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Effects of Dietary Inclusion of Brazilian Joyweed (*Alternanthera brasiliana*) Leaf Meal on Growth Performance, Haematological and Biochemical Profiles of African Catfish (*Clarias gariepinus*) Juveniles

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Highlights

- Dietary inclusion of *Alternanthera brasiliana* leaf meal at 0.5 g/100 g produced the best growth performance in *Clarias gariepinus* juveniles.
- Fish fed the 0.5 g/100 g diet recorded the highest final weight, weight gain, and improved feed conversion ratio, showing better feed utilization than the control group.
- Higher inclusion levels reduced growth performance, likely due to antinutritional factors, reduced nutrient digestibility, and lower feed palatability.
- Moderate supplementation improved haematological indices, including packed cell volume, haemoglobin concentration, red blood cells, and white blood cells, suggesting better oxygen transport and immune response.
- Biochemical parameters such as total protein, albumin, and globulin improved, indicating enhanced physiological condition and immune status in the fish.

Abstract

This study evaluated the effects of dietary inclusion of *Alternanthera brasiliana* leaf meal on growth performance, feed utilization, haematological indices, and biochemical parameters of *Clarias gariepinus* juveniles during a 56-day feeding trial. A total of 225 juveniles (initial mean weight: 9.08 ± 0.10 g) were randomly distributed into 15 glass tanks at 15 fish per tank, with three replicates per treatment. Five experimental diets containing 0.0, 0.5, 1.0, 1.5, and 2.0 g/100 g *A. brasiliana* leaf meal were formulated and fed to the fish. Fish fed the diet containing 0.5 g/100 g leaf meal (AB2) achieved the highest final weight (39.56 g), weight gain (30.48 g), and improved feed conversion ratio (1.51) compared with the control. Higher inclusion levels, however, resulted in reduced growth performance, likely due to antinutritional factors affecting nutrient digestibility and feed palatability. Haematological analysis revealed significant improvements in packed cell volume and white blood cell counts, while haemoglobin concentration increased in most supplemented groups, particularly in AB2, AB4 and AB5, indicating improved oxygen transport and enhanced immune response. Biochemical parameters, including total protein, albumin, and globulin, were also significantly improved at moderate inclusion levels, suggesting enhanced physiological and immune status. The study

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concludes that dietary inclusion of *A. brasiliana* leaf meal at 0.5 g/100 g can serve as an effective functional feed additive for improving growth performance and physiological health in *C. gariepinus* juveniles.

Keywords: *Clarias gariepinus; Alternanthera brasiliana;* growth performance; haematology; aquafeed

1. Introduction

Aquaculture remains one of the fastest-growing sectors in global agriculture, contributing substantially to food security, poverty alleviation, and economic development (FAO, 2022). African catfish (*Clarias gariepinus*) remains one of the most important cultured fish species in Nigeria and many parts of Sub-Saharan Africa because of its rapid growth, hardiness, tolerance of high stocking densities, and adaptability to intensive culture systems (Huisman and Richter, 1987; Chukwu et al., 2023). However, the high and unstable costs of conventional feed ingredients, particularly fishmeal and soybean meal, which account for a major proportion of production expenses, continue to threaten the profitability and sustainability of aquaculture (Tacon and Metian, 2015). To mitigate this challenge, increasing attention has been directed toward alternative, locally available plant-based feed resources that can reduce costs without compromising fish performance. Leaf meals from medicinal plants have gained interest due to their nutritional value and the presence of bioactive compounds with antimicrobial, antioxidant, and immunostimulatory properties (Dawood et al., 2018). These properties can enhance fish health, improve feed efficiency, and promote sustainable aquaculture practices. *Alternanthera brasiliana*, a tropical medicinal plant, contains diverse bioactive compounds, including flavonoids, terpenoids, phytosterols, and other phenolic constituents that contribute to its antioxidant and therapeutic properties (Alencar Filho et al., 2020). Medicinal plants and herbal essential oils have increasingly been investigated as functional feed additives in aquaculture due to their growth-promoting, immunostimulatory and health-enhancing properties (Dawood et al., 2022). In aquaculture nutrition, the search for alternative plant-based feed additives has intensified due to their potential to improve growth performance, health status, and sustainability of production systems (Reverter et al., 2021). Therefore, this study evaluated the effects of dietary inclusion of *A. brasiliana* leaf meal on the growth performance and physiological health of *Clarias gariepinus* juveniles.

2. Materials and Methods

Experimental Site

This research was conducted over a 56-day period at the Research Laboratory within the Department of Fisheries and Aquaculture Technology at The Federal University of Technology, Akure, Ondo State.

Plant Material

Fresh *Alternanthera brasiliana* leaves were collected from the area behind the School of Agriculture and Agricultural Technology Annex, Federal University of

Technology, Akure, Ondo State. The plant was identified and authenticated by a botanist in the Department of Crop Soil and Pest Management of the same institution. A voucher specimen (FUTA-Herbarium-AB2025-017) was deposited in the University herbarium for future reference. The leaves were air-dried at room temperature to preserve their volatile constituents, after which they were ground into a fine powder using an electric blender (Model ES 242). The resulting powder was stored in an airtight container at 4°C until further use.

Experimental Fish

Healthy *Clarias gariepinus* juveniles were obtained from the Federal University of Technology Fish Farm, Akure, Ondo State, Nigeria. The fish were acclimatized for seven days prior to the experiment. During this period, they were hand-fed a control diet containing 40% crude protein to apparent satiation twice daily, between 08:00–09:00 and 16:00–17:00 hours.

Experimental Diets

The basal diets were formulated to be isonitrogenous, but not strictly isolipidic, using standard feed formulation procedures, although crude lipid levels varied only slightly among diets (9.46–9.98%). Each basal diet had a total composition of 100 g/100 g and contained approximately 40% crude protein. Thereafter, *Alternanthera brasiliana* leaf meal was added at inclusion levels of 0.5–2.0 g per 100 g basal diet after formulation and prior to pelleting. The diets were subsequently air-dried at room temperature for 48 h, packaged in polyethylene bags, sealed, labelled according to treatment, and stored at 4°C until use.

Table 1: Gross composition of the basal experimental diets (g/100g) for *C. gariepinus* juveniles

Ingredients	**AB1 (0.0)**	**AB2 (0.5)**	**AB3 (1.0)**	**AB4 (1.5)**	**AB5 (2.0)**
Fish meal	25.4	25.4	25.4	25.4	25.4
Soybean meal	28.2	28.2	28.2	28.2	28.2
Groundnut cake	23.5	23.5	23.5	23.5	23.5
Yellow maize	13.1	13.1	13.1	13.1	13.1
Vitamin-Mineral mix	2.30	2.30	2.30	2.30	2.30
Vegetable oil	5.00	5.00	5.00	5.00	5.00
Methionine	0.30	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20	0.20
Starch	2.00	2.00	2.00	2.00	2.00
(*A. brasiliana* leaf meal g/100g)	0.00	0.5	1.0	1.5	2.0

Values represent the basal diet formulation (100 g/100 g). Alternanthera brasiliana leaf meal was added separately after feed formulation as a dietary additive.

Composition of vitamin-mineral mix (Aquamix) (quantity/kg), Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium; Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450. L- Lysine, 10 g; Selenium, 50 ppm.

Experimental Design and Management

The experiment was conducted using a completely randomized design. Following the acclimation period, all fish were weighed, and 225 healthy *Clarias gariepinus* juveniles with an average weight of 9.08 ± 0.10 g were randomly selected and distributed into 15 glass tanks (70 × 45 × 45 cm) at a density of 15 fish per tank, representing five treatments with three replicates each. The experimental diets were administered twice daily between 08:00–09:00 and 16:00–17:00 to apparent satiation over a period of 56 days. Fish were weighed biweekly to monitor growth performance. Survival percentages were calculated for each replicate tank at the end of the feeding trial and subsequently averaged across the three replicates within each treatment. To maintain water quality, partial water changes were performed every three days by siphoning out waste and replacing it with fresh water. Water parameters, including temperature, dissolved oxygen, and pH, were measured twice weekly. Haematological and biochemical assays were performed according to the procedures described by (Svobodova *et al.*, 1991; Morgan and Iwama, 1997).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to compare the means of different treatment groups. Tukey's post-hoc test was used to identify significant differences between the treatment groups. All statistical analyses were conducted using SPSS (Version 23), with a significance level set at $P < 0.05$.

3. Results and Discussion

Proximate Composition of *A. brasiliana* Leaf powder

A. brasiliana leaf powder contained a high carbohydrate content (68.24%), moderate protein (7.98%), low fat (3.56%), and notable fiber, ash, and moisture levels (Table 2). The protein content of 7.98% is moderate and comparable to levels reported for several plant-derived feed resources evaluated for aquaculture applications. Although this protein level is insufficient for use as a sole dietary protein source, the leaf meal may serve as a supplementary ingredient in aquafeeds when combined with conventional protein sources. In addition to providing nutrients, medicinal plants used as feed additives may contribute functional bioactive compounds that support fish growth, antioxidant capacity, and health status (Dadras *et al.*, 2023).

Table 2: Proximate composition of *A. brasiliana* leaf

Parameters (%)	Values
Moisture	8.94
Lipid	3.56
Protein	7.98
Fibre	4.55
Ash	6.73
Nitrogen Free Extract	68.24

Proximate Composition of Experimental Diets

Table 3 summarizes the proximate composition of the experimental diets administered to the fish. Moisture content ranged from 8.82% to 9.94%, with AB4 recording the lowest value and AB5 the highest. Ash content varied between 7.23% and 7.52%, with the highest in AB1 and the lowest in AB3. Crude lipid content ranged from 9.46% to 9.98%, with AB4 having the highest and AB1 the lowest. Crude fiber content increased progressively with higher inclusion levels of *A. brasiliana* leaf meal. The crude protein content of the diets ranged from 39.64% to 40.03%, indicating that all experimental diets provided protein levels within the range considered suitable for optimum growth and nutrient utilization in *Clarias gariepinus* juveniles. Similar dietary crude protein levels (35–40%) have been reported to support growth performance in African catfish (Ali *et al.,* 2024).

Table 3 Proximate compositions of the experimental diets

Parameters (%)	AB1 (0.0)	AB2 (0.5)	AB3 (1.0)	AB4 (1.5)	AB5 (2.0)
Moisture	9.34	9.50	9.77	8.82	9.94
Ash	7.52	7.41	7.23	7.35	7.26
Lipid	9.46	9.78	9.69	9.98	9.73
Fiber	2.91	3.13	3.18	3.22	3.25
Crude Protein	39.64	39.85	40.03	39.86	39.80
Nitrogen Free Extract	31.13	30.33	30.10	30.77	30.02

Water Quality Parameters

The water quality parameters recorded during the feeding trial remained within acceptable ranges for the culture of *Clarias gariepinus* juveniles. Dissolved oxygen ranged from 6.63 to 6.70 mg/L, temperature from 27.08 to 27.15°C, and pH from 7.55 to 7.60. These values are comparable with those reported for African catfish culture and fall within ranges suitable for normal growth and physiological

performance (Ogunji and Awoke, 2017). No significant differences (P > 0.05) were observed among treatments, indicating that dietary inclusion of *Alternanthera brasiliana* leaf meal did not negatively affect water quality during the feeding trial.

Table 4. Water quality parameters

Treatments	Dissolved oxygen (mg/L)	Temperature (°C)	pH
AB1 (0.0)	6.63±0.12[a]	27.15±0.04[a]	7.58±0.06[a]
AB2 (0.5)	6.67±0.25[a]	27.08±0.11[a]	7.55±0.03[a]
AB3 (1.0)	6.70±0.13[a]	27.13±0.01[a]	7.60±0.01[a]
AB4 (1.5)	6.65±0.11[a]	27.09±0.02[a]	7.56±0.01[a]
AB5 (2.0)	6.63±0.13[a]	27.12±0.09[a]	7.59±0.07[a]

No significant differences (P > 0.05) were observed among treatments.

Growth Performance and Nutrient Utilization of *Clarias gariepinus*

Table 5 presents the growth performance and nutrient utilization of *Clarias gariepinus* fed diets containing varying levels of *Alternanthera brasiliana* leaf meal. Fish fed the AB2 diet (0.5 g/100 g) achieved the highest final mean weight (39.56 g), significantly ($p < 0.05$) outperforming other treatments, while the control (AB1; 0.0 g/100 g) recorded the lowest values. Weight gain was significantly highest in AB2, while SGR in AB2 was statistically comparable with AB3, suggesting that moderate inclusion is optimal, whereas excessive supplementation may impair nutrient utilization. The reduced performance observed at higher inclusion levels may be associated with the limitations commonly reported for plant-derived feed ingredients, including reduced nutrient availability and impaired feed utilization when incorporated beyond optimal levels (Kheto *et al.,* 2025). Feed intake also decreased beyond AB2, likely due to reduced feed acceptance associated with increased levels of plant secondary metabolites at higher inclusion levels. Although medicinal plants contain beneficial bioactive compounds, excessive levels of certain phytochemicals may negatively affect feed utilization and feeding response in fish (Mbokane and Moyo, 2022). Feed conversion ratio was significantly improved in AB2 (1.51) compared with the control diet (1.76), while feed efficiency ratio did not differ significantly (P > 0.05) among treatments, indicating relatively similar feed utilization efficiency despite variations in growth performance. Survival remained high (86.67–93.34%) across all groups, indicating that dietary inclusion of *A. brasiliana* leaf meal up to 2.0 g/100 g was not detrimental to fish survival.

Table 5: Growth and nutrient utilization of experimental fish fed *A. brasiliana*

Parameters	AB1 (0.0)	AB2 (0.5)	AB3 (1.0)	AB4 (1.5)	AB5 (2.0)
IW (g)	9.09±0.01^{a}	9.08±0.01^{a}	9.09±0.04^{a}	9.06±0.03^{a}	9.08±0.06^{a}
FW (g)	24.62±3.65^{a}	39.56±1.03^{d}	37.39±0.01^{c}	34.81±6.44^{bc}	30.79±2.14^{b}
WG (g)	15.33±3.64^{a}	30.48±1.04^{d}	28.30±0.05^{c}	25.75±6.46^{bc}	21.71±2.09^{b}
SGR (%/day)	1.79±0.23^{a}	2.63±0.05^{c}	2.53±0.01^{c}	2.40±0.31^{b}	2.18±0.09^{b}
TFI (g/fish)	27.00±2.50^{a}	45.95±4.55^{d}	43.26±10.33^{c}	39.62±1.67^{bc}	35.23±1.47^{b}
FCR	1.76±0.08^{c}	1.51±0.08^{a}	1.53±0.37^{a}	1.54±0.39^{a}	1.62±0.08^{b}
FER	0.58±0.03^{a}	0.66±0.03^{a}	0.65±0.09^{a}	0.65±0.18^{a}	0.62±0.02^{a}
Survival (%)	91.11±3.33^{a}	93.33±6.67^{a}	86.67±6.42^{a}	88.89±0.00^{a}	93.33±6.67^{a}

Values in the same row with different superscripts differ significantly at P < 0.05.
***KEY**; IW = Initial weight, FW = Final weight, WG = Weight gain, SGR = Specific growth rate, TFI = Total feed intake, FCR = Feed conversion ratio, FER = Feed efficiency ratio*

Haematological Parameters of *Clarias gariepinus*

Table 6 presents the haematological responses of *Clarias gariepinus* fed diets containing varying levels of *Alternanthera brasiliana* leaf meal. Most blood indices differed significantly among treatments. Haemoglobin concentration increased in most supplemented groups, with significant elevations observed particularly in AB2, AB4 and AB5. Increased haemoglobin levels may indicate improved oxygen transport and physiological condition of the experimental fish. Packed cell volume increased from 20.03% in the control to a peak of 25.06% in AB3, while white blood cell counts rose from 4.92 × 10^{3}/mm^{3} to 8.85 × 10^{3}/mm^{3}, suggesting enhanced immune function. Red blood cell counts reached a maximum in AB3 before declining at higher inclusion levels. Erythrocyte indices (MCV, MCH, and MCHC) also varied significantly across treatments, indicating that dietary inclusion of *A. brasiliana* influenced erythrocyte morphology and haemoglobin synthesis. The lower MCV values observed in AB2 and AB3 suggest the production of smaller but more numerous erythrocytes, which may enhance oxygen transport efficiency, while MCH and MCHC varied among treatments, with increased values observed in AB2, AB4 and AB5, whereas AB3 recorded lower values than the control. These effects may be associated with antioxidant and immunostimulatory phytochemicals such as flavonoids, phenolics, and saponins present in medicinal plants, which can modulate immune function, physiological responses, and erythrocyte characteristics in fish. Variations in erythrocyte indices may therefore reflect adaptive changes in red blood cell morphology and haemoglobin content following exposure to plant-derived bioactive compounds, as reported in fish fed medicinal plant-based dietary additives (Yue *et al.,* 2024).

Table 6. Haematological parameters of experimental fish

Parameters	AB1 (0.0)	AB2 (0.5)	AB3 (1.0)	AB4 (1.5)	AB5 (2.0)
Hb (g/100ml)	5.10±0.01^{a}	7.67±0.03^{c}	5.75±0.01^{ab}	6.69±0.01^{b}	7.72±0.01^{c}
PCV (%)	20.03±0.07^{a}	23.20±0.12^{b}	25.06±0.32^{c}	23.01±0.24^{b}	23.08±0.68^{b}
WBC (x10^{3}/mm^{3})	4.92±0.03^{a}	5.21±0.01^{b}	8.85±0.01^{d}	8.62±0.01^{d}	7.93±0.02^{c}
RBC (x10^{6}/mm^{3})	2.01±0.02^{a}	2.85±0.02^{c}	3.10±0.03^{d}	2.23±0.01^{b}	2.49±0.01^{b}
MCV (fl)	99.65±2.09^{c}	81.40±0.80^{a}	80.84±0.64^{a}	103.18±3.11^{d}	92.69±1.42^{b}
MCH (pg)	25.37±0.37^{b}	26.91±0.14^{b}	18.55±0.36^{a}	30.00±0.12^{c}	31.00±0.19^{c}
MCHC (%)	25.46±0.36^{b}	33.06±0.24^{d}	22.94±0.16^{a}	29.07±1.04^{c}	33.45±0.31^{d}

Values in the same row with different superscripts differ significantly at P < 0.05.

Key: *PCV = Pack Cell Volume, Hb = Haemoglobin Content, WBC = White Blood Cell, RBC = Red Blood Cell, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, MCV = Mean Corpuscular Volume.*

Biochemical Profile of *Clarias gariepinus*

Table 7 presents the biochemical responses of *Clarias gariepinus* fed diets containing varying levels of *Alternanthera brasiliana* leaf meal. Serum cholesterol, glucose, total protein, albumin, and globulin differed significantly among treatments. Cholesterol increased with inclusion level up to 1.0% (AB3; 62.4 mg/dL) relative to the control (36.7 mg/dL), then declined at higher levels, suggesting that moderate supplementation may have influenced lipid metabolism and cholesterol regulation in the experimental fish. Changes in serum biochemical parameters following dietary supplementation with medicinal plants may be associated with the influence of plant bioactive compounds on metabolic processes, antioxidant activity, and physiological regulation. In *Clarias gariepinus*, dietary inclusion of herbal additives has been reported to alter serum metabolites, including glucose, protein, albumin, and globulin levels, reflecting improved metabolic condition and physiological responses (Iheanacho *et al.,* 2017). In the present study, glucose levels followed a similar pattern, rising with inclusion level and peaking at AB4 (56.3 mg/dL) before declining, indicating possible alterations in energy metabolism associated with dietary bioactive compounds. Total protein was significantly higher in AB2 and AB3 than in the control, reflecting improved nutrient utilization and enhanced physiological condition, while slight reductions at higher inclusion levels may be associated with increased dietary fibre and reduced nutrient availability. Increased albumin concentrations in supplemented groups suggest improved protein synthesis and transport functions, whereas elevated globulin levels indicate enhanced immune-related responses. These effects may be linked to bioactive constituents of medicinal plants, such as flavonoids, phenolics, and other

phytochemicals, which have been reported to modulate antioxidant activity, metabolism, and immune responses in cultured fish (Awad and Awaad, 2017).

Table 7: Biochemical profile of *C. gariepinus* of experimental fish

Parameters	AB1 (0.0)	AB2 (0.5)	AB3 (1.0)	AB4 (1.5)	AB5 (2.0)
Cholesterol (mg/dL)	36.7±0.01^{a}	54.3±0.01^{c}	62.4±0.02^{d}	44.9±0.01^{b}	39.2±0.01^{ab}
Glucose (mg/dL)	40.1±0.13^{a}	46.9±0.01^{b}	50.4±0.03^{c}	56.3±0.01^{d}	40.7±0.02^{a}
Total protein (g/dL)	3.09±0.01^{a}	5.58±0.02^{c}	5.63±0.02^{c}	4.22±0.04^{b}	4.19±0.01^{b}
Albumin (g/dL)	1.58±0.02^{a}	2.32±0.02^{c}	2.05±0.01^{b}	2.28±0.02^{c}	2.26±0.02^{c}
Globulin (g/dL)	1.51±0.02^{a}	3.26±0.01^{c}	3.58±0.32^{c}	1.94±0.02^{b}	1.93±0.03^{b}

Values in the same row with different superscripts differ significantly at P < 0.05.

4. Conclusion

The feeding trial demonstrated that moderate inclusion of *A. brasiliana* leaf meal (0.5 g/100 g) produced the most favourable outcomes in growth performance and nutrient utilization of *C. gariepinus* juveniles. This inclusion level also improved haematological indices and biochemical parameters without adverse effects on fish survival or physiological condition. The findings indicate that *A. brasiliana* leaf meal can be utilized as a functional phytogenic feed additive in aquaculture diets. Future studies should investigate the long-term effects of dietary inclusion, optimal processing methods for reducing antinutritional factors, and the mechanisms underlying the immunostimulatory properties of the plant in cultured fish species.

Author Contributions:

T.M. Oladipupo designed the study, conducted the feeding trial, performed the laboratory analyses, analyzed the data, and drafted the manuscript. N.R. Osungbemiro. supervised the research, contributed to the study design, critically reviewed the manuscript, and approved the final version for publication. All authors read and approved the final manuscript.

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Informed Consent Statement: Not applicable.

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Conflict of interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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