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Effects of Novel Futa Multipurpose Sweet Sorghum on Basic Haematology, Blood Biochemistry and Genomic Expression of African Catfish (*Clarias gariepinus*)

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ABSTRACT

This study evaluated the effects of substituting maize with the Federal University of Technology Akure multipurpose sweet sorghum (*Sorghum bicolor*) on African catfish (*Clarias gariepinus*) fingerlings. The Novel Federal University of Technology Akure Multipurpose Sweet Sorghum was obtained from the Department of Crop, Soil and Pest, FUTA Ondo State, Nigeria. A total of five experimental diets were formulated, with sweet sorghum replacing maize at varying levels, alongside an initial baseline group. The feeding trial lasted 56 days, with three replicates per treatment, water quality, haematology, blood biochemistry, whole-body proximate composition, and genomic expression were assessed. Results showed that water parameters remained within the optimal range for catfish culture, indicating no adverse environmental impact of sweet sorghum inclusion. Hematological and biochemical indices, including PCV, RBC, WBC, hemoglobin, total protein, albumin, and globulin, varied significantly ($p < 0.05$) among treatments. Fish fed sweet sorghum-based diets exhibited enhanced blood health markers compared to the control. Additionally, genomic expression analysis suggested a positive influence of sweet sorghum on physiological and immune-related gene regulation. Overall, substituting maize with sweet sorghum in catfish diets improved fish health, growth, and sustainability, while reducing reliance on conventional feedstuffs. Sweet sorghum can therefore serve as a viable, locally available, and cost-effective.

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Introduction

Sweet Sorghum is a nutrient-rich crop that has been identified as a potential feed ingredient for aquaculture [1]. However, its effects on fish hematology, blood biochemistry, and genomic expression are not well understood. Understanding these effects can inform the development of more effective and sustainable feed formulations, leading to improved fish health and productivity [2]. Furthermore, the use of *Sweet Sorghum* as a feed ingredient can reduce the aquaculture industry's reliance on non-renewable resources such as fishmeal and soybean meal, thereby promoting sustainability and environmental benefits [3]. Additionally, *Sweet Sorghum* is a locally available crop in many African countries, including Nigeria, which promotes local agriculture and contributes to economic development [4].

Understanding the effects of *Sweet Sorghum* (*Sorghum bicolor*) on the hematology, blood biochemistry, and genomic expression of African catfish is crucial, as its nutrient profile and bioactive compounds may influence various physiological processes [5]. The crop's nutrient composition may affect nutrient utilization, energy production, and metabolic pathways, while its antioxidants may reduce oxidative stress and promote overall health and well-being. Moreover, the bioactive compounds in *Sweet Sorghum* may modulate enzyme activity, regulate blood cell production, and enhance disease resistance by influencing the immune response. Its antioxidant activity may also help mitigate stress in African catfish.

The use of the novel FUTA multipurpose sweet sorghum as a feed ingredient is justified by its nutritional, economic, and ecological benefits. Traditional aquafeeds in Nigeria rely heavily on maize as the primary energy source, but rising costs, competition with human consumption, and concerns over sustainability have created a need for affordable and locally available alternatives. The FUTA-developed sweet sorghum variety addresses these challenges by offering a high-yielding, multipurpose crop that thrives under local climatic conditions with relatively low input requirements [6].

Nutritionally, sweet sorghum is rich in carbohydrates, proteins, and bioactive compounds that support metabolism, growth, and antioxidant defense in fish [7]. Unlike conventional maize, sorghum contains functional phytochemicals that may provide added immunomodulatory and stress-protective benefits, making it suitable for enhancing the resilience of African catfish (*Clarias gariepinus*). Furthermore, studies have demonstrated that plant-based diets, including sorghum, do not compromise hematological or biochemical parameters when properly balanced, thereby ensuring fish health and performance [5]. From an economic perspective, adopting FUTA multipurpose sweet sorghum in aquafeed reduces dependence on imported or costly grains, lowering feed costs which often account for 60–70% of aquaculture production expenses [2]. Since sweet sorghum is adaptable to local soils and resistant to drought, its cultivation strengthens food and feed security while creating new value chains for farmers and feed millers.

Ecologically, the crop supports sustainable aquaculture by minimizing pressure on maize supplies and reducing environmental footprints associated with overdependence on conventional grains. As Nigeria continues to expand its aquaculture sector to meet growing protein demand, integrating resilient and locally adapted feed crops such as FUTA multipurpose sweet sorghum ensures long-term productivity and aligns with climate-smart aquaculture strategies [8]. In summary, FUTA multipurpose sweet sorghum is not just an alternative to maize but a strategic innovation that addresses nutritional adequacy, cost reduction, ecological sustainability, and resilience in aquafeed production. Its introduction into catfish diets therefore represents a timely and scientifically grounded step toward sustainable aquaculture development in Nigeria.

By investigating the effects of the novel Sweet Sorghum (*Sorghum bicolor*) on blood biochemistry, hematology, and genomic expression of African catfish, this study aims to provide valuable insights into the nutritional, physiological, and molecular effects of this novel feed supplement, thereby contributing to sustainable aquaculture practices and improved fish health management. The general objective of this study is to determine the effects of Sweet Sorghum (*Sorghum bicolor*) on the hematology and blood biochemistry of African catfish (*Clarias gariepinus*).

Materials and Methods

Experimental Location

This research was carried out at the Teaching and Research Farm of the department of Fisheries and Aquaculture Technology, Federal University of Technology Akure, Ondo State, Nigeria.

Collection and Preparation of (Sweet Sorghum) *Sorghum bicolor L*

The Novel Federal University of Technology Akure Multipurpose Sweet Sorghum was obtained from the Department of Crop, Soil and Pest, FUTA Ondo State, Nigeria.

Preparation of the Sweet Sorghum

sweet sorghum grain was divided into four parts, the first part was soaked in clean water for 24hours and rinse in fresh water thereafter sun-dry for 3days, and it was then be milled into fine powder and stored. The second part was milled also into fine powder without any treatment and stored. The third part was soaked for 72hours, then spread evenly on Plantain or Banana leaves in an enclosed room for 5 days to spout thereafter sun-dry for 3 days and milled into fine powder. The last part was roasted on fire with a pan, after it was well roasted, it was left to cool, after that it was milled into fine powder.

Figure 1: Sweet Surghum (*Sorghum bicolor*)
Species: *Sorghum bicolor*
Source: Department of Crop, Soil and Pest, FUTA Ondo State, Nigeria.
MAG. X 0.05

Experimental Diets Formulation

Sweet sorghum and other ingredients were bought before the commencement of the experiment to avoid differences associated with varying batches. The feed ingredients were purchased from a reputable feed mill in Akure, Ondo State. five (5) diet of 40% crude protein (CP) containing fishmeal, groundnut cake, soybean meal, maize, sweet sorghum and fixed ingredients including vitamin and mineral premix, oil and corn starch (binder). Required weight (gram) of each ingredient was calculated using Pearson Square method with the stipulated crude protein requirement of the fish. All the diet is carefully prepared which involves grinding the ingredients, measuring, mixing together and pelletizing (2 mm diameter) in a pelleting machine. The pellets were spread under the sun to dry; after drying, it was packed in an air-tight container and store in a cool and dry place until use. The sorghum was used to replace maize at graded levels in the different diets as an alternative energy source, in order to evaluate its effect on the growth performance, hematology, and blood biochemistry of *Clarias gariepinus*.

Table 1: Ingredient's Composition of the Experimental Diets (g/100) for *Clarias gariepinus*

INGREDIENT	(control)	RSS (100%)	SSS (100%)	FSS (100%)	SS (100%)
FM (65%)	16.00	16.00	16.00	16.00	16.00
SBM (42%)	27.00	27.00	27.00	27.00	27.00
GNC (48%)	30.00	30.00	30.00	30.00	30.00
Maize	18.00	0.00	0.00	0.00	0.00
RSS	0.00	18.00	0.00	0.00	0.00
SSS	0.00	0.00	18.00	0.00	0.00
SS	0.00	0.00	0.00	0.00	18.00
FSS	0.00	0.00	0.00	18.00	0.00
Soya Oil	5.00	5.00	5.00	5.00	5.00
Vit/Min Premix	2.00	2.00	2.00	2.00	2.00
Corn Starch	2.00	2.00	2.00	2.00	2.00

KEYS
FSS= Fermented Sweet Sorghum
SSS= Soaked Sweet Sorghum
RSS= Roasted Sweet Sorghum
SS= Sweet Sorghum
GNC= Groundnut Cake
SBM= Soyabean Meal

FM= Fish Meal

Experimental Fish

African Catfish (*Clarias gariepinus*) were used as the test fish species in this research. A total of two hundred (200) juveniles were purchased from a private fish farm along FUTA south gate, Akure, Ondo State, Nigeria. The juveniles had an initial mean weight of 13.0 ± 2.0 g, which falls within the typical range for *C. gariepinus* juveniles reported in similar studies [9-11]. This ensured size uniformity and minimized variation in growth response during the feeding trial.

Experimental Design

The design of the experiment is completely randomized design (CRD) giving the hypothesis whether there is significant difference or no significant difference in the replacement of Maize with Sweet sorghum as an energy source in the experiment. The *C. gariepinus* juveniles was acclimatized to the experimental system for 14 days. The weight of the fish was taken and recorded immediately after acclimatization using an electronic weighing balance (Model PB 3002) and allotted into fifteen (15) different plastic tanks with the desired number juveniles per tank. The weighing will continue every two weeks until the experiment is terminated. The fish is fed 5% of their body weight two times daily in the morning between 8-9am and evening between 5-6pm for 56 days.

Water Quality Parameters Assessment

The plastic tanks (70cmx45cmx45cm) are filled with 30 Liters of water to avoid water quality deterioration and pollution. The water was changed twice in a week and the physic-chemical parameters (dissolved oxygen, pH, conductivity and the temperature) of the water in the tank was check every two (2) weeks to keep the water conducive for the fish using HANNA Multiparameter water checker (Poland).

Proximate Composition of Experimental Feed and Fish

Proximate analysis was carried out on the formulated feed and experimental fish according to AOAC (2005) to determine the moisture, ash, crude protein, crude lipid and Nitrogen Free Extract (NFE).

Blood Collection and Sampling Procedure

Approximately 1 mL of blood was collected into EDTA-coated tubes for hematological analysis. An additional 2 mL was collected into plain tubes, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 10 minutes to extract serum for biochemical analysis.

The Differential White Blood Cell

Differential white blood cell count is performed to quantify the circulatory levels of lymphocytes, granulocytes and monocytes. Blood samples are taken from fish from each treatment. Bleeding is performed by puncture of the caudal blood vessels. One drop f blood is smeared on each slide. Slides are air-dried at room temperature, fixed in 95% methanol and stained with Giemsa stain (Sigma chemical Co. Ltd, Poole, UK) for 20 minutes and mounted. Lymphocytes, granulocytes and monocytes are identified following standard blood description guides like Svoboda et al., (2005). The absence or presence of granules in the cytoplasm of the leucocyte cell is used to distinguish between the agranulocytes and granulocytes. A minimum of 200 white blood cells per slide is then enumerated and the values were as a percentage of the total leucocytes, unidentified cells are excluded.

Haemoglobin Estimation (Hb): Haemoglobin estimation is done with the aid of a haemoglobinometer (Sigma®, England). The apparatus and the reagents include Standard Shilonometer, N/10 HCl and 0.02ml pipette. The graduated tube is filled to the 20ml mark; 0.02 ml of blood is added and mixed thoroughly until the colour matches the standard. The amount of solution in the graduated tube gives the haemoglobin concentration and this is expressed in percentages.

Red Blood Cells Count (RBC): Counting of the red blood cells (erythrocytes) is done in the counting chambers of the Neubauer haemocytometer with the aid of the Olympus BX 50 microscope (Olympus, UK) and expressed as (10 mm) number of cells counted.

Packed Cell Volume (PCV): PCV is determined by drawing non-clotted blood by capillary action into microhaematocrit tubes; one end of the tubes is sealed with a synthetic sealant. The sealed tube is centrifuged in a microhaematocrit centrifuge. Centrifugation lasts for 5 minutes at 10500 revolutions per minute (rpm). The PCV is then measured using a microhaematocrit reader and expressed as a percentage.

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are calculated using the formulae given by Svoboda et al (2005) as follows. Mean corpuscular volume (MCV) is expressed in femtolitres (fl) and calculated as:

$$MCV = \frac{PCV \times 1000}{Erythrocyte\ count\ (Er)}$$

Mean corpuscular haemoglobin (MCH) expresses the average haemoglobin concentration in every erythrocyte cell. It is expressed in pictogram (pg).

$$MCH = \frac{\text{haemoglobin value}}{Erythrocyte\ count}$$

Mean corpuscular haemoglobin concentration (MCHC) is the concentration of haemoglobin in a unit volume of erythrocytes. It is calculated from the haemoglobin value (Hb) and the haematocrit value (PCV).
Hb/PCV x 1000

Serum Glucose: Serum glucose concentration is measured according to using Bio-La-Test oxochrome GLUCOSA (Glu 250E) [12]. Based on the oxidation of glucose catalyzed by glucose oxidase to hydrogen peroxide and gluconate, the peroxide produced is determined by midation coupling with substituted phenol and 4-amino antipyrin. The coupling is catalysed by peroxidase.

DNA Extraction Protocol

Approximately 100 mg of fungi mycellia was grinded with Dellaporta extraction buffer (100 mM Tris pH 8,51 EDTA PH 8, 500 mM NaCl, 10 mM mcrcaptoethanol) and DNA extracted as described briefly. Each sample was grinded in 1000 µl of the buffer in a sterilized sample bags. Mix was collected in sterile eppendorf tube and 40 µl of 20% SDS was then added, this was followed by brief vortexing and incubated at 65oC for 10 minutes. At room temperature, 160 µi of 5 M potassium acetate was then added vortexed and centrifuged at 10000 g for 10 minutes Supernatant where collected in another eppendorf tube and 400 µl of cola iso propanol was added mixed gently and kept at -20 OC for 60 minutes. Centrifugation was at 12000g for 10 minutes to precipitate the DNA after which supernatant was gently decana and ensured that the pellet was not disturbed. DNA was then washed with 500 μι of 70% ethanol by centrifuging 10000g for 10 minutes. Ethanol was decanted and DNA air-dried at room temperature until no trace of ethanol was seen in the tube. Pellet was then re-suspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA.

Statistical Analysis

The experiment was designed with a completely randomized design (CRD). All data collected from the experiment during the trial is subject to one-way Analysis of Variance (ANOVA) (Steel and Torrie, 1980) and using the Statistical Package for Social Sciences (SPSS) software. Differences between the mean treatments is consider using Duncan Multiple range tests (P = 0.05) (Duncan, 1955) and gel analyzer.

Results

Water Quality Parameters

The mean values of water quality parameters (temperature, pH, dissolved oxygen, and conductivity) recorded during the 56-day feeding trial are presented in Table 2. Results showed that all parameters remained within the recommended range for the culture of *Clarias gariepinus*. There were no significant differences ($p > 0.05$) among treatments, indicating that the inclusion of sweet sorghum in the diets did not adversely affect water quality throughout the experiment.

Table 2: Water Quality Parameters Recorded During the Experimental Period

Parameter	Source	Control	RSS	FSS	SSS	SS
Temp (°C)	26.05±0.01^{ab}	26.03±001^{ab}	26.30±0.01^{b}	26.40±0.01^{b}	26.00±0.01^{a}	26.16± 0.15^{ab}
pH	7.55±0.01^{c}	7.14± 0.01^{b}	7.30± 0.01^{b}	7.42±0.01^{bc}	7.04± 0.01^{a}	7.11± 0.01^{ab}
DO (mg/L)	8.38± 0.02^{ab}	8.21± 0.01^{a}	8.50±0.10^{bc}	8.80±0.10^{c}	8.80± 0.10^{c}	8.70± 0.10^{bc}
D. solids (mg/L)	171±1.00^{a}	244± 1.00^{b}	322± 1.00^{c}	294±1.00^{bc}	294± 1.00^{bc}	232± 1.00^{b}
Salinity (ppm)	83 ± 1.00^{a}	123 ± 1.00^{b}	162.5±2.50^{c}	148±1.00^{bc}	148 ± 1.00^{bc}	117 ± 1.00^{b}
Salinity (%)	0.00 ± 0.00^{a}	0.01 ± 0.01^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}

Proximate Composition of Experimental Diets

The proximate composition of the formulated diets is presented in Table 2. The result shows that: The proximate composition of the diets showed significant ($p < 0.05$) differences among treatments. Moisture content was highest in SS (T5, 6.67%) and lowest in RSS (T2, 4.51%). Crude protein content differed significantly, with SSS (T3, 52.38%) being the highest, while RSS (T2, 45.26%) had the lowest. Crude lipid followed a similar trend, with SSS (T3, 9.01%) significantly higher than all other treatments, whereas FSS (T4, 4.01%) was lowest. Ash content was significantly highest in RSS (T2, 20.51%^{a}), while the lowest value was observed in FSS (T4, 16.51%). Crude fiber content varied significantly ($p < 0.05$), with FSS (T4, 2.02%) being the highest and the Control (T1, 1.50%) the lowest. NFE was highest in the Control diet (T1, 25.44%), followed by RSS and FSS, while SSS (T3, 14.72%) had the lowest value.

Table 3: Proximate composition of experimental diets (% dry matter basis)

Parameter	Control (T1)	RSS (T2)	SSS (T3)	FSS (T4)	SS (T5)
Moisture (%)	5.21 ± 0.10^{b}	4.51 ± 0.12^{c}	5.74 ± 0.08^{b}	5.66 ± 0.15^{b}	6.67 ± 0.11^{a}
Crude Protein (%)	40.25 ± 0.14^{b}	40.26 ± 0.20^{c}	40.38 ± 0.18^{a}	39.67 ± 0.22^{b}	40.15 ± 0.17^{c}
Crude Lipid (%)	6.33 ± 0.09^{b}	5.84 ± 0.12^{c}	9.01 ± 0.14^{a}	4.01 ± 0.10^{d}	7.05 ± 0.13^{b}
Ash (%)	18.50 ± 0.11^{b}	20.51± 0.18^{a}	17.83± 0.16^{b}	16.51± 0.13^{c}	19.02± 0.15^{a}
Crude Fiber (%)	1.50 ± 0.06^{c}	1.84 ± 0.07^{b}	1.91 ± 0.05^{b}	2.02 ± 0.08^{a}	1.63 ± 0.06^{c}
NFE (%)	30.44 ± 0.13^{a}	27.04± 0.16^{b}	25.72± 0.12^{c}	31.13± 0.14^{b}	25.48± 0.15^{c}

Means in the same row with different superscripts (a, b, c, d) are significantly different at $p < 0.05$ (Duncan's multiple range test)

Table 4: Whole-Body Proximate Composition of Fish After 56 Days Feeding Trial (% Dry Matter Basis)

Parameter	Control (T1)	RSS (T2)	SSS (T3)	FSS (T4)	SS (T5)	
Moisture (%)	10.40± 0.10^{d}	5.80 ± 0.21^{e}	7.02 ± 0.01^{e}	7.80 ± 0.21^{e}	12.02±0.01^{b}	11.80±0.21^{c}
C.Protein(%)	67.32 ± 0.01^{a}	61.71± 0.01^{e}	66.74± 0.02^{b}	63.9± 0.01^{d}	64.69 ± 0.01^{c}	66.73 ± 0.01^{b}
Lipid (%)	14.02 ± 0.01^{d}	14.75 ± 0.26^{c}	12.02 ± 0.01^{e}	11.80 ± 0.21^{e}	16.02 ± 0.01^{b}	18.80 ± 0.21^{a}
Ash (%)	21.02 ± 0.01^{e}	25.80 ± 0.21^{d}	28.02 ± 0.01^{a}	27.80 ± 0.21^{a}	27.02 ± 0.01^{b}	18.51 ± 0.01^{f}

Values are means ± SE of triplicate groups (n=3). Means in the same row with different superscripts (a, b, c, d, e, f) are significantly different at $p < 0.05$ (Duncan's multiple range test).

Haematological Parameters

The hematological indices of *Clarias gariepinus* fed the experimental diets are presented in Table 4. The result shows that: RBC count increased significantly ($p < 0.05$) with sweet sorghum diets, with the highest values in T5 (SS: 3.05 ± 0.05 ×10^{6}/µL) and the lowest in control (T1: 1.85 ± 0.05 ×10^{6}/µL). WBC count also followed the same trend, with SS (T5) producing the highest WBC (8.80 ± 0.10 ×10^{3}/µL), suggesting stronger immune modulation. Hemoglobin and PCV values were significantly elevated in SS-fed fish (8.05 ± 0.05 g/dL and 26.00 ± 1.00%, respectively) compared to other treatments. MCV and MCH showed decreasing trends across treatments, with control diets maintaining higher values than experimental ones. MCHC was significantly reduced in T3 (SSS: 27.52 ± 0.01 g/dL), while T1, T2, and T4 maintained higher but statistically similar levels.

Table 5: Haematological Parameters of *Clarias Gariepinus* Fed Experimental Diets

Parameter	T1 (Control)	T2 (RSS)	T3 (SSS)	T4 (FSS)	T5 (SS)
RBC (×10^{6}/µL)	1.85 ± 0.05^{e}	2.25 ± 0.05^{d}	2.35 ± 0.05^{c}	2.75 ± 0.05^{b}	3.05 ± 0.05^{a}
WBC (×10^{3}/µL)	7.00 ± 0.00^{d}	7.50 ± 0.10^{c}	6.70 ± 0.10^{e}	7.80 ± 0.10^{b}	8.80 ± 0.10^{a}
Hemoglobin (g/dL)	7.00 ± 0.00^{c}	6.67 ± 0.01^{d}	6.33 ± 0.01^{e}	7.68 ± 0.01^{b}	8.05 ± 0.05^{a}
PCV (%)	22.00 ± 1.00^{c}	21.00 ± 1.00^{c}	22.00 ± 1.00^{c}	24.00 ± 1.00^{b}	26.00 ± 1.00^{a}
MCV (fL)	110.52 ± 0.01^{a}	94.73 ± 0.51^{c}	95.82 ± 0.01^{c}	85.20 ± 0.01^{e}	86.20 ± 0.90^{d}
MCH (pg)	36.84 ± 0.00^{a}	31.75 ± 0.00^{b}	26.29 ± 0.01^{c}	28.42 ± 0.01^{d}	25.81 ± 0.01^{e}
MCHC (g/dL)	33.33 ± 0.01^{b}	33.36 ± 0.01^{b}	27.52 ± 0.01^{e}	33.35 ± 0.01^{b}	29.63 ± 0.01^{c}

Values are means ± SE of triplicate groups (n=3). Means in the same row with different superscripts (a, b, c, d, e, f) are significantly different at $p < 0.05$ (Duncan's multiple range test **Serum Biochemistry**

The serum biochemical parameters of experimental fish are presented in Table 5. The result shows that: There were significant differences ($p < 0.05$) among treatments, indicating that the inclusion of sweet sorghum in blood biochemical analysis indicated clear differences across treatments. Fermented sorghum diet (TrC) produced the highest serum protein (7.01 g/dl), albumin (4.32 g/dl), and globulin (3.58 g/dl), reflecting enhanced protein metabolism and overall physiological status. In comparison, fish fed the control/raw sorghum diets (TrA and TrB) recorded the lowest serum protein (5.94–6.65 g/dl), albumin (2.56–2.71 g/dl), and globulin (2.49–3.30 g/dl) values. Roasted (TrD) and soaked (TrE) sorghum diets improved serum protein indices relative to raw sorghum but were less effective than fermentation.

Table 6: Serum Biochemical Parameters of Clarias Gariepinus Fed Experimental Diets

Parameter	Control	RSS	FSS	SSS	SS
T.Protein (g/dL)	6.11 ± 0.06^{c}	5.97 ± 0.04^{c}	6.73 ± 0.11^{b}	6.88 ± 0.19^{a}	6.50 ± 0.10^{b}
Albumin (g/dL)	3.15 ± 0.01^{b}	2.67 ± 0.07^{c}	2.56 ± 0.00^{c}	4.31 ± 0.02^{a}	3.24 ± 0.06^{b}
Globulin (g/dL)	3.30 ± 0.01^{c}	2.50 ± 0.01^{d}	3.31 ± 0.01^{c}	3.59 ± 0.01^{a}	3.50 ± 0.01^{ab}

Values are means ± SE of triplicate groups (n=3). Means in the same row with different superscripts (a, b, c, d, e, f) are significantly different at $p < 0.05$ (Duncan's multiple range test

DNA Band Profile (Gel Electrophoresis) of African catfish fed the experimental diets

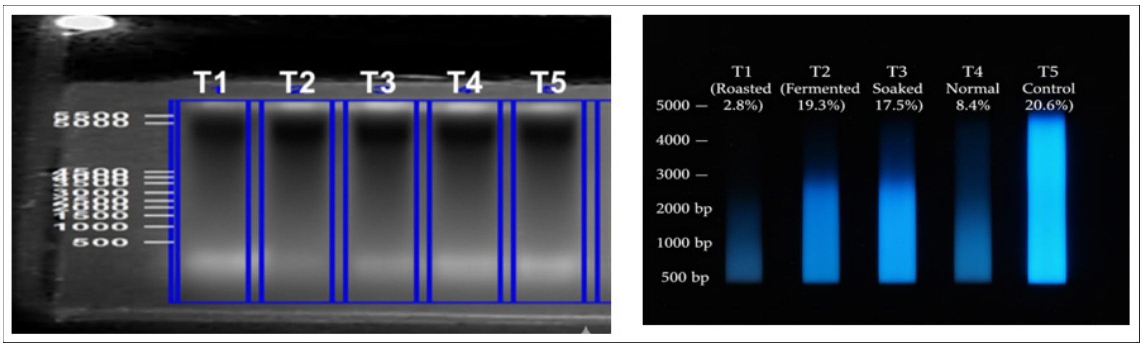

Figure 2: Gel Electrophoresis Analysis of DNA Integrity under Various Treatments

Figure 2: The DNA bands generated after PCR amplification of the mitochondrial 16S rRNA gene were analyzed by gel electrophoresis. The banding pattern showed clear, distinct bands at expected sizes, with no smearing or degradation observed. Band intensity reflects DNA quantity, with size markers (bp) on the right

T1- Roasted: Smallest DNA pile (2.8%). Roasting destroyed most of the DNA. (Fragment: 5,500 bp).

T2 -Fermented: Medium DNA pile (9.1%). This is the Reference amount. (Fragment: 5,000 bp).

T3 -Soaked: Largest DNA pile before the Control (17.5%). Soaking was the gentlest treatment. (Fragment: 4,500 bp).

T4 -Normal: Standard DNA pile (8.4%). This is the baseline amount for comparison. (Fragment: 4,000 bp).

T5 -Control: Absolute biggest DNA pile (20.6%). This is the maximum amount of untouched DNA. (Fragment: 3,500 bp).

Discussion

Water Quality Parameters

Throughout the experimental period, water quality parameters such as temperature, dissolved oxygen (DO), pH, and conductivity were maintained within optimal ranges for the culture of *Clarias gariepinus*, this suggests that FUTA sweet sorghum inclusion did not compromise water quality, which is critical for sustaining fish growth and physiological performance [13].

Proximate Composition of Experimental Diets

The proximate composition of the formulated diets showed significant differences in crude protein, lipid, ash, crude fiber, and nitrogen-free extract (NFE) values. Diets in which maize was substituted with sweet sorghum generally exhibited higher crude protein and fiber content compared to the control diet. These findings agree with Francis, who noted that alternative cereals can enhance the nutritional quality of aquafeeds when properly formulated [14]. The crude lipid values were reduced in sorghum diets, which may contribute to leaner fish flesh and reduce excessive fat deposition. The overall nutrient composition of the diets indicated that sweet sorghum is a promising feed ingredient capable of meeting the nutritional requirements of *Clarias gariepinus*.

Haematological Responses

Hematological parameters serve as sensitive indicators of the physiological and health status of fish in response to dietary changes. The results revealed significant variations ($p < 0.05$) in packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell count (RBC), and white blood cell count (WBC) across treatments. Fish fed diets in which maize was substituted with sweet sorghum showed improved RBC and Hb levels compared to the control diet, suggesting enhanced oxygen-carrying capacity and improved metabolic activity. Similar observations were reported by Gabriel, who found that alternative plant-based feed ingredients can enhance blood profile parameters when well-formulated [15].

WBC values were higher in sweet sorghum-fed groups, reflecting improved immune response and resilience against stress and pathogens. This agrees with the findings of Olayemi, who emphasized the role of plant-based antioxidants and bioactive compounds in stimulating immune function in cultured fish [16]. The variations in mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) further suggest that the dietary substitution of maize with sweet sorghum positively influenced erythropoiesis, indicating the suitability of the test ingredient for supporting hematological health.

Blood Biochemistry

The biochemical parameters, including total protein, cholesterol, glucose, and liver enzymes, further reflected the nutritional and metabolic effects of maize substitution with sweet sorghum. Serum total protein levels were significantly ($p < 0.05$) elevated in fish fed sorghum-based diets compared to the control, implying improved protein synthesis and utilization. This finding is consistent with Eyo, who reported that diets rich in quality plant protein sources enhance serum protein and support tissue development [17].

Serum glucose levels, an indicator of energy metabolism, showed moderate fluctuations across treatments, but values remained within the normal physiological ranges reported for Clarias gariepinus. The relatively stable glucose profile suggests that sweet sorghum inclusion did not induce metabolic stress. Cholesterol levels were lower in sorghum-fed groups, indicating that FUTA multipurpose sweet sorghum may play a role in lipid regulation, as supported by the findings of Oladipo and Adebayo, who observed hypocholesterolemic effects of sorghum derivatives in aquafeeds [18].

DNA and Growth Implications

Even though this study mainly examined proximate composition, hematological, and biochemical parameters, the findings also have broader implications for genetic stability and growth performance in fish. A nutritionally balanced diet does more than just maintain good health, it also influences how growth-related genes are expressed [19]. In this research, the consistent improvements in protein utilization, blood quality, and immune response among catfish fed with FUTA sweet sorghum suggest that the diet creates a stable internal environment. Over the long term, this stability can help sustain the fish's genetic potential, ensuring that genes responsible for growth are effectively expressed [7, 5]. Similar findings indicate that plant-based feeds, when balanced, enhance physiological resilience and may positively influence genetic expression linked to growth and immunity [14, 19]. Thus, replacing maize with FUTA sweet sorghum in the diet of *Clarias gariepinus* may not only improve immediate health but also promote better growth efficiency and resilience across generations [6, 21].

Plant-based diets have already been shown to regulate gene expression linked to metabolism, stress tolerance, and immunity in aquaculture species. Evidence further indicates that nutrient composition affects DNA methylation and transcriptional control of growth-related genes, thereby connecting nutrition directly with genetic stability [22-28]. These insights support the view that FUTA sweet sorghum, as a sustainable local feed, could strengthen long-term genetic expression and enhance growth performance in African catfish.

Conclusion

The findings of this study confirm that FUTA multipurpose sweet sorghum is a highly effective substitute for maize in the diets of *Clarias gariepinus*. Among the different feed options tested, the FUTA-developed sweet sorghum variety consistently delivered superior outcomes in terms of protein utilization, hematological stability, biochemical balance, and immune response. Its nutritional profile, coupled with the presence of beneficial phytochemicals, ensures that fish not only achieve steady growth but also maintain resilience against stress and disease.

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Competing Interests

The authors declared that they have no competing interests.

Authors' Contributions

FL and GOK conceived the study, supervised the research, and coordinated all project components. SB and AO conducted the experimental work, assisted in fish husbandry, collected and analysed data, and contributed to manuscript preparation. They

also performed phytochemical identification and analysis of bromelain and lignan, also contributed to laboratory work.

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