

Haematological and Serum Biochemical Responses of African Catfish, *Clarias gariepinus* juveniles to Dietary Replacement of Soybean Meal with Fermented *Albizia lebbbeck* Seed Meal

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<https://doi.org/10.67601/njbls.v3i2b.72>

Article info

Date received

15/08/2026

Date accepted

04/09/2026

Available online

18/09/2026

Keywords

Albizia lebbbeck;
Clarias gariepinus;
hematology;
serum
biochemistry;
fish feed

Abstract

Rising costs and limited supply of conventional soybean meal have intensified interest in alternative plant-based protein sources for aquafeeds yet the physiological safety of *Albizia lebbbeck* seed meal, a locally available legume by-product, has not been systematically evaluated in African catfish. This study therefore evaluated the effect of fermented *A. lebbbeck* seed meal to replace soybean meal at graded levels on the hematological and serum biochemical profile of *Clarias gariepinus* juveniles. One hundred and fifty juveniles (mean initial weight 15.20 ± 0.85 g) were fed five isonitrogenous (40% crude protein) diets containing fermented *A. lebbbeck* seed meal at 0, 25, 50, 75 and 100% inclusion in a completely randomized design with three replicates for eight weeks. Blood was analysed for haematological and serum biochemical indices and data were subjected to one-way ANOVA with means separated by LSD ($P < 0.05$). Hematological analysis showed significant differences ($P < 0.05$) among treatments for haemoglobin, white blood cells, red blood cells, neutrophils, eosinophils and lymphocytes, while packed cell volume did not differ significantly ($P > 0.05$). The highest haemoglobin (10.00 g/dL) occurred at 75% inclusion, the highest white blood cell count ($10.0 \times 10^9/L$) at 25% inclusion and the highest red blood cell count ($11.40 \times 10^{12}/L$) at 100% inclusion. Biochemical analysis revealed significant differences ($P < 0.05$) among treatments for total protein, albumin, alanine aminotransferase, globulin, aspartate aminotransferase and glucose with no significant differences in potassium or sodium ($P > 0.05$). The highest albumin (20.00 g/L) occurred at 0% and 100% inclusion, the highest alanine aminotransferase (27.00 U/L) at 75% inclusion, the highest aspartate aminotransferase (31.00 U/L) at 25%



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inclusion and the highest glucose (7.10 mmol/L) at 75% inclusion. These findings indicated that fermented *A. lebbeck* seed meal can fully replace soybean meal in the diets at 75% without adverse hematological or biochemical effects, therefore inclusion at or below 75% is recommended.

Introduction

Fish supplies roughly 40% of animal protein consumed by the average Nigerian and blood parameters are widely used to monitor fish health because blood tissue reflects physiological and metabolic changes in response to diet, environment and stress (Adesina, 2017; Ayoola et al., 2014). African catfish, *Clarias gariepinus*, is a preferred aquaculture species across Africa due to its hardiness, fast growth, tolerance of poor water quality and acceptance of formulated feed (Al-Khalaifah et al., 2020; Oluwaniyi et al., 2017). Rising feed costs have driven interest in alternative, locally available protein sources, including *Albizia lebbeck*, a leguminous tree whose seeds contain appreciable protein, carbohydrate, fat and micronutrient content, alongside bioactive compounds with antioxidant and antimicrobial properties (Oyewole et al., 2020). However, its use in aquafeeds is constrained by anti-nutritional factors that may affect fish health if not adequately reduced through processing such as fermentation.

Soybean meal is the principal conventional plant protein source used in Nigerian aquafeeds because of its favourable amino acid profile and relatively low content of antinutritional factors but Nigerian feed mills rely heavily on imported soybean meal and this dependence drives persistent price volatility and periodic scarcity (Iheanacho et al., 2025). This dependence on soybean meal exposes catfish farmers to input-cost shocks and has motivated the search for locally available legume seeds such as *A. lebbeck* that can replace soybean meal in whole or in part without compromising fish health, provided that their antinutritional load is adequately reduced by processing such as fermentation.

Hematological and serum biochemical indices are sensitive non-lethal tools for assessing the physiological impact of novel feed ingredients (Witeska et al., 2023; Falaye et al., 2018). Despite

growing interest in *A. lebbeck* as a fish feed ingredient, its effect on the hematology and biochemistry remains poorly documented. This study was therefore designed to evaluate the effect of *A. lebbeck* seed meal on the hematological and serum biochemical parameters of the species.

Nutritionally, *A. lebbeck* seed compares favourably with several conventional plant protein sources used in aquafeeds. Reported crude protein values for the whole seed range from approximately 10% to as high as 27–43% depending on variety, maturity and analytical basis, alongside appreciable ether extract, crude fibre and mineral content, particularly potassium, magnesium and calcium (Balkrishna et al., 2022; Muhammad et al., 2010). The seed also contains lysine and arginine-rich protein fractions that compare favourably with the amino acid profiles required in fish diets. These attributes make *A. lebbeck* seed meal an attractive candidate ingredient for reducing reliance on soybean meal which face persistent price volatility and supply constraints in Nigeria.

Despite this nutritional promise, raw *A. lebbeck* seed contains antinutritional factors, including saponins, tannins, phytates, oxalates and cyanogenic compounds, that can depress feed intake, impair nutrient digestibility and disturb normal physiological function if not adequately reduced before inclusion in animal diets (Muhammad et al., 2010; Malik et al., 2022). Fermentation is a widely used, low-cost processing method capable of substantially lowering these antinutrient concentrations through microbial and enzymatic activity, while simultaneously improving protein solubility and mineral bioavailability (Francis et al., 2021). However, partial or complete replacement levels has not been evaluated using hematological and serum biochemical parameters

Blood-based biomarkers are particularly well suited to this evaluation because they respond

rapidly and sensitively to dietary, environmental and toxicological stressors, often before overt clinical signs appear. Erythrocyte indices such as PCV, haemoglobin and RBC count reflect oxygen-carrying capacity and are frequently depressed under nutritional stress or toxicant exposure (Witeska et al., 2023). Leucocyte counts and differentials indicate immune and inflammatory status, while serum enzymes such as ALT and AST serve as sensitive markers of hepatocellular integrity and total protein, albumin and globulin reflect the liver's synthetic capacity and general nutritional status (Melefa and Okoloye, 2023; Idowu et al., 2017). Reported baseline hematological values for healthy juveniles vary considerably across studies and localities, with PCV typically in the range of 25–40%, haemoglobin around 8–11 g/dL and RBC counts of roughly $2\text{--}4.5 \times 10^{12}/\text{L}$ (Melefa and Okoloye, 2023; Idowu et al., 2017) underscoring the importance of using an internal control diet for meaningful comparison.

Building on this background, the present study was designed not only to determine whether fermented *A. lebbeck* seed meal is physiologically well tolerated by *C. gariepinus* juveniles, but also to characterize the dose-response pattern across a full replacement gradient of soybean meal, from 0% (control) to 100% inclusion. Such a gradient allows identification of any threshold beyond which hematological or biochemical indices depart markedly from control values, information that is directly useful for recommending safe practical inclusion levels to fish farmers and feed millers.

Materials and methods

Study Area. This study was conducted at the Department of Fisheries, University of Maiduguri, Borno State, Nigeria (latitude 11.8261°N, longitude 13.1810°E within the Sahelian semi-arid zone characterized by wet (June–September), dry-cold (October–January) and dry-hot (February–May) seasons, with temperatures ranging 20–45°C (NOAA, 2016). Juveniles of uniform size (mean weight 15.20 ± 0.85 g) were obtained from a certified hatchery and acclimatized in a 500 L holding tank for 14 days prior to the start of the feeding trial, during which they were fed a commercial diet to standardize nutritional status.

Throughout acclimatization and the trial, water quality parameters were monitored twice weekly using a multiparameter water quality meter and maintained within ranges suitable for culture (temperature 26–29°C; dissolved oxygen > 5.0 mg/L; pH 6.8–7.6). Water in each experimental unit was exchanged every 48 hours to maintain acceptable quality and minimize the buildup of nitrogenous waste.

Preparation of *Albizia lebbeck* Seed Meal. Seeds of *A. lebbeck* were collected locally, removed from their pods and ground into flour. This flour was fermented by mixing with water in an airtight container and incubating in the dark for 48 hours, after which it was dried and re-ground into a fine powder.

Feed formulation. Five isonitrogenous (40% crude protein) diets were formulated with fermented *A. lebbeck* seed meal at 0, 25, 50, 75 and 100% using Pearson square methods with fish meal, soybean meal, yellow maize, vitamin/mineral premix, lysine, methionine, calcium, vitamin C, salt, starch and oil. Dry ingredients were milled to a fine particle size, sieved, weighed according to the formulation, mixed to homogeneity and pelleted (0.2 mm diameter) before air-drying and storage. The gross composition of the experimental diets is presented in Table 1. **Experimental Design and Feeding.** A total of one hundred and fifty juveniles were randomly allocated into 15 units (5 treatments $\times$ 3 replicates, 10 fish per replicate) in a completely randomized design. Feeding was carried out at 5% body weight daily and adjusted weekly in two equal rations at 07:30 and 17:30 h for 8 weeks.

Blood Collection and Hematological Analysis. At the end of the eight-week feeding trial, fish were fasted for 24 hours before sampling to minimize the confounding effect of recent feed intake on blood chemistry. Blood samples were collected from three replicate fish per treatment via the caudal vein into EDTA bottles to prevent clotting (Whiteman, 2004) and transported on ice for analysis. Packed cell volume (PCV) was determined by the microhaematocrit method (12,000 rpm, 5 min); haemoglobin (Hb) by Sahli's method; red blood cells (RBC) and white blood cells (WBC) by haemocytometer counts following standard

dilution procedures; and differential leucocyte counts from stained blood smears examined microscopically. Mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated using standard formulae (Wickham et al., 1990; Kori-Siakpere and Ubogu, 2008).

Serum Biochemical Analysis. From the remaining pooled blood collected without anticoagulant, serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at -20°C until analysis. Commercial kits (Randox, UK) were used to assay serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), based on the methods of Reitman and Frankel (1957) and Klein et al. (1960), with absorbance read at 540 nm. Serum albumin was determined separately by the bromocresol green (BCG) dye-binding method (Dumas et al., 1971), while total protein was determined by the Biuret method and globulin obtained by subtracting albumin from total protein. Serum glucose was measured by the glucose oxidase method and electrolytes (potassium and sodium) were determined by flame photometry.

Behavioural and Mortality Observations. Fish in all treatments were observed daily throughout the trial for mortality, feed acceptance and abnormal swimming or respiratory behaviour, which can indicate acute physiological stress not always captured by terminal blood sampling. Any mortality was recorded and dead fish were removed promptly and excluded from subsequent biomass calculations. **Data Analysis.** All data obtained were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics (version 25.0) and treatment means were separated using least significant difference (LSD) at $P < 0.05$. Results are presented as mean $\pm$ standard deviation (SD).

Results and Discussion

Results

Throughout the eight-week feeding trial, fish across all treatments, including the 100% fermented *A. lebbeck* seed meal diet, readily accepted the experimental feeds and no treatment-related

mortality or overt abnormal behaviour was observed, suggesting that fermentation had reduced residual antinutritional load to a level compatible with normal feeding and survival even at full soybean meal replacement.

Hematological Profile. No significant difference ($P > 0.05$) was observed among treatments for packed cell volume. Haemoglobin was highest at 75% inclusion (10.00 g/dL) and lowest at 100% inclusion (8.00 g/dL), with all treatments significantly different from one another ($P < 0.05$). For white blood cell count, the highest value occurred at 25% inclusion ($10.0 \times 10^9/\text{L}$) and the lowest at 0% inclusion ($8.00 \times 10^9/\text{L}$); the 0% and 75% levels did not differ significantly ($P > 0.05$), while all other treatments did ($P < 0.05$). Red blood cell count peaked at 100% inclusion ($11.40 \times 10^{12}/\text{L}$) and was lowest at 75% inclusion ($8.00 \times 10^{12}/\text{L}$); the 0% and 50% levels did not differ significantly ($P > 0.05$).

Values for neutrophils were highest at 100% inclusion (40.50%) and lowest at 25% inclusion (16.00%), with significant differences among all treatments ($P < 0.05$). Eosinophils recorded their highest value at 0% inclusion (4.00%), significantly higher than all other treatments ($P < 0.05$), which ranged from 0.00–1.00%. Lymphocytes were highest at 25% inclusion (84.0%) and lowest at 100% inclusion (64.0%), with significant differences among treatments ($P < 0.05$). Neither monocytes nor basophils were detected in any treatment (Table 2).

Serum Biochemical Profile. A significantly higher total protein value ($P < 0.05$) was recorded at 100% inclusion (34.0 g/L) than at 25% inclusion (30.0 g/L), with no significant differences among the 0%, 50%, 75% and 100% levels. Albumin peaked at 0% and 100% inclusion (20.00 g/L each, not significantly different from each other) and was lowest at 75% inclusion (17.50 g/L). Meanwhile, ALT reached its highest value at 75% inclusion (27.00 U/L), significantly different from all other treatments (24.00 U/L at 0%, 50% and 100%; 23.00 U/L at 25%; $P < 0.05$).

Concentrations of globulin were highest at 75% inclusion (16.00 g/L) and lowest at 25% inclusion (12.00 g/L), with the 75% and 100% levels not

significantly different from each other. For AST, the highest value occurred at 25% inclusion (31.00 U/L) and the lowest at 75% inclusion (16.00 U/L), with the 0% and 50% levels not significantly different ($P > 0.05$) but all other treatments significantly different ($P < 0.05$). Glucose values were highest at 75% inclusion (7.10 mmol/L) and lowest at 100% inclusion (2.50 mmol/L), with significant differences among all treatments ($P < 0.05$). No significant differences ($P > 0.05$) were observed among treatments for potassium or sodium, which ranged 4.10–4.50 mmol/L and 135.0–140.0 mmol/L, respectively (Table 3).

Discussion

The observed values for PCV (0.24–0.30), haemoglobin (8.00–10.00 g/dL) and RBC ($8.00\text{--}11.40 \times 10^2/\text{L}$) across all *A. lebbeck* treatments fall broadly within, or close to, ranges previously reported for apparently healthy juveniles under normal culture conditions, including PCV of roughly 25–40% and haemoglobin of about 8–11 g/dL (Melefa & Okoloye, 2023; Idowu et al., 2017). This baseline comparison supports the interpretation that fermentation was largely successful in reducing the seed's antinutritional load, given that raw *A. lebbeck* seed is known to contain saponins, tannins, phytates and cyanogenic compounds capable of provoking anaemia-like reductions in erythrocyte indices if consumed at high, unprocessed concentrations (Muhammad et al., 2010). Fermented *A. lebbeck* seed meal appears

to have supported red blood cell production and oxygen-carrying capacity at moderate-to-high inclusion levels without impairing erythropoiesis, given the absence of significant treatment effects on PCV alongside elevated haemoglobin at 75% inclusion. This agrees with Adesina (2017), who reported improved hematological parameters in *C. gariepinus* juveniles fed sunflower seed meal-based diets. At the same time, the decline in haemoglobin and RBC at 75–100% inclusion for some indices may reflect the cumulative effect of residual anti-nutritional factors at higher inclusion levels, consistent with the general concern that anti-nutritional compounds in plant-based ingredients can affect fish physiology if not adequately reduced by processing (Francis et al., 2021).

A mild immune or stress response to increasing dietary *A. lebbeck* content is suggested by the elevated neutrophil count at 100% inclusion alongside reduced lymphocyte and eosinophil values at higher inclusion levels, since differential leucocyte counts are sensitive indicators of physiological stress in fish (Seibel et al., 2021). This pattern of neutrophilia accompanied by relative lymphopenia is consistent with a classical stress leucogram, commonly reported in fish exposed to sub-lethal chemical or nutritional stressors, in which neutrophils are mobilized from marginal pools in response to circulating cortisol while lymphocyte trafficking to peripheral blood is simultaneously suppressed (Witeska et al., 2023).

Table 1. Gross composition (%) of experimental diets with graded levels of fermented *Albizia lebbeck* seed meal

Ingredient	0%	25%	50%	75%	100%
Fish meal	35.38	35.38	35.38	35.38	35.38
Soybean meal	35.38	26.55	17.69	8.85	0.00
FALSM	0.00	8.85	17.69	26.55	35.38
Yellow maize	24.74	24.74	24.74	24.74	24.74
Premix	1.00	1.00	1.00	1.00	1.00
Lysine	0.40	0.40	0.40	0.40	0.40
Methionine	0.40	0.40	0.40	0.40	0.40
Calcium	0.50	0.50	0.50	0.50	0.50
Vitamin C	0.20	0.20	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50	0.50
Starch	0.50	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0	100.0

FALSM = Fermented *Albizia lebbeck* seed meal

Table 2. Effect of *Albizia lebbek* seed meal on hematological profile of *Clarias gariepinus*

Parameter	0%	25%	50%	75%	100%
PCV	0.25 ±0.03 ^a	0.28 ±0.03 ^a	0.26 ±0.03 ^a	0.30 ±0.03 ^a	0.24 ±0.03 ^a
HB (g/dL)	8.30 ±0.26 ^{cd}	9.30 ±0.26 ^b	8.60 ±0.26 ^c	10.00 ±0.26 ^a	8.00 ±0.26 ^d
WBC (×10 ⁹ /L)	8.00 ±0.33 ^c	10.00 ±0.33 ^a	9.20 ±0.33 ^b	8.40 ±0.33 ^c	9.60 ±0.33 ^{ab}
RBC (×10 ¹² /L)	10.40 ±0.29 ^b	11.00 ±0.29 ^{ab}	10.60 ±0.29 ^b	8.00 ±0.29 ^c	11.40 ±0.29 ^a
Neutrophils (%)	18.00 ±5.44 ^{bc}	16.00 ±5.44 ^c	29.00 ±5.44 ^b	20.00 ±5.44 ^{bc}	40.50 ±5.44 ^a
Eosinophil (%)	4.00 ±0.77 ^a	0.00 ±0.77 ^b	1.00 ±0.77 ^b	0.00 ±0.77 ^b	0.00 ±0.77 ^b
Lymphocytes (%)	78.0 ±1.55 ^b	84.0 ±1.55 ^a	70.0 ±1.55 ^c	80.0 ±1.55 ^b	64.0 ±1.55 ^d
Monocytes (%)	0	0	0	0	0
Basophils (%)	0	0	0	0	0

Means with the same superscript within a row are not significantly different ($P > 0.05$). PCV = packed cell volume; HB = haemoglobin; WBC = white blood cells; RBC = red blood cells; SD = standard deviation

Table 3. Effect of *Albizia lebbek* seed meal on serum biochemical profile of *Clarias gariepinus*

Parameter	0%	25%	50%	75%	100%
TP (g/L)	33.00 ±1.08 ^a	30.00 ±1.08 ^b	32.50 ±1.08 ^a	33.00 ±1.08 ^a	34.00 ±1.08 ^a
ALB (g/L)	20.00 ±1.12 ^a	18.00 ±1.12 ^{ab}	19.00 ±1.12 ^{ab}	17.50 ±1.12 ^b	20.00 ±1.12 ^a
ALT (U/L)	24.00 ±1.13 ^b	23.00 ±1.13 ^b	24.00 ±1.13 ^b	27.00 ±1.13 ^a	24.00 ±1.13 ^b
GLO (g/L)	13.00 ±1.13 ^b	12.00 ±1.13 ^b	13.00 ±1.13 ^b	16.00 ±1.13 ^a	14.00 ±1.13 ^{ab}
AST (U/L)	27.00 ±0.99 ^b	31.00 ±0.99 ^a	28.00 ±0.99 ^b	16.00 ±0.99 ^d	20.00 ±0.99 ^c
GLU (mmol/L)	6.70 ±0.28 ^{ab}	5.60 ±0.28 ^c	6.30 ±0.28 ^b	7.10 ±0.28 ^a	2.50 ±0.28 ^d
Potassium (mmol/L)	4.30 ±0.31 ^a	4.50 ±0.31 ^a	4.20 ±0.31 ^a	4.40 ±0.31 ^a	4.10 ±0.31 ^a
Sodium (mmol/L)	136.00 ±4.10 ^a	139.00 ±4.10 ^a	138.00 ±4.10 ^a	135.00 ±4.10 ^a	140.00 ±4.10 ^a

Means with the same superscript within a row are not significantly different ($P > 0.05$). TP = total protein; ALB = albumin; ALT = alanine aminotransferase; GLO = globulin; AST = aspartate aminotransferase; GLU = glucose; SD = standard deviation

The comparatively low eosinophil counts recorded at inclusion levels of 25% and above, relative to the control, may further reflect a shift in leucocyte allocation away from eosinophil-mediated responses as neutrophils assumed a more prominent role in the circulating differential count. Notably, the absence of monocytes and basophils across all treatments, including the control, suggests these cell types were either present below the detection threshold of the differential counting method used or genuinely rare in the circulating blood of *C. gariepinus* juveniles under the culture conditions of this study, a pattern that has also been reported by other workers using similar staining and enumeration protocols in this species. Protein status at complete replacement remained comparable between the control (0%) and full-inclusion (100%) diets. This is consistent with Inya et al. (2022), who reported improved biochemical

parameters in *C. gariepinus* fed sesame seed meal-based diets. Treatment-dependent shifts in hepatic enzyme activity are indicated by elevated ALT at 75% inclusion and AST at 25% inclusion, broadly consistent with the enzymatic responses reported by Tukur et al. (2022) for fingerlings fed fermented *Moringa oleifera* kernel meal. Because ALT and AST are intracellular enzymes released into circulation primarily when hepatocyte membrane integrity is compromised, the moderate, non-monotonic fluctuations observed across treatments, rather than a progressive dose-dependent rise, argue against overt hepatotoxicity and instead suggest transient, treatment-specific shifts in metabolic enzyme activity consistent with dietary adaptation rather than cellular damage. This interpretation is reinforced by the parallel finding that globulin, another hepatic synthetic product and key component of the immune

system's humoral response, was highest at 75% inclusion (16.00 g/L) rather than declining, which would be expected if hepatocellular function were being progressively impaired by the test ingredient.

Altered carbohydrate metabolism or reduced feed intake at full replacement may explain the marked reduction in glucose at 100% inclusion (2.50 mmol/L, compared with 6.70 mmol/L in the control), a finding that warrants further metabolic investigation. Electrolyte balance and osmoregulation, on the other hand, do not appear to have been adversely affected by *A. lebbeck* inclusion, given the lack of significant differences in potassium and sodium across all treatments, in line with Adesina (2017). From a practical standpoint, the absence of mortality, the maintenance of hematological indices within literature-reported reference ranges and the comparable protein status between the control and full-inclusion diets together suggest that fermented *A. lebbeck* seed meal is well tolerated by *C. gariepinus* juveniles even at complete replacement of soybean meal. Nonetheless, the marked reduction in glucose and the shifts in RBC observed specifically at 100% inclusion for some parameters indicate that physiological adaptation may be reaching its limit at this level, reinforcing the recommendation that inclusion be capped at or below 75% pending further metabolic and growth-performance verification. These findings are broadly consistent with the wider literature on fermented plant-based ingredients in catfish diets, where moderate inclusion levels typically support normal physiology while very high or complete replacement levels occasionally reveal subtle metabolic trade-offs not evident at lower doses (Inya et al., 2022; Tukur et al., 2022).

Limitations and Future Directions

This study focused specifically on hematological and serum biochemical responses over an eight-week feeding period and did not concurrently assess growth performance, nutrient digestibility, or carcass composition, all of which are essential complementary endpoints for a complete evaluation of *A. lebbeck* seed meal as a soybean meal replacer. In addition, blood sampling was

conducted only at the end of the trial; a time-course sampling design, with blood drawn at intervals throughout the feeding period, would have allowed the transient stress-related shifts observed in some leucocyte and enzyme parameters to be distinguished more clearly from persistent, dose-dependent physiological effects.

Future work should therefore combine hematological and biochemical monitoring with detailed growth performance trials, nutrient digestibility assays and histopathological examination of the liver, kidney and intestine, to build a more complete physiological picture of how graded *A. lebbeck* seed meal inclusion affects them. Investigating the residual antinutrient content of the fermented meal used in this study, rather than relying on fermentation duration alone as an indirect proxy for detoxification, would also strengthen confidence in the safety margins proposed here. Finally, given the pronounced shifts observed at 100% inclusion for glucose and some hematological indices, dose-response trials with finer increments between 75% and 100% inclusion would help pinpoint the precise threshold at which physiological tolerance begins to decline.

Conclusion

Fermented *Albizia lebbeck* seed meal influenced the hematological and biochemical profile in a dose-dependent manner, with haemoglobin, ALT and glucose peaking at 75% inclusion and RBC and total protein highest at 100% inclusion. Electrolyte balance (potassium, sodium) and PCV were unaffected across all inclusion levels. These findings suggest fermented *A. lebbeck* seed meal is a viable alternative protein source diets, though inclusion beyond 75% should be approached cautiously given the marked decline in glucose and RBC observed at 100% inclusion for some parameters. Complete replacement of soybean meal with fermented *A. lebbeck* seed meal did not compromise fish health, indicating that this locally available, low-cost legume can replace soybean meal at 75% inclusion.

Long-term feeding trials are also recommended to assess chronic effects on immune function, disease resistance and fillet quality.

Acknowledgement

The authors are grateful to the Department of Fisheries, University of Maiduguri, for providing laboratory facilities and technical support during the course of this study.

Conflict of Interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

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