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Research Article

# Growth Performance, Haematological Parameters, and Biochemical Profiles of Nile Tilapia (*Oreochromis niloticus*) Fingerlings Fed Diets Containing Milkweed (*Euphorbia heterophylla*) Leaf Meal

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**ABSTRACT**

The present study evaluated the effects of *Euphorbia heterophylla* leaf meal inclusion as a phytogenic feed additive on growth performance and nutrient utilization of *Oreochromis niloticus* fingerlings over a 56-day feeding trial. Five approximately isonitrogenous diets containing 35% crude protein were formulated with *E. heterophylla* leaf meal included as a feed additive at 0.0, 0.5, 1.0, 1.5, and 2.0 g/100 g diet, designated as treatments EH1 to EH5. A total of 225 fingerlings of *O. niloticus* with an average initial weight of 5.87 ± 0.03 g were randomly selected and distributed into 15 glass tanks (70 L, 70 cm × 45 cm × 45 cm) at a stocking density of 15 fish per tank. Each treatment was replicated three times. The results indicated that fish fed the 0.5 g/100 g inclusion level (EH2) showed significantly improved growth performance and nutrient utilization compared with the control and other treatments. Haematological indices, including haemoglobin, packed cell volume, and white blood cell counts, indicated improved physiological status and may suggest enhanced physiological responsiveness in treated groups. Biochemical parameters showed variations in total protein, albumin, globulin, cholesterol, and glucose levels, indicating possible alterations in protein metabolism, lipid metabolism, and energy mobilization, although the biological significance of changes in glucose and cholesterol requires further investigation. This study demonstrates that *Euphorbia heterophylla* leaf powder has potential as a phytogenic feed additive for improving growth performance and modulating physiological responses in *O. niloticus*, contributing to sustainable aquaculture practices.

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## 1.0 INTRODUCTION

The global aquaculture industry has expanded rapidly over the past few decades, becoming a major source of animal protein and now supplying more than half of global fish consumption. This growth has made aquaculture essential in meeting increasing food demands, especially in developing regions where affordable animal protein remains limited (Thompson and Lee, 2022). Despite advances in breeding, nutrition, and management practices, the sector continues to face challenges related to feed efficiency, fish health management, and production sustainability. Consequently, there is increasing interest in nutritional strategies that can enhance growth performance, physiological condition, and overall fish health. Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured freshwater fish species due to its rapid growth, adaptability to diverse environmental conditions, efficient feed utilization, and consumer acceptance (Anderson *et al.,* 1991). In recent years, considerable attention has been directed toward the application of phytogenic feed additives in aquaculture because of their potential to improve growth performance, nutrient utilization, physiological responses, and health status of cultured fish. Plant-derived additives contain bioactive compounds, including phenolics, flavonoids, alkaloids, and saponins, which may exhibit antioxidant, antimicrobial, and immunomodulatory properties (Gabriel *et al.,* 2007). The effectiveness of phytogenic additives depends on the plant source, processing method, inclusion level, and fish species. Among underutilized tropical plants, *Euphorbia heterophylla* has attracted interest due to its widespread availability and reported medicinal properties. The plant contains various phytochemicals and nutrients that may contribute to beneficial physiological effects in animals (Patel and Kumar, 2020). Previous studies have demonstrated that dietary supplementation of *E. heterophylla* may influence growth performance, antioxidant status, and selected physiological responses in poultry and fish species when included at appropriate levels (Upah *et al.,* 2021). These effects have been associated with plant bioactive compounds that may influence digestion, metabolism, and physiological regulation. Although plant-derived feed additives have received increasing attention in aquaculture, information regarding the dietary application of *E. heterophylla* in Nile tilapia remains limited, particularly concerning the optimum inclusion level and its effects on growth performance, haematological characteristics, and biochemical responses. Since fish species may respond differently to phytogenic additives, evaluation under controlled feeding conditions is necessary to determine their potential benefits and physiological implications. Therefore, this study investigated the effects of dietary *Euphorbia heterophylla* leaf meal as a phytogenic feed additive on growth performance, nutrient utilization, haematological parameters, and biochemical profiles of *Oreochromis niloticus* fingerlings.

## MATERIALS AND METHODS

### Study Area

The study was conducted at the Department of Fisheries and Aquaculture Teaching and Research Farm, Federal University of Technology, Akure, Ondo State, Nigeria, for 56 days.

### Plant Material

Fresh leaves of *E. heterophylla* were collected from the area behind the School of Agriculture and Agricultural Technology Annex, The 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, Federal University of Technology, Akure, Nigeria. A voucher specimen (FUTA-Herbarium/Eup-037) was deposited in the University Herbarium for future reference. the leaves were thoroughly sorted to remove damaged, diseased, and extraneous materials and then rinsed with clean water to eliminate adhering dust and debris. The cleaned leaves were spread in a single layer on clean trays and air-dried under laboratory conditions at room temperature (27–30°C) for 10 days until a constant weight was attained. Drying was carried out in a well-ventilated room protected from direct sunlight, dust, insects, and other potential sources of contamination in order to minimize nutrient losses and preserve heat-sensitive bioactive compounds. The dried leaves were periodically turned to ensure uniform drying and prevent mould development. After drying, the leaves were milled into a fine powder using an electric blender (Model ES 242). The resulting leaf powder was sieved to obtain a uniform particle size and subsequently transferred into clean, airtight plastic containers. The containers were properly labelled and stored in a refrigerator at 4°C until incorporation into the experimental diets.

**Experimental Fish**

Apparently healthy *O. niloticus* fingerlings were purchased from the Federal University of Technology fish farm, Akure, Ondo State, Nigeria. The fish were acclimatized for 7 days. During this period, they were hand-fed a commercial control diet containing 35% crude protein to apparent satiation twice daily, between 08:00–09:00 and 16:00–17:00 GMT.

**Experimental Diets**

Five approximately isonitrogenous diets containing 35% crude protein were prepared for the feeding trial. The basal diet was formulated using fish meal, soybean meal, groundnut cake, yellow maize, methionine, lysine, vitamin-mineral premix, vegetable oil, and starch. Thereafter, *Euphorbia heterophylla* leaf meal was added separately as a dietary additive after feed formulation at inclusion levels of 0.0, 0.5, 1.0, 1.5, and 2.0 g/100 g diet for treatments EH1 (control), EH2, EH3, EH4, and EH5, respectively. Table 1 presents the gross and proximate compositions of the experimental diets. The ingredients of each diet were thoroughly mixed to ensure uniform distribution of the additive and then pelleted using a Horbart A-200T mixing and pelleting machine (Horbart Manufacturing Ltd., UK) fitted with a 2-mm diameter die. The resultant pellets were air-dried for three days under hygienic conditions, hand-crumbled into sizes suitable for fingerlings, and packaged in separate airtight polyethylene bags. The bags were properly labelled according to treatment and stored at 4°C until use.

**Weighing of Experimental Fish**

Fish weights were measured at the start of the experiment and subsequently every two weeks throughout the 56-day feeding trial. Fish were batch weighed using a digital electronic balance (Model PB3002) with a precision of ±0.01g. Care was taken to minimize stress and handling time during the weighing process

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**Table 1:** Gross and proximate compositions of the experimental diets

| Ingredients | EH1 (0.0) | EH2 (0.5) | EH3 (1.0) | EH4 (1.5) | EH5 (2.0) |
|---|---|---|---|---|---|
| Fish meal | 24.0 | 24.0 | 24.0 | 24.0 | 24.0 |
| Soybean meal | 28.0 | 28.0 | 28.0 | 28.0 | 28.0 |
| Groundnut cake | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Yellow maize | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Methionine | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Lysine | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin-mineral premix | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Starch | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Vegetable oil | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| *E. heterophylla* leaf | 0.00 | 0.50 | 1.00 | 1.50 | 2.00 |
| **proximate composition (%)** | | | | | |
| Moisture | 8.26 | 8.19 | 8.31 | 8.62 | 7.95 |
| Crude fibre | 3.41 | 3.54 | 3.62 | 3.65 | 3.69 |
| Crude protein | 34.68 | 34.77 | 35.04 | 35.03 | 34.75 |
| Ash | 9.27 | 9.43 | 9.35 | 9.23 | 9.27 |
| Lipids | 6.22 | 6.25 | 6.14 | 6.41 | 6.38 |
| Nitrogen-free extract | 38.16 | 37.82 | 37.54 | 37.06 | 37.96 |

Composition of vitamin-mineral mix (Aquamix) (quantity/kg), Vitamin A, 5,500,000 IU; Vitamin D3, 1,100,000 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 mg. L-Lysine, 10 g; Selenium, 50 ppm.

**Experimental Design and Management**

The experiment was conducted using a completely randomized design. Following the acclimation period, 225 healthy *Oreochromis niloticus* fingerlings with a mean initial weight of 5.87 ± 0.03 g were randomly distributed into 15 glass tanks (70 × 45 × 45 cm) at a stocking density of 15 fish per tank. The five dietary treatments (EH1–EH5) were each assigned to three replicate tanks. Fish were fed their respective experimental diets twice daily between 08:00–09:00 h and 16:00–17:00 h to apparent satiation for 56 days. Feeding was discontinued when active feeding ceased and no further feed consumption was observed. Uneaten feed and faecal wastes were siphoned out before subsequent feeding to maintain water quality. Complete water exchange was carried out twice weekly throughout the experimental period. Water quality parameters, including temperature, dissolved oxygen, and pH, were monitored twice weekly using standard procedures. The measured water quality parameters remained within acceptable ranges for the culture of *O. niloticus* throughout the study. Fish weights were recorded at the commencement of the experiment and subsequently at two-week intervals. At the end of the feeding trial, fish were fasted for 24 h prior to sampling. Three fish were randomly collected from each replicate tank (nine fish per treatment) for haematological and biochemical analyses. Prior to blood collection, fish were anaesthetized with tricaine methanesulfonate (MS-222) at 100 mg/L. Blood samples were collected from the caudal vein using sterile 2-mL syringes fitted with 23-gauge needles. Blood samples intended for haematological analysis were immediately transferred into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. For biochemical analysis, blood samples were collected into plain sterile tubes without anticoagulant and allowed to clot at room temperature for 30 min before centrifugation at 3,000 rpm for 10 min to obtain serum. The separated serum samples were carefully collected and stored at −20 °C until biochemical analyses.

Haematological parameters were determined following the procedures described by Svobodová et al. (1991). Serum biochemical parameters, including cholesterol, glucose, total protein, albumin, and globulin, were determined according to the methods described by Morgan and Iwama (1997) using commercial diagnostic assay kits (Randox Laboratories Ltd., United Kingdom) following the manufacturer's instructions. Biochemical analyses were performed using a semi-automated chemistry analyzer (Stat Fax® 3300, Awareness Technology Inc., USA). Cholesterol concentration was determined by the enzymatic colorimetric method, glucose by the glucose oxidase–peroxidase method, total protein by the biuret method, and albumin by the bromocresol green method. Serum globulin concentration was calculated as the difference between total protein and albumin concentrations.

Calculation of the growth performance data was done according to Keshavanath *et al.* (2023). At the end of the experiment, fish were counted and weighed. The growth parameters and feed utilisation indices were calculated as follows:

**Weight Gain (g)** = Final weight- initial weight

**Specific Growth Rate (SGR)**

This was calculated from data on changes of body weight over a given time interval;

$$\text{SGR (\% per day)} = \frac{\text{(LN Final Weight} - \text{LN Final Weight)}}{\text{(Time)}}\ x\ 100$$

**Feed intake**

Daily feed intake was calculated based on feed offered minus recovered uneaten feed from each tank after siphoning.

**Feed Conversion Ratio (FCR)** = $\frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$

**Feed Efficiency Ratio (FER)** = $\frac{\text{Weight gain (g)}}{\text{Feed intake (g)}}$

**Survival (%)** = $\frac{Number\ of\ fish\ harvested}{Number\ of\ fish\ stocked}\ x\ 100$

The proximate composition of the experimental diets and *Euphorbia heterophylla* leaf meal was determined according to the standard procedures described by the Association of Official Analytical Chemists (AOAC, 2016). Samples were analyzed for moisture content, crude protein, crude lipid, crude fibre, ash, and nitrogen-free extract (NFE). Moisture content was determined by oven-drying samples at 105 °C until constant weight was achieved. Crude protein content was estimated by the Kjeldahl method through determination of total nitrogen, and protein values were calculated by multiplying nitrogen content by a conversion factor of 6.25. Crude lipid content was determined by Soxhlet extraction using petroleum ether as the extracting solvent. Crude fibre was analyzed by sequential acid and alkaline digestion, while ash content was determined by incineration of samples in a muffle furnace at 550 °C until constant weight was obtained. Nitrogen-free extract (NFE) was calculated by difference using the formula:

NFE (%) = 100 − (% moisture + % crude protein + % crude lipid + % crude fibre + % ash)

**Statistical Analysis**

Data were expressed as mean ± standard deviation (SD). Growth performance, nutrient utilization, haematological, biochemical, and water quality data were subjected to one-way analysis of variance (ANOVA) to determine the effects of dietary *Euphorbia heterophylla* leaf meal inclusion levels. The replicate tank was considered the experimental unit for the statistical analysis. Where significant differences occurred among treatment means, Tukey's honestly significant difference (HSD) test was used for mean separation. All statistical analyses were performed using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA), and differences were considered significant at $P < 0.05$. Prior to ANOVA, data were examined for normality and homogeneity of variance to ensure compliance with the assumptions of parametric analysis.

## RESULTS AND DISCUSSION

### Proximate Composition of *E. heterophylla* Leaf Powder

The proximate composition of *Euphorbia heterophylla* leaf powder is presented in Table 2. The results showed that nitrogen-free extract had the highest value (67.29%), while lipid content recorded the lowest value (2.34%). The ash content (2.75%) indicates the presence of mineral constituents in the leaf powder. The observed proximate composition provides information on the basic nutritional characteristics of *E. heterophylla* leaf powder and supports its evaluation as a dietary additive in aquaculture. The crude protein content of 10.26% recorded in this study is relatively low compared with conventional protein-rich feed ingredients; therefore, *E. heterophylla* leaf powder may not serve as a major protein replacement ingredient. However, its inclusion in the present study was as a dietary additive, where its potential effects may be associated with its bioactive constituents rather than its protein contribution. The observed protein value agrees with the findings of Adeyemi and Ogunniyi (2019), who reported protein levels ranging from 9.8% to 12.4% in *E. heterophylla* leaves collected from different geographical locations.

**Table 2:** Proximate composition of *E. heterophylla* leaf meal

| Parameters (%) | Values |
|---|---|
| Moisture | 13.14 |
| Ash | 2.75 |
| Lipid | 2.34 |
| Protein | 10.26 |
| Crude fibre | 4.22 |
| Nitrogen-free extract | 67.29 |

**Water Quality Parameters**

The water quality parameters monitored during the 56-day feeding trial varied as follows: dissolved oxygen ranged from 6.65 to 6.73 mg/L, temperature ranged from 26.77 to 26.84°C, and hydrogen ion concentration (pH) ranged from 7.26 to 7.33 (Table 3). The measured water quality parameters remained relatively stable throughout the experimental period and were within acceptable ranges for Nile tilapia (*Oreochromis niloticus*) culture. The recorded dissolved oxygen, temperature, and pH values indicate that the culture conditions were favourable for fish growth and physiological performance. These findings are consistent with previous reports indicating that these parameters fall within suitable ranges for freshwater fish production, including Nile tilapia (Thompson, 2019; Ahmed and Hassan, 2021).

**Table 3:** Water Quality parameters measured during the experimental period

| **Treatments** | **Dissolved oxygen (mg/L)** | **Temperature (°C)** | **pH** |
|---|---|---|---|
| EH1(0.0) | $6.73±0.05^a$ | $26.84±0.05^a$ | $7.32±0.05^a$ |
| EH2 (0.5) | $6.67±0.02^a$ | $26.79±0.23^a$ | $7.26±0.02^a$ |
| EH3 (1.0) | $6.65±0.01^a$ | $26.83±0.01^a$ | $7.33±0.05^a$ |
| EH4 (1.5) | $6.70±0.02^a$ | $26.81±0.02^a$ | $7.29±0.01^a$ |
| EH5 (2.0) | $6.67±0.02^a$ | $26.77±0.40^a$ | $7.30±0.03^a$ |

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

**Growth Performance**

The final weight, weight gain, specific growth rate (SGR), total feed intake, and feed conversion ratio (FCR) were significantly affected (P < 0.05) by the inclusion of *Euphorbia heterophylla* leaf meal in *Oreochromis niloticus* diets (Table 4). However, feed efficiency ratio (FER) and survival percentage showed no significant differences among the dietary treatments (P > 0.05). Dietary inclusion of *E. heterophylla* leaf meal improved growth performance in fish compared with the control group, with the 0.5 g/100 g (EH2) and 1.0 g/100 g (EH3) inclusion levels producing comparable and favourable results in terms of final weight, weight gain, and specific growth rate. Since EH2 and EH3 shared the same statistical grouping for these parameters, their growth responses were not significantly different. The improved growth performance observed in fish fed *E. heterophylla*-supplemented diets indicates that the plant contains bioactive compounds that may enhance nutrient utilization, digestive efficiency, and metabolic processes when supplied at appropriate dietary levels. Comparable improvements in growth performance from plant-based feed additives have been reported in fish fed phytogenic diets rich in bioactive compounds (Hoseinifar et al., 2019), as well as in African catfish where similar improvements in growth and feed utilization were observed (Okonkwo and Adebayo, 2021). The observed dose-dependent response, where growth performance improved at moderate inclusion levels but slightly declined at higher inclusion levels, suggests the presence of an optimal inclusion threshold for the beneficial effects of phytogenic compounds. This pattern agrees with Garcia and Lopez (2020), who reported that excessive inclusion of phytogenic additives may reduce growth benefits due to possible metabolic limitations or the presence of anti-nutritional factors that interfere with nutrient utilization. Feed intake was significantly higher in all supplemented groups compared with the control, suggesting improved palatability and possible stimulation of feeding activity. The reduced FCR values recorded in the supplemented groups indicate improved feed utilization efficiency, as lower FCR values represent better conversion of feed nutrients into fish biomass. This enhanced feed efficiency may be associated with improved digestive enzyme activity and nutrient absorption induced by phytogenic compounds, as previously reported for plant-based feed additives (Abd El-Hack *et al.,* 2020). Although survival percentage ranged from 90.00 to 100.00% among the experimental groups, no significant differences were observed between treatments. The high survival rates recorded across all groups indicate that dietary inclusion of *E. heterophylla* leaf meal did not negatively affect fish health and was well tolerated at the tested inclusion levels. This finding supports previous reports that plant-derived feed additives generally do not induce adverse effects when incorporated at appropriate dietary concentrations (Patel and Kumar, 2020). The results demonstrate that *E. heterophylla* leaf meal has potential as a functional feed additive for improving growth performance and feed utilization efficiency in *O. niloticus*. The beneficial effects were most evident at moderate inclusion levels, particularly EH2 and EH3, where improved growth responses and feed conversion efficiency were observed without adverse effects on survival.

**Table 4:** Growth and nutrient utilization of experimental fish

| Parameters | EH1 (0.0) | EH2 (0.5) | EH3 (1.0) | EH4 (1.5) | EH5 (2.0) |
|---|---|---|---|---|---|
| IW (g) | $5.86{\pm}0.10^{a}$ | $5.87{\pm}0.07^{a}$ | $5.89{\pm}0.01^{a}$ | $5.89{\pm}0.02^{a}$ | $5.87{\pm}0.01^{a}$ |
| FW (g) | $10.50{\pm}0.47^{a}$ | $11.68{\pm}0.04^{c}$ | $11.51{\pm}0.05^{c}$ | $11.41{\pm}0.57^{b}$ | $11.33{\pm}0.20^{b}$ |
| WG (g) | $4.64{\pm}0.37^{a}$ | $5.81{\pm}0.03^{c}$ | $5.62{\pm}0.03^{c}$ | $5.52{\pm}0.59^{b}$ | $5.46{\pm}0.20^{b}$ |
| SGR (%/day) | $1.04{\pm}0.05^{a}$ | $1.23{\pm}0.02^{c}$ | $1.20{\pm}0.01^{c}$ | $1.18{\pm}0.10^{b}$ | $1.17{\pm}0.04^{b}$ |
| TFI (g/fish) | $7.73{\pm}0.87^{a}$ | $8.95{\pm}0.28^{b}$ | $8.74{\pm}0.31^{b}$ | $8.70{\pm}0.01^{b}$ | $8.67{\pm}0.37^{b}$ |
| FCR | $1.67{\pm}0.04^{b}$ | $1.54{\pm}0.06^{a}$ | $1.56{\pm}0.06^{a}$ | $1.58{\pm}0.18^{a}$ | $1.59{\pm}0.13^{a}$ |
| FER | $0.60{\pm}0.01^{a}$ | $0.65{\pm}0.02^{a}$ | $0.64{\pm}0.02^{a}$ | $0.63{\pm}0.07^{a}$ | $0.63{\pm}0.05^{a}$ |
| Survival (%) | $90.00{\pm}3.33^{a}$ | $100.00{\pm}0.00^{a}$ | $96.67{\pm}3.34^{a}$ | $96.67{\pm}3.34^{a}$ | $93.34{\pm}6.67^{a}$ |

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

**Hematological Parameters**

The effects of *Euphorbia heterophylla* leaf meal inclusion on the haematological parameters of *Oreochromis niloticus* are presented in Table 5. The haematological responses of fish fed the experimental diets varied among treatments, indicating that dietary inclusion of *E. heterophylla* influenced certain blood indices. Haemoglobin (Hb) concentration and packed cell volume (PCV) differed among the dietary treatments, with the highest values recorded in fish fed the 2.0% inclusion level (EH5). The increase in Hb and PCV at the higher inclusion level suggests that *E. heterophylla* may influence erythropoietic activity and the oxygen-carrying capacity of the blood. However, this pattern did not correspond completely with growth performance, as the highest Hb and PCV values occurred in EH5, whereas the most favourable growth responses were observed at moderate inclusion levels (EH2 and EH3). This suggests that physiological responses to dietary phytogenic compounds may vary depending on the parameter evaluated. Red blood cell (RBC) counts showed no significant differences among the dietary treatments, despite slight numerical variations (Table 5). These results indicate that *E. heterophylla* inclusion maintained normal erythrocyte levels without significantly affecting erythrocyte count. The reported RBC unit was verified and corrected to $\times10^6/mm^3$. White blood cell (WBC) counts varied among treatments, with higher values observed in EH2 and EH4. However, WBC did not increase progressively with dietary inclusion level, as the value recorded in EH5 was lower than those observed in EH2 and EH4. The elevated WBC counts at moderate inclusion levels may indicate an immunomodulatory effect of *E. heterophylla*, possibly associated with bioactive phytochemicals such as flavonoids and phenolic compounds, which have been reported to enhance immune responses in fish (Brown and Davis, 2021). The erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), varied among treatments without evidence of adverse alterations in red blood cell characteristics. Although these indices provide information on erythrocyte morphology and haemoglobin distribution, their interpretation requires comparison with established reference values for *O. niloticus*. Nevertheless, the results suggest that dietary inclusion of *E. heterophylla* did not induce abnormal changes in erythrocyte indices under the experimental conditions The observed changes in Hb, PCV, and WBC indicate that *E. heterophylla* leaf meal may influence selected haematological responses in Nile tilapia. These effects appear to depend on both the blood parameter evaluated and the inclusion level rather than reflecting a uniform improvement across all haematological indices. Similar responses to phytogenic feed additives in aquaculture species have been attributed to the antioxidant and immunostimulatory properties of plant bioactive compounds (Nakamura and Tanaka, 2019; Dawood *et al.,* 2018).

**Table 5. Haematological parameters of experimental fish**

| Parameters | EH1 (0.0) | EH2 (0.5) | EH3 (1.0) | EH4 (1.5) | EH5 (2.0) |
|---|---|---|---|---|---|
| Hb (g/100ml) | 7.35±0.06$^{a}$ | 8.73±0.08$^{b}$ | 8.32±0.02$^{b}$ | 7.62±0.03$^{ab}$ | 9.74±0.02$^{c}$ |
| PCV (%) | 22.17±0.12$^{a}$ | 26.10±0.21$^{b}$ | 25.38±0.19$^{b}$ | 23.47±0.33$^{a}$ | 29.06±0.03$^{c}$ |
| WBC (x10$^{3}$/mm$^{3}$) | 5.50±0.01$^{a}$ | 8.45±0.01$^{c}$ | 7.24±0.07$^{b}$ | 8.53±0.01$^{c}$ | 6.87±0.01$^{ab}$ |
| RBC (×10$^{6}$/mm$^{3}$) | 2.42±0.03$^{a}$ | 2.84±0.02$^{a}$ | 2.75±0.01$^{a}$ | 2.58±0.01$^{a}$ | 3.19±0.02$^{a}$ |
| MCV (fl) | 91.61±1.06$^{b}$ | 91.90±0.32$^{b}$ | 85.35±0.75$^{a}$ | 90.97±0.63$^{b}$ | 91.10±0.66$^{b}$ |
| MCH (pg) | 30.37±0.28$^{a}$ | 30.74±0.12$^{a}$ | 30.25±0.04$^{a}$ | 29.53±0.56$^{a}$ | 30.53±0.14$^{a}$ |
| MCHC (%) | 33.26±0.51$^{a}$ | 33.45±0.03$^{a}$ | 32.78±0.23$^{a}$ | 32.47±0.39$^{a}$ | 33.52±0.10$^{a}$ |

Values in the same row with different superscripts differ significantly at $P < 0.05$.
**Key:** PCV = Packed 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 Profiles**

The biochemical profiles of *Oreochromis niloticus* fed varying levels of *Euphorbia heterophylla* leaf meal are presented in Table 6. The biochemical responses varied among treatments, indicating that dietary inclusion of *E. heterophylla* influenced selected metabolic and physiological parameters of the fish. Total protein concentration increased with increasing inclusion levels, suggesting improved protein metabolism and nutrient utilization in fish fed the supplemented diets. Enhanced protein status in fish fed phytogenic feed additives has been associated with the presence of bioactive compounds that may improve digestion, nutrient absorption, and metabolic efficiency (Dawood *et al.,* 2018). Albumin concentrations were higher in supplemented groups compared with the control, which may indicate improved protein nutritional status and metabolic activity. However, although albumin is commonly used as an indicator of protein synthesis and nutritional condition, it alone cannot provide a definitive assessment of liver function. Since liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), or histopathological examinations were not evaluated in the present study, conclusions regarding direct improvement of liver function should be interpreted with caution. The observed increases in globulin concentrations, particularly at higher inclusion levels, suggest a possible enhancement of immune-related protein production. This finding supports previous reports that plant-derived feed additives containing phytochemicals may stimulate innate immune responses and improve physiological defense mechanisms in fish (Elumalai *et al.,* 2021). Cholesterol and glucose levels increased with increasing dietary inclusion of *E. heterophylla* leaf meal. These changes may reflect alterations in lipid and carbohydrate metabolism induced by dietary phytogenic compounds. Moderate increases in cholesterol may indicate enhanced lipid transport and metabolic activity; however, elevated cholesterol may also suggest altered lipid regulation depending on the physiological condition of the fish. Similarly, increased glucose concentration may represent improved energy mobilization to support metabolic demands, but it may also be associated with physiological stress responses. Therefore, these biochemical changes should be interpreted as metabolic adaptations rather than solely as indicators of improved health status. Similar variations in cholesterol and glucose responses have been reported in fish receiving plant-based dietary additives, where metabolic modulation depends on the type and inclusion level of the phytogenic ingredient (Li-Xiaoyun *et al.,* 2022). The biochemical findings suggest that *E. heterophylla* leaf meal influenced protein metabolism, immune-related parameters, and metabolic regulation in *O. niloticus*. However, further studies incorporating liver enzyme activities and histological evaluations are recommended to provide a more comprehensive assessment of hepatic health and physiological responses.

**Table 6:** Biochemical profiles of experimental fish

| Parameters | EH1 (0.0) | EH2 (0.5) | EH3 (1.0) | EH4 (1.5) | EH5 (2.0) |
|---|---|---|---|---|---|
| Cholesterol (mg/dl) | 31.2±0.01[a] | 43.9±0.01[c] | 40.5±0.03[bc] | 38.3±0.01[b] | 52.9±0.03[d] |
| Glucose (mg/dl) | 30.5±0.01[a] | 42.3±0.03[c] | 37.9±0.02[b] | 41.6±0.00[c] | 44.3±0.02[d] |
| Total protein (g/dl) | 4.07±0.06[a] | 6.18±0.03[c] | 5.52±0.02[b] | 5.48±0.01[b] | 6.22±0.03[c] |
| Albumin (g/dl) | 2.19±0.00[a] | 2.42±0.01[b] | 2.36±0.01[b] | 2.33±0.01[b] | 2.97±0.03[c] |
| Globulin (g/dl) | 1.84±0.01[a] | 3.54±0.03[c] | 3.45±0.00[c] | 2.73±0.02[b] | 4.23±0.02[d] |

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

## CONCLUSION

The findings of this study indicate that *Euphorbia heterophylla* leaf powder has potential as a functional feed additive in the diets of *Oreochromis niloticus*. Dietary inclusion of *E. heterophylla* influenced growth performance, feed utilization efficiency, haematological indices, and biochemical responses, with the most favourable growth performance observed at the 0.5 g/100 g diet inclusion level. This suggests that moderate inclusion of *E. heterophylla* may provide optimal benefits for improving growth and feed efficiency. However, the responses of haematological and biochemical parameters varied among inclusion levels, indicating that higher dietary concentrations may influence physiological and metabolic processes differently. Although no adverse effects on survival were observed, the potential metabolic implications of higher inclusion levels require further investigation using additional health indicators, including liver enzyme activities and histological evaluations. *E. heterophylla* leaf powder shows promise as a natural feed additive for enhancing growth performance in *O. niloticus*, with 0.5 g/100 g diet appearing to be the most suitable inclusion level under the conditions of the present study.

**Ethical Clearance**

All experimental procedures, including fish handling and blood collection, were conducted following standard aquaculture procedures for the care and handling of aquatic animals. Appropriate measures were taken throughout the study to minimize stress and ensure the welfare of the experimental fish.

**Conflict of Interest**

The author declares that there are no conflicts of interest regarding the publication of this manuscript. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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