ABSTRACT

The Nile tilapia, *Oreochromis niloticus*, is a widely cultivated fish throughout the world. The fish is particularly important for aquaculture in Nigeria due to its popularity. The objectives of this study are to investigate the growth performance, proximate composition, and DNA fingerprint and genetic variation of *O. niloticus* containing foreign fish DNA. A study was conducted to enhance the growth performance of the fish by transferring purified DNA into the fish's somatic tissue. Different concentrations of DNA (0, 10, 20, 30, and 40 µg/µl) extracted from the Nile perch (*Lates niloticus*) were injected into the somatic tissue of the tilapia fingerlings. The injected fish were then raised for 120 days in a polyethylene mobile fish pond (2.5 x 1.5 x 1.2 deep). The results show that Fingerlings injected with 40 µg/µl have significantly ($P<0.05$) better final weight, weight gain, average daily weight gain, specific growth rate, crude protein, ether extract, and metabolizable energy. Gel electrophoresis revealed distinct banding patterns, suggesting differential integration or expression of *L. nioloticus* DNA fragments among the treated fish. The number of effective alleles (Ne), Shannon's Information Index (I), Expected Heterozygosity (He), and Unbiased Expected Heterozygosity (uHe) were observed to be high in fish containing a higher concentration of the *L. niloticus* DNA. RAPD analysis indicated that each fish exhibited unique banding patterns, indicating complete genetic variation among the treatments. This study revealed that fragmented DNA materials from *Lates niloticus* have the potential to improve growth in *Oreochromis niloticus*. Fragment of *L. niloticus* DNA moderately integrated into the *O. niloticus* genome. The DNA of *L. niloticus* has significantly altered the proximate composition of Nile tilapia, especially by enhancing protein content and metabolizable energy. These changes could offer nutritional, economic, and production benefits in Tilapia aquaculture, though further investigation into safety, long-term performance, and consumer acceptance is essential.

Keywords: Nile Perch, DNA, Somatic cell, Enhancement, Fish, Aquaculture

INTRODUCTION

Nile tilapia, *Oreochromis niloticus*, is one of the world's most widely cultured fish species, particularly in Africa, due to its high adaptability, rapid growth, and ability to thrive in diverse aquatic environments. According to FAO (2025), global Tilapia production reached about 6.2 million tonnes in 2023, making it the second most farmed freshwater fish after carp, while Roderick (2025) reported that the Rabobank Global Seafood Specialist, Nikolik, estimated global Tilapia production will surpass 7 million metric tonnes in 2024. China remains the largest global producer, followed by Indonesia, while Egypt leads Africa, contributing nearly 1.5 million tonnes (60% of the continent's total production), followed by Nigeria with about 300,000 tonnes and

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Wha to do: Revise title to reflect the actual intervention ("Somatic Injection of Nile Perch Genomic DNA into Nile Tilapia Fingerlings"

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Commented [H3]: **Conflation of Somatic Injection with Genetic Transformation, you repeatedly refere to this as "DNA transfer," "transgenesis," and "genetic modification" without evidence that the injected DNA crossed cell membranes, entered nuclei, or integrated into chromosomes.**
You need to know here that naked DNA Intramuscular injection typically results in:
1.Degradation by nucleases in the tissue
2.Uptake by a small fraction of cells (if any)
3.No germline transmission

What to do:
1.Reframe the study as investigating "somatic DNA injection effects" rather than transgenesis
2.Remove claims of "genetic transformation" or "genome integration" unless substantiated with molecular evidence
3.Discuss the difference between transient DNA presence and genomic integration in the body of the manuscript

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Commented [H5]: These were not dealt with in your methodology and no result to back up your claims here , please remove

Commented [H6]: Numerical inconsistencies exist between abstract and tables (e.g., growth metrics). No confidence intervals or exact p-values for key claims.

What to do: Rewrite the abstract with precise methods, verified molecular outcomes (or note their absence), and tempered claims. Include 95 % CIs and effect sizes.

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Several references are outdated (1990s–early 2000s). More recent advances in fish transgenesis and CRISPR/Cas9 applications should be cited to situate the study in current research.

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other sub-Saharan countries like Ghana, Uganda, and Kenya (FAO 2025). Africa's estimated Tilapia production, therefore, is about 2.0–2.5 million tonnes (FAO, 2025). According to Steffens (2024), the Nile Tilapia plays an important global role in supporting food security, provides affordable, high-quality protein, helping combat malnutrition in developing countries, and provides income for smallholder farmers and fishers, supporting rural economies (Wasso *et al.*, 2024). Because of its affordability, the Nile Tilapia is sometimes considered a food for the poor, or the fish for the masses (El-Sayed, 2020).

Nile tilapia, *Oreochromis niloticus*, remains a key species in global aquaculture, but its growth performance has declined in many farmed populations due to genetic stagnation and inbreeding. Traditional selective breeding has been done with limited success in overcoming these challenges. As demand for faster-growing, more efficient fish increases, there is a growing need for advanced techniques like DNA transfer to enhance growth and other economic traits. However, the potential of DNA transfer in improving the growth performance of Nile tilapia is still underexplored, particularly about how it may influence other traits such as growth and nutritional composition. This creates a vital research gap that warrants investigation. The genus *Lates* belongs to the family Latidae and is of the order Perciform, comprising eleven (11) species that occur in fresh and brackish water. The Nile Perch, *Lates niloticus*, belongs to the family Latidae of order Perciformes, and is a freshwater species indigenous to African rivers and lakes, including the Nile, Chad, Senegal, Niger, and Congo River basins. The fish is one of the largest (sixth largest) freshwater fish Kaufman (1992) could serves as valuable genetic materials Haruna *et al.* (2025) for enhancing growth and other useful traits in fish, taking advantage of its size (growth vigour).

Many approaches can be used to improve the growth of the fish. One of the methods can be done through transgenesis (gene/DNA transfer). The most common method used to date is the microinjection of DNA/genes into the pronuclei of zygotes of fish Shakweer *et al.* (2023). DNA/gene can be manually injected into the skeletal muscles of fish (Wolff *et al.*, 1990). According to Sudha *et al.* (2001), a foreign gene can be transferred into fish *in vivo* by introducing DNA into embryos or directly into the somatic tissues of young or adult fish. Assem & El-Zaeem (2005) opined that the direct insertion of DNA into fish muscle is a simple approach that provides faster results and can eliminate the need for screening transgenic individuals and selecting germline carriers. Gene transfer and expression through intramuscular injection of foreign DNA into the skeletal muscles of fish has been achieved by Hansen *et al.* (1991), Rahman & Maclean (1992), Anderson *et al.* (1996), Tan & Chan (1997), Xu *et al.* (1999). El-Zaeem (2004), El-Zaeem & Assem (2004), and Hemeida *et al.* (2004). Moreover, Sudha *et al.* (2001) stated that the expression of muscular injection of DNA was evident in several nonmuscle tissues, such as skin epithelia, pigment cells, blood vessel cells, and neuron-like cells.

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Several genes have now been introduced into various fish species to enhance growth, resistance to disease, tolerance to freezing, etc (Shears *et al*., 1991; Chatakondi *et al*., 1995; El-Zaeem, 2001, 2004; Dunham *et al*., 2002; El-Zaeem & Assem, 2004). According to Dunham (2011), DNA transfer techniques have shown promising results in improving the growth performance of various fish species, including Nile Tilapia. In a study by El-Zaeem & Assem (2004), it was found that injecting shark DNA into the skeletal muscles of *Oreochromis niloticus* resulted in accelerated growth and improved body composition. Another study by El-Zaeem *et al*. (2011) observed higher body weight, feed utilization, and specific growth rate in *Oreochromis niloticus* injected with Seabream and *Artemia salina* DNA to enhance salt tolerance. According to El-Zaeem *et al*. (2012a), growth performance and body composition in red Tilapia injected with shark DNA were superior compared to non-injected *Coptodon* (*Tilapia*) *zilli*. El-Zaeem (2012b) reported improvements in growth performance and body composition of grey mullet fingerlings injected with shark DNA. Additionally, Martinez *et al.* (2000) reported a 290% food conversion efficiency in transgenic tilapia compared to the control group. Genetically modified *O. niloticus* showed more than 80% weight increase compared to non-modified strains (El-Zaeem, 2011). Rahman (2001) reported a mean mass of 653 g in *O. niloticus* containing an exogenous fish growth hormone gene after 7 months of culture, representing a 2.5-fold increase in growth compared with non-transgenic siblings. Rahman *et al*. (2013) reported significant improvements in growth rates by 30%, feed conversion efficiency, and overall biomass production in *O. niloticus* produced through gene transfer.

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Genetically modified or transgenic Nile tilapia (*Oreochromis niloticus*) has been developed to enhance growth rates, disease resistance, and feed efficiency. Studies indicate that genetic modifications can influence the proximate composition of the fish, including moisture, protein, lipid, and ash content. Studies comparing transgenic and non-transgenic tilapia suggest that transgenic variants may exhibit higher protein content and altered lipid profiles, potentially due to enhanced metabolic activity. Several authors reported the proximate composition of Nile Tilapia: Job *et al*. (2015), Olopade *et al*. (2016), and Otene *et al*. (2024). The proximate composition of genetically modified Tilapia has been documented by El-Hawarry (2012), Kumara *et al*. (2020), Suwannatrai *et al*. (2023), and El-Zaeem *et al*. (2023).

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Random Amplified Polymorphic DNA (RAPD) has been successfully used to differentiate species and subspecies of tilapia, such as *Oreochromis niloticus*, using polymorphic banding patterns generated by random primers (Bardakci & Skibinski, 1994). RAPD is a powerful molecular tool used to generate DNA fingerprints for fish species. It works by amplifying random segments of genomic DNA using short primers, producing unique banding patterns that reflect genetic variation (Dinesh *et al*., 1993; Bardakci & Skibinski, 1994; Ali *et al*., 2004). RAPD serves as both forward and reverse primers, and is usually able to amplify fragments from 1–10 genomic sites simultaneously. They are quick and easy to assay, require a low quantity of DNA with no sequence data for primer construction (Welsh & McCelland, 1990).

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Microinjection of Genomic DNA (gDNA) into another fish could affect the proximate composition or nutritional profile of the recipient. Proximate analysis of fish helps determine the nutritional profile of the fish and is one of the major indicators of fish quality for human consumption (Jannatun *et al*., 2023). Determining the proximate composition of genetically modified fish ensures that the fish meat meets food high standards Otene *et al*., 2024) and ensures that genetic modification does not negatively impact the nutritional profile, making it a healthy protein source.

The constraints for the development and growth of Tilapia farming in Nigeria are slow growth, early maturity, and high prolificacy. The high prolificacy leads to overpopulating the pond, resulting in stunted fish and a poor market. The improvement of the growth of Tilapia via selective breeding takes a longer period (5-10 years). The use of synthetic hormones to control reproduction (sex reversal) to obtain fast-growing all-male Tilapia has been successful. However, the technology is cumbersome, coupled with global concern about the effect of this steroid (hormone) in fish flesh on humans and the environment. The knowledge of the fate of residuals of the steroids in fish is still low, Mlalila *et al*. (2015), or unclear. The use of hormones, especially 17α-Methyltestosterone in Nile tilapia farming, has been of concern to consumers as it involves potential risks to human health and environmental contamination by its residue Hoga *et al*. (2018) as cited by Karaket *et al*. (2023). Additionally, the effects of the hormone and its residue on the vital organs of man are also uncertain. Natural hormonal sex reversal to produce all males Tilapia as an improvement in the growth of Tilapia has been attempted by several authors, Andri *et al*. (2010), Chukwu *et al*. (2012), Diyaware *et al*. (2016). Mohammed *et al*. (2024), but with limited success (less than 100% male). The improvement of through DNA transfer may reduce the dependence on imported Tilapia strains. The enhancement of *Oreochromis niloticus* growth through DNA transfer has not been reported in Nigeria. The objectives of this study are to investigate the growth performance, proximate composition, and DNA fingerprint of *O. niloticus* containing foreign fish DNA.

MATERIALS AND METHODS

Ethics Consideration

The experiment was conducted following the approval of the Health Research Ethics Committee (HREC) No. UNIMAID/HREC/VOL.1/2022/04 guiding the use of animals in scientific research at the University of Maiduguri, Nigeria.

Study Area

The study was conducted at the Teaching and Research Fish Farm of the Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Nigeria. The study area is situated between latitude 11° 05'N and longitude 13° 05'E, with hot and cool periods as well as rainy (wet) and dry seasons. The wet season has a short duration of erratic rainfall of 3 - 4 months per year with an annual rainfall of 630 mm. During the dry season, ambient temperatures are lower in December and January, ranging from 15

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Commented [H26]: What is this for?

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Commented [H29]: You claimed novelty ("enhancement of Oreochromis niloticus growth through DNA transfer has not been reported in Nigeria") but ignores decades of somatic DNA injection literature (Wolff et al. 1990; Rahman & Maclean 1992; El-Zaeem & Assem 2004, etc.). No clear hypotheses.

What to do: rephrase in relation to paucity of information on the study

Commented [H30]: State testable hypotheses (e.g., "Higher DNA doses will increase growth via stable integration").
Alongside your objective.s.

Commented [H31]: Ethical approval cited, but no biosafety level, containment, or risk assessment for GM fish

What to do:
1. Provide full biosafety and animal ethics details compliant with international GM animal guidelines (e.g., OECD, EFSA).
2. If claiming genetic modification, provide evidence of containment and environmental risk assessment
3. Address food safety concerns regarding consumption of fish with potentially integrated foreign DNA
4. Clarify whether these fish were contained according to national biosafety regulations (Nigeria's NBMA guidelines)

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- 20 °C (night), and higher in March to June at 33 - 47 °C (day). The relative humidity ranges between 33.5 - 34.5%.

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Source of Donor Fish

The Nile Perch, *Lates niloticus* (300 - 456.0 g) were obtained from Karabonde landing site (Figure 1), Kainji Lake, Niger State, Nigeria. Karabonder landing site is situated between latitudes 9.899403° and longitudes 4.564203°

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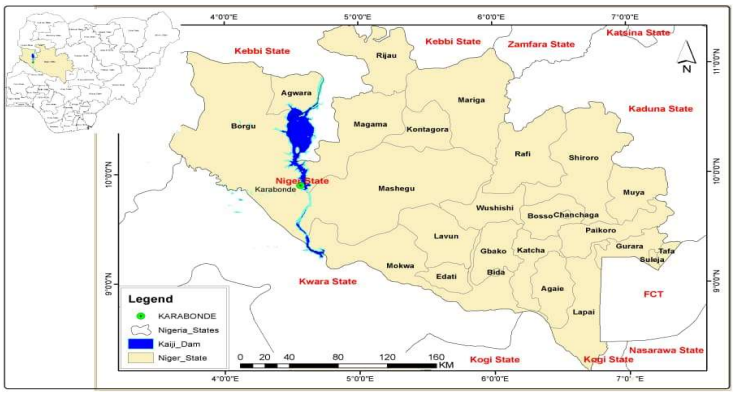

Figure 1: Map of Niger State showing Karabonde landing site, Kainji Dam, Nigeria.

DNA Extraction and Quantification

Fifty (50) mg fin tissue samples of the Nile Perch, *Lates niloticus*, were collected, preserved in DNA-grade ethanol, and transported to the Northeast Biotechnology Centre, University of Maiduguri, Nigeria. Genomic DNA was extracted from the tissue of *Lates niloticus* following the protocol described by Baradakci & Skibinski (1994). The fin tissues were crushed into smaller pieces using a laboratory pestle and mortar and homogenized using a tissue homogenizer. The crushed tissues were then transferred into a microcentrifuge tube containing 50 mM Tris Bis buffer, 100 mM EDTA (pH 8.0), 100 mM NaCl, 0.1 % SDS, and 0.5 mg/ml proteinase K, and incubated overnight for the samples to digest. After incubation, DNA was extracted twice for 15 - 20 minutes with a 1:1 ratio of phenol and chloroform and again twice for 15 minutes with 24:1 volume of chloroform and isoamyl alcohol. The aqueous phase was then precipitated with 2.5 volumes of 100% DNA grade ethanol in the presence of one-tenth volume of 3.0 M sodium acetate (pH 6.0). DNA pellets were washed with 70 % DNA-grade ethanol and dissolved in 0.1 ml saline sodium citrate (SSC) buffer. The purity and concentration of genomic DNA were determined using an Ultra Violate (UV) Spectrophotometer (Model: Nanodrop 2000/2000c, Thermo-Scientific, USA) at an optical density measurement of 260/280 nm.

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Commented [H37]: No verification of DNA integration/expression (only RAPD, which cannot confirm transgene presence). No Southern blot, qPCR, or sequencing. No germline transmission assessment.

What to do: Add molecular confirmation (qPCR for foreign DNA, RT-qPCR for expression, or sequencing of integration sites)

Experimental Design

A total of 675 juveniles of *Oreochromis niloticus* (mean weight of 104.47 g and 5.47 cm length) were divided into five groups, with 135 juveniles per group (treatment). Different concentrations of SSC buffer-based DNA: 0 as control, 10, 20, 30, and 40 µg/µl with a final volume of 0.1ml were injected into the dorsal somatic tissue of 45 fingerlings using a thermodynamic micro-syringe and needle in three replications in a complete randomized design manner. The fingerlings containing the DNA were reared in a 3 x 2 x 1.2 m deep polyethylene fish pond at 7.5 fish/m^2 in three replications. The fish were fed with a 35 % crude protein commercial diet for 3 months at 5 % of their body weight twice daily at 9:00 am and 3:00 pm, respectively.

Growth Performance of *O. niloticus* Containing Exogenous DNA

At the end of the three-month rearing period, the final weight (g), final length (cm), fish mortality, and quantity of feed applied were recorded. We estimated the following growth indices for each treatment:

i) Weight gain (g) = $W_2 - W_1$, where W_2 and W_1 are the final and initial weights of the fish, respectively.

ii) Percentage weight gain = $W_2 - W_1$ x 100, where W_2 and W_1 are the final and initial weights of the fish, respectively.

iii) Mean Daily Weight Gain (MDWG) in grams = $\frac{W2-W}{t}$, where = W_2 and W_1 are the final and initial weight of the fish, respectively, and t = the culture period (days).

iv) Specific Growth Rate (SGR % per day) = $\frac{\text{InlogW2}-\text{InlogW1}}{t}$ x 100, where In. logW_2 = natural log of final weight, In.log W_1 = natural log of initial weight, and t = culture period (Busacker *et al.*, 2012).

v) Feed conversion Ratio (FCR) = $\frac{\text{Dry weight of feed}}{\text{weight gain ( )of fish}}$

vi) Survival (%) = $\frac{\text{No.of fish survived}}{\text{.of fish stocked}}$ x 100.

Monitoring of Water Quality

The temperature (°C), pH, electrical conductivity (µS), and total dissolved solids (ppm) were measured using a Multifunction 4-in-1 Temp./EC/ TDS/pH Meter (Model: 9908, China), and dissolved oxygen was recorded using a portable digital DO meter (Model: BLE-9100).

Proximate Composition of *O. niloticus* Improves Through DNA Transfer.

Fifty (50) g of fish muscle from each treatment were collected and placed in an icepack containing ice flakes and shipped to the Animal Care quality control laboratory, Kano State, Nigeria, for proximate composition. The proximate composition was conducted in triplicate using a digital Near Infrared (NIR) Multi-Checker (Model: 21MC11309A06, China) according to AOAC (2005).

RAPD Protocol/Fingerprint Analysis

At the end of the experiment, 50 mg tissue from each treatment (including the control) in three replicates was collected after sedation according to the method

Commented [H39]: You used five DNA concentrations but lacks justification for the chosen range (0–40 µg/µl).

What to do: A rationale based on prior studies or pilot experiments is needed.

The control group is appropriate, but additional controls (e.g., sham injection with buffer only) should be included to rule out injection effects.

What to do: Indicate this under limitation

Where is the DNA source control:, No analysis of whether detected "Nile perch DNA" in tilapia is merely residual injected DNA persisting in muscle tissue (not integrated)

Commented [H40]: If all 45 fish per DNA concentration were raised together, this represents pseudoreplication (tank = experimental unit, not individual fish)

Commented [H41]: Injection protocol lacks details (needle gauge, injection site depth, anaesthesia). No sham-injection or buffer-only controls.

Even if DNA integrated into somatic cells (which is not proven), this would not be heritable. The study makes no distinction between somatic mosaicism and germline transmission, and provides no evidence that the "modifications" are heritable.

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Commented [H42]: No information on tank effects or randomization of treatments across tanks/pond

Commented [H43]: Remove, you already indicate what W2 and W1 stands for above

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Commented [H45]: Growth differences could stem from differential feed consumption rather than DNA effects. Feed intake was not measured or reported.

... [2]

Commented [H46]: Only single time-point water quality data presented. Fluctuations in temperature, DO, ammonia could confound growth results.

... [3]

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Commented [H48]: Use of Near Infrared (NIR) spectroscopy for proximate analysis is acceptable, but calibration against standard methods (AOAC wet chemistry) is not reported.

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Commented [H50]: RAPD protocol uses only three primers; scoring and reproducibility not described.

... [6]

Commented [H51]: RAPD banding patterns are not reliable evidence of foreign DNA integration.
Band differences can arise from PCR artifacts, primer binding variations, epigenetic changes, or statistical no

... [5]

described by Diyaware *et al.* (2017). Three tissue samples were also collected from the donor fish (*L. niloticus*). The samples were stored at −20°C until DNA extraction. Genomic DNA was extracted from the tissues following the protocol described by Baradakci & Skibinski (1994). Each tissue was crushed individually into smaller pieces using a laboratory pestle and mortar and homogenized using a tissue homogenizer. The crushed tissues were then transferred into separate microcentrifuge tubes containing 50 mM Tris Bis buffer, 100 mM EDTA (pH 8.0), 100 mM NaCl, 0.1 % SDS, and 0.5 mg/ml proteinase K, and incubated overnight for the samples to digest. After incubation, DNA was extracted twice for 15 - 20 minutes with a 1:1 ratio of phenol and chloroform and again twice for 15 minutes with 24:1 volume of chloroform and isoamyl alcohol. The aqueous phase was then precipitated with 2.5 volumes of 100 % DNA grade ethanol in the presence of one-tenth volume of 3.0 M sodium acetate (pH 6.0). DNA pellets were washed with 70 % DNA-grade ethanol and dissolved in 0.1 ml saline sodium citrate (SSC) buffer. The purity and concentration of genomic DNA were determined using an Ultra Violate (UV) Spectrophotometer (Model: Nanodrop 2000/2000c, Thermo Scientific, USA) at an optical density measurement of 260/280 nm.

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Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) reactions were performed in 25 µL volumes containing 10 × PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 1.0 µM primer, 1 U Taq DNA polymerase, and 20–50 ng template DNA. Three RAPD primers modified from Ogbuebunu & Awodiran (2017) were used for the PCR (Table 1). All primers were amplified at an initial denaturation of 94 °C for 3 minutes, 40 cycles, denaturation temperature of 94 °C for 30 seconds, annealing temperature of 36 °C for 1 minute, extension at 72 °C for 1 minute, final extension at 72 °C for 10 minutes, and holding temperature of 4 °C.

Table 1: Random Amplified Polymorphic DNA (RAPD) Primer Sequences

Primers	Primer Sequence
OPH-05	AGTCGTCCCC
OPT-06	CAAGGGCAGA
OPT-20	GACCAATGCC

Gel Electrophoresis

Polymerase chain reaction products were resolved on 1.5 % agarose gels stained with ethidium bromide. The gels were visualized under Ultraviolet (UV) light. Banding patterns were compared between the fish that recieved the DNA and the control fish. The presence of unique bands in injected fish was interpreted as evidence of foreign DNA integration.

Data Analysis

Data obtained on growth performance indices, proximate composition, and water quality were subjected to a one-way analysis of variance. The RAPD fingerprints

Commented [H53]: The study performs multiple pairwise comparisons (0 vs 10, 0 vs 20, etc.) without Bonferroni correction or similar adjustment, increasing Type I error risk.

Growth Data Interpretation

1.Initial weights: Not statistically compared (though appear similar). ANCOVA should be used with initial weight as covariate, not just final weight ANOVA.
2.Survival data**:** Not reported in Table 2 despite being mentioned in methods. Mortality differences could confound growth results (if smaller fish died, average weight increases artifactually).

Commented [H54]: To do what and at what P value

generated for all samples were scored using a Gel analyzer. The genetic diversity was determined using GenAlex (Peakall & Smouse, 2012). . Phylogenetic trees to check similarity indices were constructed using Nei's (1972) Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Euclidean Cluster analysis with the aid of PAST 4.03.

RESULTS

Water Quality Parameters

Table 1 shows the water quality parameters observed during the study. Dissolved Oxygen (DO) ranged from 6.50 to 6.82 mg/l, temperature 26 - 26.6 °C, while TDS and EC ranged between 79.00 - 81.16 ppm and 159 - 166 µs/cm, respectively.

Table 1: Water quality parameters observed during the study

Parameters	Deoxyribonucleic Acid (DNA) Concentration (µg/µl)				
	0	10	20	30	40
DO (mg/L)	6.54±0.15^{b}	6.06±0.26^{a}	6.83±0.24^{ab}	6.52±0.04^{b}	6.51±0.06^{b}
Temp. (°C)	26.59±0.00	26.55±0.00	26.50±0.00	26.52±0.02	26.60±0.00
pH	8.17±0.12	8.15±5.18	8.16±0.14	8.18±0.15	8.16±0.13
TDS (ppm)	80.16±2.22	79.96±1.81	82.00±4.543	81.16±5.73	79.17±3.17
EC (µs/cm)	164.50±3.45	163.53±3.00	166.00±27.94	165.50±10.73	159.83±6.10

Growth Performance of *O. niloticus* Containing Exogenous Fish DNA

Table 2 shows the growth performance of *O. niloticus* injected with *L. niloticus* DNA. Final weight, weight gain, average daily weight gain, and percentage weight gain, as well as specific growth rates, were significantly ($p<0.05$) better in fish injected with 40 µg/µl *L. niloticus* DNA. The final length was statistically similar across the treatments. However, Feed Conversion Ration (FCR) was higher (1.46±0.07) in fish containing 20 (µg/µL) DNA. No significant variations ($p>0.05$) were observed between the FCR of fish injected with 10 and 30 (µg/µL) DNA. Similarly, there was no significant difference ($p>0.05$) observed between the FCR of fish treated with 40 (µg/µL) DNA compared to those containing 10- 30 (µg/µL) *L. niloticus* DNA.

Table 2: Growth Performance of *O. niloticus* Containing Exogenous Fish DNA

Parameters	DNA Concentrations (µg/µL)				
	0	10	20	30	40
IW (g)	103.29±0.33	103.03±0.73	103.18±0.75	102.21±0.70	101.88±0.84
FW(g)	653.35±12.55^{b}	587.01±6.11^{c}	573.53±34.50^{c}	658.91±5.20^{b}	751.90±18.50^{a}
IL (cm)	5.21±0.14	5.28±0.064	5.80±0.42	5.70±0.12	5.34±0.09
FL (cm)	11.37±0.34	11.38±0.59	12.28±0.70	12.35±0.38	13.51±0.26
WG (g)	550.06±12.34^{b}	483.97±6.54^{c}	470.35±35.24^{c}	556.69±4.62^{b}	650.02^{a}
ADWG (g/day)	4.58±0.10^{b}	4.030.05^{c}	3.92±0.29^{c}	4.64±0.04^{b}	5.41±0.16^{a}
% WG	375.40±23.57^{b}	388.67±53.78^{a}	366.42±45.02^{a}	397.39±2.99^{b}	432.96±10.34^{a}
SGR (%/Day)	2.79±0.01^{b}	2.74±0.02^{c}	2.74±0.02^{c}	2.80±0.01^{b}	2.86±0.01^{a}
FCR	1.19±0.02^{c}	1.38±0.06^{ab}	1.46±0.07^{a}	1.37± 0.01^{ab}	1.31±0.03^{bc}

Commented [H55]: The phylogenetic tree (Figure 4) based on RAPD data is inappropriate for showing "integration". UPGMA clustering of RAPD bands shows genetic distance, not transgene integration.

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Commented [H57]: You claimed to use one way ANONA, none of these Tables in result indicate such results. Also, ANOVA is mentioned, but post-hoc tests (e.g., Tukey's HSD) are not reported. Confidence intervals should be included.

Commented [H58]: Should be: µS/cm

Commented [H59]: This should be Table 2

Commented [H60]: Table 1 shows significant differences in Dissolved Oxygen (DO) between treatments (6.06±0.26 vs 6.83±0.24 mg/L, p<0.05). DO differences can significantly affect growth performance independently of any genetic treatment. This confounding variable is not addressed.

The superscripts leter a, b, ab, what do they signifiy, how did you arrived a these?

Commented [H61]: Should be: µS/cm

Commented [H62]: Should be Table 3

Commented [H63]: The data show significant improvements at 40 µg/µl DNA, but the interpretation is overly strong. The possibility of transient expression or epigenetic effects should be acknowledged.

Means with the same superscript in the same row are not significantly different ($p>0.05$).
Key**:** IW **=** Initial weight, FW = Final weight, initial length, final length, WG = Weight gain, ADWG = Average daily weight gain, SGR = Specific growth rate, FCR = Feed conversion ratio.

However, the Feed Conversion Ratio (FCR) increased with an increase in the DNA concentration, with a slight decrease in fish containing 30 - 40 µg/µl DNA. Feed conversion ratio was significantly ($p<0.05$) better in fish treated with 20 µg/µl DNA compared to others, except the control.

Commented [H64]: You did not discuss this appropriately in your discussion section, Elaborate on the likely cause of the disparity in this findings

The Growth Pattern of *O. niloticus,* Containing Exogenous Fish DNA

The growth pattern of *O. niloticus,* containing exogenous fish DNA, is illustrated in Figure 2. There was a significant ($p<0.05$) progressive growth increase with the increase in the DNA concentration throughout the study. It appears that the DNA transfer had a dose-dependent relationship, as 10 and 20 µg/µl did not significantly improve growth (Fig. 1).

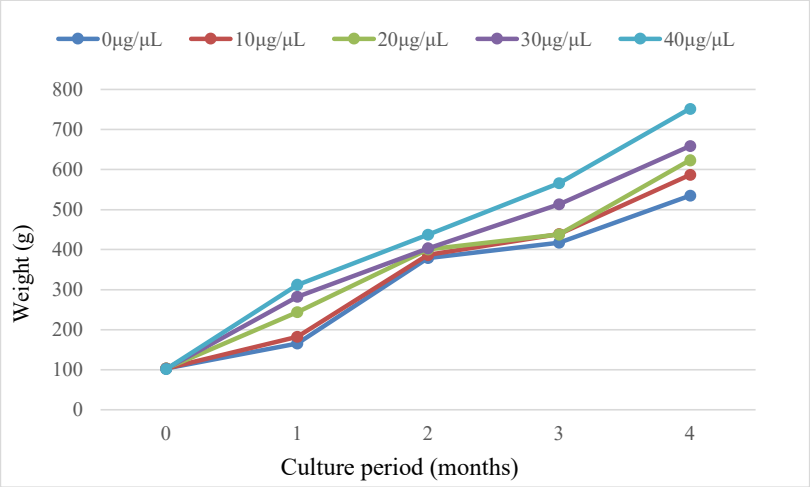

Figure. 2: Growth pattern of *O. niloticus* containing exogenous fish DNA

Proximate Composition of *O. niloticus* containing exogenous fish DNA.

Table 3 shows the proximate composition of *O. niloticus* containing exogenous fish DNA. Crude protein (CP) content increased significantly ($p<0.05$) with an increase in the DNA concentration. A higher CP of 39.05 % was observed in fish injected with 40 µg/µl of DNA, followed by 36.36 % CP in fish injected with 30 µg/µl of DNA. A lower CP of 27.90 % was observed in the control group, suggesting that the foreign DNA might have enhanced muscle protein synthesis in the Nile Tilapia.

Commented [H65]: Should be Table 4

Commented [H66]: The increase in crude protein and metabolizable energy is interesting, but the mechanism is speculative. Without gene expression analysis (e.g., muscle protein synthesis markers), the conclusion is weak.
Also, Ash and moisture data are presented but not meaningfully discussed.

Table 3: Proximate Composition of *O. niloticus* Containing Exogenous Fish DNA.

Nutrients	DNA Concentration (µg/µl)				
(%)	0	10	20	30	40

CP	27.90±0.33^{e}	33.28±0.39^{d}	34.76±0.21^{c}	36.36±0.33^{b}	39.05±0.49^{a}
Ash	0.89±0.05	0.86±0.08	0.94±0.03	0.63±0.32	0.88±0.06
EE	10.19±0.4^{c}	13.12±0.03^{a}	11.13±0.02^{c}	9.16±0.03^{e}	12.47±0.02^{a}
CF	1.34±0.00^{b}	1.64±0.03^{a}	0.98±0.01^{d}	1.06±0.00^{c}	0.92±0.03^{e}
Moisture	60.83±0.17^{b}	58.63±0.25^{c}	60.44±0.96^{b}	61.40±0.00^{b}	63.94±0.02^{a}
ME(Kcal)	2560.40±3.73^{d}	3000.60±13.68^{b}	2754.40±100.60^{c}	2970.20±3.09^{b}	3474.10±91.36^{a}

Means in the same group having similar superscripts are not significantly different ($p>0.05$).
Key: CP = Crude protein, EE = Ether extract, CF = Crude fiber, ME = Metabolizable energy.

Commented [H67]: metabolizable energy -ME (Kcal) was indicated but metabolizable energy is typically calculated, not measured directly by NIR- calculation method not specified in methodology.

Commented [H68]: How did you determine this?

Ash

Ash levels showed no significant variation ($p>0.05$) among the entire treatments. This suggests that DNA transfer had minimal effect on mineral content.

Ether Extract

Ether Extract (EE) was significantly ($p<0.05$) higher (13.12±0.3 and 12.47±0.02 %) in fish treated with 10 and 40 µg/µl DNA, respectively, indicating an increase in lipid deposition at these concentrations. The lowest EE (9.16±0.03 %) value was observed in fish containing 30 µg/µl DNA concentration. Generally, there were significant variations ($p<0.05$) among the mean values of the EE across all the treatments. The ether extract content, which reflects the lipid concentration in fish, showed noticeable ($p<0.05$) variations across the treatments.

Crude fiber

Crude fibre decreased with an increase in the DNA levels. Crude fibre was observed to be significantly ($p<0.05$) higher (1.64±0.03%) in fish injected with 10 µg/µl DNA compared to the entire treatment. The lowest (0.92±0.01%) crude fibre content was observed in the group injected with 40 µg/µl DNA.

Moisture Content

Moisture content increases with an increase in DNA concentration, with the significantly ($p<0.05$) highest (63.94%) mean value in fish that received 40 µg/µl DNA. This trend suggests a positive correlation between DNA concentration and moisture retention in fish. Significant variation ($p<0.05$) was observed between the mean moisture contents of the fish treated with 10 µg/µl DNA compared to the entire treatments. However, no significant variations were observed among the moisture contents of fish treated with 0, 20, and 30 µg/µl DNA.

Metabolizable Energy

Metabolizable Energy (ME) of the *O. niloticus* containing *L. niloticus* DNA increased significantly ($p<0.05$) with an increase in the DNA concentration levels, peaking at 3474.1 Kcal in fish treated with 40 µg/µl DNA, followed by *O. niloticus* injected with 10 and 30 µg/µl DNA (3000.60 and 2970.20 Kcal, respectively). The control had a lower 2560.40 (Kcal) ME.

Fingerprint Random Amplified Polymorphic DNA Analysis

Commented [H69]: RAPD analysis is outdated and prone to reproducibility issues. A more modern techniques such as microsatellites, SNP genotyping, or whole-genome sequencing.

The DNA purity used in this study was 1.76 ng/µL at A260/A280 and 1.09 at A260/A230. Figure 3 is the representative random amplified polymorphic DNA (RAPD) gel of *O. niloticus* injected with *L.niloticus* DNA. Gel electrophoresis revealed distinct banding patterns: the control fish had 5 bands, while fish injected with 10, 20, 30, and 40 µg/µL had 7, 6, 8, and 9 bands, respectively, suggesting probable differential integration or expression of *L. niloticus* DNA fragments among the treated fish. The donor fish DNA fragment is approximately 300 bp in size, based on its position relative to the 250 bp and 500 bp ladder bands.

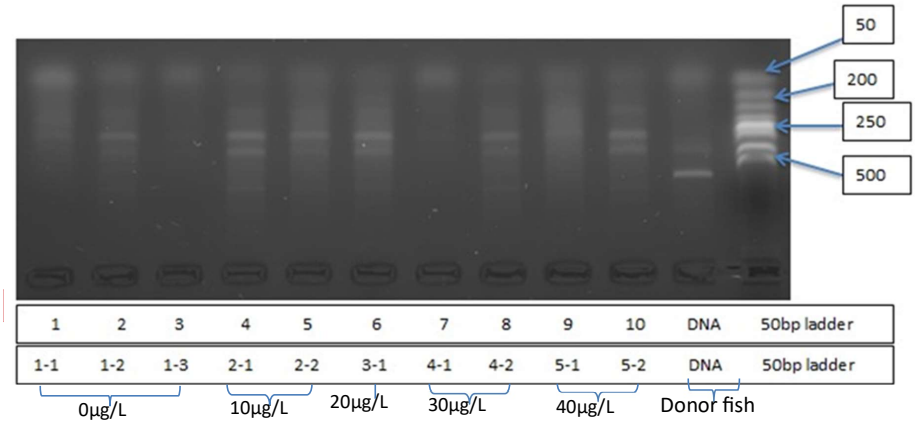

Figure 3: Gel representative of *Oreochromis niloticus* containing *Lates niloticus* DNA

Commented [H70]: Gel electrophoresis results lack molecular weight markers and quantification.

Genetic Similarity Indices

The dendrogram generated from gel electrophoresis banding profiles using UPGMA (Figure 4) cluster revealed distinct groupings among fish that received *L. niloticus* DNA. The control fish (0 µg/µL of DNA) clustered separately from the donor fish, indicating genetic dissimilarity from their sibling that received DNA. In contrast, samples that received 10 to 20 µg/µL DNA showed progressive clustering toward *L. niloticus*, suggesting a dose-dependent assimilation of *L. niloticus* genetic material. *Oreochromis niloticus* injected with 30 and 40 µg/µL DNA formed a close cluster with *L. niloticus*, supported by high bootstrap values of 100, indicating close similarity. The *O. niloticus* containing 10 µg/µL exhibited moderate bootstrap, indicating that it is closer to *L. niloticus* than those injected with 30 and 40 µg/µL DNA, suggesting partial uptake or variability in transformation efficiency.

Commented [H71]: Bootstrap values of 100 suggest artificial clustering due to experimental artifacts rather than true genetic relationships.

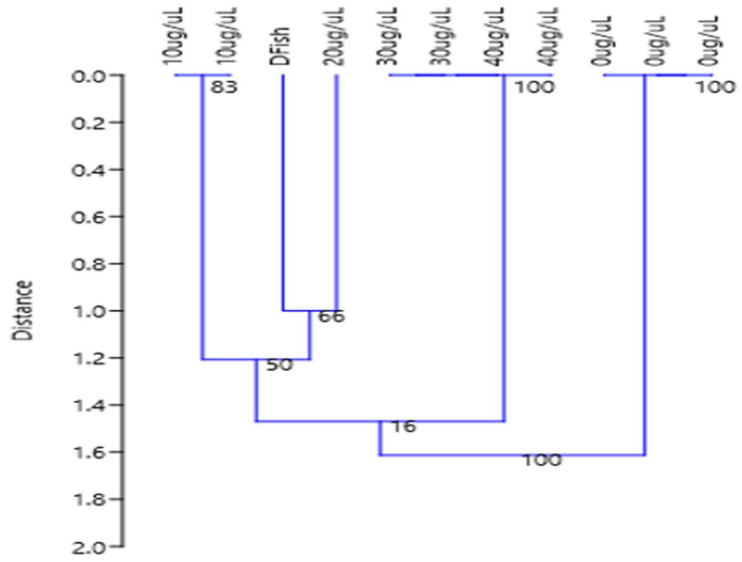

Figure 4: Phylogenetic tree based on Nei's (1972) standard genetic distances using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method generated from RAPD data. **Key**: DFish = Donor fish (*Lates niloticus*).

Overall, the dendrogram supports the hypothesis that the increasing DNA concentration enhances genetic similarity to the source organism, with 30–40 µg/µL representing a potential threshold for effective transformation.

DISCUSSION

Water quality parameters

The water quality parameters recorded during this study are within the range for tilapia culture suggested by Romana-Eguia *et al.* (2020).

Growth Performance of *O. niloticus* Containing Exogenous Fish DNA

The increase in growth performance of *O. niloticus* injected with DNA of *L. niloticus* is similar to that obtained by several workers, including El-Zaeem (2004), El-Zaeem & Assem (2004), Hemeida *et al.* (2004) and Assem & El-Zaeem (2005), who demonstrated that administering 40 µg/µl fish of shark DNA enhanced growth performance, body composition, and immune traits in *O. niloticus* and *T. zillii* (*Coptodon zilli*). Kusa *et al.* (2025) also observed better final weight, mean daily weight gain, and specific growth in *Oreochromis niloticus* injected with 40µg/µL *Heterotis niloticus* DNA. El-Zaeem *et al.* (2011, 2012a) also observed higher body weight and specific growth rate in *Oreochromis niloticus* injected with Seabream and *Artemia salina* DNA. El-Zaeem (2012b) also reported improvements in growth performance and body composition of grey mullet fingerlings injected with shark DNA. Thabet *et al* (2025) also report higher

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The manuscript does not adequately compare its findings with similar DNA transfer studies in tilapia or other aquaculture species.

Commented [H73]: Move before Table 1 as discussion to the result

final weight, weight gain, specific growth rate, and survival rate in *O. nilotiucs* induce with Salmon DNA via the organs.

The FCR ranges of *O. niloticus* injected with DNA observed in this study are lower than 1.88 and 1.89 reported by El-Zaeem *et al*. (2011) for fish containing Shark DNA. Similarly, the FCR is also lower than 1.5 reported by Krivins (2024) for *O. niloticus* fed a commercial diet. In the same fish species injected with sea bream (*Sparus aurata*) and Artemia (*Artemia salina*), respectively, and 1.83 reported by El-Zaeem *et al*. (2023) for *Coptodon zilli* injected with Shark DNA. The variation may be due to the differences in the fish species and the sources of the DNA.

Commented [H74]: Feed conversion ratio (FCR) results are inconsistent and not fully explained. The discussion should address why FCR worsened at intermediate concentration

Commented [H75]: Move to the corresponding section under the reault.

Growth Pattern of *O. niloticus* Containing Exogenous Fish DNA

The better progressive growth pattern observed in the study might be an indication that the fish containing high concentrations of the DNA might have efficiently utilized the diet given to them. Zhu (1993) also observed similar results after microinjecting foreign DNA into fertilized fish eggs. Fu *et al.* (1998) reported that transgenics were more efficient in utilizing dietary protein compared to their non-transgenic siblings. In a similar vein, Rahman *et al*. (2001) confirmed that transgenic tilapia are also more efficient in utilizing protein and energy.

Commented [H76]: You cites "transgenic tilapia" studies (Zhu.,1993) involving microinjection of gene constructs into fertilized eggs, which is fundamentally different from injecting fragmented genomic DNA into juvenile somatic tissue. These are not comparable methodologies.

Proximate Composition of *O. niloticus* Containing Exogenous Fish DNA

The protein contents observed in this study were higher than 14.62 % CP observed in red belly tilapia (*Coptodon zilli*) injected with Shark DNA, as observed by El-Zaeem *et al*. (2023), and 11.32, 13.10, and 9.80 % in *O. niloticus*, *O. aureus*, and their hybrids, respectively, as documented by El-Hawarry (2012). Suwannatrai *et al*. (2023) reported 14.27% crude protein of *O. niloticus* from Sakon Nakhon province, Thailand. While Kumara *et al.* (2020) noticed 7.85 % crude protein in *O. niloticus* from Sri Lanka. The variation in the crude protein level may be due to the differences in the fish species, the source of the DNA, and the age of the fish. However, Mukti *et al*. (2020) reported 85.70 and 87.00 % crude protein in triploid male and female *O. niloticus*, respectively. The greater variation observed in the crude protein levels of the sterile Tilapia compared to the tilapia containing foreign DNA could be due to the complete utilization of feed for growth only by the fish, rather than growth and reproduction as occurs in the fertile Tilapia containing foreign DNA. Martinez *et al*. (2000) reported that transgenic Tilapia demonstrated an increased protein synthesis and growth rate concomitant with enhanced glycolysis and oxidation of amino acids in juvenile transgenic Nile tilapia. The increase in crude protein level observed in the fish injected with 40 µg/µl may be due to the effective conversion of sparing nutrients from fish feed by the fish. A similar trend was also reported by Yuvarajan *et al*. (2019) in Genetically Improved Farmed Tilapia (GIFT), which recorded a remarkable increment in protein and lipid contents. Such an increase implies improved nutritional quality, which is important for Tilapia aquaculture profitability (Cebeci *et al*., 2020) and improves the public health of consumers.

Commented [H77]: The observed changes in proximate composition (higher protein, moisture, EE) are interpreted as genetic improvements. However, you should know that:

1. Increased moisture content (63.94% as against 60.83%) often indicates edema or stress, not improved quality
2. Injection trauma could alter metabolic rates, explaining apparent "improved FCR" without genetic change
3. No histological examination of muscle tissue to confirm these are normal compositional changes

Ash

The significance of ash contents among the treatments aligns with studies that note that ash is less sensitive to genetic or dietary manipulation (Abdel-Tawwab *et al*., 2010). The ash contents observed in this study are lower than 1.36 % for *O. niloticus* and hybrid red tilapia reported by Olopade *et al*. (2016).

Ether Extract

High lipid levels, especially in fish containing 10 and 40 µg/µl DNA, may indicate altered lipid metabolism due to foreign DNA expression. The observed (9 - 13 %) EE may indicate that the foreign DNA could have influenced lipid metabolism in the Nile tilapia. Higher ether extract levels can be beneficial by improving the energy density, flavor, and consumer acceptability of the fish (Shearer, 1994). However, excessively high lipid deposition could pose risks such as reduced shelf-life due to lipid oxidation (Raatz *et al*., 2013) and may not align with consumer preferences for leaner fish in some markets. Genetic modification, particularly through DNA/gene transfer, is known to impact lipid regulation pathways. A similar trend was observed in transgenic mice containing silencing genes (*SREBP-1c* and *PPARα*), which significantly changes lipid synthesis and fatty acid oxidation (Horton *et al*., 2002). Devlin *et al*. (2001) demonstrated that transgenic salmon exhibited increased lipid accumulation due to enhanced growth signals, suggesting that similar mechanisms might explain the ether extract changes observed in this study. Therefore, while moderate increases in EE could enhance nutritional value, careful optimization is required to balance consumer health concerns and product quality. Similarly, higher ether extract in the fish containing the foreign DNA may result in beneficial fatty acids such as Omega-3, which can enhance the fish's health value as suggested by Tocher (2003).

Crude fibre

The crude fibre observed in this study is higher than 0.98 % reported by Yuvarajan *et al*. (2019) in Genetically Improved Farmed Tilapia (GIFT) and 0.54 and 0.59 % reported by Olapode *et al*. (2016) for *O. niloticus* and hybrid red tilapia, respectively. The variation in the fibre content may be due to strain variation (GIFT, normal tilapia, and the hybrids). Although fish naturally contain low fiber, these changes may reflect alterations in gut microbiota or feed digestion due to genetic influence. Bolawa *et al*. (2011) reported very low crude fibre, while Otene *et al.* (2024) found fibre values consistently to be below 1 %, emphasizing that fish muscle is not a source of dietary fibre.

Moisture contents

The higher moisture contents observed in this are lower than 81.39 and 80.09 % observed by Olopade *et al*. (2016) for *O. niloticus* and hybrids of red Tilapia, respectively. El-Hawarry (2012) reported 79.09, 79.12, and 80.45% moisture contents of fillets of *O. niloticus*, *O. aureus*, and their hybrids. The increase in the Metabolizable

energy (ME) with an increase in concentration of the DNA may indicate improved nutrient density and energy utilization, possibly reflecting metabolic shifts influenced by the transferred DNA sequences related to growth or feed efficiency. According to Thabet *et al.* (2025), foreign DNA can upregulate metabolic efficiency, thereby increasing metabolizable energy concentration. Konnert *et al.* (2022) opined that high ME indicates efficient nutrient conversion in the fish, which translates to better protein and fat availability for human consumption.

RAPD Fingerprint

The 100% polymorphism observed across all loci in this study is consistent with the report of Ogbuebunu & Awodiran (2017), who also reported similar polymorphism values in *L. nilotiucs* populations from three water bodies in Nigeria. The exhibition of different banding patterns may indicate genetic variation among the fish. This may suggest successful genomic integration and expression of exogenous DNA, resulting in distinct genetic profiles for each treated fish. The 100% polymorphism observed in this study may be an indicator of genetic variability likely induced by the injected *L. niloticus* DNA. Such complete polymorphism is uncommon in natural populations and may point to the effectiveness of DNA-mediated genetic transformation. Polymorphism in RAPD profiles typically reflects underlying genetic differences, which may arise from mutations, recombination, or integration of foreign DNA (Williams *et al.*, 1990).

The 250 bp band appears to correlate with increasing DNA concentration in *O.niloticus*, suggesting a gene or DNA fragment being expressed or integrated more efficiently at higher concentration **(Kusa *et al.*, 2026)**. The 500 bp band, observed in fish injected with 30 and 40 µg/µL, might be a unique gene fragment only expressed or detectable at higher DNA concentrations, and is possibly a dose-sensitive gene or regulatory element activated at higher DNA loads.

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The presence of distinct bands in the injected fish and their absence in controls indicated the integration of the foreign DNA. Assem & El-Zaeem (2005) reported integration of Shark DNA into the genome of *Tilapia zilli* (*Coptodon zill*ii) after direct injection. In a similar vein, El-Zaeem *et al.* (2011) reported the random integration of sea bream and Artemia DNA into *O. niloticus* genomes. They claimed that the integrations could be a functional or a silent integration, as suggested by Yaping *et al.* (2001). Fujimura & Kocher (2011) demonstrated efficient transgenesis in *O. niloticus* using the Tol2 transposon system, achieving approximately 30% germline transmission and strong Green Fluorescent Protein (GFP) expression. Similarly, Olabode *et al.* (2014) reported successful integration of growth hormone genes using Tol2-mediated microinjection, with PCR confirmation and enhanced growth in transgenic tilapia.

In this case, the exogenous DNA likely introduced novel alleles or disrupted existing loci, leading to unique RAPD fingerprints in each DNA-treated *O. niloticus*. Similar findings were reported by Ikpeme *et al*. (2015), who used RAPD markers to assess genetic diversity in *Clarias gariepinus* and found higher polymorphism in wild populations compared to cultured ones, emphasizing the role of genetic enrichment in diversity. The complete polymorphism observed in this study may also reflect the concentration-dependent integration efficiency of the injected DNA. Higher DNA concentrations could facilitate more frequent or diverse integration events, as suggested by Rahman *et al*. (1998), who reported enhanced growth and genetic variation in transgenic tilapia expressing exogenous growth hormone genes. In a similar vein, Bera (2024) highlighted that increased gene copy via higher DNA input facilitated more frequent integration events, leading to enhanced growth traits in transgenic fish. While Cebeci *et al.* (2020) noted that higher DNA doses improve integration.

Genetic Variability

This pattern of closer cluster formation of fish that recieved higher DNA concentration implies that higher DNA concentrations could have enhanced genomic integration or expression, which is consistent with previous findings in transgenic fish by Zhang *et al*. (2010) and Agha *et al*. (2018). The moderate bootstrap exhibited by fish injected with DNA closer to the donor fish, *L. niloticus,* suggests partial uptake or variability in transformation efficiency. The distinct clustering between *O. niloticus* treated with 10 - 20 µg/µL and 40 µg/µL DNA further validated the reproducibility of the gel scoring and the robustness of the clustering method. These findings align with earlier reports by Sneath & Sokal (1973), Rohlf (2000), Fu *et al*. (2021), and Georgieva (2022) that hierarchical clustering can effectively resolve genetic relationships in electrophoretic data.

Conclusion

This study revealed that fragmented DNA materials from *Lates niloticus* may potentially improve growth in *Oreochromis niloticus*. Fragment of *L. niloticus* DNA moderately integrated into the *O. niloticus* genome. The DNA of *L. niloticus* has significantly altered the proximate composition of Nile tilapia, especially by enhancing protein content and metabolizable energy. These changes could offer nutritional, economic, and production benefits in Tilapia aquaculture, though further investigation into safety, long-term performance, and consumer acceptance is essential. It suggests that genomic DNA from fish species like *L. niloticus* can be used to induce genetic diversity in *O. niloticus*, potentially improving traits such as growth, disease resistance, or environmental tolerance.

Commented [H79]: Your study does not show the molecular biology test to back this assertion, pleae remove it

Page 6: [1] Commented [H41] **HomePC** **4/12/2026 7:49:00 PM**

Injection protocol lacks details (needle gauge, injection site depth, anaesthesia). No sham-injection or buffer-only controls.

Even if DNA integrated into somatic cells (which is not proven), this would not be heritable. The study makes no distinction between somatic mosaicism and germline transmission, and provides no evidence that the "modifications" are heritable.

Replication is mentioned (three replicates), but statistical power analysis is missing.

What to do: The sample size justification should be provided using GI Power

The method of somatic tissue injection is described, but there is no evidence of stable integration into the genome. The claim of "integration" is speculative without sequencing or Southern blot confirmation.

Page 6: [2] Commented [H45] **HomePC** **4/13/2026 7:10:00 AM**

Growth differences could stem from differential feed consumption rather than DNA effects. Feed intake was not measured or reported.

What to do:

Measure and report feed intake per tank. Calculate feed conversion ratio (FCR) with intake data to distinguish growth efficiency from consumption effects.

Page 6: [3] Commented [H46] **HomePC** **4/13/2026 7:08:00 AM**

Only single time-point water quality data presented. Fluctuations in temperature, DO, ammonia could confound growth results.

What to do:

Provide time-series water quality data or acknowledge as limitation. Ensure all tanks maintained within acceptable ranges throughout.

Page 6: [4] Commented [H48] **HomePC** **4/13/2026 6:17:00 AM**

Use of Near Infrared (NIR) spectroscopy for proximate analysis is acceptable, but calibration against standard methods (AOAC wet chemistry) is not reported.

NIR requires species-specific calibration; was the instrument calibrated for genetically modified tilapia, or did the "modifications" alter spectral properties in ways that could artifactually affect readings? Indicate all these.

Page 6: [5] Commented [H51] **HomePC** **4/13/2026 7:01:00 AM**

RAPD banding patterns are not reliable evidence of foreign DNA integration.

Band differences can arise from PCR artifacts, primer binding variations, epigenetic changes, or statistical noise.

RAPD cannot distinguish between integration, transient presence, or methodological artifacts.

What to do:

Either (a) replace RAPD with robust molecular methods (whole-genome sequencing, targeted PCR, FISH), OR

(b) substantially temper conclusions to state that RAPD detected "genetic polymorphism" without claiming integration.

Page 6: [6] Commented [H50] **HomePC** **4/12/2026 6:20:00 PM**

RAPD protocol uses only three primers; scoring and reproducibility not described.

What to do: Validate RAPD with sequencing of bands or switch to transgene-specific PCR.

RAPD banding patterns alone are insufficient to prove integration; they only suggest polymorphism.

What to do: More robust molecular techniques (e.g., qPCR, sequencing, fluorescence in situ hybridization) are expected or else indicate in your limitations