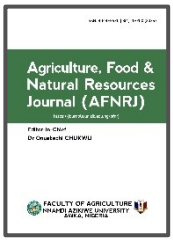
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Original Article

Haematological profile and growth performance of *Clarias gariepinus* fed diet fortified with cotton (*Gossypium herbaceum*) leaf extract

OPEN ACCESS

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ABSTRACT

High reliance on synthetic antibiotics in fish production has contributed to concerns about antibiotic resistance, creating interest in natural feed additives. This study investigated the effect of cotton leaf extract (CLE) on the haematological profile and growth performance of Clarias gariepinus. A total of 270 fingerlings (0.82 ± 0.13 g) were randomly distributed into 18 rectangular tanks (15 fish per tank) in a completely randomized design comprising six treatments replicated three times. The treatments were a basal commercial diet without additive (0.0 g/kg), an Aquaceryl reference diet containing 0.15 g/kg Aquaceryl+, and diets supplemented with 0.1, 0.2, 0.3 and 0.4 g CLE/kg diet. Fish were fed twice daily to apparent satiation for 60 days. Fish growth variables and blood samples were collected from three fish per replicate at the end of the feeding trial and analysed using standard haematological procedures. Data were analysed using one-way analysis of variance (ANOVA) and Duncan's multiple range test at $P < 0.05$. Dietary CLE significantly improved final weight, weight gain and specific growth rate, with the strongest responses generally observed at 0.2 and 0.3 g CLE/kg diet. Fish fed 0.2–0.4 g CLE/kg diet had significantly higher lymphocyte counts ($P < 0.05$), whereas red blood cell counts did not differ significantly among treatments ($P > 0.05$). Haemoglobin concentration differed significantly among treatments, with the highest value recorded at 0.2 g CLE/kg diet. Cotton leaf extract at 0.2 g/kg diet is recommended based on the combined growth and haematological responses observed in this study.

KEYWORDS: *Aquaculture, Fish nutrition, Haematology. Sustainable fish production*

INTRODUCTION

Fish remains an important source of animal protein worldwide, particularly in developing countries where it contributes substantially to people's dietary needs. The fisheries sector plays a major role in global food security, supplying a considerable proportion of the protein consumed by the world's population. However, the capacity of capture fisheries to meet the growing demand for fish is becoming increasingly limited. This situation has created a need to expand aquaculture production as an alternative means of increasing the availability of fish for human consumption (FAO, 2024). The rising demand for fish, coupled with the declining potential for further expansion of wild fish catches, has therefore increased global interest in aquaculture development (FAO, 2026). Among the species commonly cultured in West Africa, including Nigeria, the African catfish (*Clarias gariepinus*) is one of the most important because of its adaptability and suitability to farming conditions. The aquaculture industry has continued to expand, with farmers increasingly adopting intensive production

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systems to improve fish yield. However, intensification can also create conditions that favour the occurrence and spread of infectious diseases. Disease outbreaks remain a major challenge in fish farming because they can result in considerable mortality and significant economic losses. Effective disease management therefore requires a combination of measures, including appropriate use of antibiotics when necessary, maintaining suitable stocking densities, careful handling of fish, good water and environmental management, the provision of quality pelleted feeds, stocking during cooler periods, and vaccination where applicable (Wright *et al*., 2023; World Organisation for Animal Health [WOAH], 2025).

Antibiotics have long been used in aquaculture to promote fish growth and improve resistance to diseases. However, their excessive and continuous use, particularly for the prevention and treatment of bacterial infections, has contributed to the development and spread of antibiotic-resistant bacteria, creating concerns about their long-term use in aquaculture (Ferri *et al*., 2022; Deekshit *et al*., 2023). As a result, increasing attention has been given to natural alternatives, including medicinal plants, herbs, and spices, which can be incorporated into fish diets as feed additives to improve feed utilization and overall production performance. A growing body of research indicates that plant-derived feed additives and phytogenic compounds can positively influence several aspects of fish performance and health. Their inclusion in aquaculture diets has been associated with improved growth and feed utilization, enhanced appetite, modulation of physiological stress, and stimulation of both innate and adaptive immune responses (Citarasu, 2010; Vijayaram *et al*., 2022; Hossain *et al*., 2024). Plant bioactive compounds have also been reported to improve haematological and biochemical characteristics, antioxidant capacity, gut health, and resistance to bacterial and other pathogenic challenges in cultured fish (Vijayaram *et al*., 2022; Zare *et al*., 2023; Kuebutornye *et al*., 2024).

Recent experimental evidence further demonstrates that phytogenic supplementation can improve growth performance, immune-related parameters, and survival following pathogen challenge, supporting its potential as a functional nutritional strategy in aquaculture (Adeniyi *et al*., 2025; Gruber *et al*., 2025). These effects are generally attributed to the diverse bioactive constituents of plants, including polyphenols, flavonoids, terpenoids, essential oils and other phytochemicals with antioxidant, antimicrobial, anti-inflammatory and immunomodulatory properties (Vijayaram *et al*., 2022; Adeniyi *et al*., 2023, 2024; Hossain *et al*., 2024). Consequently, increasing scientific attention has been directed toward herbs, plant extracts and other phytogenic products as functional feed additives that may contribute to healthier and more environmentally sustainable aquaculture while reducing reliance on conventional chemotherapeutic agents and antibiotics.

Cotton (*Gossypium herbaceum*) is an economically important crop cultivated in Nigeria, primarily for its fibre, while the plant has also been associated with traditional medicinal applications. In Nigeria, *G. herbaceum* has been reported to have medicinal uses, including the traditional use of its leaves for the treatment of diarrhoeal conditions (Adeniyi & Lawal, 2017). Phytochemical investigations of *G. herbaceum* leaves have identified several bioactive constituents, including flavonoids, tannins, saponins, steroids, phenolic compounds and terpenoids, although the composition may vary with the extraction solvent and analytical method employed (Larayetan *et al*., 2021; Oloruntola *et al*., 2024). Flavonoids and other phenolic compounds are of particular interest because they possess documented antimicrobial and antioxidant properties, with evidence of antibacterial and antifungal activities reported for flavonoid-rich plant extracts (Panche *et al*., 2016; Sharma *et al*., 2025). Extracts of *G. herbaceum* leaves have also demonstrated wound-healing activity in experimental animal models, including enhanced wound contraction, reduced epithelialization time and increased wound-breaking strength (Velmurugan *et al*., 2012; Velmurugan, 2013). More recently, experimental studies have further demonstrated antioxidant, anti-inflammatory and other nutraceutical properties of *G. herbaceum* leaf powder, supporting its potential as a source of biologically active plant compounds (Oloruntola *et al*., 2024). Importantly, the application of *G. herbaceum* in aquaculture has received comparatively little attention, although Adeniyi and Lawal (2017) reported that dietary supplementation with air-dried cotton leaf powder improved growth performance and nutrient utilization in African catfish (*Clarias gariepinus*). Therefore, further investigation is warranted to establish the effects of cotton leaf extract, particularly on growth performance, nutrient utilization and haematological responses of *C. gariepinus*.

MATERIALS AND METHOD

Experimental Site

The experiment was conducted at the Teaching and Research Farm of the College of Agriculture, Kwara State University, Malete, Nigeria. The Malete campus lies within approximately 8.6979–8.7284°N latitude and 4.4595–4.5030°E longitude and is located within the Southern Guinea Savanna ecological zone. The area has distinct wet and dry seasons, with the rainy season generally occurring from April to October and the dry season from November to March. The mean annual temperature is approximately 27°C, while average annual rainfall is about 1,100 mm. The vegetation is characterized by a mixture of trees, shrubs, grasses and seasonal herbs. The study area has an average elevation of about 300 m, with local variation across the campus (Suleiman *et al*., 2018).

Plant Collection and extraction

Fresh leaves of cotton were collected at Ganmo Area, Kwara State, Nigeria. Fresh cotton leaves were gathered in a sack, weighed, cleaned, and air-dried at room temperature (25°C) for 2 weeks. It was further oven-dried at 50°C in the laboratory for 2 hours and ground into a powdery meal using a blender. Soxhlet extraction was carried out for 6 hours at 1: 10w/v of

the cotton leaf meal and ethanol. The extracts obtained were concentrated and tried to remove the solvent used for the extraction. The extracts were collected in a beaker and placed in a water bath at a constant temperature of 78.37°C. The dried sample was labeled and refrigerated at -25°C for further use.

Experimental Design

The experiment was arranged using a completely randomized design (CRD), consisting of six dietary treatments, each replicated three times. The six treatments were: (T1) basal commercial diet without additive (0.0 g/kg); (T2) basal commercial diet supplemented with 0.15 g/kg Aquaceryl+; (T3) basal commercial diet supplemented with 0.1 g CLE/kg; (T4) basal commercial diet supplemented with 0.2 g CLE/kg; (T5) basal commercial diet supplemented with 0.3 g CLE/kg; and (T6) basal commercial diet supplemented with 0.4 g CLE/kg. Aquaceryl+ was treated as an antibiotic reference treatment, not as a second control, because it was included to provide a benchmark against a commercial antibiotic-containing feed additive.

Preparation of Experimental Diets

A commercial Skretting feed (1.0 mm) was used as the basal diet; no feed formulation was performed in this study. The basal commercial pellets were weighed into separate batches for each treatment. For the CLE treatments, the required quantity of cotton leaf extract (0.1, 0.2, 0.3 or 0.4 g) was first dissolved in a small measured volume of the extraction solvent and then evenly sprayed onto 1 kg of basal feed while the pellets were continuously mixed to obtain a uniform coating. The treated feed was air-dried for 24 hours at room temperature to remove residual solvent before feeding. For the Aquaceryl reference treatment, 0.15 g Aquaceryl+ was similarly dispersed and uniformly applied to 1 kg of basal feed according to the manufacturer's recommended inclusion rate, followed by 24 hours of air-drying. Aquaceryl+ contains oxytetracycline, neomycin sulphate, erythromycin stearate and dextrose. The treated diets were packed separately in labelled polythene bags and stored in a dry place until use. The basal Skretting feed was subjected to proximate analysis before the feeding trial.

Experimental Protocol

Fingerlings of *Clarias gariepinus* were obtained from a reputable fish hatchery in Ilorin, Kwara State, Nigeria, and transported to the Teaching and Research Farm of the College of Agriculture, Kwara State University, Malete. Upon arrival, the fish were allowed to acclimatize for 14 days in a 300-L plastic tank and were fed with commercial Skretting feed (0.8 mm) throughout the acclimation period. At the end of the acclimation period, 270 fingerlings with an initial mean weight of 0.82 ± 0.13 g were randomly selected and distributed among 18 experimental tanks measuring 45 × 35 × 30 cm³. Each tank was filled with 40 L of water and stocked with 15 fingerlings, with each treatment replicated three times. To minimize the risk of the fish jumping out of the tanks, all experimental units were securely covered with mosquito netting.

Before the feeding trial commenced, the fish were deprived of feed for 24 hours to allow gastrointestinal clearance and stimulate feeding response. Following this period, fish were fed the respective experimental diets to apparent satiation twice daily, between 8:00 and 9:00 a.m. and between 5:00 and 6:00 p.m., throughout the 60-day experimental period. Fish biometric measurements, including body weight and total length, were taken at the beginning of the experiment and subsequently at weekly intervals to monitor growth. To maintain suitable conditions for growth and survival, water in each experimental tank was replaced every three days.

Data on Fish Growth Performance

At the end of the feeding trial, growth performance and nutrient utilization were assessed using standard indices. The equations used were as follows (Viagem *et al*., 2026):

$$Survival\ (\%) = (NF \div NI) \times 100 \quad (1)$$
$$SGR\ (\%\ day^{-1}) = [ln(FW) - ln(IW)] \div t \times 10 \quad (2)$$
$$PER = WG \div PI \quad (3)$$
$$PI = FI \times CP\ fraction \quad (4)$$
$$FCR = FI \div WG \quad (5)$$
$$RGR\ (\%) = [(FW - IW) \div IW] \times 100 \quad (6)$$
$$WG = FW - IW \quad (7)$$

Where: NF = number at final, NI = number at initial, SGR = specific growth rate, FW = final body weight, IW = initial body weight, t = time of experiment, PER = protein efficiency ratio, WG = weight gain, PI = protein intake, FI = feed intake, CP = crude protein, FCR = feed conversion ratio, RGR = relative growth rate.

Haematological Analysis

Blood collection and analysis

At the end of the feeding trial, three fish were randomly selected from each replicate for blood collection. Blood samples were drawn from the caudal vein of each fish using a 2 mL syringe and transferred into heparinized sample bottles to prevent clotting. Samples were immediately kept on ice in a cooler and transported to the Central Research Laboratory, Ilorin, Nigeria, for analysis. Standard fish haematological procedures described by Blaxhall and Daisley (1973) were followed. Haemoglobin concentration was estimated using the cyanmethemoglobin method, packed cell volume (PCV) was determined using the microhaematocrit technique, and red blood cell indices were calculated following Dacie and Lewis (2001). The haematological parameters assessed included red blood cell (RBC) count, haemoglobin (HGB), PCV, lymphocyte (LYM), platelet-related parameters and red blood cell indices. The equations for MCV, MCH and MCHC were expressed as follows:

$$MCHC = [HGB\ (g/dL) \times 100] \div Hematocrit\ (\%) \quad (8)$$
$$MCH = [HGB\ (g/dL) \times 10] \div RBC\ (\times 10^{12}/L) \quad (9)$$

$MCV = [Hematocrit\ (\%) \times 10] \div RBC\ (\times 10^{12}/L)$ (10)
Where: MCHC = mean corpuscular haemoglobin concentration, MCH = mean corpuscular haemoglobin, MCV = mean corpuscular volume, HGB = haemoglobin concentration, RBC = red blood cell counts

Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA), and Duncan's multiple range test was applied to determine significant differences among the treatment means. Polynomial regression analysis was also performed to assess the relationship between the treatments and weight gain. All statistical analyses were conducted at a 5% probability level using SPSS (Statistical Package for the Social Sciences), Version 23.

RESULTS

Experimental Diet Proximate Composition

The proximate composition of the basal commercial diet is presented in Table 1. The Skretting feed (1.0 mm) was subjected to proximate analysis before administration to *Clarias gariepinus*. The feed contained 45.23% crude protein and a gross energy value of 1644.61 kJ/100 g.

Effect of cotton leaf extract on nutrient utilization and growth performance of *Clarias gariepinus*

The growth and feed utilization parameters of African catfish fed the test diets are presented in Table 2. Final weight, weight gain and SGR were significantly higher ($P < 0.05$) in several CLE treatments than in the basal and Aquaceryl reference diets, with the highest final weight and weight gain recorded at 0.2 g CLE/kg diet. Feed intake and FCR were generally lower in fish fed CLE-supplemented diets. PER was significantly higher at 0.2 and 0.4 g CLE/kg diet. Relative growth rate was significantly higher at 0.2 and 0.3 g CLE/kg diet (Figure 1). Survival was numerically highest at 0.4 g CLE/kg diet, although the differences among treatments were not statistically significant ($P > 0.05$).

Table 1: Proximate composition of the basal commercial diet (Skretting, 1.0 mm) fed to *Clarias gariepinus*.

Parameters	Composition
Moisture (%)	7.15
Ash (%)	7.19
Gross energy (KJ/100g)	1644.61
Nitrogen-free extract (%)	26.26
Crude Protein (%)	45.23
Crude Fibre (%)	2.22
Crude Lipids (%)	11.95

Cotton leaf extract effect on the haematological parameters of *Clarias gariepinus*

Table 3 presents the haematological responses of *Clarias gariepinus* fed the experimental diets. Fish receiving 0.2 g CLE/kg diet recorded the highest haemoglobin concentration. Lymphocyte counts were significantly higher ($P < 0.05$) in fish fed diets containing 0.2–0.4 g CLE/kg than in the basal and Aquaceryl reference treatments. Red blood cell counts did not differ significantly among treatments ($P > 0.05$). Dietary CLE also had no significant effect on PCV, RDW-SD or RDW-CV. Similarly, MCV, MCH and MCHC did not vary significantly among the experimental groups

Table 2: Growth performance, nutrient utilization and survival of *Clarias gariepinus* fed diets supplemented with cotton leaf extract over a 60-day feeding period.

Parameter	Dietary treatment					
	Basal (0.0 g/kg)	Aquaceryl+ (0.15 g/kg)	CLE (0.1 g/kg)	CLE (0.2 g/kg)	CLE (0.3 g/kg)	CLE (0.4 g/kg)
Initial weight (g)	0.79 ± 0.03	0.84 ± 0.03	0.83 ± 0.04	0.81 ± 0.03	0.79 ± 0.04	0.84 ± 0.06
Final weight (g)	12.07 ± 0.17^{b}	11.64 ± 0.14^{a}	13.03 ± 0.18^{c}	13.50 ± 0.24^{d}	13.39 ± 0.16^{d}	13.22 ± 0.15^{cd}
Weight gain (g)	11.26 ± 0.13^{b}	10.79 ± 0.11^{a}	12.18 ± 0.14^{c}	12.67 ± 0.26^{d}	12.61 ± 0.19^{d}	12.37 ± 0.10^{cd}
SGR (g/day)	4.54 ± 0.04^{b}	4.39 ± 0.03^{a}	4.60 ± 0.05^{bc}	4.69 ± 0.09^{c}	4.71 ± 0.09^{c}	4.60 ± 0.09^{bc}
Feed Intake (g)	12.85 ± 0.06^{c}	12.34 ± 0.15^{d}	10.52 ± 0.09^{b}	10.31 ± 0.10^{ab}	10.77 ± 0.22^{c}	10.16 ± 0.05^{a}
FCR	1.14 ± 0.01^{c}	1.14 ± 0.01^{c}	0.86 ± 0.003^{b}	0.81 ± 0.02^{a}	0.85 ± 0.03^{b}	0.82 ± 0.003^{a}
PER	1.94 ± 0.01^{a}	1.93 ± 0.01^{a}	2.56 ± 0.01^{b}	2.72 ± 0.08^{c}	2.59 ± 0.09^{b}	2.69 ± 0.01^{c}
RGR	1420.75 ± 39.13^{b}	1289.77 ± 26.20^{a}	1475.11 ± 46.59^{b}	1566.42 ± 90.11^{c}	1592.25 ± 93.34^{c}	1482.30 ± 86.85^{b}
S (%)	88.89 ± 3.85	88.89 ± 3.85	91.11 ± 3.85	91.11 ± 3.85	93.33 ± 0.00	95.56 ± 3.85

Values are presented as treatment means ± standard deviation. Different superscript letters within a row indicate significant differences among treatments at $P < 0.05$. A = Aquaceryl+ reference treatment; CLE = cotton leaf extract; FCR = feed conversion ratio; RGR = relative growth rate; S = survival; PER = protein efficiency ratio; SGR = specific growth rate.

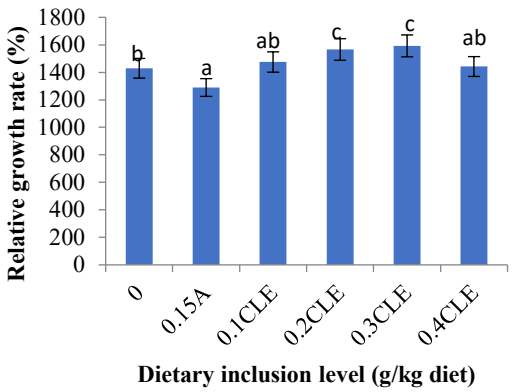


Figure 1: Relative growth rate of Clarias gariepinus fed diets supplemented with cotton leaf extract over a 60-day feeding period. *A = Aquaceryl+ reference treatment; CLE = cotton leaf extract.*

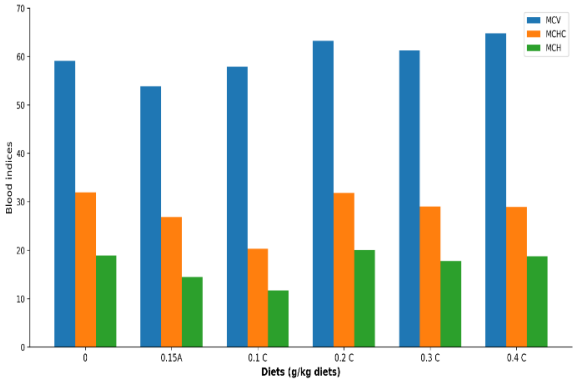


Figure 2: Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) of *Clarias gariepinus* after feeding diets supplemented with cotton leaf extract for 60 days.

Table 3: Haematological parameters of *C. gariepinus* fed diets containing cotton leaf extract for 60 days.

Parameters / Parameter	Dietary treatment					
	Basal (0.0 g/kg)	Aquaceryl+ (0.15 g/kg)	CLE (0.1 g/kg)	CLE (0.2 g/kg)	CLE (0.3 g/kg)	CLE (0.4 g/kg)
LYM (cells/liter)	56.75 ± 3.10^{a}	64.50 ± 2.65^{a}	68.75 ± 2.22^{a}	96.25 ± 1.89^{b}	96.50 ± 1.29^{b}	97.50 ± 1.29^{b}
RBC (cells/µL)	7.80 ± 0.36	7.40 ± 0.43	8.45 ± 0.47	7.28 ± 0.17	8.37 ± 0.36	6.89 ± 0.22
HGB (g/dl)	14.70 ± 0.37^{c}	10.65 ± 0.54^{ab}	9.85 ± 0.45^{a}	14.90 ± 0.80^{c}	14.85 ± 0.40^{c}	12.85 ± 0.54^{b}
PCV (g/L)	46.00 ± 1.83	39.75 ± 1.71	48.75 ± 1.71	46.00 ± 1.41	51.25 ± 2.21	44.50 ± 1.29
RDWSD (fL)	22.60 ± 0.71	22.05 ± 0.82	24.95 ± 1.90	23.95 ± 0.82	22.95 ± 0.61	23.65 ± 0.57
RDWCV (fL)	15.40 ± 0.36	14.25 ± 0.54	13.85 ± 0.24	14.60 ± 0.24	15.25 ± 0.48	14.15 ± 0.33

Values are presented as treatment means ± standard deviation. Different superscript letters within a row indicate significant differences among dietary treatments at $P < 0.05$. A = Aquaceryl+ reference treatment; CLE = cotton leaf extract; PCV = packed cell volume; RBC = red blood cell; HGB = haemoglobin; LYM = lymphocyte; RDW-SD = red blood cell distribution width standard deviation; RDW-CV = red blood cell distribution width coefficient of variation.

DISCUSSION

The increasing dependence on synthetic antibiotics has contributed to the growing problem of antibiotic resistance, creating a need to explore safer and more natural alternatives. As a result, considerable attention has been given to the use of plant-based additives in aquaculture feeds. Several herbs and spices have been investigated and successfully incorporated into fish diets because of their potential to enhance growth, improve overall health, and strengthen the immune system of cultured fish (Bondad-Reantaso *et al*., 2023; Wang *et al*., 2024; Gruber *et al*., 2025).

The significantly higher WG, SGR, RGR and PER recorded in fish fed 0.2 and 0.3 g CLE/kg diet may be attributed to improved utilization of the diets and the bioactive constituents present in cotton leaves. This observation is consistent with Dada & Ikuerowo (2009), who reported improved growth performance in *C. gariepinus* broodstock fed diets supplemented with ethanolic Garcinia kola seed extract. Similarly, Adeniyi & Lawal (2017) observed enhanced growth performance in *C. gariepinus* fed diets containing cotton leaf powder. The present findings also agree with Adeniyi *et al*. (2018; 2023; 2024), who reported improved growth performance, nutrient utilization or health-related responses following dietary supplementation with *Tamarindus indica*, *Moringa oleifera* and *Jatropha gossypiifolia* plant products in cultured fish.

The red blood cell (RBC) counts recorded in this study showed no significant difference among the treatment groups. Red blood cells are important for oxygen transport and carbon dioxide exchange in fish (Harter & Brauner, 2021; Chen & Luo, 2023). Therefore, the absence of significant variation in RBC values suggests that the dietary treatments did not substantially alter erythrocyte-related oxygen transport. Packed cell volume (PCV), also referred to as haematocrit, also showed no significant variation among treatments. Haemoglobin concentration, however, differed significantly, with the highest value observed at 0.2 g CLE/kg diet. Lymphocyte counts were significantly higher in fish fed 0.2–0.4 g CLE/kg diet. Dietary

CLE had no significant effect on PCV, RDW-SD or RDW-CV. These findings indicate that the tested CLE inclusion levels produced measurable changes in some blood parameters, particularly haemoglobin and lymphocytes, without causing broad alterations in the erythrocyte profile.

The values of MCV, MCH and MCHC did not differ significantly among the treatment groups. This finding differs from Olasunkanmi (2011), who reported changes in MCH in *C. gariepinus* fed diets containing raw *Mucuna utilis* seed meal. MCV provides information about erythrocyte size and can be useful in assessing abnormalities associated with erythropoiesis. Changes in MCV may occur with alterations in erythrocyte size, including those associated with macrocytic or microcytic anaemia (Maner *et al*., 2024; Killeen & Adil, 2025). The absence of significant differences in MCV, MCH and MCHC in the present study suggests that the tested CLE inclusion levels did not cause detectable alterations in these red blood cell indices.

CONCLUSION AND RECOMMENDATIONS

The study demonstrated that dietary cotton leaf extract influenced growth performance, nutrient utilization and selected haematological parameters of *C. gariepinus* fingerlings. The most favourable overall responses were observed at 0.2 g CLE/kg diet, while 0.3 g CLE/kg also improved several growth indices. The findings support the potential of cotton leaf extract as a phytogenic feed additive and an inclusion level of 0.2 g CLE/kg feed is therefore recommended under the conditions of this study. Further research should investigate the phytochemical composition, antimicrobial activity, safety, histological effects and dose-response relationship of cotton leaf extract in *C. gariepinus.*

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Authors Contributions

OVA, TAO & JOO managed data collection, data interpretation, data presentation, material support, and wrote the first draft of the manuscript. ATA & JBZ managed the literature review and data analysis. AYA & BF managed the development of methodology and management of the experimental setup. All authors read and approved the final manuscript.

Ethical Statement

All institutional ethical guidelines for handling of experimental animals were adhered to during the study.

AI Declaration Statement

The authors declare that Artificial Intelligence (AI) tools, ChatGPT and Grammarly, were used for improving the structure of the manuscript and language editing/grammar correction, respectively. All outputs were critically reviewed, verified, and approved by the authors, who accept full responsibility for the content of the manuscript.

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