ASSESSMENT OF HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL, 1822) INJECTED WITH NILE PERCH, *Lates niloticus* (LINNAEUS, 1758) DNA

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ABSTRACT

The study aims to assess the impact of direct intramuscular injection of Nile perch DNA on the hematological and biochemical indices of African catfish. A total of 225 African catfish fingerlings were divided into five DNA concentrations: 0 (T1), 1 (T2), 2 (T3), 3 (T4), and 4 µg (T5) per fish, with each group replicated three times in a completely randomized design. The experiment took place in 15 plastic tanks, each housing 15 fish, and the fish were fed a commercial diet for a 20-week period. The data collected on haematological and biochemical indices were subjected to one-way analysis of variance. Results on hematological analysis revealed that red blood cell counts significantly increased ($p \leq 0.05$) with higher DNA doses, peaking at 2 µg (2.15 × 10^12/L), while white blood cell counts peaked at 4 µg (37.18 × 10^12/L). Furthermore, higher DNA doses were associated with significant increases ($p \leq 0.05$) in mean corpuscular volume, hemoglobin levels and lymphocyte counts. Biochemical analysis indicated notable differences ($p \leq 0.05$) in total protein levels, albumin, globulin, and the albumin/globulin ratio, with the highest total protein concentration (31.93 g/L) observed in the 3 µg group. These findings suggest that the hematological and biochemical indices of C. gariepinus fingerlings can be enhanced through the injection of Nile perch DNA, within the normal range for optimal physiological functioning in fish.

Keyword: African catfish, Biochemical indices, Direct injection, DNA concentrations, haematological parameters, Nile perch

INTRODUCTION

African catfish, *Clarias gariepinus* (Burchell, 1822) is an important aquaculture species that is cultured in various regions in the world. Nigeria is the largest producer of *Clarias gariepinus* in the world followed by the Netherlands, Brazil, Hungary, Kenya, Syrian Arab Republic, South Africa, Cameroon, and Mali (FAO, 2016). It is Africa's second most cultured species and the world's third most cultured catfish species (FAO, 2017). It is a crucial species in global aquaculture, renowned for its rapid growth, resilience, and adaptability to various farming conditions (FAO, 2016). However, there is continuous interest in further enhancing its physiological and biochemical traits to optimize production.

Genetic engineering, specifically through the direct injection of exogenous DNA, has emerged as a promising technique for such enhancements (El-Zaeem, 2012). Foreign genes can be introduced into fish in vivo by either introducing DNA into embryos or directly into somatic tissues of adults. Common methods for introducing 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 straightforward method that yields rapid results and eliminates the need for screening transgenic individuals and selecting germline carriers (El-Zaeem et al., 2012a). Studies have shown successful gene transfer and expression through intramuscular direct injection of foreign DNA into skeletal muscles of fish, suggesting a potentially simple and rapid method for enhancing fish characteristics (El-Zaeem et al., 2012b).

Nile perch, *Lates niloticus* (Linnaeus, 1758) is the world's largest freshwater fish, reaching a mature weight of up to 200 kg and a length of 2 meters, but the majority of them are caught before attaining such size (Asnake, 2018). Nile perch mature at about three years of age and can live up to 16 years, with each individual capable of spawning multiple times and producing a large number of eggs up to 16 million eggs at a time (Shinkafi, 2013). It is notable for its impressive growth rates and robust physiological traits, making its DNA an attractive candidate for genetic enhancement in other fish species (Asnake, 2018).

Hematological parameters such as such as red blood cell count, white blood cell count, and haemoglobin concentration, provide valuable insights into the fish's immune response and overall health. Biochemical indices like serum protein levels, including albumin and globulin, serve as indicators of metabolic function and immune system activity (Yu et al., 2020). These indices are critical indicators of fish health and are widely used to monitor the physiological state of aquaculture species (Jimoh *et al*., 2020a). Previous research has demonstrated that the introduction of foreign DNA into fish can lead to significant physiological and biochemical changes, potentially improving traits such as growth, disease resistance, and stress tolerance (Devlin *et al*., 2014; Maclean, 2015). However, the effects of such genetic interventions on the haematological and biochemical profiles of *Clarias gariepinus* remain underexplored. The main objective of this study was to evaluate the effect of direct intramuscular injection of Nile perch DNA on the haematological and biochemical indices of African catfish.

MATERIALS AND METHODS

Experimental Site

The experiment took place at the Fish Hatchery Complex of the Department of Fisheries and Aquaculture, Federal University Dutse, Jigawa State, located at latitude 11° 42' North, longitude 9° 22' East, and an altitude of 436m above sea level (Google, 2024).

Experimental Fish

African catfish fingerlings, averaging a total length of 9.51 ± 0.20 cm and a weight of 6.18 ± 0.32 g, were obtained from A4 Global Fisheries in Kano and transported to the study site in a 50-liter jerrican filled halfway with fresh water. The fish were acclimated to the experimental conditions in a 1,500-liter plastic tank for two weeks and fed a commercial diet (Blue Crown, 2mm) containing 45% crude protein at 5% of their body weight twice daily, with equal rations given at 9:00 and 17:00 hours.

Source of Foreign DNA

Nile perch, averaging a total length of 25.45 ± 2.19 cm and a weight of 244.51 ± 62.21 g, were acquired from local fisherfolk at Dingare landing site of the River Hadejia. 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 undergoing DNA extraction processing. After 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.

Molecular Analysis

DNA was extracted from the liver of Nile perch following the protocols of Promega (2023). The quantity and purity of the extracted DNA were assessed using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, USA) at an absorbance of 260/280 nm with TE buffer as the blank. This involved placing 1 µl of

the DNA samples on the Nanodrop after blanking with TE buffer used for DNA elution. The quality of the DNA was evaluated through 1% agarose gel electrophoresis (Invitrogen, California, USA). The DNA was stored at -20°C until further analysis. Subsequently, the extracted DNA was cleaved using the EcoR1 type II restriction enzyme (New England Biolabs® Inc. R0101S) to generate DNA fragments of varying sizes, enabling their integration into the host genome as per the manufacturer's instructions. EcoRI recognizes and cleaves DNA at the sequence 5'-GAATTC-3', resulting in sticky ends with overhanging single-stranded DNA. Agarose gel electrophoresis was performed following the procedure outlined by Williams et al. (1993) to confirm the restriction enzyme digestion of the genomic DNA. The digested DNA fragments were analyzed on a 2% agarose gel (Invitrogen, California, USA) and stained with 10 µl of ethidium bromide. The lengths of the DNA fragments were compared with a 100 bp DNA marker. The amplified pattern was visualized under a UV transilluminator and captured using a 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, 1, 2, 3 and 4 µg/0.1ml/fingerlings) and injected into the target host in vivo using a hypodermic needle (a disposable insulin syringe of 1 ml). The injection was administered intramuscularly at a 45° angle at the beginning of the fin, just above the lateral line, as described by Woynarovich & Horvath (1980).

Experimental Design

Two hundred and twenty-five (225) African catfish were randomly allocated into five treatments (T1, T2, T3, T4, and T5), with each treatment replicated three times in a completely randomized design (CRD). The feeding experiment utilized fifteen 100-liter plastic bowls, each containing 15 fish per experimental unit.

Experimental Management

The experimental fish were fed a commercial diet (Blue crown, 2 mm) containing 45% crude protein at 5% of their body weight twice daily in two equal rations at 9:00 and 17:00 hours for a total culture period of twenty weeks (140 days). The amount of feed given was adjusted biweekly based on the new weight of the fish. Any remaining feed was removed from the culture medium, and an equal amount of water was replaced immediately on a daily basis. The entire water in the system was completely renewed every three days, and the bottoms of the bowls were cleaned to remove any dirt from the medium. Dissolved oxygen, pH, and temperature levels were monitored weekly using a dissolved oxygen meter (Model: Hanna H19146) and a pH/temperature pen (Model: Comboly Hanna H198130) throughout the duration of the study.

Haematological and Biochemical Analysis

Upon completion of the study, blood samples were obtained from the caudal vein fin of the experimental fish, following the protocol outlined by Klontz & Smith (1986). These samples were then transported to the Haematology Laboratory at the Federal University Dutse Teaching Hospital for haematological analysis, utilizing techniques established in fish haematology as per Ivanova (1983) and Haghighi (2010).

Serum was obtained by centrifugation of blood at 3000 rpm for 20 minutes and stored at –20°C for biochemical analysis as follows;

I) Serum total protein was measured by Biuret method, as described by Armstrong and Carr (1964).

ii) Albumin concentration was determined according to the method of Doumas *et al.* (1971).

iii) Globulin concentration was estimated by subtraction of albumin concentration from serum total protein value (Assem & El-Zaeem, 2005).

iv) Albumin/Globulin ratio was estimated by dividing albumin by globulin (Assem & El-Zaeem, 2005).

Statistical Analysis

The data collected on haematological and biochemical indices underwent one-way analysis of variance (ANOVA), and the significant mean differences among the various groups were compared using the Duncan multiple range test ($p < 0.05$). The analysis was conducted using IBM SPSS Statistics 27.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA), and GraphPad Prism 10 was utilized for creating the figures.

RESULTS

Haematological Parameters of African Catfish injected with Varying Concentrations of Nile Perch DNA

The results of the haematological parameters of African catfish injected with varying concentrations of Nile perch DNA is presented in Table 1. The red blood cell (RBC) count exhibited a significant difference ($p \leq 0.05$), with T3 demonstrating the highest RBC count (2.15 × 10^12/L). However, a decline in RBC count was noted in T5 (1.19 × 10^12/L). The white blood cell (WBC) count also rose significantly ($p \leq 0.05$) in response to higher doses of DNA, peaking at T5 with a count of 37.18 × 10^9/L, while the lowest count was observed in T1 at 13.64 × 10^9/L. Mean corpuscular volume (MCV) values remained relatively stable across the treatment groups, with minor fluctuations. T2 recorded the highest MCV at 145.47 fL, while T3 recorded the lowest MCV at 137.40 fL. Mean corpuscular hemoglobin (MCH) values increased progressively from T1 to T5, with T5 achieving the highest value of 32.47 pg and T1 the lowest value of 30.87 pg. However, these values were not significantly different ($p > 0.05$). Mean corpuscular hemoglobin concentration (MCHC) values displayed slight variations but remained consistent ($p > 0.05$) across all groups, with T2 showing the highest MCHC at 22.80 g/dL and T4 the lowest at 22.20 g/dL. Hematocrit (HCT) values revealed significant differences ($p \leq 0.05$) among the treatment groups; a trend of higher values was observed in T3 (29.47%) and T4 (27.87%), with a lower value in T5 (16.75%). Hemoglobin (HGB) levels exhibited a slight increase with higher DNA doses, with T4 presenting the highest level at 6.57 g/dL, followed by T5 at 6.17 g/dL, and the lowest observed in T1 (3.80 g/dL). Red cell distribution width-coefficient of variation (RDW-CV) values varied significantly among the groups, with T5 showing the highest value of 17.65% and the lowest in T1 (12.20%); however, this did not significantly differ from the other injected groups. There was a significant difference ($p \leq 0.05$) in the RDW-standard deviation (RDW-SD) values across the treatment groups, with T4 recording the highest value (94.20 fL) and the lowest value in T1 (81.93 fL). Lymphocyte (LYM) counts increased significantly ($p \leq 0.05$) with higher DNA doses, with T5 recording the highest count at 35.73 × 10^9/L and T1 the lowest at 13.26 × 10^9/L.

Table 1: Haematological Parameters of African catfish Injected with Varying Concentrations of Nile Perch DNA

Parameter	Treatment groups					
	T1 (Control)	T2 (1 µg)	T3 (2 µg)	T4 (3 µg)	T5 (4 µg)	P-value
RBC (10^12/L)	1.55±1.14^{e}	1.79±0.14^{c}	2.15±0.48^{a}	1.96±0.21^{b}	1.19±0.74 ^{d}	0.043
WBC (10^9/L)	13.64±12.08^{e}	24.14±5.41^{d}	26.95±36.87^{c}	34.70±17.86^{b}	37.18±14.79^{a}	0.023
MCV (fl)	138.60±7.66^{c}	145.47±9.58^{a}	137.40±5.75^{c}	142.47±2.83^{b}	140.55±0.35^{b}	0.045
MCH (pg)	30.87±1.01^{a}	30.73±1.56^{a}	31.50±1.13^{a}	31.63±0.60^{a}	32.47±1.86^{a}	0.545
MCHC (g/dL)	22.33±0.51^{a}	22.80±0.92^{a}	22.37±1.16^{a}	22.20±0.26^{a}	22.45±0.78^{a}	0.907
HCT (%)	22.07±16.09^{b}	24.23±1.96^{b}	29.43±5.66^{a}	27.87±3.09^{a}	16.75±10.39^{c}	0.034
HGB (g/dL)	3.80±2.40^{c}	4.97±3.74^{b}	5.50±0.61^{b}	6.57±1.10^{a}	6.17±0.60^{a}	0.026
RDW-CV (%)	12.20±0.87^{b}	12.23±0.40^{b}	12.43±0.55^{b}	12.97±1.02^{b}	17.65±0.21^{a}	0.031
RDW-SD (fl)	81.93±2.23^{e}	90.67±4.75^{b}	84.80±5.81^{d}	94.20±12.94^{a}	88.90±0.99^{c}	0.001
LYM (10^9/L)	13.26±12.11^{d}	25.50±5.45^{c}	25.59±35.12^{c}	27.59±24.98^{b}	35.73±13.75^{a}	0.042

Means having similar superscripts within raw are not significantly different ($p > 0.05$)

Key: RBC= Red Blood Cells, WBC= White Blood Cells, MCV= Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration, HCT= Hematocrit, HGB= Hemoglobin, RDW-CV= Red Cell Distribution Width - Coefficient of Variation, RDW-SD= Red Cell Distribution Width - Standard Deviation, LYM= Lymphocytes.

Biochemical Indices of African Catfish injected with Varying Concentrations of Nile Perch DNA

The results of the biochemical indices of African catfish injected with varying concentrations of Nile Perch DNA is presented in Figure 1. Total protein levels exhibited significant variation ($p \leq 0.05$) among the treatment groups. The T4 (3 µg) group recorded the highest concentration at 31.93 g/l, while the control group (T1) demonstrated a lower concentration of 24.87 g/l. Albumin levels were relatively stable across T1, T2, T3, T4, and T5, with no significant difference observed ($p > 0.05$); however, T1 had a higher level of 15.83 g/l compared to T3, which had a lower level of 14.90 g/l. Globulin concentrations also varied significantly ($p \leq 0.05$), with T4 exhibiting a higher level of 16.45 g/l, while T1 showed lower levels at 12.37 g/l. The albumin/globulin (A/G) ratio was highest in the T2 group at 1.20, decreasing in T5 to 0.89, with significant differences ($p \leq 0.05$) noted across all treatment groups.

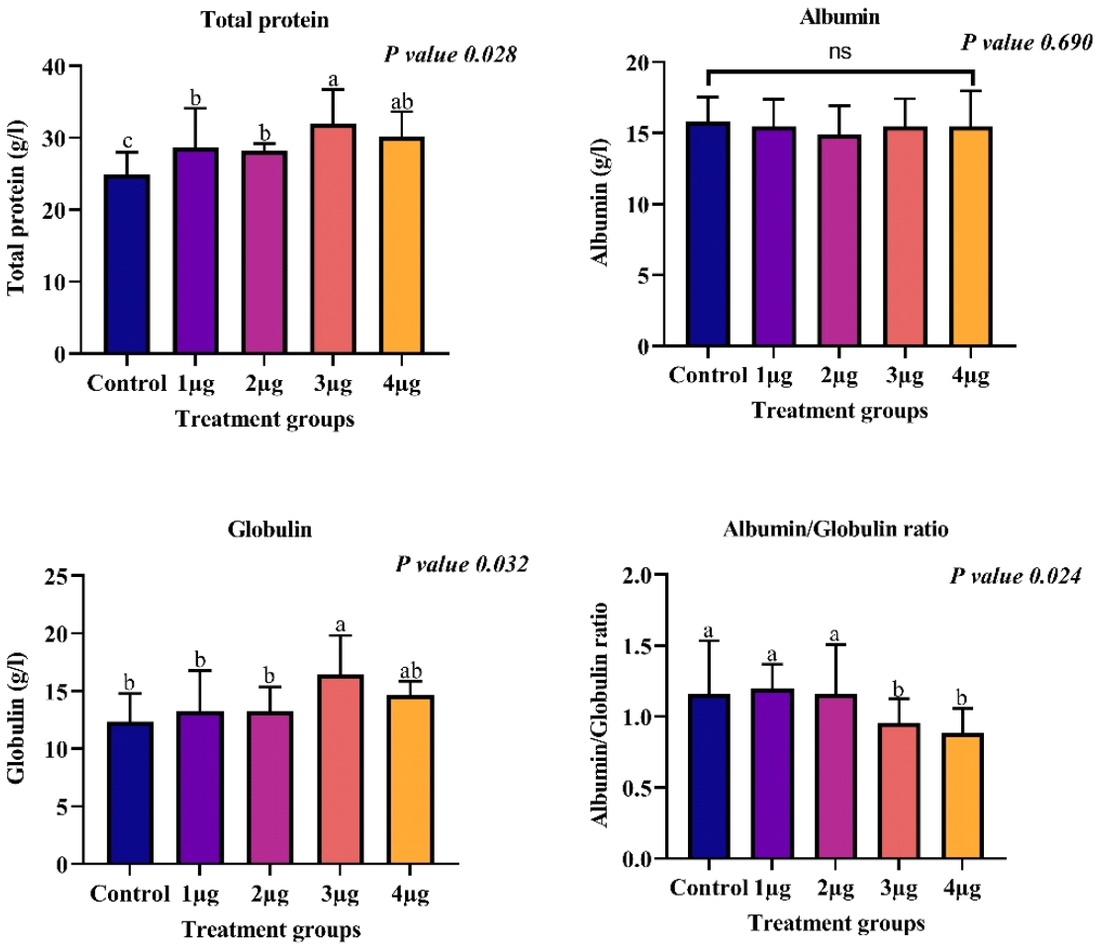

Figure 1: Biochemical Analysis of African Catfish Injected with Varying Concentrations of Nile Perch DNA

DISCUSSION

Haematological Parameters of African Catfish injected with Varying Concentrations of Nile Perch DNA

Haematological parameters are useful resources for assessing fish health in relation to dietary manipulations and tracking physiological and pathological changes in fish as a result of such feed manipulations (Jimoh *et al.*, 2016). According to Jimoh *et al.* (2020a) and Jimoh *et al.* (2020b), dietary manipulations, malnutrition, and disease conditions can all alter blood composition. The analysis indicates both improvements and variations in these parameters based on the DNA dosage administered. The red blood cell (RBC) count increased progressively from the control group (T1) to T3, suggesting enhanced erythropoiesis at moderate DNA doses. This finding is consistent with Adegbesan *et al.* (2018), who noted significant increases in RBC counts with specific treatments. The white blood cell (WBC) count also rose with increasing DNA doses, indicating an improved immune response crucial for fish health and disease resistance. These results align with findings from Adegbesan *et al.* (2018) and Jimoh *et al.* (2022), who reported increased WBC counts in fish subjected to various nutritional and

environmental treatments, suggesting enhanced immunomodulatory effects. Mean corpuscular volume (MCV) values remained stable across treatment groups, with T2 recording the highest MCV (145.47 fl). Mean corpuscular hemoglobin (MCH) values increased from T1 to T5, reaching a maximum of 32.47 pg in T5. Mean corpuscular hemoglobin concentration (MCHC) showed slight variations but remained consistent, with T2 exhibiting the highest MCHC (22.80 0.92 g/dL). The stability of MCV and MCHC suggests that DNA injections do not significantly alter the size or hemoglobin concentration of individual RBCs. This is consistent with Okore *et al*. (2016), who observed variations in MCV based on dietary treatments. Haematocrit (HCT) values did not show significant differences among treatment groups, although T3 and T4 exhibited higher trends. Hemoglobin (HGB) levels slightly increased with higher DNA doses, with T4 presenting the highest level (6.57 g/dL), indicating that moderate doses of Nile perch DNA can enhance the blood's oxygen-carrying capacity without significantly altering overall blood volume. Red cell distribution width-coefficient of variation (RDW-CV) values varied significantly, with T5 showing the highest value (17.65%), indicating greater variation in RBC size at higher DNA doses. In contrast, red cell distribution width-standard deviation (RDW-SD) values remained consistent across groups, suggesting uniform RBC size distribution within each treatment. Lymphocyte (LYM) counts increased with higher DNA doses, with T5 recording the highest count (35.73 x 10^9/L), indicating enhanced immune cell production, consistent with findings by Adegbesan *et al*. (2018).

Biochemical Indices of African Catfish injected with Varying Concentrations of Nile Perch DNA

Immunological or physiological parameters from healthy fish may be obtained from blood samples as indirect measurements of disease resistance. The levels of serum proteins that are present in the blood serum indicate the progress of immune system development (Assem & El-Zaeem, 2005). The observed variation in total protein levels among treatment groups is consistent with the findings of Assem & El-Zaeem (2005), who noted increased total protein concentrations in *Tilapia zillii* following Shark DNA injections. T4 (3 µg) exhibited the highest total protein concentration at 31.93 g/l, suggesting that higher doses of Nile perch DNA may enhance protein synthesis and metabolic function in the fish. In contrast, the lower total protein levels in the control group (T1) at 24.87 g/l, indicating that the dose may be insufficient to stimulate protein synthesis. Albumin, a negatively charged, relatively small protein synthesized by liver cells, is the most abundant protein in extracellular fluid, contributing to approximately 70% of the plasma colloid osmotic pressure (Bernadi et al., 2014). Albumin levels were relatively stable across most treatments. The control group recorded the highest albumin level at 15.83 g/l. This elevated albumin level in the control group may indicate better overall health and optimal liver function, as albumin is primarily synthesized in the liver (Levitt & Levitt, 2016). The absence of foreign DNA injection in the control group likely resulted in less physiological stress, allowing the liver to function more efficiently and maintain higher albumin production. In contrast, globulin concentrations varied significantly, with T4 reaching 16.45 g/l, suggesting an immune response triggered by the DNA injection. This increase in globulin aligns with findings from Magnadottir (1998) and Assem & El-Zaeem (2005), which reported elevated IgM and globulin levels in teleosts and *Tilapia zillii* following DNA injection, indicating a heightened immune response. The albumin/globulin (A/G) ratio is a key indicator of nutritional status and overall health in fish. The T2 group exhibited the highest A/G ratio, reflecting a balanced protein profile. However, the A/G ratio significantly decreased in T5, suggesting a shift towards a more immune-dominated response. This reduction is

consistent with previous studies indicating that increased globulin levels, often due to immune stimulation, can lead to a lower A/G ratio (Assem & El-Zaeem, 2005).

Conclusion

The haematological and biochemical indices of *Clarias gariepinus* fingerlings showed improvement when injected with 4 and 3 µg of Nile perch DNA, as evidenced by higher white blood cell counts and total protein levels, respectively. This suggests that the haematological and biochemical indices of *C. gariepinus* fingerlings can be enhanced through the direct intramuscular injection of Nile perch DNA.

Acknowledgement

The authors express their gratitude to the Tertiary Education Trust Fund Institutional Based Research (TETFund IBR 2024) for supporting this research.

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