival in *Tilapia zilli* injected with shark DNA, suggesting that DNA injections can be safe and effective for improving fish survival. However, this contrasts with Barde *et al*. (2020), who reported higher survival in the control group.

Length-weight Relationship of African Catfish injected with Varying Concentrations of Nile Perch DNA

The length-weight relationship (LWR) is a key indicator for assessing the growth patterns of fish population. The results of this study indicate that all treatment groups of African catfish

exhibited negative allometric growth (b < 3). The positive correlation coefficients (R^2) demonstrate a strong relationship between length and weight in these fish. Our findings are consistent with Shittu and Oguntoye (2020) who reported b values of 1.63 and 2.74 for *C. gariepinus* reared in ponds in Ilaro, Ogun State, confirming a negative allometric growth pattern. Similarly, Banikannda *et al.* (2023) found b values of 0.18, 0.35, and 0.42 in populations from Adjohoun, Dangbo, and Porto-Novo in the Oueme Valley, Benin Republic, all indicating negative allometric growth supported by high R^2 values. Sadauki *et al.* (2023) reported b values of 2.80 for males, 2.89 for females, and 2.85 for both sexes combined from the Zobe reservoir in Katsina State, further confirming this pattern. These findings were echoed by Saduaki *et al.* (2024) in *C. gariepinus* from the Ajiwa Reservoir, Katsina State, revealing 'b' values of 2.81 for males, 2.93 for females, and 2.85 for both sexes combined, indicating a negative allometric growth pattern. This suggests that African catfish adhere to the cube law, showing a positive correlation between total length and body weight, with an "r" value greater than 0.50 for all African catfish studied.

In contrast, some studies have reported positive allometric growth in *C. gariepinus*. Davies *et al.* (2013) reported b values of 7.74 for males, 6.96 for females, and 7.87 for both sexes combined, indicating positive allometric growth, which is significantly higher than the values observed in our study. Keyombe *et al.* (2015) noted similar results in Lake Baringo.

CONCLUSION AND RECOMMENDATION

This study concludes that the growth and survival of African catfish fingerlings can be enhanced by injecting the fish with 4 µg of Nile perch DNA. Also, the growth pattern (length-weight relationship) of African catfish fingerlings did not change after the injection of Nile perch DNA.

Based on the results of this study, the following recommendations are proffered:

1. Direct intramuscular injection of Nile perch DNA should be utilized to enhance the growth and overall performance of African catfish.

2. Further studies should be carried out on optimal dosing using higher concentrations of Nile perch DNA and long-term effects in aquaculture.

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Authors' Contribution

Author MAH conceived the idea, provided material support and reviewed the manuscript. IBS developed the methodology, conducted literature searches, and reviewed the manuscript. UBZ managed data collection, data analysis and interpretation, and writing the initial draft of the manuscript. All authors read and approved the final manuscript.

Ethical Approval

The biosafety and bioethics committee of Federal University Dutse have duly observed and approved all the experimental procedures used in this study. The approval was based on the approved scientific research protocols, and all relevant local and/or international animal welfare laws, guidelines, and policies for the care and use of animals.

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