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HAEMATOLOGICAL AND BIOCHEMICAL RESPONSES OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*, BURCHELL, 1822) TO GRADED DIETARY LEVELS OF FERMENTED *Albizia lebbeck* SEED MEAL

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ABSTRACT

This study evaluated the haematological and serum biochemical responses of African catfish (*Clarias gariepinus*) juveniles fed graded dietary levels of fermented *Albizia lebbeck* seed meal (ALSM). Five isonitrogenous diets were formulated with ALSM replacing conventional protein at 0% (T1, control), 25% (T2), 50% (T3), 75% (T4), and 100% (T5). One hundred and fifty (150) fingerlings (mean initial weight: 15.3 ± 0.4 g) were randomly distributed into 15 tanks (10 fish/tank, 3 replicates/treatment) and fed at 5% body weight twice daily for 8 weeks. Haematological analysis revealed significant differences ($P < 0.05$) in haemoglobin (Hb), white blood cell count (WBC), red blood cell count (RBC), neutrophil, eosinophil, and lymphocyte percentages across treatments, while packed cell volume (PCV) showed no significant variation. The highest Hb (10.0 g/dL) was recorded at T4, whereas the highest WBC (10.0×10^9/L) and RBC (11.4×10^{12}/L) were recorded at T2 and T5, respectively. Serum biochemical analyses showed significant differences ($P < 0.05$) in total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), globulin, and glucose. Elevated ALT at T4 and AST at T2, combined with declining lymphocyte percentages and hypoglycaemia at T5, suggest dose-dependent hepatic stress and immunosuppression at high inclusion levels. These findings indicate that fermented ALSM can be incorporated up to 50% replacement level without compromising fish health, but inclusion beyond 75% should be approached cautiously in practical feed formulation.

Keywords: *Albizia lebbeck*; African catfish; anti-nutritional factors; biochemical profile; *Clarias gariepinus*; fermentation; haematology; alternative protein

1.0 INTRODUCTION

Global aquaculture production has expanded rapidly in recent decades, placing immense pressure on conventional protein sources, particularly fishmeal, which currently accounts for over 60% of production costs in many tropical aquaculture systems (FAO, 2022). In Nigeria, fish contributes approximately 40% of total animal protein intake, with *Clarias gariepinus* (African catfish) representing one of the most economically important cultured species owing to its fast growth rate, hardiness, tolerance of high stocking densities, and ability to utilize formulated feeds (Al-Khalaifah *et al.*, 2020). The increasing cost and limited availability of fishmeal have necessitated extensive research into cost-effective, locally available alternative protein sources for aquafeed formulation (Francis *et al.*, 2021).

Albizia lebbeck (Benth.), commonly known as white siris or Indian walnut, is a multipurpose leguminous tree (family Fabaceae) widespread across sub-Saharan Africa, South Asia, and the Caribbean. Its seeds contain 20–28% crude protein, significant quantities of essential amino acids, and appreciable levels of lipids and minerals (Jha *et al.*, 2012; Ali *et al.*, 2023). However, the nutritional utility of raw *A. lebbeck* seed meal is limited by anti-nutritional factors (ANFs) including tannins, saponins, trypsin inhibitors, and phytates, which impair digestibility and nutrient bioavailability (Francis *et al.*, 2021; Akinwande *et al.*, 2024). Fermentation has been widely demonstrated as an effective low-cost technique to reduce ANFs, improve protein digestibility, and enhance amino acid availability in legume-based feed ingredients (Oyewole *et al.*, 2020).

Fish haematology and serum biochemistry are sensitive, non-lethal diagnostic tools for assessing nutritional status, immune competence, and overall health in cultured fish (Adeyemo *et al.*, 2003; Ayoola *et al.*, 2014). Parameters such as haemoglobin, packed cell volume, red and white blood cell counts, serum transaminases (ALT and AST), albumin, and glucose provide reliable indicators of metabolic homeostasis, hepatic integrity, and immunological responses to dietary manipulation (Rehulka, 2002; Falaye *et al.*, 2011). Elevated transaminase activities, in particular, are widely employed as biomarkers of hepatocellular damage resulting from dietary ANFs or nutritional imbalances (Tukur *et al.*, 2022).

Despite growing interest in *A. lebbeck* as a feed ingredient, comprehensive data on its effects on the haematological and serum biochemical profiles of *C. gariepinus* remain limited, particularly for fermented preparations. This study therefore investigated the dose-dependent effects of fermented *A. lebbeck* seed meal on the haematological and serum biochemical profiles of *C. gariepinus* juveniles, with the aim of identifying safe dietary inclusion thresholds for practical aquaculture application.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted at the Aquaculture Research Unit, Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria. Maiduguri is situated in the semi-arid Sahelian ecological zone at latitude 11.8261°N and longitude 13.1810°E, at an elevation of 354 m above sea level, with mean annual temperatures of 27–35°C (NOAA, 2016).

2.2 Collection and Processing of *Albizia lebbeck* Seed Meal

Albizia lebbeck seeds were harvested from mature, naturally growing trees near the University of Maiduguri campus. Seeds were manually separated from pods, air-dried under shade for 72 hours, and finely ground using an attrition mill. Fermentation was conducted by mixing seed flour with distilled water (1:2 w/v) in sealed polyethylene containers and incubating at 28 ± 2°C for 48 hours in darkness. Post-fermentation, the material was oven-dried at 60°C for 24 hours, re-milled to a uniform fine powder, and analysed for proximate composition and anti-nutritional factors using AOAC (2005) standard methods.

2.3 Diet Formulation and Experimental Design

Five isonitrogenous experimental diets (35% crude protein) were formulated with fermented *A. lebbeck* seed meal (ALSM) replacing the conventional protein component at 0% (T1, control), 25% (T2), 50% (T3), 75% (T4), and 100% (T5). Diets were pelleted using a motorized feed pelletizer, air-dried for 48 hours, and stored at 4°C until use. One hundred and fifty (150) *C. gariepinus* fingerlings (mean weight: 15.3 ± 0.4 g) were randomly assigned to 15 plastic tanks (10 fish/tank, 3 replicates/treatment) in a completely randomized design (CRD). Fish were fed at 5% body weight twice daily (07:30 and 17:30 h) for 8 weeks; rations were adjusted weekly based on individually recorded body weights.

2.4 Blood Sampling and Haematological Analysis

At the end of the feeding trial, three fish per replicate were randomly selected and anaesthetized with clove oil (50 mg/L). Blood was collected from the caudal vein using 5 mL heparinised syringes and divided into K_2EDTA-coated tubes for haematological analysis and plain tubes for serum separation (Whiteman, 2004). Packed cell volume (PCV) was determined by the micro-haematocrit method; haemoglobin (Hb) by Sahli's acid haematin method; red blood cell count (RBC) and white blood cell count (WBC) by haemocytometry following dilution with formal-citrate and Türk's solutions, respectively. Differential WBC counts (neutrophils, eosinophils, lymphocytes, monocytes, basophils) were performed on Giemsa-stained blood smears at 100× magnification. Derived erythrocyte indices — mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) — were calculated using standard formulae.

2.5 Serum Biochemical Analysis

Serum was separated by centrifugation (3000 rpm, 10 min) and stored at −20°C until analysis. Total protein was determined by the Biuret method; albumin by the bromocresol green (BCG) dye-binding method at 540 nm; globulin was calculated as the arithmetic difference between total protein and albumin. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the Reitman-Frankel colorimetric method using Randox diagnostic kits (Randox Laboratories, UK). Alkaline phosphatase (ALP) was determined by hydrolysis of p-nitrophenyl phosphate; glucose by the glucose oxidase–peroxidase (GOD-POD) enzymatic method. Serum electrolytes (sodium, Na^+; potassium, K^+) were determined by flame photometry. All analyses were conducted in triplicate, and results expressed as mean ± SEM.

2.6 Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics v.26. Significant differences among treatment means were separated using the

Least Significant Difference (LSD) post-hoc test at α = 0.05. Homogeneity of variance was verified by Levene's test.

3. RESULTS

3.1 Effect of Fermented Albizia lebbeck Seed Meal on Haematological Profile

The haematological responses of *C. gariepinus* to dietary fermented ALSM are summarized in Table 1. PCV did not differ significantly ($P > 0.05$) across treatments, ranging from 0.24 (T5) to 0.30 (T4). Haemoglobin was significantly influenced by dietary treatment ($P < 0.05$), with the highest value (10.00 g/dL) at T4 and the lowest (8.00 g/dL) at T5. WBC peaked at T2 (10.00×10^9/L) and was lowest at T1 (8.00×10^9/L). RBC was highest at T5 (11.40×10^{12}/L) and lowest at T4 (8.00×10^{12}/L). Neutrophil percentage was lowest at T2 (16.00%) and highest at T5 (40.50%), with a notable elevation at T3 (29.00%). Eosinophils were detected only in T1 (4.00%) and T3 (1.00%), being absent in T2, T4, and T5. Lymphocyte percentage was highest at T2 (84.0%) and declined with increasing inclusion beyond 25%, reaching 64.0% at T5.

Table 1: Haematological parameters of *Clarias gariepinus* juveniles fed diets containing graded levels of fermented *Albizia lebbeck* seed meal.

Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	SEM
PCV	0.25^{a}	0.28^{a}	0.26^{a}	0.30^{a}	0.24^{a}	0.017
Hb (g/dL)	8.30^{cd}	9.30^{b}	8.60^{c}	10.00^{a}	8.00^{d}	0.148
WBC ($\times 10^9$/L)	8.00^{c}	10.00^{a}	9.20^{b}	8.40^{c}	9.60^{ab}	0.189
RBC ($\times 10^{12}$/L)	10.40^{b}	11.00^{ab}	10.60^{b}	8.00^{c}	11.40^{a}	0.167
Neutrophils (%)	18.00^{bc}	16.00^{c}	29.00^{b}	20.00^{bc}	40.50^{a}	3.139
Eosinophils (%)	4.00^{a}	0.00^{b}	1.00^{b}	0.00^{b}	0.00^{b}	0.447
Lymphocytes (%)	78.00^{b}	84.00^{a}	70.00^{c}	80.00^{b}	64.00^{d}	0.894
Monocytes (%)	0	0	0	0	0	0
Basophils (%)	0	0	0	0	0	0

$^{a-d}$Means with different superscripts within a row differ significantly ($P < 0.05$). PCV = Packed Cell Volume; Hb = Haemoglobin; WBC = White Blood Cells; RBC = Red Blood Cells; SEM = Standard Error of Mean.

3.2 Effect of Fermented Albizia lebbeck Seed Meal on Serum Biochemical Profile

Serum biochemical parameters are presented in Table 2. Total protein (TP) was highest at T5 (34.00 g/L) and lowest at T2 (30.00 g/L). Albumin differed significantly ($P < 0.05$) among treatments, with the highest values at T1 and T5 (20.00 g/L each) and the lowest at T4 (17.50 g/L). Globulin was significantly elevated at T4 (16.00 g/L) compared with all other treatments. ALT activity was highest at T4 (27.00 U/L), significantly exceeding all other treatments ($P < 0.05$). AST activity peaked at T2 (31.00 U/L) and was markedly reduced at T4 (16.00 U/L) and T5 (20.00 U/L). Glucose concentration was highest at T4 (7.10 mmol/L) and lowest at T5 (2.50 mmol/L). Serum Na^+ and K^+ showed no significant differences ($P > 0.05$) among treatments.

Table 2: Serum biochemical parameters of *Clarias gariepinus* juveniles fed diets containing graded levels of fermented *Albizia lebbeck* seed meal.

Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)	SEM
TP (g/L)	33.00^{a}	30.00^{b}	32.50^{a}	33.00^{a}	34.00^{a}	0.625
ALB (g/L)	20.00^{a}	18.00^{ab}	19.00^{ab}	17.50^{b}	20.00^{a}	0.648
ALT (U/L)	24.00^{b}	23.00^{b}	24.00^{b}	27.00^{a}	24.00^{b}	0.655
GLO (g/L)	13.00^{b}	12.00^{b}	13.00^{b}	16.00^{a}	14.00^{ab}	0.651
AST (U/L)	27.00^{b}	31.00^{a}	28.00^{b}	16.00^{d}	20.00^{c}	0.571
GLU (mmol/L)	6.70^{ab}	5.60^{c}	6.30^{b}	7.10^{a}	2.50^{d}	0.161
K^+ (mmol/L)	4.30^{a}	4.50^{a}	4.20^{a}	4.40^{a}	4.10^{a}	0.179
Na^+ (mmol/L)	136.00^{a}	139.00^{a}	138.00^{a}	135.00^{a}	140.00^{a}	2.366

$^{a-d}$Means with different superscripts within a row differ significantly ($P < 0.05$). TP = Total Protein; ALB = Albumin; ALT = Alanine Aminotransferase; GLO = Globulin; AST = Aspartate Aminotransferase; GLU = Glucose; K^+ = Potassium; Na^+ = Sodium; SEM = Standard Error of Mean.

4. DISCUSSION

4.1 Haematological Responses

The non-significant variation in PCV across treatments in this study suggests that the dietary inclusion of fermented ALSM at all tested levels did not compromise the overall erythropoietic capacity of *C. gariepinus*. This finding is consistent with Adesina (2017), who reported stable PCV values in *C. gariepinus* fed sunflower seed meal diets, and with Inya *et al.* (2022), who observed that fish can maintain haematocrit within physiologically normal ranges (22–35%) when dietary protein quality is adequate.

The significantly elevated Hb at T4 (75% ALSM) is noteworthy. Haemoglobin concentration reflects the oxygen-carrying capacity of blood and is sensitive to dietary iron and copper bioavailability (Wickham *et al.*, 1990). Legume-based ingredients including *Albizia* species are known to contain appreciable concentrations of iron and haematinic precursors (Jha *et al.*, 2012), and fermentation has been reported to enhance mineral bioavailability by reducing phytate, the primary chelator of divalent cations (Francis *et al.*, 2021). The decline in Hb at T5 relative to T4 may indicate that at 100% replacement, the cumulative burden of residual ANFs or amino acid imbalances begins to impair erythropoiesis.

The highest WBC count at T2 (25%) reflects stimulated innate immune activity at a moderate inclusion level. White blood cells, particularly lymphocytes and neutrophils, are frontline defenders against pathogen invasion, and their modulation by dietary components has been well documented in *C. gariepinus* (Al-Khalaifah *et al.*, 2020). The progressive decline in lymphocyte percentage from T2 (84.0%) through T5 (64.0%), accompanied by a reciprocal increase in neutrophil percentage (16.0% to 40.5%), is indicative of a shift from adaptive to innate immune dominance, a pattern typically associated with chronic dietary stress (Rehulka, 2002). Elevated neutrophilia at T5 may signal an inflammatory response triggered by residual saponins or condensed tannins in ALSM, which are known to induce mucosal irritation in the gastrointestinal tract (Francis *et al.*, 2021). The absence of eosinophils at T2, T4, and T5 may reflect an absence of parasitic challenge during the trial or their suppression at elevated dietary inclusion levels.

The elevated RBC at T5 contrasts with the low Hb at the same treatment, suggesting microcytic or hypochromic erythrocytes — a pattern consistent with subclinical iron-deficiency anaemia or impaired haem synthesis due to protein–amino acid imbalance at the highest ALSM inclusion level. Similar divergence between RBC and Hb parameters in catfish fed high levels of unconventional plant proteins has been reported by Falaye *et al.* (2011) and Tukur *et al.* (2022).

4.2 Serum Biochemical Responses

Serum total protein and albumin are reliable indicators of nutritional status and hepatic synthetic capacity (Whiteman, 2004). The maintenance of TP and albumin within comparable ranges across T1–T4, with recovery at T5, suggests adequate protein synthesis at most inclusion levels. The transient decline in albumin at T4 (17.50 g/L) may reflect competition between immune globulin synthesis (elevated globulin of 16.00 g/L at T4) and albumin production during periods of sub-clinical inflammatory challenge, a well-recognized negative acute-phase response in teleost fish (Rehulka, 2002).

The significantly elevated ALT at T4 (27.00 U/L) and the relatively high AST at T2 (31.00 U/L) are the most clinically significant biochemical findings of this study. ALT is highly liver-specific in teleosts, and elevated serum ALT is the primary biomarker of hepatocellular injury (Tukur *et al.*, 2022). The peak ALT at 75% inclusion corresponds with elevated globulin and a high neutrophil count, together pointing to hepatic inflammation at this level. In contrast, the peak AST at T2 reflects early transient hepatocyte stress that resolves at moderate-to-high inclusion levels, possibly as an adaptive hepatic response or due to compartmentalization of AST in cardiac and skeletal muscle (Rehulka, 2002). These patterns align with findings by Tukur *et al.* (2022) who reported elevated transaminases in *C. gariepinus* fed fermented *Moringa oleifera* kernel meal beyond 50% inclusion. Saponins and tannins present even in fermented ALSM have been documented to cause hepatocyte swelling and lysosomal destabilization in fish at high dietary doses (Francis *et al.*, 2021; Akinwande *et al.*, 2024).

Serum glucose reflects both dietary carbohydrate supply and metabolic homeostasis regulated by insulin and cortisol. The hypoglycaemia observed at T5 (2.50 mmol/L) is of particular concern and may indicate impaired gluconeogenesis due to insufficient glucogenic amino acid supply or hepatic dysfunction at 100% ALSM inclusion. Fish glucose values below 3.0 mmol/L are generally considered indicative of nutritional stress (Adesina, 2017). Conversely, the hyperglycaemia at T4 (7.10 mmol/L) may reflect a cortisol-mediated stress response or the glycolytic effects of moderate saponin intake stimulating gluconeogenesis (Hou *et al.*, 2020). The stability of Na^+ and K^+ across all treatments confirms that electrolyte homeostasis and osmoregulatory function were not compromised at any inclusion level, consistent with findings by Adesina (2017) and Inya *et al.* (2022).

4.3 Practical Implications for Feed Formulation

Taken collectively, the haematological and serum biochemical data indicate that fermented *A. lebbeck* seed meal can be incorporated into *C. gariepinus* diets at up to 50% replacement of conventional protein (T3) without evidence of significant hepatic stress, immune compromise, or electrolyte disruption. At 75% inclusion (T4), marginal hepatic stress signals (elevated ALT, globulin, neutrophilia, hyperglycaemia) suggest approaching a toxicological threshold, while at 100% (T5), immunosuppression, hypoglycaemia, and altered erythrocyte morphology indicate clear nutritional inadequacy. These findings are broadly consistent with those of

Akinwande *et al.* (2024), who recommended a maximum 40–50% ALSM inclusion for Nile tilapia (*Oreochromis niloticus*) to avoid growth depression and hepatotoxic effects. Future studies should investigate complementary detoxification strategies — including autoclaving, enzymatic treatment with phytase and tannase, or combined fermentation–soaking — to further reduce ANF burden and expand the safe incorporation range of ALSM in commercial aquafeeds.

5. CONCLUSION

This study demonstrated that fermented *Albizia lebbeck* seed meal is a viable alternative protein source for African catfish (*Clarias gariepinus*) at dietary inclusion levels of up to 50% without compromising haematological integrity or serum biochemical homeostasis. Beyond this threshold, evidence of hepatic stress, altered immune cell differentials, and metabolic disturbance increasingly manifests. A 75% inclusion level represents a critical inflection point, characterized by elevated ALT activity, neutrophilia, and hyperglycaemia, while 100% inclusion is associated with lymphocytopenia, hypoglycaemia, and likely subclinical hepatopathy. These health indices suggest that fermentation alone is insufficient to render ALSM safe at very high dietary proportions, and complementary processing methods are recommended. The findings provide a practical scientific basis for the partial substitution of conventional protein sources with fermented ALSM in cost-effective aquafeed formulations for *C. gariepinus* production in Nigeria and comparable tropical aquaculture systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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