

EVALUATION OF SERUM BIOCHEMICAL CHANGES IN *Clarias gariepinus* EXPOSED TO DIFFERENT CONCENTRATIONS OF DRIED POULTRY DROPPINGS

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ABSTRACT

This study assessed the levels of serum biochemical changes in *C. gariepinus* juveniles exposed to varying concentrations of dried poultry droppings. The dried poultry droppings were collected from Green Gold Construct Layers Unit in Ikole-Ekiti, Ekiti State, Nigeria. Ten (10) fish were randomly allocated to five treatments (T1, T2, T3, T4, and T5) in triplicate for 8 weeks. Blood samples were collected to assess serum protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, and urea. The changes observed were concentration dependent, with the highest biochemical alterations recorded in fish exposed to 6.0 g/L (T5) showing increased ALP (97.60 IU/L), urea (0.88 mmol/L), and creatinine (8.20 mmol/L), while the lowest changes were observed in the control group (T1) with stable biochemical values. The results revealed significant changes in the biochemical indicators in exposed treatments compared to the control. Increase in AST, ALT, urea, and creatinine concentration could be hepatic and renal dysfunction, while decrease in total protein and albumin suggested nutritional imbalance. Findings showed that *C. gariepinus* exposure to dried poultry droppings may affect physiological functions even at lower concentration. Unregulated disposal of dried poultry droppings in aquatic environments posed a serious ecological implication; hence, stringent waste management practice is encouraged.

Keywords: *Clarias gariepinus*, Serum biochemistry, Dried poultry droppings, Aquatic pollution, Fish toxicology.

1. INTRODUCTION

Aquaculture has emerged as one of the fastest-growing sectors of the global food production industry and offers humans all over the world a source of high-quality protein (Burić *et al.*, 2020; Young *et al.*, 2020). The aquaculture industry's explosive rise has contributed to reducing human over-reliance on natural fish stocks. Global aquaculture production will need to triple in the coming ten years to meet the demand for fish for human consumption due to the world's population expansion (Food and Agriculture Organisation, 2018). Global fisheries pressure, however, will also keep rising (Organisation for Economic Co-operation and Development, 2018). The rising demand for fish has prompted fish farmers to either expand their feeding production or increase the stocking rate. Nevertheless, the high price of feed has slowed down aquaculture's expansion and made the need for lower-cost sources. By promoting planktonic growth, releasing nitrogen and phosphorus, and supplying organic carbon through the heterotrophic cycle, animal manures are used in intensive cultures to increase fish production

(Ajiboye *et al.*, 2017). For thousands of years, fish farming has used chicken droppings as a widespread practice all throughout the world. Among the many substances found in poultry droppings include pathogens, antibiotics, heavy metals, uric acid, and ammonia. The main harmful component, ammonia, can harm fish by degrading the quality of the water, which impacts breathing, growth, and general health. A useful method for assessing the physiological state of cultured fish and figuring out how chicken droppings affect fish blood health is blood analysis. Fish exposed to chemicals may experience an increase or decrease in their haematological index values (Adesina *et al.*, 2017). Fish alterations in their haematological responses to stressors are markers of their stressful stages and provide helpful hints for halting any unfavourable situation that could endanger their health (Adesina, 2017). *Clarias gariepinus* is one of the most popular cultural species among Nigerian fish farmers and it is highly valued commercially in the market and is highly appreciated

by fish growers both in Nigeria and throughout Africa (Akinsorotan *et al.*, 2024). Prior research using dried poultry droppings assessed *Clarias gariepinus* growth, microbial properties, anaemia, haematological abnormalities, and nutrient utilisation (Omitogun *et al.*, 2006; Orji & Udonwu, 2006; Ajiboye *et al.*, 2017). The possible detrimental impacts of dried poultry droppings on the serum biochemistry of *C. gariepinus*, however, have received little to no consideration in studies. Therefore, the aim of the study is to assess the *C. gariepinus* juveniles' serum biochemical changes.

2. MATERIALS AND METHODS

2.1 Poultry Droppings

Fresh poultry droppings were collected from green gold construct Nigeria Limited's layers unit in Ikole Ekiti, Ekiti State. The poultry droppings were spread on a clean roofing sheet, covered with a net with a mesh size of 1.2 millimeters, and sun-dried for three weeks to prevent the growth of moulds that could be hazardous to the fish. After drying, the poultry droppings, were grinded and stored at room temperature in a plastic bag.

2.2 Sample Collection

Two hundred and fifty (250) healthy samples of *C. gariepinus* were obtained from a reputable fish farm in Ado Ekiti with mean weight of 23.51g and mean length of 13.9 cm. The fish samples were conditioned and transported to the wet laboratory of Federal University Oye-Ekiti, Ikole Campus inside a 50-liter container filled with clean, oxygenated water. The fish were acclimatized for 14 days period. Their water was changed every two days to remove faecal and uneaten feed. Fish were fed twice a day a commercial feed at 08.00am and 17.00pm to ad libitum. Feeding was stopped 24 hours before the beginning the experiment.

2.3 Experimental Procedure

After 14 days acclimatization period, the fish were randomly divided into five treatments in triplicate with 10 fish per treatment group in a

transparent 50 litre volume dimension tank and exposed to different concentrations of dried poultry droppings (0g, 3g, 4g, 5g, and 6g) in control T1, T2, T3, T4, and T5 Respectively. The feeding trial was eight (8) weeks. Fish were fed at 5% body weight. Fish samples were weighed bi-weekly, and the new weight was used to calculate the amount of feed provided to the fish samples.

2.4 Serum Biochemical Analysis

Fish from each treatment group were sample gently at random after the feeding trial. With 2.5 ml sterile plastic syringes, blood sample was withdrawn from caudal vein located ventrally of the vertebral column. 2ml of blood sample was placed in a clean test tube, allowed for 30 minutes to clot and centrifuged at 2000g for 10 minutes and the serum harvested according to the method of Tomlinson *et al.* (2013).

The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by colorimetric method using RAM diagnostic kit according to the method of (Reitman *et al.*, 1957) at 530–550 nm. The serum alkaline phosphatase activity was measured according to the modified method of (Moss, 1982). The serum total protein levels were estimated according to (Grant, 1987). The serum albumin level was evaluated according to (Doumas *et al.*, 1997). According to Doumas and Biggs (1971), the serum globulins levels were calculated mathematically by subtracting albumin values from total protein values. The total serum lipids, total cholesterol, triglyceride, and high-density lipoprotein (HDL-c) were estimated according to the methods of (Zöllner and Kirsch, 1966), respectively.

2.5 Statistical Analysis of Data

The data collected were Analysed using One way analysis of variance (ANOVA) SPSS version 21. Separation of means was carried out using Duncan's Multiple Range Test was used to compare the differences among the means.

3. RESULTS

The results of the biochemical reactions of *Clarias gariepinus* juveniles subjected to different concentrations of dried poultry droppings revealed levels of dependent changes in a number of physiological function indicators (Table 1). The Alkaline Phosphatase (ALP) values range from 79.65 ± 2.55 IU/L in T3 which is the lowest to 97.60 ± 0.00 IU/L in T5 as the highest value. A significant difference was observed across treatments (p<0.05). However, the results reduce in T3 compare to the control T1 which could be as a result of stress or altered enzymes synthesis due to moderate exposure of the fish to poultry droppings. Total protein concentration increases significantly in T2 (70.5 ± 0.50 g/L) as compared to T1 (46.6 ± 8.80 g/L)

the lowest. A decline was observed as poultry dropping concentration increases in T3 to T5. The decrease in protein levels may reflect toxicant stress or systemic inflammation. Serum urea and creatinine concentrations shows no significant differences (p>0.05) across treatments. However, a slight increase was observed in T3 (0.90 ± 0.01 mmol/L) for Urea and (11.25 ± 1.05 mmol/L) for creatinine. Across all treatments, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was below detectable thresholds (<2 and <4 IU/L) respectively, showing that the exposure concentrations may not have caused overt hepatic necrosis or cytoplasmic enzyme leakage that these investigations could have detected.

Table 1: Alkaline Phosphatase Activity (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Concentrations of Total Protein, Urea, Creatinine.

Treatments	ALP (iu/L)	Total Protein (g/L)	Urea (mmol/L)	Creatinine (mmol/L)	AST	ALT (iu/L)
1	97.10± 0.50 ^b	46.6± 8.80 ^a	0.83± 0.075 ^a	10.2± .000 ^a	<2	<4
2	86.65± 9.55 ^{ab}	70.5± 0.50 ^b	0.84± 0.060 ^a	10.25±1.050 ^a	<2	<4
3	79.65± 2.55 ^a	53.9± 0.90 ^{ab}	0.90± 0.010 ^a	11.25±1.050 ^a	<2	<4
4	91.20± 1.30 ^{ab}	61.4± 4.75 ^{ab}	0.83± 0.065 ^a	10.25±1.050 ^a	<2	<4
5	97.60± .000 ^b	61.5± 1.00 ^{ab}	0.88±0.005 ^a	8.20± .000 ^a	<2	<4

Mean ±SD. Values with the same superscript across the column are not significantly different at P≤0.05 DMRT.

The concentrations of serum lipids and lipoproteins in *Clarias gariepinus* juveniles exposed to different concentrations of dried poultry droppings are presented in Table 2. Total cholesterol (TC) slightly decreases with increased concentration of poultry droppings, as observed in T3 to T5. Triglyceride levels range from 3.48 to 3.83 mg/dL, with the highest concentration in T3. low-density lipoprotein (LDL) level decreases with increasing concentration, particularly in Treatments 3 to 5. LDL decreases from 4.27 ± 0.46 mg/dL to 2.94 ± 0.91 mg/dL. High-density lipoproteins (HDL) decrease slightly with an increase in

poultry droppings concentration, with the highest value in T1 (1.46 ± 0.15) and the lowest recorded in T5 (1.33 ± 0.03). No significant differences (P>0.05) were observed among lipids and lipoproteins observed in *C. gariepinus* juveniles. Because of the toxic components of the poultry droppings, like uric acid and ammonia, these decreases in total cholesterol (TC) and low-density lipoprotein (LDL) may indicate hepatic dysfunction or disruption in lipid metabolism, whereas high-density lipoproteins (HDL) suggest a degree of physiological compensation, perhaps reflecting the fish's adaptive metabolic response.

Table 2: Concentration of Lipids and Lipoproteins

Treatments	TC	Triglyc.	HDLipo.p	LDLipo.p
1	7.14 ^a ± 0.65	3.69 ^a ± 0.30	1.46 ^a ± 0.15	4.09 ^a ± 0.39
2	7.12 ^a ± 0.67	3.57 ^a ± 0.09	1.38 ^a ± 0.02	4.27 ^a ± 0.46
3	6.49 ^a ± 0.26	3.83 ^a ± 0.35	1.33 ^a ± 0.07	3.42 ^a ± 0.17
4	5.84 ^a ± 0.91	3.48 ^a ± 0.09	1.33 ^a ± 0.03	2.94 ^a ± 0.91
5	6.62 ^a ± 0.13	3.74 ^a ± 0.26	1.35 ^a ± 0.05	3.57 ^a ± 0.06

TC = Total Cholesterol; HDLipo.p =High Density lipo-protein; LDP= Low Density lipo protein; Triglyc. = Triglyceride. Uses Harmonic mean sample size = 2.00. The value in each row having the same superscript is not significantly different.

Table 3 presents serum electrolyte balance in *C. gariepinus* juveniles exposed to dried poultry droppings. Results show that potassium (K⁺) concentration increases in T2 (3.18±0.75 mmol/L) and T3 (3.33±0.21 mmol/L), respectively, compared to T1 (2.17±0.05

mmol/L). Sodium (Na⁺) and chloride (Cl⁻) levels remained relatively stable across treatments. Bicarbonate (HCO₃⁻) levels showed little variation (1.60–2.10 mmol/L), indicating that acid-base balance and buffering capacity were generally maintained.

Table 3: Electrolyte Levels

Treatments	K	Na	Cl	HCO ₃
1	2.17± 0.05	151.5± 0.60	150.0±0.65	1.70± 0.50
2	3.18± 0.75	150.5± 0.45	151.1± 1.35	2.05± 0.25
3	3.33± 0.21	150.4± 0.85	154.4±1.15	1.60± 0.50
4	2.65± 0.47	151.2± 1.15	150.2± 0.07	2.10± 0.20
5	2.08± 0.02	152.3± 0.00	149.9±1.30	1.75 ± 0.35

Mean ±SD. Values with the same superscript across the column are not significantly different at $p \leq 0.05$ DMRT.

4.1 DISCUSSION

In aquatic ecotoxicology studies, fish are good bioindicators because of their great sensitivity to changes in the aquatic environment. Fish that comes into contact with organic pollutants like poultry droppings and other livestock waste are susceptible to a number of biochemical, physiological, and behavioural changes, frequently before any obvious symptoms appear (Zhang *et al.*, 2023). The abundance of nitrogenous chemicals, antibiotics, and pathogens in these animal waste products can interfere with aquatic species' vital metabolic functions and organ function (Raza *et al.*, 2022). Fish serum biochemical variables are sensitive indications of liver function, metabolic health, and systemic stress (Rani *et al.*, 2022). It is particularly pertinent to evaluate these markers in the widely cultivated African catfish species *C. gariepinus* because of its ecological and

commercial significance and its demonstrated resilience to a variety of environmental stressors (Nwani *et al.*, 2021). Specifically, the levels of Alkaline phosphatase (ALP) in this research shows treatment dependent variation across treatments. The enzyme alkaline phosphatase (ALP) has been utilised extensively as a biomarker for organ stress and membrane damage and is connected with intestine and liver function. According to Banaee *et al.* (2013), variations in ALP levels across treatments are indicative of variations in hepatic stress, enzyme induction, or membrane permeability. The ALP fluctuation observed in this study is similar to the results of Yilmaz *et al.* (2020), who found that rainbow trout exposed to chicken dropping effluent had changed ALP levels. They attributed this fluctuation to hepatobiliary stress. This study's decrease in ALP at T3 and increase at T5

observed are consistent with the biphasic response pattern described by Ekanem *et al.* (2021), who discovered that ALP levels were suppressed at moderate exposure to cassava wastewater but induced at higher dosages as a result of escalating liver strain. The concentration of total serum protein showed a biphasic response, increasing at moderate exposure (T2) and then declining in T3 through T5. The first rise could be an immunological response or acute phase protein synthesis, but the following fall at greater exposure levels most likely indicates immunosuppression, hepatic dysfunction, or protein degradation (Adeyemo, 2008; Kumar *et al.*, 2016). The findings in fish exposed to different agro-waste effluents, where toxicant stress damages protein metabolism and disturbs homeostasis, are in agreement with the finding of Raza *et al.* (2022). Although not statistically significant, serum urea and creatinine levels indicated low increases in T3. Because being around organic waste usually doesn't kill aquatic species, these changes might be early signs of kidney problems or less effective waste removal (Zhang *et al.*, 2023). According to Wang *et al.* (2021) and Zhang *et al.* (2023), fish exposed to livestock and poultry manure showed dose-dependent increases in these parameters. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were below detectable thresholds (<4 and <2 IU/L, respectively), indicating that neither cytoplasmic enzyme leakage nor considerable hepatocellular necrosis was caused by the exposure concentrations. However, the undetectable AST and ALT levels in this study differ from those of Eze *et al.* (2020) and Rani *et al.* (2022), who found that *O. niloticus* exposed to cassava and industrial effluents, respectively, had markedly higher levels of these enzymes. It's possible that the study's lack of measurable AST/ALT indicates that the exposure levels were below necrotic thresholds. Given that *C. gariepinus* is known to have a high level of xenobiotic resilience, it might also be a reflection of species-specific sensitivity (Adeyemo, 2008). The theory of modified hepatic lipid metabolism, which may be brought on by hepatocellular injury or the regulation of cholesterol production pathways, is supported by the decrease in total cholesterol

levels and low-density lipoproteins (LDL) with increasing concentrations of poultry droppings. This result is consistent with that of Eze *et al.* (2020) and Rani *et al.* (2022), who found that exposure to cassava and chicken manure decreased blood lipids associated with oxidative stress and liver damage in *Oreochromis niloticus* and *Clarias gariepinus*. High-density lipoprotein (HDL) and triglyceride levels were comparatively constant across treatments, indicating either selective targeting of lipid components or some degree of metabolic compensation. However, as LDL and TC are sensitive indicators of hepatic and endocrine disturbance in fish, the biological significance of regular declines in these parameters cannot be discounted (Raza *et al.*, 2022). However, this study's constant HDL and triglyceride levels contrast with those of Rani *et al.* (2022), who found that fish exposed to textile effluents had decreased levels of all lipid markers. This variance might be the result of variations in the kinds of contaminants, the length of exposure, or physiologically unique adaptation reactions of *C. gariepinus*. The significant rise of serum potassium (K^+) in T2 and T3 is indication of gill epithelial malfunction since K^+ is strictly controlled by kidney and gill ion transport systems. This is line with the findings of Nwani *et al.* (2021), catfish exposed to nitrogen-rich agro-effluents showed increased K^+ . Gill damage or ionic leakage as a result of membrane destabilisation are frequently linked to such increases (Banaee *et al.*, 2013). Across treatments, serum sodium (Na^+) and chloride (Cl^-) stayed relatively constant, indicating that fundamental osmoregulatory processes were not considerably compromised. Similar to this, the fish-maintained acid-base balance based on the small changes in bicarbonate (HCO_3^-), however the little increase could be buffering reactions to internal acid load (Chavan *et al.*, 2017). The stability of sodium and chloride levels, however, differs from the results of Chavan *et al.* (2017), who found that freshwater fish exposed to heavy metals saw a marked decrease in Na^+ and Cl^- levels, suggesting decreased ion uptake. Under the tested exposure regime, this implies that although chicken droppings affect K^+

regulation, they might not substantially alter the Na⁺/Cl⁻ balance. Nwani *et al.* (2021) observed increased potassium (K⁺) in catfish exposed to nitrogen-rich agro-effluents, and the rise in K⁺ levels in Treatments 2 and 3 is consistent with their findings. Gill damage or ionic leakage as a result of membrane destabilisation are frequently linked to such increases (Banaee *et al.*, 2013). However, relative stability of sodium and chloride levels contrasts with findings by Chavan *et al.* (2017), who found that exposure to heavy metals in freshwater fish significantly decreased the levels of Na⁺ and Cl⁻, indicating reduced ion absorption. Under the tested exposure regime, this implies that although chicken droppings affect K⁺ regulation, they might not substantially alter the Na⁺/Cl⁻ balance.

5. CONCLUSION

The present findings have established that exposure to different concentrations of dried poultry droppings induces significant serum biochemical changes in *Clarias gariepinus* juveniles. The highest alterations were observed in fish exposed to the highest concentration, with notable increases in alkaline phosphatase, urea, and creatinine, indicating early physiological stress, particularly affecting hepatic and osmoregulatory functions. Conversely, the lowest changes were recorded in the control group, where parameters remained stable. Some parameters, such as AST, ALT, and electrolytes like sodium and chloride, were largely unaffected. Considering the ecological and economic importance of *C. gariepinus*, these results underscore the urgent need for sustainable waste disposal strategies and stricter policies to mitigate pollution-related impacts on aquatic ecosystems.

ETHICAL APPROVAL FOR ANIMAL USE

Experimental animal care was carried out in accordance with the relevant guidelines/regulation in Nigeria.

CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this manuscript.

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