

## ASSESSMENT OF *Moringa oleifera* LEAF-MEAL EXTRACTS DIETS USING DIFFERENT SOLVENTS ON GROWTH AND HAEMATOLOGICAL PARAMETERS OF *Clarias gariepinus*

\*<sup>1</sup>SULEIMAN, A.M., <sup>2</sup>A. M. ORIRE, <sup>1</sup>Z. R. MUDI, <sup>1</sup>H. ABDUL-AZEZZ, <sup>1</sup>M. U. AMINU AND <sup>1</sup>F. B. ABUBAKAR

<sup>1</sup>Department of Fisheries and Aquaculture, Faculty of Agriculture, Bayero University Kano, Kano, Nigeria.

<sup>2</sup>Department of Fisheries, Aquaculture and Wildlife, University of Abuja, Abuja, Nigeria

\*Corresponding Author: samohammed.faq@buk.edu.ng, +2347032867661, 0000-0001-9915

### ABSTRACT

This study evaluated the effects of combined *Moringa oleifera* leaf meal extracts, solvent-extract combinations, and inclusion ratios on the growth performance and haematological parameters of *Clarias gariepinus*. A total of 960 juveniles ( $9.8 \pm 0.1$  g) were used in a 56-day feeding trial arranged in a  $3 \times 5$  factorial design, comprising three extract combinations (aqueous + ethanol, MAE; ethanol + hexane, MEH; aqueous + hexane, MAH) and five inclusion ratios (0:100, 25:75, 50:50, 75:25, and 100:0%), resulting in fifteen treatments with three replicates each. Fish were fed at 5% body weight, twice daily. Both extract combination and inclusion ratio significantly affected ( $p < 0.05$ ) growth and feed utilization parameters, except apparent net protein utilization and survival, while interaction effects were not significant. Diets containing MAE and MEH at 100:0% produced the highest mean weight gain, specific growth rate ( $3.84\text{--}3.96\%$  day<sup>-1</sup>), protein efficiency ratio (2.14), and survival (88.33–100%), and the lowest feed conversion ratio (1.01–1.06). Haematological indices were significantly influenced ( $p < 0.05$ ), with superior treatments recording higher red blood cell counts (up to  $4.10 \times 10^{12}$  L<sup>-1</sup>), haemoglobin (11.00 g dL<sup>-1</sup>), and packed cell volume (33.00%). The results indicate that polar solvent combinations, particularly MAE at higher inclusion ratios, enhance growth performance and physiological status of *C. gariepinus*, confirming *M. oleifera* leaf meal extract as an effective and sustainable aquafeed additive. The study concludes that *M. oleifera* leaf extracts, particularly when extracted with polar solvents like water and ethanol, serve as effective growth enhancers and immunostimulants, optimizing both the growth and health status of *C. gariepinus*.

**Keywords:** Aquaculture, Medicinal, Nutrition, Solvent and Plant

### INTRODUCTION

Aquaculture has to do with the rational rearing of fish in an enclosed and reasonably shallow water body where all life processes of fish can be controlled. The world aquaculture production grew by 2.7 percent in 2020 compared with that of 2019, and an all-time low rate of annual growth in over 4 decades (Action, 2020). The net increase of 2.3 million tonnes in the same period was comparable to some years in the last decade. In Africa, Nigeria is the largest producer of aquaculture products in sub-Saharan Africa, but its production has been declining since 2016 due to competitiveness in procurement of feed raw materials (FAO, 2022).

In Nigeria and the rest of the world, aquaculture is seen as a means of meeting fish demand shortly since the stocks from the wild are showing signs of depletion (Stefanie, 2014). The aquaculture industries in Nigeria have focused attention on the *Clarias species* because they occupy a unique position in commercial aquaculture due to their nature of being hardy, tasty, tolerance to poor water quality conditions, highly fecund, early growth performance, efficient feed conversion ratio, especially in the male and thereby attract high market value (Afi and Ofor, 2016).

Nutrition plays an indispensable role in aquaculture, requiring precise optimization to ensure the efficient production of fish for human consumption. Fish nutrition

is fundamentally the study of essential nutrients and energy sources required to sustain health, maximize growth, and support reproductive success (Stefanie, 2014). Driven by these nutritional advancements, global aquaculture production has maintained a steady growth trajectory, recently expanding at an annual rate of 9.2% (Eteng and Opeh, 2023). The rising demand for fish fuelled by its importance in the human diet is colliding with a rapid decline in captured fish stock. As such, aquaculture venture is predicted to reliably provide an alternative to the dwindling captured fisheries to meet the expanding protein need of the global populace (Sadiku, 2014).

Feed in aquaculture production is the most important factor for determining success in both semi-intensive and intensive fish farming system. It is supplied to the fish with the aim to increase growth and maintain the survival rate of the fish and any feed that is not in accordance with the quantity, quality and nutrient balance needs for the target fish will cause growth inhibition of the fish fed (Octavia et al., 2019). This may lead to unsatisfactory result of fish farming production.

In aquaculture enterprise, it is recognised as the primary cost extended during a production cycle. It is one of the factors in the equation determining the profitability and viability of aquaculture products. It contributes between 60% and 70% to the variable cost of fish production,

which consumes a lot of profit generation by the farmer (Orire and Sadiku, 2012; Ganguly *et al.*, 2013; Bake *et al.*, 2014). Ajiboye *et al.* (2012) also states that, in aquaculture the acceptability of diets, feeding rate and the nutritional components of aqua feeds are also essential to boost the growth performance of farmed fish species. Thus, formulating a diet that meets the nutritional requirements of culturable fish species is imperative for aquaculture species' rapid growth and survival rate (Orire and Abubakar, 2013).

*Moringa oleifera* belongs to the family of Moringaceae and it can survive in harsh climatic condition including impoverished soil without being affected by drought. It has beneficial properties from the root to the leaves and the leaf contain various constituents such as crude protein, amino acids, vitamins, minerals, fatty acids and other nutrient that are beneficial to fish growth and good health condition (Ajantha *et al.*, 2020). According to Eteng and Opeh, (2023), the tree can be established in slightly alkaline soils up to a pH of 9 as well as acidic soils of as low as a pH of 4.5 and is well suited for a wide range of adverse environments that would not be suitable for other fruit, nut and tree crops. This research seek to assess *Moringa oleifera* leaf meal extracts diets made from aqueous solution, ethanol and hexane solvents on growth performance and haematological parameters of *Clarias gariepinus* juveniles.

## MATERIALS AND METHODS

### Procurement and preparation of *Moringa oleifera* leaf meal

Fresh *M. oleifera* leaves were sourced from Kure Market along the Dutsen Kura Western Bypass, Minna, Niger State. To ensure freshness, leaves were purchased early in the morning before exposure to market conditions. The plant species was authenticated at the Department of Plant Biology, Federal University of Technology, Minna, Nigeria.

The leaves were detached from the stems, washed thoroughly with distilled water to remove dust and debris (Suleiman *et al.*, 2023), and air-dried at ambient temperature for seven (7) days at the Department of Water Resources, Aquaculture and Fisheries Technology Laboratory, FUT Minna. The dried leaves were steam heated in an autoclave at 60°C for 15 minutes to reduce residual moisture content. The material was then crushed using an aluminium mortar and pestle, followed by fine milling with a hammer mill. The powdered leaf meal was packaged in labelled polythene bags and refrigerated until extraction.

### Extraction of *Moringa Oleifera* leaf meal

Leaf meal extraction was carried out using Soxhlet apparatus and three solvents aqueous, ethanol, and hexane following the method of Suleiman *et al.* (2018). A pre-dried (fat-free) thimble was weighed ( $W_1$ ), and 50 g of the sample was added and reweighed ( $W_2$ ). A 500 ml round-bottom flask was weighed ( $W_3$ ) and filled to two-thirds with each solvent. The Soxhlet extractor was assembled and connected to a reflux condenser, with temperature

maintained at 60°C to ensure a gentle boil. The extraction process was allowed to run for 5–6 hours until the siphoning solvent became transparent.

After extraction, the thimble was removed, and the solvent was distilled off. The residue was oven-dried at 100°C for 5 minutes, cooled in a desiccator, and weighed ( $W_4$ ). The thimble was further dried at 50°C to a constant weight ( $W_5$ ). Extraction efficiency was calculated as:

$$\% \text{ Extraction} = \frac{W_4 - W_3}{W_2 - W_1} \times 100$$

The extracts were then refrigerated for subsequent feed formulation (Idowu, 2016; Chakraborty *et al.*, 2018).

### Feed ingredients and proximate analysis and feed formulation

Feed ingredients including soybean and maize were purchased from Janguza Market along Gwarzo Road, Kano, while vitamin premix was obtained from Kazy Feed and Fisheries, Kado Fish Market, Life Camp, Abuja. Dried clupeid (fishmeal source) was obtained from Tiga Dam Fish Market, oven-dried, and milled into fine particles. Soybean was heat-treated at 100°C for 10 minutes to deactivate trypsin inhibitors (Azubuike and Nwoye, 2021), allowed to cool, and milled. Maize was similarly milled into fine powder. Proximate compositions of all feed ingredients and formulated diets were determined using standard procedures of the Association of Official Analytical Chemists (AOAC, 2010). Analyses included moisture, crude protein, crude lipid, crude fibre, ash, and nitrogen-free extract (NFE). Carcass and faecal samples were also analysed to evaluate nutrient utilization.

Sixteen experimental diets comprising varying inclusion levels of combined *M. oleifera* leaf extracts were formulated to contain 40% crude protein (Table 1). Diet 1 served as the control, while Diets 2-16 contained the *M. oleifera* leaf extracts derived from different solvent combinations as described as: Aqueous + Ethanol Extracts (MAE): 0:100% (D2), 25:75% (D3), 50:50% (D4), 75:25% (D5), and 100:0% (D6). Ethanol + Hexane Extracts (MEH): 0:100% (D7), 25:75% (D8), 50:50% (D9), 75:25% (D10), and 100:0% (D11). Aqueous + Hexane Extracts (MAH): 0:100% (D12), 25:75% (D13), 50:50% (D14), 75:25% (D15), and 100:0% (D16). All ingredients were weighed using an Ohaus digital balance (MP 300) and mixed thoroughly for homogeneity before pelleting. Extracts were dissolved in 250 ml warm water (60°C) and incorporated into a 1 kg dough for each diet. The doughs were pelletized to 2 mm diameter, oven-dried at 60°C for 24 hours (MINI/75/DIG oven), labelled, and stored at 0°C prior to feeding trials (Suleiman *et al.*, 2023).

### Experimental design and set-up

A total of 960 *C. gariepinus* juveniles with average weight of 9.8 g ± 0.1 were procured from a reputable hatchery in Abuja and acclimatized to laboratory conditions for two weeks, before the feeding trial. Fish were distributed into



48 calibrated plastic tanks (50L capacity) randomly. Each tank was stocked with fifteen (15) fish, randomly assigned to the treatments. Tanks were filled to 30L mark and covered with mesh nets to prevent fish from jumping out.

The experiment was laid out in a 3 × 5 factorial design, consisting of two experimental factors. The first factor

comprised three solvent-extract combination methods of *M. oleifera* leaf meal, namely: combined aqueous and ethanol extracts (MAE), combined ethanol and hexane extracts (MEH), and combined aqueous and hexane extracts (MAH). The second factor involved five inclusion ratios of the combined extracts, namely 0:100, 25:75, 50:50, 75:25, and 100:0%.

**Table 1:** Feed Formulation of *M. oleifera* Leaf Extracts Using Aqueous, Ethanol and Hexane for *C. gariepinus* Production

Ingredients	Inclusion Levels															
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16
Fish Meal (g)	25.71	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63	24.63
Soybean Meal (g)	51.42	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26	49.26
Maize Meal (g)	17.87	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11	17.11
EMA (g)	0.00	0.0	1.25	2.50	3.75	5.00	0.00	1.25	2.50	3.75	5.00	0.00	0.00	0.00	0.00	0.00
EME (g)	0.00	5.0	3.75	2.50	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	2.50	3.75	5.00
EMH (g)	0.00	0.00	0.00	0.00	0.00	0.00	5.00	3.75	2.50	1.25	0.00	5.00	3.75	2.50	1.25	0.00
Lipid (g)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone Meal (g)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
VMP (g)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
<b>Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>
<b>Proximate Compositions of the Diets</b>																
MC (%)	7.05	8.82	8.80	6.93	7.02	7.32	7.01	7.33	6.92	7.10	7.10	6.98	7.25	6.85	7.02	7.28
Crude Protein (%)	43.22	40.01	40.00	41.01	39.99	40.12	40.00	40.02	41.04	39.99	40.01	41.0	40.05	40.04	40.3	40.07
Crude Lipid (%)	16.52	17.39	17.41	16.52	17.42	17.51	17.53	17.52	16.98	17.55	17.54	17.61	16.95	17.23	16.99	17.12
Crude Fibre (%)	4.07	2.90	3.02	3.91	4.02	4.01	4.02	4.04	4.92	4.12	4.04	3.99	4.05	5.12	5.10	5.09
NFE (%)	16.51	25.14	25.79	26.38	26.28	25.68	26.45	25.84	23.33	26.17	26.33	23.64	26.19	25.79	25.58	25.44
Ash (%)	12.63	5.74	4.98	5.25	5.27	5.36	4.99	5.25	6.75	5.07	4.98	6.78	5.51	4.97	5.01	5.00
<b>Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>

D = Diet, EMA = Extract of Moringa Aqueous, EME = Extract of Moringa Ethanol, EMH = Extract of Moringa Hexane, VMP = Vitamin and Mineral Premix, MC = Moisture Content, and NFE = Nitrogen Free Extract.



### Feeding procedure and data collection

Fish were fed twice daily (8:00 - 4:00pm) at 5% body weight. Sampling was conducted fortnightly to record weight gain and adjust feed rations accordingly. Water quality parameters temperature (31-33°C), dissolved oxygen (3.5-5.5 mg/l), pH (7.5 ± 0.01 – 7.8 ± 0.03), electrical conductivity (198 ± 0.03 – 203 ± 0.03 µS/cm), and total dissolved solids (300 ± 0.05 – 320 ± 0.05 ppm) were measured daily to ensure optimal rearing conditions.

### Growth performance indices

Growth indices were computed following the methods of De Silva & Anderson (1995), Bbole *et al.* (2016), and Suleiman *et al.* (2023). Parameters determined included

Mean Initial Weight (g) =

$$\frac{\text{Total Initial Fish Carcass Weight (g)}}{\text{Total number of Fish per Tank at Initial}}$$

Mean Final Weight (g) =

$$\frac{\text{Total Final Fish Carcass Weight (g)}}{\text{Number of Fish at the end of experiment}}$$

Mean Weight Gain (g) = Mean Final fish Carcass Weight (g) – Mean Initial fish Carcass Weight (g)

$$\text{Specific Growth Rate (\%)} = \frac{(\ln W_2 - \ln W_1)}{T} \times 100$$

Where, W2 and W1 = mean final and initial weight

ln = Natural Logarithms

T = Number of days in the experiment

$$\text{Feed Conversion Ratio} = \frac{\text{Feed Fed (gram/dry weight)}}{\text{Live Weight gain (g)}}$$

$$\text{Protein Efficiency Ratio} = \frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g)}}$$

Apparent Net Protein Utilization (%) =

$$\frac{(P_2 - P_1)}{\text{Total protein consumed (g)}} \times 100$$

P1 = Protein in fish carcass at the beginning of the experiment

P2 = Protein in the carcass at the end of the experiment

Survival Rate (%) =

$$\frac{\text{Initial Number of Fish Stocked} - \text{Mortality}}{\text{Number of Fish stocked}} \times 100$$

### Haematological analysis

Blood samples were collected from the caudal vein using 2 ml syringes inserted at a 45° angle near the anal fin, following Argungu *et al.* (2017). Samples were transferred into EDTA-coated tubes, gently mixed, and stored in ice-filled sample boxes prior to analysis (Lawal *et al.*, 2019; Suleiman *et al.*, 2022). Sampling was done in the morning to minimize diurnal variations (Oyegbile *et al.*, 2017). Haematological parameters analysed following Kelly (1979) and Schalm *et al.* (1975) included total red blood cell count (RBC), total white blood cell

count (WBC), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

### Statistical analysis

Weekly growth measurements were used to generate growth performance curves. Data on growth performance indices, haematological parameters, and survival rates were subjected to two way analysis of variance (ANOVA) to evaluate the main and interaction effects of solvent extract combination methods and inclusion ratios. All statistical analyses were performed using R statistical software, and differences were considered statistically significant at  $p < 0.05$ . Where significant effects were detected, Tukey's honestly significant difference (HSD) test was applied as a post hoc procedure for pairwise mean comparisons.

### RESULTS

The effects of combined *M. oleifera* leaf extract types, their inclusion ratios, and the interaction between both factors on the growth performance and feed utilization of *Clarias gariepinus* are presented in Table 2. The results showed that the different combined *Moringa* extracts and their inclusion ratios exerted a significant effect ( $p < 0.05$ ) on all growth and feed utilization parameters, except for apparent net protein utilization (ANPU) and survival rate. Fish fed diets containing combined aqueous and ethanol extracts (MAE) and combined ethanol and hexane extracts (MEH) at the 100:0% inclusion ratio exhibited the highest mean final weight, mean weight gain, specific growth rate, protein efficiency ratio, and percentage survival. Correspondingly, the lowest feed conversion ratio (0.96) was recorded in these two treatments. However, the interaction between extract type and inclusion ratio did not exert a statistically significant effect ( $p > 0.05$ ) on any of the growth, feed utilization, or survival parameters evaluated.

The effects of combined *Moringa oleifera* leaf extract types, inclusion ratios, and their interactions on the haematological parameters of *C. gariepinus* are shown in Table 3. Significant main and interaction effects ( $p < 0.05$ ) were observed for all the haematological indices measured, except mean corpuscular haemoglobin concentration (MCHC). Notably, the treatments that recorded superior growth performance (MAE and MEH at the 100:0% inclusion ratio) also produced the highest red blood cell counts ( $4.10 \times 10^{12} \text{ L}^{-1}$ ) and MCHC values (33.35%), alongside the lowest white blood cell counts ( $10.6 \times 10^9 \text{ L}^{-1}$ ), mean corpuscular volume (73.24 fL), and mean corpuscular haemoglobin (24.4 pg).

**Table 2:** Growth response of *C. gariepinus* Fed Diets Supplemented with Various Solvent Interaction Ratios of *M. oleifera* Leaf Extracts

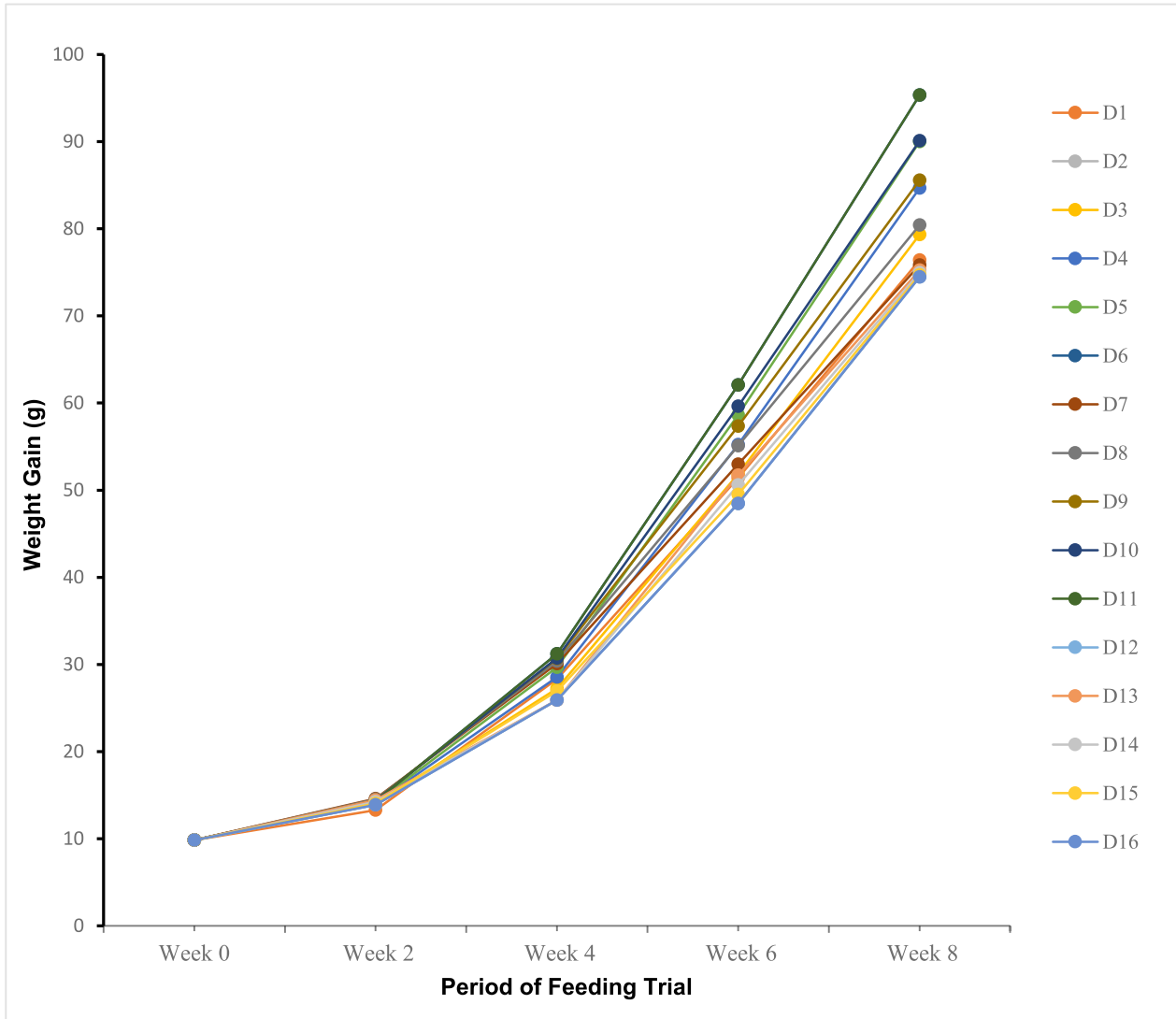
Extract combined	Ratio of combination	SGR						
		MFW (g)	MWG (g)	(%/Day)	FCR	PER	% ANPU	% Survival
MAE	0:100	74.25 ± 5.75 <sup>b</sup>	64.42 ± 5.74 <sup>b</sup>	3.61 ± 0.14 <sup>b</sup>	1.09 ± 0.10 <sup>ab</sup>	1.62 ± 0.14 <sup>b</sup>	86.40 ± 10.94 <sup>a</sup>	96.67 ± 5.77 <sup>a</sup>
		79.19 ± 4.10 <sup>b</sup>	69.36 ± 4.10 <sup>ab</sup>	3.72 ± 0.09 <sup>ab</sup>	1.06 ± 0.07 <sup>ab</sup>	1.73 ± 0.11 <sup>ab</sup>	88.23 ± 7.20 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
	25:75	84.58 ± 4.54 <sup>ab</sup>	74.75 ± 4.53 <sup>ab</sup>	3.84 ± 0.09 <sup>ab</sup>	1.03 ± 0.05 <sup>ab</sup>	1.87 ± 0.11 <sup>ab</sup>	90.07 ± 3.91 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
		89.98 ± 6.24 <sup>ab</sup>	80.14 ± 6.24 <sup>b</sup>	3.95 ± 0.12 <sup>ab</sup>	1.01 ± 0.06 <sup>ab</sup>	2.00 ± 0.16 <sup>a</sup>	91.90 ± 3.24 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
	50:50	95.33 ± 8.48 <sup>a</sup>	85.50 ± 8.47 <sup>a</sup>	4.05 ± 0.16 <sup>a</sup>	0.96 ± 0.06 <sup>a</sup>	2.14 ± 0.21 <sup>a</sup>	93.73 ± 6.10 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>
		75:25	75.25 ± 7.16	65.89 ± 7.17 <sup>b</sup>	3.64 ± 0.17 <sup>b</sup>	1.18 ± 0.10 <sup>ab</sup>	1.65 ± 0.18 <sup>b</sup>	94.90 ± 2.22 <sup>a</sup>
MEH	0:100	80.63 ± 6.21 <sup>ab</sup>	70.80 ± 6.23 <sup>ab</sup>	3.75 ± 0.14 <sup>ab</sup>	1.04 ± 0.03 <sup>ab</sup>	1.77 ± 0.16 <sup>ab</sup>	95.1 ± 1.97 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
		85.88 ± 5.72 <sup>ab</sup>	75.97 ± 5.73 <sup>ab</sup>	3.87 ± 0.12 <sup>ab</sup>	1.08 ± 0.00 <sup>ab</sup>	1.90 ± 0.14 <sup>ab</sup>	93.32 ± 1.97 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
	25:75	90.48 ± 7.03 <sup>ab</sup>	80.65 ± 7.01 <sup>ab</sup>	3.96 ± 0.14 <sup>ab</sup>	1.06 ± 0.02 <sup>ab</sup>	2.20 ± 0.18 <sup>a</sup>	94.03 ± 4.03 <sup>a</sup>	95.00 ± 8.66 <sup>a</sup>
		95.33 ± 8.48 <sup>a</sup>	85.50 ± 8.47 <sup>a</sup>	4.05 ± 0.15 <sup>a</sup>	0.96 ± 0.06 <sup>a</sup>	2.14 ± 0.21 <sup>a</sup>	93.73 ± 6.10 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>
	50:50	74.25 ± 5.75 <sup>b</sup>	64.42 ± 5.74 <sup>b</sup>	3.61 ± 0.14 <sup>b</sup>	1.10 ± 0.09 <sup>ab</sup>	1.61 ± 0.14 <sup>b</sup>	94.98 ± 2.21 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
		75.35 ± 3.94 <sup>b</sup>	65.52 ± 4.10 <sup>b</sup>	3.64 ± 0.09 <sup>b</sup>	1.14 ± 0.06 <sup>ab</sup>	1.73 ± 0.21 <sup>ab</sup>	92.78 ± 3.95 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
MAH	0:100	74.99 ± 0.74 <sup>b</sup>	65.16 ± 0.75 <sup>b</sup>	3.63 ± 0.12 <sup>b</sup>	1.13 ± 0.05 <sup>ab</sup>	1.87 ± 0.11 <sup>ab</sup>	90.65 ± 6.18 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
		74.62 ± 2.53 <sup>b</sup>	64.79 ± 2.52 <sup>b</sup>	3.62 ± 0.05 <sup>b</sup>	1.14 ± 0.06 <sup>ab</sup>	2.00 ± 0.16 <sup>ab</sup>	88.53 ± 8.54 <sup>a</sup>	93.33 ± 5.77 <sup>a</sup>
	25:75	74.25 ± 5.75 <sup>b</sup>	64.42 ± 5.74 <sup>b</sup>	4.05 ± 0.16 <sup>a</sup>	1.09 ± 0.09 <sup>ab</sup>	1.61 ± 0.14 <sup>b</sup>	86.40 ± 10.94 <sup>a</sup>	96.67 ± 5.77 <sup>a</sup>
		74.25 ± 5.75 <sup>b</sup>	64.42 ± 5.74 <sup>b</sup>	4.05 ± 0.16 <sup>a</sup>	1.09 ± 0.09 <sup>ab</sup>	1.61 ± 0.14 <sup>b</sup>	86.40 ± 10.94 <sup>a</sup>	96.67 ± 5.77 <sup>a</sup>
	50:50	74.25 ± 5.75 <sup>b</sup>	64.42 ± 5.74 <sup>b</sup>	4.05 ± 0.16 <sup>a</sup>	1.09 ± 0.09 <sup>ab</sup>	1.61 ± 0.14 <sup>b</sup>	86.40 ± 10.94 <sup>a</sup>	96.67 ± 5.77 <sup>a</sup>
		74.25 ± 5.75 <sup>b</sup>	64.42 ± 5.74 <sup>b</sup>	4.05 ± 0.16 <sup>a</sup>	1.09 ± 0.09 <sup>ab</sup>	1.61 ± 0.14 <sup>b</sup>	86.40 ± 10.94 <sup>a</sup>	96.67 ± 5.77 <sup>a</sup>
<b>Two-Way ANOVA</b>								
Extract combined(P-Value)		0.000	0.000	0.000	0.003	0.000	0.109	0.695
Ratio (P-Value)		0.000	0.000	0.000	0.006	0.000	0.997	0.205
Interaction (P-Value)		0.086	0.085	0.095	0.318	0.085	0.602	0.999

Treatments in the same column having the same superscripts are not significantly different ( $P > 0.05$ ). MFW (Mean Final Weight), MWG (Mean Weight Gain), SGR (Specific Growth Rate), FCR (Food Conversion Ratio), PER (Protein Efficiency Ratio), ANPU (Apparent Net Protein Utilization), MAE (Moringa Aqueous Ethanol), MEH (Methanol Hexane) and MAH (Moringa Aqueous Hexane).

**Table 3:** Haematological parameters of *C. gariepinus* Fed Diets Supplemented with Various Solvent Interaction Ratios of *M. oleifera* Leaf Extracts

Solvents Combined	Ratio of Interaction	RBC x	WBC x	PCV (%)	Hb	MCV	MCH	MCHC (%)
		10 <sup>12</sup> /l	10 <sup>9</sup> /l		(g/dl)	(fl)	(pg)	
<b>MAE</b>	0:100	3.60 ± 0.20 <sup>bcd</sup>	13.73 ± 0.31 <sup>de</sup>	33.00 ± 1.00 <sup>a</sup>	11.00 ± 0.10 <sup>a</sup>	91.81 ±4.55 <sup>a</sup>	30.61 ± 1.60 <sup>a</sup>	33.35 ± 0.71 <sup>a</sup>
	25:75	3.73 ± 0.16 <sup>bcd</sup>	12.95 ± 0.28 <sup>f</sup>	32.25 ± 0.50 <sup>ab</sup>	10.75 ± 0.05 <sup>ab</sup>	87.17 ±3.50 <sup>ab</sup>	29.06 ± 1.28 <sup>abc</sup>	33.35 ± 0.34 <sup>a</sup>
	50:50	3.85 ± 0.13 <sup>ab</sup>	12.17 ± 0.25 <sup>g</sup>	31.51 ± 0.00 <sup>abc</sup>	10.50 ± 0.00 <sup>bc</sup>	82.52 ± 2.98 <sup>abc</sup>	27.51 ± 1.01 <sup>bcd</sup>	33.35 ± 0.34 <sup>a</sup>
	75:25	4.10 ± 0.10 <sup>a</sup>	10.60 ± 0.20 <sup>g</sup>	30.00 ± 1.00 <sup>bcd</sup>	10.00 ± 0.10 <sup>de</sup>	73.23 ± 4.23 <sup>c</sup>	24.40 ± 0.84 <sup>e</sup>	33.35 ± 0.78 <sup>a</sup>
	100:0	4.10 ± 0.10 <sup>a</sup>	10.6 ± 0.20 <sup>h</sup>	30.00 ± 1.00 <sup>bcd</sup>	10.00 ± 0.10 <sup>dc</sup>	73.24 ± 4.23 <sup>c</sup>	24.40 ± 0.83 <sup>c</sup>	33.35 ± 0.78 <sup>a</sup>
<b>MEH</b>	0:100	2.80 ± 0.00 <sup>g</sup>	15.93 ± 0.15 <sup>a</sup>	25.00 ± 1.00 <sup>g</sup>	8.30 ± 0.20 <sup>de</sup>	89.29 ± 3.57 <sup>a</sup>	29.64 ± 0.71 <sup>abc</sup>	33.21 ± 0.53 <sup>a</sup>
	25:75	3.00 ± 0.05 <sup>fg</sup>	15.38 ± 0.19 <sup>ab</sup>	27.00 ± 1.00 <sup>fg</sup>	8.98 ± 0.12 <sup>g</sup>	89.92 ± 2.98 <sup>a</sup>	29.89 ± 0.55 <sup>abc</sup>	33.25 ± 0.57 <sup>a</sup>
	50:50	3.45 ± 0.05 <sup>cde</sup>	13.27 ± 0.18 <sup>ef</sup>	27.50 ± 0.00 <sup>efg</sup>	9.15 ± 0.05 <sup>g</sup>	81.26 ± 0.33 <sup>abc</sup>	27.02 ± 0.06 <sup>cde</sup>	33.28 ± 0.13 <sup>a</sup>
	75:25	3.78 ± 0.08 <sup>abc</sup>	11.93 ± 0.18 <sup>h</sup>	28.75 ± 0.50 <sup>def</sup>	9.58 ± 0.03 <sup>f</sup>	77.25 ± 2.23 <sup>bc</sup>	25.71 ± 0.45 <sup>dc</sup>	33.32 ± 0.045 <sup>a</sup>
	100:0	4.10 ± 0.01 <sup>a</sup>	10.6 ± 0.20 <sup>h</sup>	30.00 ± 1.00 <sup>bcd</sup>	10.00 ± 0.10 <sup>dc</sup>	73.24 ± 4.23 <sup>c</sup>	24.40 ± 0.84 <sup>e</sup>	33.35 ± 0.78 <sup>a</sup>
<b>MAH</b>	0:100	2.80 ± 0.00 <sup>g</sup>	15.93 ± 0.15 <sup>a</sup>	33.00 ± 1.00 <sup>a</sup>	8.30 ± 0.20 <sup>h</sup>	89.29 ± 3.57 <sup>a</sup>	29.64 ± 0.71 <sup>abc</sup>	33.31 ± 0.53 <sup>a</sup>
	25:75	3.00 ± 0.05 <sup>fg</sup>	15.38 ± 0.19 <sup>ab</sup>	27.00 ± 1.00 <sup>fg</sup>	8.975 ± 0.12 <sup>g</sup>	89.91 ± 2.98 <sup>a</sup>	29.89 ± 0.55 <sup>abc</sup>	33.25 ± 0.57 <sup>a</sup>
	50:50	3.20 ± 0.10 <sup>ef</sup>	14.83 ± 0.23 <sup>bc</sup>	29.00 ± 1.00 <sup>cdef</sup>	9.65 ± 0.18 <sup>ef</sup>	90.55 ± 2.98 <sup>a</sup>	30.13 ± 0.76 <sup>ab</sup>	33.28 ± 0.62 <sup>a</sup>
	75:25	3.40 ± 0.15 <sup>dc</sup>	14.28 ± 0.26 <sup>cd</sup>	31.00 ± 1.00 <sup>abcd</sup>	10.33 ± 0.13 <sup>cd</sup>	91.18 ± 3.59 <sup>a</sup>	30.37 ± 1.15 <sup>ab</sup>	33.31 ± 0.66 <sup>a</sup>
	100:0	3.60 ± 0.20 <sup>bcd</sup>	13.73 ± 0.31 <sup>de</sup>	33.00 ± 1.00 <sup>a</sup>	11.00 ± 0.01 <sup>a</sup>	91.81 ± 4.56 <sup>a</sup>	30.61 ± 1.60 <sup>a</sup>	33.35 ± 0.71 <sup>a</sup>
<b>Two-Way ANOVA</b>								
Extract	(P-Value)	0.000	0.000	0.000	0.000	0.000	0.000	0.936
Ratio	(P-Value)	0.000	0.000	0.000	0.000	0.000	0.000	0.998
Interaction	(P-Value)	0.000	0.000	0.000	0.000	0.000	0.000	1.000

Treatments in the same column having the same superscripts are not significantly different (P>0.05). RBC (Red Blood Cells), WBC (White Blood Cells) PCV (Pack Cell Volume), Hb (Haemoglobin), MCV (Mean Cell Volume), MCH (Mean Corpuscular Haemoglobin) MCHC (Mean Corpuscular Haemoglobin Concentration).



**Figure 1:** Biweekly Growth Performance of *C. gariepinus* Fed *M. oleifera* Leaf Extracts Based Diets of Different Ratios for 56 Days

**DISCUSSION**

The growth performance response and haematological parameters of *C. gariepinus* in this study signifies that the bioactivity of *M. oleifera* leaf meal extracts is significantly influenced by the solvent extraction ratio. The growth performance and nutrient utilization of *C. gariepinus* in this study demonstrate that the inclusion of *Moringa oleifera* leaf meal extracts is highly effective, particularly when processed with polar solvents (aqueous and ethanol).

The improved SGR and FCR at the 100:0 ratios indicate that pure extracts may contain a higher concentration of bioactive compounds, such as flavonoids, which enhance metabolic efficiency as observed by Fagbenro *et al.* (2013). According to Yusuf *et al.* (2021), *M. oleifera* extracts contain potent antioxidants that reduce physiological stress, thereby allowing more energy to be diverted toward muscle tissue accretions.

The uniform response in PER, ANPU, and Survival Rate across all the treatments groups is a critical finding. It

indicates that while different solvents affect the rate of fish growth, better utilization of protein and the overall safety of the plant extract remain high. The high survival rates are in accordance with the findings of Omitoyin *et al.* (2019), who reported that Moringa supplements in aquafeed do not exert toxic effects on the vital organs of African Catfish, even at varying inclusion levels.

The haematological parameters in this research serve as a physiological mirror of the fish internal environment. The higher RBC, Hb, and PCV in the MAE (75:25 and 100:0) dietary groups suggest that these solvent ratios optimize the extraction of iron and essential amino acids required for erythropoiesis. In the recent research by Omitoyin *et al.* (2019) notes that improved erythrocyte profiles in catfish are directly linked to enhanced oxygen carrying capacity which supports higher metabolic demands during rapid growth phases.

Conversely, the elevated WBC counts in the hexane-dominant groups (MEH 0:100 and MAH 0:100) indicate a state of leucocytosis, likely a defensive response to



dietary-induced stress or the presence of residual anti-nutritional factors that non-polar solvents failed to neutralize. As noted by Adegbesan and Abdulraheem (2020), stabilized (lower) WBC counts in fish fed plant-based additives are an indicator of high biocompatibility and low inflammatory response. The consistency of MCHC across all treatments further proves that the diets did not disrupt haemoglobin saturation, maintaining homeostasis throughout the experimental period (Fazio, 2019).

## CONCLUSION

Based on the findings of this study, it is concluded that the inclusion of *Moringa oleifera* leaf meal extracts in the diets of *Clarias gariepinus* significantly enhances both growth performance and physiological health. The effectiveness of the extract is highly dependent on the solvent used, with polar solvents (Aqueous and Ethanol) outperforming non-polar solvents (Hexane). Pure polar extracts diet (MAE 100:0 and MEH 100:0) yielded the best specific growth rates and feed conversion efficiency, promoted higher RBC and Hemoglobin levels, ensuring superior oxygen transport and metabolic vigor. The stabilized (lower) WBC counts in polar extract groups (aqueous and ethanol) indicate reduced dietary stress and high biocompatibility, while hexane-based diets induced leukocytosis. The high survival rates across all groups confirm that *M. oleifera* extracts are nontoxic and serve as safe, sustainable, and eco-friendly immunostimulants in aquafeed productions.

## REFERENCES

- Action, S. I. (2020). World fisheries and aquaculture. *Food and Agriculture Organization, 2020*, 1-244. <https://openknowledge.fao.org/server/api/core/bitsstreams/f985caed-cc7a-457e->
- Adegbesan, S. I. and Abdulraheem, I. (2020). Growth Performance, Nutrient Utilization, Haematology and Serum Biochemistry of African Catfish (*Clarias gariepinus*) Broodstock Fed Varying Inclusion Levels of *Aspilia africana* Leaves-Paste. *Nigeria Journal of Animal Production*. 47(1):129-139.
- Adewolu, M. A., Adoti, A. J. and Akinyemi, O. (2008). Some Haematological Parameters of African Catfish Fed Diets Containing Different Levels of Cassava Leaf Meal. *African Journal of Biotechnology*, 7(22), 4112-4115.
- Afia, O. E. and Ofor C. O. (2016). Haematological Indices of the *Clarias gariepinus Heterobranchus longifilis* (hybrid catfish - Heteroclaris) reared at different feeding levels. *Nigerian Journal of Agriculture, Food and Environment.*; 12(3), 6-11.
- Ajiboye, O. O., Yakubu A. F. and Adams T. E. (2012). A Perspective on the Ingestion and Nutritional Effects of Feed Additives in Farmed Fish Species. *World Journal of Marine Sciences*, 4(1), 87-101
- AOAC. (2010). Official Method of Analysis 18<sup>th</sup> Edition, *Association of Official Analytical Chemists*, Washington, D.C. U.S.A.
- Argungu, L. A., Siraj, S. S., Christianus, A., Amin, M. S. N., Abubakar, M. S. and Abubakar, I. A. (2017). *A simple and rapid method for blood collection from walking catfish, Clarias batrachus (Linnaeus, 1758)*. 16(3), 935-944.
- Ayotunde, E. O., & Ofem, B. O. (2020). Growth Response and Nutrient Utilization of African Catfish fed Moringa-Based Diets. *Journal of Aquatic Science*, 35(1), 45-54.
- Azubiike, A. and Nwoye, E. (2021). Effect of Raw and Toasted Soybean Diets on the Growth of *Clarias gariepinus*. *Merit Research Journal of Microbiology and Biological Sciences*. 9(3), 021-031
- Bake, G. G., Martins, E. I. and Sadiku, S. O. E. (2014). Nutritional evaluation of varying levels of cooked flamboyant seed meal (*Delonix regia*) on the growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) fingerlings. *International Journal of Agriculture, Forestry and Fisheries*, 3(4), 233-239.
- Bbole, L., Chanda, M., Nephter, M. and As, K. (2016). Analysis of Growth Performance and Haematological of *Oreochromis niloticus* fed on a Varying Diet of *Moringa oleifera* Lam. Leaf meal as an additive Protein Source. *International Journal of Fisheries and Aquaculture*. 8(11), 105-111.
- Bondi, A. A. (1987). *Animal Nutrition*. John Wiley & Sons Ltd New York. 432-433.
- Chakraborty, S. B., Hancz, C., Chosal, I. and Deborsee, M. (2018). Dietary Administration of Plant Extracts for Production of Monosex Tilapia: Searching a Suitable Alternative to Synthetic Steroid in Tilapia Culture. *Turkish Journal of Fisheries and Aquatic Sciences*. 18, 267-275.
- De-Silva, S. S. and Anderson, T. A. (1995). *Fish Nutrition in Aquaculture*. Chapman and Hall, London. *Sciences*, 320.
- Ede, F. R., Nwanna, L. C. and Obasa, S. O. (2020). Utilization of Moringa Leaf Extract as Dietary Supplement in *Clarias gariepinus*. *Aquaculture Reports*, 18, 100452.
- Ekanem, A. P., Udoh, J. P. and Akpabio, G. T. (2017). Haematological Responses of *Clarias gariepinus* Fed Diets Supplemented with Phyto-genic Additives. *Journal of Fisheries and Aquatic Science*, 12(3), 101-108.
- Eteng, S. U. and Opeh, P. B. (2023). Growth performance of the African catfish, *Clarias gariepinus* (Burchell, 1822) Juveniles fed diet containing *Moringa oleifera*. *International Journal of Fisheries and Aquaculture Research*. 5(4), 40-45.
- Fagbenro, O. A., Adeparasu, E. O. and Jimoh, W. A. (2013). Haematological Profile of Blood of African Catfish (*Clarias gariepinus*, Burchell, 1822) Fed Sunflower and Sesame Meal Based Diets. *Academic Journal of Fisheries and Aquatic Science*. 8(1), 80-86.
- Fahey, J. W. (2005). *Moringa oleifera: A review of the Medical Evidence for Its Nutritional, Therapeutic, And Prophylactic Properties*. *Trees for Life Journal*, 1, 5-12.
- FAO (2022) *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation*. Rome by



- Food and Agriculture Organisation.
- Fazio, F. (2019). Fish hematology Analysis as an Important tool of Aquaculture: A Review. *Journal of Aquatic Animal Health*, 31(1), 3-13.
- Gabriel, U. U., Numbere, A. O., and Alagoa, K. J. (2019). Effects of Moringa leaf Inclusion on Growth and Haematological Parameters of Catfish. *International Journal of Fisheries and Aquatic Studies*. 7(5), 56–63.
- Ganguly S, Krushna C, Sarkar S, and Chowdhury S (2013). Supplementation of prebiotics in fish feed: a review. *Rev Fish Biology Fisheries*. 23, 195-199.
- Halver, J. E. (1989). Progress in Nutrition and Diet Development Research. Nutrition Consultancy Report, 30<sup>th</sup> April–31<sup>st</sup> May 1989, 66pp.
- Idowu, T. A., Williams, S., Adedeji, S. A. and Sogbesan, H. A. (2016). Effects of Different Additives on The Survival and Haematology of *Clarias gariepinus* (Burchell, 1822) Fingerlings during Transportation. *Nigerian Journal of Tropical Agriculture*. 16, 1-11.
- Kelly, W. R. (1979). Veterinary Clinical Diagnosis (2nd Edition). Balliere, Tindall, London.
- Lawal, Aderolu, A.Z., Adewumi, G.A. and Mudiaga, M. O., and A. (2019). Growth, Nutrient Utilization, Haematology and Biochemical Parameters of African Catfish (*Clarias gariepinus*, BURCHELL, 1822) Fed with Varying Levels of *Bacillus Subtilis*. *Agrosearch*, 19(1), 13–27.
- Moyo, B., Masika, P. J., Hugo, A., and Muchenje, V. (2011). Nutritional Characterization of Moringa (*Moringa oleifera* Lam.) Leaves. *African Journal of Biotechnology*, 10(60). 12925–12933.
- Octavia, D. Nurcahyani, R., Setya Rahardja, B., and Anam Al Arif, M. (2019). Moringa Leaf Flour (*Moringa oleifera*) As an Alternative for the Replacement of Crude Fat and Energy in the Commercial Feed of Siamese Catfish (*Pangasius Hypotalamus*). *International Journal of Civil Engineering and Technology (IJCIET)*, 10(05), 404-412.
- Olagunju, A. A., Oladipo, A. A., and Osho, F. (2018). Effect of Graded Levels of *Moringa oleifera* Leaf Meal on Performance of *Clarias gariepinus*. *Nigerian Journal of Fisheries*, 15(1), 145–154.
- Olaniyi, A. A., Oyeleye, O. O., and Adeyemi, A. S. (2021). Phyto-genic Feed Additives and their Effects on Growth and Immunity of African Catfish. *Aquaculture International*, 29, 1769–1782.
- Omitoyin, B. O., Tiamiyu, L. O. and Oladimeji, A. (2019). Effect of Moringa Leaf Meal on The Growth and Haematological Parameters of *Clarias Gariepinus* Juveniles. *West African Journal of Applied Ecology*, 27(2), 60–72.
- Orire, A. M. and Sadiku, S. O. E. (2012). Development of Farm Made Floating Feed for Aquaculture Species. *Journal of International Scientific Publications: Agriculture and Food*, 2(4): 579-583.
- Orire, A. M. and Sadiku, S. O. E. (2012). Development of Farm Made Floating Feed for Aquaculture Species. *Journal of International Scientific Publications: Agriculture and Food*, 2(4): 579-583.
- Orire, A. M. and Salihu, Z. (2021). Effects of Nutrient Inclusion levels on Aquafeed Buoyancy. *Tanzania Journal of Agricultural Sciences*. 19 (2), 161-166
- Orire, A.M. and Abubakar, S. (2013). Graded level inclusion of melon shell in the diet of hybrid catfish (*Heteroclarias*) as energy source. *Continental journal of fisheries and Aquatic Science*. 7(1), 8–17.
- Orire, A.M. and Abubakar, S. (2013). Graded level inclusion of melon shell in the diet of hybrid catfish (*Heteroclarias*) as energy source. *Continental journal of fisheries and Aquatic Science*. 7(1), 8–17.
- Oyegbile, B., Abdullahi, B. A. and Yola, I. A. (2017). Haematological Profile of *Heterobranchius bidorsalis* Fingerlings Fed Processed *Delonix regia* Seeds at Different inclusion Levels of Diets. *International Journal of Fisheries and Aquaculture*. 9(8), 81-85.
- Sadiku, S. O. E. (2014). Fish Nutrient: The First-Class Nutrient for All. *Inaugural Lecture Series* 30<sup>th</sup> of the Federal University of Technology, Minna, Nigeria.
- Schalm, O. W., Jain, O. N. and Carrol, E. J. (1975). Veterinary Haematology. 3rd Edition., Lea and Febiger, Philadelphia. 144- 156.
- Siddhuraju, P. and Becker, K. (2003). Antioxidant Properties of Various Solvent Extracts of *Moringa oleifera* Leaves. *Food Chemistry*, 85(4), 497–507.
- Stefanie, M. H. (2014). Fish nutrition and current issues in aquaculture; the balance in providing safe and nutritious seafood in an environmentally sustainable manner. *Journal of Aquaculture Research and Development (JARD)*. 5(3), 1-10.
- Suleiman, A. M., Orire, A. M. and Sadiku, S. O. E. (2018). Effects of Moringa *oleifera* Leaves, Bark Stem of *Lannea barteri* and Antibiotic (Oxytetracycline) on Haematological Parameters of *Clarias gariepinus* Fingerlings. *Fisheries and Aquaculture Journal*. 9 (2), 246-250.
- Suleiman, A. M., Orire, A. M. and S. O. E. Sadiku (2022) Effects of Processed Selected Medicinal Plants Dites on Haematological Parameters of *Clarias gariepinus* (Burchell, 1822). *Proceedings of the 2<sup>nd</sup> International Conference of Agriculture and Agricultural Technology (ICAAT)*. 472-479.
- Suleiman, A. M., Orire, A. M., Sadiku, S. O. E. and Bake, G. G. (2023). Growth Performance, Nutrient Utilization and Survival Rate of *Clarias gariepinus* Fed Varied Inclusion of Processed *Moringa oleifera* diets. *International Journal of Fisheries and Aquatic Studies* 2023; 11(1), 36-40
- Yusuf, A. A., Eyo, J. E., and Mohammed, A. (2021). Effect of *Moringa oleifera* Leaf Extract on Growth Performance of African Catfish. *Nigerian Journal of Fisheries and Aquaculture*, 9(2), 15–23.