ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26may2076

Dietary Inclusion of Milkweed (*Euphorbia heterophylla*) Leaf Meal on the Carcass and Mineral Composition of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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Publication Date: 2026/06/13

Abstract: This study assessed the effects of dietary inclusion of *Euphorbia heterophylla* leaf meal on the carcass composition and mineral profile of *Oreochromis niloticus*. Five isonitrogenous diets containing (35% crude protein) were formulated with graded inclusion levels of 0.0, 0.5, 1.0, 1.5, and 2.0 g/100 g (EH1–EH5). Using a completely randomized design, 225 fingerlings (5.87 ± 0.03 g) were stocked into 15 glass tanks with three replicates per treatment and fed for 56 days. Carcass proximate composition and mineral contents were then evaluated. Results showed significantly increased crude protein content in fish fed the supplemented diets, while moisture, ash, lipid, and nitrogen-free extract values remained largely unaffected. Mineral analysis revealed improved levels of calcium, sodium, potassium, and iron in treated groups, indicating enhanced mineral absorption and utilization. These findings demonstrate that *Euphorbia heterophylla* leaf powder may serve as an effective natural feed additive for improving carcass quality and mineral composition in *O. niloticus*, thereby contributing to more efficient and sustainable aquaculture practices.

Keywords: *Euphorbia heterophylla; Oreochromis niloticus; Carcass Composition; Mineral Composition.*

How to Cite: Oladipupo T. M. (2026) Dietary Inclusion of Milkweed (*Euphorbia heterophylla*) Leaf Meal on the Carcass and mineral Composition of Nile Tilapia (*Oreochromis niloticus*) Fingerlings. *International Journal of Innovative Science and Research Technology*, 11(5), 4225-4229. https://doi.org/10.38124/ijisrt/26may2076

I. INTRODUCTION

Aquaculture is an important source of animal protein worldwide and has expanded significantly in recent years to meet the growing demand for food, particularly in response to the decline in wild fisheries (FAO, 2020). Nile tilapia (*Oreochromis niloticus*) is one of the most important tropical fish species native to Africa and widely cultured across the globe. It is primarily herbivorous but may exhibit omnivorous feeding habits under culture conditions. This species efficiently converts a wide range of feedstuffs into high-quality protein and thrives on artificial diets (FAO, 2022). The use of antibiotic growth promoters in aquaculture has increasingly been restricted due to concerns about antimicrobial resistance and their potential risks to human health and the environment (World Health Organization, 2020; Cabello *et al.*, 2022). Consequently, regulatory bodies and consumers are advocating for safer and more sustainable alternatives. In line with this shift, there is growing interest in the use of medicinal plants and herbal products as natural feed additives. These plant-based additives are considered environmentally friendly and are widely considered effective alternatives for enhancing feed utilization, growth performance, and fish health (Abdel-Latif *et al.,* 2021). Among underutilized plant resources, *Euphorbia heterophylla* has gained attention as a potential feed ingredient in aquaculture. The plant is widely distributed across tropical regions, exhibits rapid growth, and contains appreciable levels of protein along with various bioactive compounds that may confer health benefits (Ogunji *et al.*, 2021; Adeyemi *et al.*, 2022). Previous studies in poultry and catfish nutrition indicate that its dietary inclusion can enhance growth performance, improve antioxidant capacity, and stimulate immune responses when used at appropriate levels (Adeniyi *et al.*, 2023; Oladele *et al.*, 2021). Despite these promising findings, information on its utilization in *Oreochromis niloticus* remains limited, particularly with respect to optimal dietary inclusion levels and its effects on nutrient composition. Therefore, this study aimed to evaluate the effects of dietary supplementation with *Euphorbia heterophylla* leaf meal on the carcass composition and mineral profile of *O. niloticus* fingerlings.

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II. 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, over a period of 56 days.

➢ *Plant Material*

Fresh leaves of *Euphorbia heterophylla* were collected from the area behind the School of Agriculture and Agricultural Technology Annex, Federal University of Technology, Akure, Ondo State. The plant material was identified and authenticated by a botanist from the Department of Crop Soil and Pest Management of the same institution. The leaves were air-dried at room temperature to prevent the loss of heat-sensitive volatile compounds. After drying, they were milled into a fine powder using an electric blender (Model ES-242) and stored in an airtight container at 4°C until further use.

➢ *Experimental Fish*

Apparently healthy *Oreochromis niloticus* fingerlings were obtained from the Federal University of Technology fish farm, Akure, Ondo State, Nigeria. The fish were acclimatized for 7 days prior to the commencement of the experiment. During acclimatization, the fish were 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 isonitrogenous diets (35% CP) were formulated for the fish to contain *E. heterophylla* leaf at graded inclusion levels (0.0, 0.5, 1.0, 1.5 and 2.0g/100g) and labelled EH1-EH5, respectively. The diet containing 0.0g/100g of *E. heterophylla* leaf meal served as the control without any inclusion of additives. Other ingredients were fish meal, soya bean meal, groundnut cake, yellow maize, methionine, lysine, vitamin-mineral mix, vegetable oil and starch. Table 1 shows gross and proximate composition of the experimental diets. The constituents of each diet were thoroughly mixed and pelleted using a Hobart A-200T mixing and pelleting machine (Hobart Manufacturing Ltd. UK) using a 2 mm diameter die. The resultant strands obtained were sun-dried for 3 days and hand crumbled into smaller sizes that can be swallowed by the fingerlings, after which they were packed in separate airtight polyethylene bags, sealed and marked according to treatments and stored at 4°C until feeding.

➢ *Weighing of Experimental Fish*

Fish weights were recorded at the beginning of the experiment and every two weeks thereafter over the 56-day feeding trial. Batch weighing was carried out using a digital electronic balance (Model PB3002) with a precision of ±0.01 g. Efforts were made to reduce handling time and minimize stress to the fish during each weighing session.

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 mix	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, 450mg. L-Lysine, 10 g; Selenium, 50 ppm.

➢ *Experimental Design and Management*

The experiment was arranged in a completely randomized design. After the acclimation period, the fish were weighed, and 225 healthy *O. niloticus* fingerlings with a mean weight of 5.87 ± 0.03 g were selected at random and stocked into 15 glass tanks (70 L, 70 × 45 × 45 cm^3) at a density of 15 fish per tank. This setup comprised five dietary treatments, each with three replicates. The experimental diets were fed twice daily, between 08:00–09:00 and 16:00–17:00 hours, to apparent satiation throughout the 56-day trial.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26may2076

Complete water exchange was carried out twice weekly. Water quality parameters, including temperature, dissolved oxygen, and pH, were also monitored twice weekly. Proximate analysis was carried out on the plant and experimental diets, and fish according to AOAC (1990). At the end of the feeding trial, four fish were randomly selected from each replicate tank for carcass and mineral analyses. The selected fish were euthanized humanely, and whole-body samples were collected. The mineral composition was carried out using an Atomic Absorption Spectrophotometer (AAS) as described by (AOAC (2016). Mineral analysis was conducted by weighing 0.5 g of a finely ground sample into a conical flask, followed by the addition of 10 ml of nitric acid (HNO_3). The mixture was heated at 60°C for 15 minutes. Subsequently, 5 ml of perchloric acid was added, and the mixture was reheated at 60°C for another 15 minutes. The flask was then placed on a hot plate and heated until the sample volume was reduced to approximately 1 ml. The digest was diluted to 100 ml with distilled water. Sodium (Na) and potassium (K) concentrations were determined using a flame photometer, while calcium (Ca), iron (Fe), and magnesium (Mg) were analyzed using an atomic absorption spectrophotometer.

➢ *Statistical Analysis*

Data were analyzed using one-way analysis of variance (ANOVA) to test differences among treatment means. Tukey's post-hoc test was used to separate significantly different means. All analyses were performed using SPSS (Version 23.0), with significance accepted at $p < 0.05$.

III. RESULTS AND DISCUSSION

➢ *Proximate Composition of E. heterophylla Leaf Powder*

The proximate composition of *E. heterophylla* leaf meal showed that nitrogen-free extract was the most abundant component at 67.29%, while fat had the lowest value at 2.34%, as presented in Table 2. The ash content of 2.75% indicates a reasonable mineral presence in the leaf material. The proximate analysis of *E. heterophylla* leaf powder highlights key nutritional attributes that suggest its potential use as a feed additive in aquaculture. The crude protein content of 10.26% recorded in this study is consistent with the findings of Adeyemi and Ogunniyi (2019), who reported protein levels ranging from 9.8% to 12.4% in *E. heterophylla* leaves sourced from different 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*

During the 56-days feeding trial, the measured water quality parameters showed slight variation: dissolved oxygen ranged from 6.65 to 6.73 mg/L, temperature ranged from 26.77 to 26.84°C, and pH ranged from 7.26 to 7.33 (Table 3). These values remained within the optimal range for Nile tilapia culture throughout the study, indicating that environmental conditions were stable and unlikely to have influenced the treatment effects. The consistency of these parameters suggests that observed differences in fish performance were primarily due to dietary treatments rather than fluctuations in water quality. Overall, the recorded values complied with acceptable standards for freshwater fish culture, including *O. niloticus*, and are consistent with the reports of (Thompson, 2019) and 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 $P < 0.05$.

➢ *Carcass Composition*

The carcass composition analysis of fish fed diets supplemented with *E. heterophylla* revealed a significant increase in crude protein content, while moisture, ash, lipid, and nitrogen-free extract (NFE) values remained largely unchanged across treatments (Table 4). This pattern suggests that dietary inclusion of the plant primarily enhances protein deposition and muscle growth without markedly altering overall carcass proximate composition. The increase in crude protein content indicates improved nutrient utilization and more efficient conversion of dietary protein into muscle tissue. This observation aligns with Gasco *et al.* (2020), who reported that functional feed ingredients enhance protein retention and carcass quality in aquaculture species through improved digestive efficiency and metabolic utilization. Similarly, plant-based bioactive compounds have been shown to promote muscle protein synthesis and improve body composition in fish (Bae *et al.*, 2022). Comparable trends

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26may2076

have also been documented in terrestrial livestock, where phytogenic feed additives improved carcass yield and muscle accretion by enhancing nutrient partitioning toward lean tissue formation. For instance, recent livestock nutrition studies have shown that phytogenic compounds improve growth performance and carcass traits through better protein synthesis and digestive efficiency (Abd El-Hack *et al.*, 2020; Valenzuela-Grijalva *et al.*, 2019). These consistent findings across species suggest that phytogenic compounds primarily influence protein metabolism rather than fat or mineral deposition, supporting their role as functional growth-promoting additives in both aquaculture and terrestrial animal production systems (Hoseinifar *et al.*, 2019).

Table 4 Whole Body Composition of Experimental Fish

Parameters (%)	**EH1 (0.0)**	**EH2 (0.5)**	**EH3 (1.0)**	**EH4 (1.5)**	**EH5 (2.0)**
Moisture	8.43±0.02^{a}	8.72±0.02^{a}	8.92±0.03^{a}	8.90±0.01^{a}	8.54±0.06^{a}
Ash	10.5±0.03^{a}	10.4±0.01^{a}	10.9±0.09^{a}	10.3±0.03^{a}	10.8±0.05^{a}
Lipid	13.7±0.09^{a}	12.5±0.00^{a}	12.8±0.06^{a}	12.4±0.07^{a}	12.7±0.03^{a}
Crude protein	54.39±0.02^{a}	56.58±0.10^{b}	56.23±0.02^{b}	57.03±0.05^{b}	56.51±0.11^{b}
NFE	12.98±0.04^{a}	11.80±0.08^{a}	11.15±0.15^{a}	11.37±0.10^{a}	11.45±0.12^{a}

Values in the same row with different superscripts differ significantly at P < 0.05.
Key: NFE = Nitrogen free extract

- ➢ *Mineral Composition*

The mineral profile of *Oreochromis niloticus* fed *E. heterophylla*-supplemented diets showed significantly increased calcium, sodium, potassium, and iron contents, indicating improved mineral absorption and retention. This supports the report of Santos *et al.* (2021) that phytogenic feed additives enhance mineral uptake through improved digestive efficiency and intestinal absorption. Calcium increased significantly in all supplemented diets (EH2–EH5), suggesting enhanced bioavailability even at low inclusion levels. Phytogenic additives have been associated with improved calcium retention and bone mineralization via enhanced digestive enzyme activity and gut morphology in *O. niloticus* (El-Sayed and Lebdah, 2019; Abdel-Tawwab *et al.*, 2020). Sodium increased (3.35 to 4.90 g/100 g), reflecting improved ionic balance and osmoregulatory efficiency, consistent with enhanced intestinal transport and nutrient uptake in plant-fed fish (Dawood *et al.*, 2018). Potassium rose from 1.94 to 3.25–3.39 g/100 g with no significant differences among treatments, indicating a plateau effect and physiological saturation, as potassium homeostasis in tilapia is tightly regulated (El-Sayed, 2022). Iron showed the greatest increase (3.82 to 5.50–5.66 g/100 g), likely due to improved bioavailability and absorption mediated by plant phytochemicals. Similar enhancements in trace mineral retention have been linked to improved gut morphology, enzyme activity, and mineral chelation in fish fed plant-based additives (Gabriel *et al.*, 2018; Olusola and Nwanna, 2020). In contrast, magnesium remained largely unchanged except for a slight increase at EH2, indicating strong physiological regulation. Stable magnesium levels under dietary variation have also been reported in tilapia due to tight electrolyte homeostasis (Li *et al.*, 2021).

Table 5 Minerals Composition of Experimental Fish

Parameters g/100g	**EH1 (0.0)**	**EH2 (0.5)**	**EH3 (1.0)**	**EH4 (1.5)**	**EH5 (2.0)**
Calcium	4.23±0.08^{a}	5.35±0.04^{b}	5.42±0.08^{b}	5.26±0.29^{b}	5.49±0.05^{b}
Sodium	3.35±0.12^{a}	4.87±0.06^{b}	4.83±0.01^{b}	4.77±0.01^{b}	4.90±0.09^{b}
Magnesium	0.63±0.03^{a}	1.05±0.02^{b}	0.65±0.15^{a}	0.83±0.02^{a}	0.86±0.05^{a}
Potassium	1.94±0.06^{a}	3.36±0.04^{b}	3.34±0.03^{b}	3.25±0.08^{b}	3.39±0.19^{b}
Iron	3.82±0.32^{a}	5.54±0.02^{b}	5.62±0.04^{b}	5.50±0.02^{b}	5.66±0.02^{b}

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

IV. CONCLUSION

In conclusion, dietary inclusion of *Euphorbia heterophylla* leaf meal improved the carcass quality of *Oreochromis niloticus* by increasing crude protein content while maintaining stable levels of other proximate components. It also enhanced mineral composition, particularly calcium, sodium, potassium, and iron, indicating better nutrient absorption and utilization. Overall, the results suggest that *E. heterophylla* is a promising phytogenic feed additive that supports protein deposition and mineral bioavailability without negatively affecting carcass composition, making it suitable for sustainable aquaculture production.

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