ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

Assessment of the Proximate and Mineral Compositions of *Moringa Oleifera* Leaf Extracts, the Carcass and Eggs of African Clariid Catfish, *Heterobranchus bidorsalis*

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Abstract:- The need to optimize feed efficiency, feed digestibility and improve metabolism, growth, and reproductive performance of fish has necessitated the use of phytogenic feed additives in aquaculture. A Study was carried out to assess the Proximate and mineral compositions of aqueous and ethanolic *Moringa oleifera* leave extracts (AMOLE and EMOLE respectively) according to standard procedures. Consequently, these two extracts were used to formulate test diets containing 0.00/100g (control), 1.0g/100g, 2.0g/100g and 3.0g/100g inclusion levels. *H. bidorsalis* samples (n=216; 800.00±150.00g; 37.50±1.5cm) were randomly distributed in triplicate into 24 concrete tanks of size 6m x 4m x1.3m in a completely randomized 2x4 factorial design and fed at 5% body weight twice daily for 16 weeks. The proximate and mineral compositions of the diets was determined while the carcass and the eggs nutrients compositions were subsequently assessed. The proximate composition of the eggs were significantly higher in crude protein contents than the CP in the MOLE (34.14±0.05), AMOLE (38.84±0.17) and EMOLE (38.44±0.07) diets and the Carcass (37.46±0.28) with range (72.32±0.41 (control) - 78.78±0.58 % (3.0/100g)) while the ash (1.44±0.10 (3g/100g - 2.41±0.10% (control)) and ether (2.88±0.09 (3.0/100g) - 3.25±0.9% (control)) contents where significantly lower than the highest contents of ash (20.01±0.27%) recorded in fish fed 2.0/100g AMOLE diet and (7.94±0.05%) ether recorded in the control diet. Aqueous extraction method had the highest significant (P<0.05) retention of mineral concentrations: Ca (21.04), P (14.89), Mg (16.40), Na (23.33), K (65.57), Fe (10.67), Cu (6.48) and Zn (5.55) compare to ethanolic extraction method: Ca (2.87), P (12.65), Mg (7.65), Na (19.24), K (13.76), Fe (9.07), Cu (2.40) and Zn (2.32). For the carcass, only Manganese, Iron and Copper compositions were significant (P<0.05) among all treatment groups and the control. These findings have far reaching nutritional importance in the healthcare system and will help to address undernutrition in fish broodstock management in a cost effective manner. Thus, the use of M. *oleifera leaves* extracts as phytogenic feed additives and nutrients booster should be encouraged and sustained towards sustainable aquaculture development.

Keywords:- *Proximate, Mineral, Heterobranchus bidorsalis, Moring oleifera Leave Extract.*

I. INTRODUCTION

Clariid catfish are favorite food fish and suitable aquaculture candidate especially in African and Asian continents (Ajani *et al.*, 2011). *H. bidorsalis* is one of the five most cultured clariid catfish in Africa, especially in Nigeria. The culture potential of *H. gariepinus* is derived from the fact that it is hardy, can survive out of water for several hours, grows up to 30 kg in the wild, and accepts a wide variety of food including pelleted diets. It is one of the easiest and the commonest fish raised in ponds with a remarkable fast growth and its ability to adapt to crowded pond conditions have enabled it to gain tremendous popularity in sub-Saharan Africa (Reed *et al.*, 1967; Olatunde, 1983). Phytogenic Feed Additives (PFAs) are substitutes of plant origin such as essential oil, herbs, extracts and spices that are added to animal diets at recommended levels with the aim of improving animal performance. Most of growth promoting feed additives includes, extracts, hormones, ionospheres and probiotics (Abdelhadi, 2010). It is worthy to note that the metabolism, growth and reproduction of fish broodstock and progeny is a function of feed optimization, nutrient quality and utilization. Hence, minerals perform a wide variety of structural, biochemical and physiological functions in fish (DeSilva and Anderson, 1995).

This underscores the high importance attached to the knowledge of nutrients profiles through proximate and mineral analyses of feed, fish broodstock and progeny. Calcium

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diphosphate is rich in calcium, phosphorus, iron, magnesium and zinc. All these minerals present in the selected ingredients may be responsible for the high mineral content of formulated diet. Nonetheless, the significant elements are noted for their functions in fish physiology, calcium is important in skeleton and scale formation in bony fish. It is also important for metabolism, nerve and muscle functioning, as well as, osmoregulation (De Silva and Anderson, 1995). Calcium, according to Lovell (1989), is essential for hard tissue structure, blood clotting, muscle contraction, nerve impluse transmission, osmoregulation, and serve as a cofactor for enzymatic processes. Its percentage in fresh (wet) body of finfish ranges from 0.5 to 1%. Iron, on the other hand, is a vital element necessary for blood cells production, as it is a component of haemoglobin known for oxygen carriage, and exchange round animal body. It belongs to the less soluble minerals group; hence it is transported in fish body in protein-bound form. Calcium, which however belongs to the highly soluble group, can be easily exchanged between the body fluids and the surrounding water across the gill membranes. Most fresh water fish can absorb sufficient calcium from water unless calcium carbonate content of the water is below 5mg/l, therefore, supplemental calcium is not required in mineral premixes of fish feeds.

Minerals perform a wide variety of structural, biochemical and physiological functions in fish (De Silva and Anderson, 1995, Ugwu *et al*., 2007). Six (6) major elements (Fe, Zn, Mn, Ni, I, Mb and Co) have been identified as essential for animal life (Underwood, 1977). Although most of these elements might be required by fish, only six dietary minerals have been shown to be required or utilized by Salmonids (De Silva and Anderson, 1995). Most fish species derive their minerals from food or water in which they live. Several studies and reports show that all parts of the plant *Moringa oleifera* and their extracts are rich sources of a number of vitamins and minerals, which may contribute significantly to its antioxidant, therapeutic, and nutritional value. The leaves, seeds and green immature pods serve as a good source of natural antioxidants due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids. The high concentrations of ascorbic acid, iron, calcium, phosphorus, copper, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, βcarotene, protein, and in particular essential amino acids such as methionine, cystine, tryptophan and lysine present in Moringa leaves and pods make it a virtually ideal dietary supplement (Anwar *et al*., 2007).

Moringaceae a popular plant family with high nutritional quality has a specie, *M. oleifera* which has a wide distribution and application in Africa and Asia and is described by (Olson M.E, 2010) as a horseradish tree (it roots taste like horseradish), drumstick tree (due to its slender, long, triangular seed pods) and ben oil or benzoil tree (because of the oil derived from the seeds). Nutritional analysis indicates that *Moringa* leaves contain a wealth of essential disease preventing nutrients which make it suitable to be included in fish diets as food supplement (Krishnaiah *et al*., 2009). Recent study has revealed that *M. oleifera* leaves extracts have immense nutritional values and can be used to improve animal growth and reproductive performance, as well as the quality and nutritional composition of the carcass (Onuoha S.O. *et al*., 2024). Studies have equally showed that *M. oleifera* contain high levels of essential amino acids, crude protein (above 30.29% Dry leaf nutritive content), minerals, amino acids, vitamins and fatty acid properties (Moyo *et al.*, 2011). It has also been found that extract obtained from the leaves of *Moringa* in 80% ethanol contains growth enhancing properties for higher plants (Makkar and Becker, 1996). Several authors have documented positive findings on the effect of *M. oleifera* on fish growth and survival. Eyo and Ivon (2017) recommended 15 % inclusion level of *M. oleifera* leaf meal (MLM) for optimal growth of *H. longifilis.* Ochang *et al*., (2015) recommended 20% *M oleifera* leaf meal (MLM) for optimal growth of *C. gariepinus.* However, limited studies have been documented on the nutrient compositions of *M. oleifera* leave extracts as feed additives and its effect on nutrient compositions of the carcass and eggs of *H. bidorsalis* broodstock.

II. MATERIALS AND METHODS

A. Collection, Identification and Authentication of plant material and Experimental Fish

Moringa Oleifera leave samples were collected from Adedayo Moringa Farm, Ibadan, identified and further authenticated by a plant taxonomist at Department of Botany, University of Ibadan, Nigeria where a Voucher Specimen is deposited. Whereas, the experimental fish, Heterobranchus bidorsalis samples were collected from Nigerian Institute for Oceanography and Marine Research, Sapele, Delta State.

- *Processing of Plant Materials*

The processing of the plant was carried out in the Aquaculture and Fisheries Management Laboratory, Faculty of Renewable Natural Resources, University of Ibadan.

- *Processing: Air Drying*

The leaves were spread on cotton sheet and kept in well ventilated room at a room temperature. The leaves were shade dried for a period of 7 – 10 days (Isitua *et al*., 2015) for proper drying to be achieved. Upon drying, the leaves were pulverized under aseptic conditions using an Orange Legend Gold electric grinder into fine powdery form, weighed, sieved, labelled and stored in dry dark airtight sealed glass jar and kept at −20 ^{0}C for phytochemical, proximate and mineral analyses.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

➢ *Preparation of plant extracts for Proximate and Mineral Analyses*

The nutrient composition analyses followed the method of Association of Official Analytical Chemists (AOAC, 2012) at the Department of Pharmaceutical Chemistry, University of Ibadan.

➢ *Experimental Diets Composition and Formulation*

The feed ingredients used for the composition of the experimental diets containing each of the Aqueous and Ethanolic *Moringa oleifera* leave extracts were procured from Kesmac Feed Industry within Ibadan Metropolis. The diets were prepared following the method of feed formulation described by (Ekanem *et al.,* 2010). This was done bi-weekly so as to maintain quality throughout the trial period. The experimental diets were as presented in tables 2.1 – 2.2. The experimental diets were formulated to contain 0.0/100g (control), 1.0/100g, 2.0/100g and 3.0 g/100g of the extracts (Tiamiyu *et al*., 2019).

➢ *Experimental Design*

The study followed a Completely Randomized Design in a 2 x 4 factorial design. The sub-adults on arrival were acclimatized in earthen pond for 30 days and fed a 40% Crude protein conventional diet afterwards the weight of the fish samples was determined. At the end of acclimatization, 216, eight-month old *H. bidorsalis* weighing 800.00g±150.00g were randomly distributed into 24 treatment groups in outdoor concrete tanks of size 6mx4mx1.3m each replicated thrice and fed at 5% body weight twice daily for 16 weeks. The fish were kept in the tank of 0.45m water level (Hect *et al.*, 1988), at the stocking rate of 1 fish/m, with sex ratio of 3 males: 6 females. The tanks were divided into treatments, representing varying levels of both aqueous and ethanolic extracted dietary *Moringa oleifera* leave inclusion. The fish were fed the test diets containing 0.0 g/100g (control), 1.0, 2.0 and 3.0 g/100g inclusion levels of *M. Oleifera* leave extract at a daily rate of 5% body weight (BW), twice a day (09:00 and 16:00 h) (Adesina, 2017) for 16 weeks.

➢ *Selection of Brood Stock for Induced Breeding*

24 Female broodstocks of *Heterobranchus bidorsalis* of average weight, 1300±150g and length, 26.5±1.5cm were collected for hormonal treatment (0.5ml/kg body weight of ovaprim) according to the treatment levels of the experiment. Ovulation and stripping of eggs took place after 12 hrs of injection and egg samples were collected in triplicates for proximate analysis.

➢ *Proximate Analysis of Moringa Oleifera Leaf Extract, Carcass and Egg Samples*

Moringa oliefera leaf extract and other samples from fish carcass and eggs were analyzed according to the method of AOAC (2012). The following nutrients were determined: Moisture, Ash, Fat, Fibre, Protein and Carbohydrate (Nitrogen Free Extract):

$$\text{Moisture \%} = \frac{\text{Wet weight – dry Weight}}{\text{Wet weight of sample}} 100$$

$$\text{\% Ash} = \frac{\text{Weight of sample remaining}}{\text{Weight of original Sample}} 100$$

$$\text{Fat \%} = \frac{(W2 - W1)\ Vc}{(VA \times SW)} 100$$

Where: W2 is the weight of glass tube and dried extract (g), W1 is the weight of empty dried glass tube (g), Vc is the total volume of chloroform (ml), VA is the volume of extract dried (ml), and SW is the weight of the sample in grammes.

$$\text{\% Fibre} = \frac{W1 - W2}{\text{Weight of the Sample}} 100$$

Where: W1= Weight of initial dried sample, W2= Weight of final dried sample

➢ *Crude Protein (%)*

The percentage of protein content was calculated according to the AOAC, 2000.

➢ *Carbohydrate (%)*

Carbohydrate content was calculated as a difference from the amount of other proximate parameters measured (AOAC, 2000). Carbohydrate = 100% - (% Moisture + % Ash + % Protein + % Fat)

➢ *Determination of mineral composition in the Extracts, carcass and egg samples*

Analyses of iron (Fe), copper (Cu), manganese (Mn), cadmium (Cd), nickel (Ni), lead (Pb), zinc (Zn), chromium (Cr), magnesium (Mg), sodium (Na), phosphorus (P), potassium (K), calcium (Ca), and sulfur(S) contents in sample were carried out using the inductively coupled plasma optical emission spectrophotometry (OPTIMA 4300 DV, Perkin Elmer, Shelton, Conn., USA). Briefly, Teflon digestion vessel was washed overnight in a solution of 2% nitric acid (v/v) prior to use. Sample was dissolved in 10 ml of 70 % nitric acid. The mixture was heated on the hot plate until digestion was completed. The digested samples was added in 5 ml of 2% nitric acid and filtered using filter paper. Sample was massed up to 100 ml with 2% nitric acid in a volumetric flask and sample run in triplicate. The concentration of mineral was then calculated and expressed as mg/100g sample.

B. Statistical Analysis

All tests were run in triplicate for this investigation, and the data were provided as the mean ± SE. The difference between groups was analyzed using a one-way analysis of variance (ANOVA) and the least significant difference (LSD) test at 5% threshold of significance ($p < 0.05$). All analysis was done using SPSS 2020 software.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

III. RESULTS

A. *Proximate Composition of Moringa Oleifera Leave Extract (Mole), Aqueous Moringa Oleifera Leave Extract, Ethanolic Moringa Oleifera Leave Extract, Carcass And Eggs*

The analysis of the proximate compositions on dry matter basis across the treatment groups considered the effects of the extraction methods on *Moringa oleifera* leave extracts as presented in table 3. The extraction methods values for Aqueous extracts and Ethanolic extracts are respectively as follows: the crude protein: 34.14±0.05, 31.8±0.13, the ash: 10.59±0.17, 8.68±0.30, the ether extract: 15.46±0.45, 12.39±0.11, the crude fibre: 7.00±0.13, 9.13±0.12, the moisture: 12.04±0.04, 11.09±0.03, the carbohydrate: 7.69±0.50, 13.00±0.04, respectively.

While the Proximate composition of experimental diets (EMOLE and AMOLE) in the various inclusion levels of MOL based diets (0g/100g (control), 1g/100g, 2g/100g, 3g/100g) in table 4 is respectively as follows: the crude protein: 35.73±0.17, 37.04±0.09, 38..44±0.07, 38.64±0.27, the ash content: 16.71±0.18, 17.08±0.13, 18.33±0.04, 17.20±0.17, the ether extract: 7.94±0.05, 7.66±0.02, 7.48±0.04, 7.91±0.01, the crude fibre: 3.73±0.01, 4.02±0.05, 4.35±0.09, 4.00±0.06, nitrogen free extract: 35.89±0.14, 34.21±0.24, 31.41±0.25, 32.25±0.18 and the moisture content: 93.22±0.08, 90.28±0.25, 90.00±0.06, 92.43±0.05.

While the result of the analysis of the proximate composition of experimental diet containing graded levels of aqueous extracted MOL in the various inclusion levels of MOL based diets (0g/100g (control), 1g/100g, 2g/100g, 3g/100g) in table 5 is respectively as follows: the crude protein: 37.24±0.50, 37.81±0.79, 38.84±0.17, 38.83±0.35, the ash content: 16.00±0.15, 17.34±0.14, 20.01±0.27, 17.27±0.09, the ether extract: 6.77±0.09, 7.08±0.09, 7.21±0.06, 6.72±0.06, the crude fibre: 3.16±0.07, 3.45±0.04, 3.19±0.05, 3.59±0.08, nitrogen free extract: 36.84±0.66, 34.31±0.99, 30.75±0.31, 33.60±0.41 and the moisture content: 92.43±0.05, 93.22±0.08, 90.28±0.25, 90.00±0.06. Moreover, on the proximate composition of *H. bidorsalis* carcass the main effect of Aqueous extraction methods on the inclusion levels of MOL based diets (0g/100g (control), 1g/100g, 2g/100g, 3g/100g) depicted respectively values as follows: the crude protein: 35.03±0.28, 35.50±0.28, 35.20±0.28, 37.46±0.28, the ash content: 9.45±1.42, 14.35±1.42, 9.76±1.42, 12.13±1.42, the ether extract: 7.33±0.35, 7.71±0.35, 6.74±0.35, 7.01±0.35, the crude fibre: 0.88±0.21, 1.33±0.21, 0.76±0.21, 1.25±0.21, the moisture content: 67.54±1.13, 69.37±1.13, 67.94±1.13, 69.76±1.13 and nitrogen free extract: 47.31±1.57, 41.12±1.57, 47.54±1.57, 42.14±1.57.

While the result of the analysis of the effect of inclusion levels (0g/100g (control), 1g/100g, 2g/100g, 3g/100g) of *Moringa oleifera* leave extract based diets on the proximate composition of *H. bidorsalis* eggs depicted respectively the mean values as follows: the crude protein: 72.09±0.58, 75.55±0.58, 73.13±0.58, 78.78±0.58, the ash content: 2.41±0.10, 2.07±0.10, 2.06±0.10, 1.44±0.10, the ether extract: 3.25±0.09, 3.13±0.09, 3.17±0.09, 2.88±0.09, the moisture content: 81.50±1.50, 80.91±1.50, 87.43±1.50, 83.35±1.50, nitrogen free extract: 22.25±0.47, 19.24±0.47, 21.64±0.47, 16.90±0.47 and the crude fibre: 0.00±0.00 for all treatments respectively.

B. *Mineral Composition of Moringa Oleifera Leave Extract (Mole), Aqueous Moringa Oleifera Leave Extracted Diet, Ethanolic Moringa Oleifera Leave Extracted Diet and Carcass*

The minerals analysed include calcium (Ca), phosphorous (P), magnesium (mg), sodium (Na), potassium (K), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) (Tables). The values of the minerals retained in the *Moringa oleifera* Leave Extracts ranged as follows: Ca: 2.87±0.07 - 21.04±0.47, P: 12.65±0.72 - 14.89±0.54, Mg: 7.24±0.07 - 16.40±0.11, Na: 16.30±0.16 - 23.33±0.27, K: 13.76±0.68 - 65.57±0.60, Mn: 0.01±0.00 - 0.06±0.01, Fe: 8.35±0.05 - 10.67±0.69, Cu: 0.94±0.04 - 6.48±0.38 and Zn: 1.35±0.27 - 5.55±0.22

Table 1: Proximate Composition of *Moringa oleifera* Leaves Extract Subjected to Different Extraction Methods

	Aqueous	**Ethanol**
Moisture (%)	12.04±0.04	11.09±0.03
Ether Extract	15.46±0.45^{a}	12.39±0.11^{b}
Ash content	10.59±0.17^{a}	8.68±0.30^{b}
Crude fiber	19.96±0.13^{c}	23.04±0.12^{b}
Crude Protein	34.14±0.05^{a}	31.8±0.13^{b}
Nitrogen Free Extract content	7.69±0.50^{c}	13.00±0.04^{b}

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

Table 2: Composition (g/100g) of *Moringa oliefera* Leave Extract 1 Diet Fed to Experimental *H. bidorsalis* Fish Samples

Feed Ingredients	**T_0 (Control)**	**T1 (1g/100g)**	**T2 (2g/100g)**	**T3 (3g/100g)**
Yellow maize	25.6	25.6	25.6	25.6
Groundnut cake	15.50	15.50	15.50	15.50
Fish meal	21.40	21.40	21.40	21.40
Cod liver Oil	2.00	2.00	2.00	2.00
Rice bran	5.80	5.80	5.80	5.80
Soya bean meal	23.20	23.20	23.20	23.20
Oyster shell	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.50	1.50	1.50	1.50
Vit/Min. premix	2.00	2.00	2.00	2.00
Cassava starch	2.00	2.00	2.00	2.00
Common salt	0.50	0.50	0.50	0.50
AMOLE	**0.00**	**1.00**	**2.00**	**3.00**
Total	100.00	100.00	100.00	100.00

- *Fish premix (Optimix Aqua) - An Animal Care Product Containing the Following Per Kg of Feed:*

Vit. A, 20000000i.u; Vit. D3, 2000000i.u, Vit. E, 200 000mg; Vit. K_3, 10, 000mg; Folic acid, 2 000mg; Niacin, 80 000mg; Calpan, 25 000mg; Vit. B_2, 9mg; Vit. B_1, 6 000mg; Vit. B_6, 11 000mg; Biotin, 100mg; Vit. C, 50 000mg; Selenium, 100mg; Iodine, 1 000mg; Iron, 30 000mg; Manganese, 60 000mg; Copper, 5 000mg; Zinc, 30 000mg; Antioxidant, 125 000mg; plus Maxigrai, a multi-enzyme (Cellulase, Beta-Glucose, Xylanase, Phytase)

KEY: AMOLE= Aqueous *Moringa Oleifera* Leave Extract

Table 3: Composition (g/100g) of *Moringa oliefera* Leave Extract 2 Diet Fed to Experimental *H. bidorsalis* Fish Samples

Feed Ingredients	**T_0 (Control)**	**T1 (1g/100g)**	**T2 (2g/100g)**	**T3 (3g/100g)**
Yellow maize	25.6	25.6	25.6	25.6
Groundnut cake	15.50	15.50	15.50	15.50
Fish meal	21.40	21.40	21.40	21.40
Cod liver Oil	2.00	2.00	2.00	2.00
Rice bran	5.80	5.80	5.80	5.80
Soya bean meal	23.20	23.20	23.20	23.20
Oyster shell	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.50	1.50	1.50	1.50
Vit/Min. premix	2.00	2.00	2.00	2.00
Cassava starch	2.00	2.00	2.00	2.00
Common salt	0.50	0.50	0.50	0.50
EMOLE	**0.00**	**1.00**	**2.00**	**3.00**
Total	100.00	100.00	100.00	100.00

- *Fish Premix (Optimix Aqua) - An Animal Care Product Containing the Following Per Kg of Feed:*

Vit. A, 20000000i.u; Vit. D3, 2000000i.u, Vit. E, 200 000mg; Vit. K_3, 10, 000mg; Folic acid, 2 000mg; Niacin, 80 000mg; Calpan, 25 000mg; Vit. B_2, 9mg; Vit. B_1, 6 000mg; Vit. B_6, 11 000mg; Biotin, 100mg; Vit. C, 50 000mg; Selenium, 100mg; Iodine, 1 000mg; Iron, 30 000mg; Manganese, 60 000mg; Copper, 5 000mg; Zinc, 30 000mg; Antioxidant, 125 000mg; plus Maxigrai, a multi-enzyme (Cellulase, Beta-Glucose, Xylanase, Phytase)

KEY: EMOLE= Ethanolic *Moringa Oleifera* Leave Extract

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

Table 4: Proximate Composition of Experimental Diet Containing Graded Levels of Aqueous Extract of *Moringa oleifera* Leaves

Proximate Composition	0g/100g	1g/100g	2g/100g	3g/100g
Crude protein	37.24±0.50	37.81±0.79	38.84±0.17	38.83±0.35
Ash	16.00±0.15^{c}	17.34±0.14^{b}	20.01±0.27^{a}	17.27±0.09^{b}
Ether extract	6.77±0.09^{bc}	7.08±0.09^{ab}	7.21±0.06^{a}	6.72±0.06^{c}
Crude fiber	3.16±0.07^{c}	3.45±0.04^{ab}	3.19±0.05^{bc}	3.59±0.08^{a}
Nitrogen Free Extract	36.84±0.66^{a}	34.31±0.99^{ab}	30.75±0.31^{c}	33.60±0.41^{bc}
Moisture	92.43±0.05^{b}	93.22±0.08^{a}	90.28±0.25^{c}	90.00±0.06^{c}

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$

Table 5: Proximate Composition of Experimental Diet Containing Graded Levels of Ethanolic Extract of *Moringa oleifera* Leaves

Proximate Composition (%)	0g/100g	1g/100g	2g/100g	3g/100g
Crude protein	35.73±0.17^{c}	37.04±0.09^{b}	38.44±0.07^{a}	38.64±0.27^{a}
Ash	16.71±0.18^{b}	17.08±0.13^{b}	18.33±0.04^{a}	17.20±0.17^{b}
Ether extract	7.94±0.05^{a}	7.66±0.02^{b}	7.48±0.04^{c}	7.91±0.01^{a}
Crude fiber	3.73±0.01^{c}	4.02±0.05^{b}	4.35±0.09^{a}	4.00±0.06^{b}
Nitrogen Free Extract	35.89±0.14^{a}	34.21±0.24^{b}	31.41±0.25^{c}	32.25±0.18^{c}
Moisture	93.22±0.08^{a}	90.28±0.25^{c}	90.00±0.06^{c}	92.43±0.05^{b}

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$

Table 6: Main Effect of Extraction Method and Inclusion Levels of Dietary *Moringa oleifera* Leaves on the Proximate Composition of *H. bidorsalis broodstock* Carcass

	Extraction Method		Inclusion Level			
Proximate Composition (%)	Aqueous	Ethanol	0g/100g	1g/100	2g/100g	3g/100g
Crude protein	35.84±0.20	35.76±0.20	35.03±0.28^{b}	35.50±0.28^{b}	35.20±0.28^{b}	37.46±0.28^{a}
Ash	11.39±1.00	11.45±1.00	9.45±1.42	14.35±1.42	9.76±1.42	12.13±1.42
Ether extract	7.17±0.25	7.22±0.25	7.33±0.35	7.71±0.35	6.74±0.35	7.01±0.35
Crude fiber	0.88±0.15^{b}	1.22±0.15^{a}	0.88±0.21	1.33±0.21	0.76±0.21	1.25±0.21
Moisture	66.85±0.80^{b}	70.46±0.80^{a}	67.54±1.13	69.37±1.13	67.94±1.13	69.76±1.13
Nitrogen Free Extract	44.71±1.11	44.35±1.11	47.31±1.57^{ab}	41.12±1.57^{b}	47.54±1.57^{a}	42.14±1.57^{ab}

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$ on the proximate composition of fish eggs

Table 7: Main Effect of Extraction Method and Inclusion Levels of Dietary Moringa oleifera Leaves

	Extraction Method		Inclusion Level			
Proximate Composition (%)	Aqueous	Ethanol	0g/100g	1g/100	2g/100g	3g/100g
Crude protein	72.32±0.41^{b}	77.46±0.41^{a}	72.09±0.58^{c}	75.55±0.58^{b}	73.13±0.58^{c}	78.78±0.58^{a}
Ash	2.09±0.07^{a}	1.90±0.07^{b}	2.41±0.10^{a}	2.07±0.10^{a}	2.06±0.10^{a}	1.44±0.10^{b}
Ether extract	3.05±0.06^{b}	3.17±0.06^{a}	3.25±0.09^{a}	3.13±0.09^{ab}	3.17±0.09^{ab}	2.88±0.09^{b}
Crude fiber	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Moisture	83.80±1.06^{a}	82.80±1.06^{b}	81.50±1.50^{ab}	80.91±1.50^{b}	87.43±1.50^{a}	83.35±1.50^{ab}
Nitrogen Free Extract	22.55±0.33^{a}	17.47±0.33^{b}	22.25±0.47^{a}	19.24±0.47^{b}	21.64±0.47^{a}	16.90±0.47^{c}

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

Table 8: Mineral Composition of *Moringa oleifera* Leaves Extract Subjected to Different Extraction Methods (mg/g)

	Aqueous Extraction	**Ethanolic extraction**
Calcium	21.04±0.47[a]	2.87±0.07[c]
Phosphorous	14.89±0.54[a]	12.65±0.72[b]
Magnesium	16.40±0.11[a]	7.65±0.21[b]
Sodium	23.33±0.27[a]	19.24±0.20[b]
Potassium	65.57±0.60[a]	13.76±0.68[c]
Manganese	0.01±0.00[b]	0.06±0.01[a]
Iron	10.67±0.69[a]	9.07±0.02[b]
Cupper	6.48±0.38[a]	2.4±0.18[b]
Zinc	5.55±0.22[a]	2.32±0.29[b]

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$

Table 9: Mineral Composition Analysis of *H. bidorsalis* Carcass Fed Inclusion Levels of Aqueous *Moringa oleifera* Leaves Extract Diet (mg/g)

	Inclusion Level			
	0.00g/100g	**1.00g/100g**	**2.00g/100g**	**3.00g/100g**
Calcium	161.84±0.35	161.37±1.15	161.72±1.40	160.73±0.15
Phosphorus	141.15±1.10	143.06±0.50	143.03±0.35	142.99±0.65
Magnesium	32.61±0.54	34.23±0.25	34.30±0.40	34.13±0.47
Sodium	43.17±0.50	45.48±0.50	45.40±0.50	45.15±0.50
Potassium	6.38±0.55	6.48±0.30	6.51±0.25	6.50±0.25
Manganese	0.05±0.00[b]	0.44±0.02[a]	0.43±0.01[a]	0.47±0.00[a]
Iron	11.16±0.50[b]	15.13±0.55[a]	15.10±0.80[a]	14.91±0.55[a]
Copper	0.14±0.02[b]	0.22±0.01[a]	0.22±0.01[a]	0.21±0.01[a]
Zinc	0.59±0.10	0.43±0.01	0.46±0.01	0.46±0.00

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$
AMOLE – Aqueous *Moringa oliefera* Leave Extract

Table 10: Mineral Composition Analysis of *H. bidorsalis* Broodstock Fed Inclusion Levels of Ethanolic *Moringa oleifera* Leaves Extract Diet (mg/g)

	Inclusion Level			
	0.00g/100g	**1.00g/100g**	**2.00g/100g**	**3.00g/100g**
Calcium	162.18±0.35[a]	158.10±0.15[b]	158.28±0.45[b]	158.05±0.40[b]
Phosphorus	141.07±1.00	141.35±0.51	140.91±0.65	141.132±0.55
Magnesium	33.06±0.60	34.72±1.15	34.58±1.35	34.42±0.95
Sodium	42.96±0.50	43.07±0.50	42.74±0.50	43.26±0.50
Potassium	6.17±0.75	6.68±0.20	6.79±0.10	6.81±0.05
Manganese	0.05±0.00[b]	0.63±0.05[a]	0.63±0.05[a]	0.60±0.05[a]
Iron	10.82±0.15	13.38±0.60	13.12±0.55	13.08±0.45
Cupper	0.15±0.02	0.15±0.0	0.17±0.02	0.18±0.03
Zinc	0.57±0.11	0.37±0.02	0.37±0.01	0.36±0.00

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$
EMOLE – Ethanolic *Moringa oliefera* Leave Extract

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

IV. DISCUSSION

- *Proximate and Mineral compositions analyses of Moringa oleifera Leave (MOL) of Aqueous and Ethanolic Moringa oleifera leave (AMOLE and EMOLE) extracted diets, Carcass and Eggs.*

The extraction methods had significant influence on the proximate composition of the MOL. There was no significant difference ($p > 0.05$) in the ether extract of MOL between the aqueous and ethanol extraction methods. The ash and crude protein contents in ethanol extraction methods were significantly lower ($p < 0.05$) than the aqueous extraction method. However, the lowest significant ($p < 0.05$) crude fiber content in MOL were recorded in aqueous extraction method compared to the ethanol extraction method. Moreover, the crude protein and crude ether values of the MOLE diets in this study which ranges from 35.73 - 38.83 and 6.77 - 7.91 respectively are similar but lower to the values of the findings of Tijani (2017) where the crude protein ranges 39.93 - 42.01 and lipid content of the diets ranged from 11.45 to 13.02 % which could be as a result of the replacement of the fish oil with vegetable oil in the compounded diet. These are comparable to and within the value range of 38.28 - 42.23, 11.43 - 13.65 reported for crude protein and crude lipid respectively by Ochang (2007) for African Catfish (C. *gariepinus*) broodstock. De Graaf and Janssen (1996) gave the range of 35 - 42%, being ideal protein content that should be in formulated feed for intensively monocultured *C. gariepinus.* This relatively high dietary protein enhances growth and food conversion. De Silva (1995), however, stated that required lipid content should be within, 10 - 20%, and protein contents between 16 - 48%, in fish diets to give optimal growth rates without producing an excessively fat carcass.

While, the main effect of extraction methods and inclusion levels of MOL based diets on the proximate composition of eggs is presented in table 7. The extraction methods and the levels of inclusion of MOL significantly influenced ($p <0.05$) the proximate composition of the egg of fish. The highest significant ($p < 0.05$) crude protein in the egg was recorded when MOL based-diet was included at 3g/100g. There was no significant difference ($p > 0.05$) in the ash content of egg obtained from fish fed 0g/100g, 1g/100g and 2g/100g MOL based-diet. The ash content in these treatment groups were however significantly higher ($p < 0.05$) than the ash content of egg obtained from fish fed 3g/100g MOL base-diet.

The proximate composition of the eggs were significantly higher in crude protein contents than the CP in the MOLE (34.14±0.05), AMOLE (38.84±0.17) and EMOLE (38.44±0.07) diets and the Carcass (37.46±0.28) with range (72.32±0.41 (control) - 78.78±0.58 % (3.0/100g)) while the ash (1.44±0.10 (3g/100g - 2.41±0.10% (control)) and ether (2.88±0.09 (3.0/100g) - 3.25±0.9% (control)) contents where significantly lower than the highest contents of ash (20.01±0.27%) recorded in fish fed 2.0/100g AMOLE diet and (7.94±0.05%) ether recorded in the control diet. This shows that the eggs of fish contain higher crude protein levels probably due to the activities of gonadotropin hormones which induce bio chemicals processes in the ovary to synthesize protein needed for egg development. The lower quantity of ash in the eggs indicate lower mineral contents which was seen to be higher in the aqueous *Moringa oleifera* diet indicating higher mineral contents. The chemical composition of the experimental fish carcass indicated values ranges as follows: the crude protein: 35.20±0.28 - 37.46±0.28, the ash content: 9.45±1.42 - 14.35±1.42, the ether extract: 6.74±0.35 - 7.71±0.35, the crude fibre: 0.76±0.21 - 1.33±0.21, the moisture content: 66.85±0.80 - 70.46±0.80, and nitrogen free extract: 41.12±1.57 - 47.54±1.57. While Opey *et al.,* (2018) reported the proximate composition of *M. oleifera* leaf meal (MLM) with a nutritional profile of crude protein level of 27.80 ± 0.02% (which was lower than the findings of this study), ash (6.45 ± 0.02%), crude fibre (18.50 ± 0.01%), crude fat (2.72 ± 0.02%), moisture (8.36 ± 0.03%) and nitrogen-free extract (36.17 ± 0.02%) for African catfish, *H. longifilis* fingerlings. Wasiu *et al.,* (2017) on the other hand, reported proximate composition of 66.79 ± 2.91 crude protein in the carcass higher than the present study; this was possibly because of the quality of the feed ingredients used in raising the catfish as well as the age of the fish which was younger in age than the fish used in this study. However, the ash content in this study is higher 14.35±1.42 than 3.41 ± 9.12 reported for *Heterobranchus bidorsalis* by Wasiu *et al.,* (2017). This shows that the fish raised with Aqueous MOLE and Ethanolic MOLE have higher mineral contents. While protein content will manifest in weight and length gains of the fish, the lipid content will supply more energy for the fish, aside from the supply from carbohydrate ingredients, for them to be able to cope with their activities in the water medium. Sodomade *et al.,* (2017) reported higher, crude protein, 43.71%. The nitrogen-free extract (sugars and starches) in aqueous and ethanolic extracted MOL diets were determined to be in the range 30.75 – 36.84 which were higher than the findings of 14.05 ± 2.68% by Mostafa *et al.,* (2023). The total lipids were determined to be 13.55 ± 1.10%. The total protein value reported by Olugbemi *et al.,* (2010) was lower (27.44%) than the value obtained in this study which ranges from 35.73 - 38.83 %, but Mutayoba *et al.,* (2011) reported very closer and higher crude protein (30.65%) *in Moringa oleifera* leaves. Similarities in chemical composition with the other study may be an indication that environmental factors such as season, geographical location and stage of maturity plays a minor role in determining nutritive values of *Moringa oleifera* Leave. The ash was determined to be within the range of 16.00 – 20.01 %, and the crude fiber was 3.16 – 4.35% which were higher than 11.65 reported by Mostafa *et al.,* (2023). These findings were much higher than those reported by Mutayoba *et al.,* (2011). According to Faizi (1994), water, protein, sugar, mineral salts, and fatty acids are the main components of *M oliefera* leaves and due to its high nutritional content and beneficial physiological characteristics, it is regarded as a good food source (Faizi, 1994).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

On the mineral composition analyses, the extraction methods had significant ($p < 0.05$) influence on the mineral compositions of the MOL. Aqueous extraction method had the highest significant ($p < 0.05$) retention of all the minerals relative to ethanol except in manganese that was significantly higher ($p < 0.05$) than the aqueous extraction method. For the mineral composition of the carcass of *H. bidorsalis* fed inclusion levels of Aqueous and Ethanolic *M. oleifera* Leave extracted diet (Table 9-10), there are no significant difference ($P>0.05$) in the calcium. phosphorous, magnesium, sodium, potassium and zinc compositions in *H. bidorsalis* fed inclusion levels of aqueous extracted of MOL diet unlike the manganese, iron and copper which differed significantly ($P<0.05$) across the treatments. The fish fed control diet (0.00g/100g) had higher calcium content compared to other treatments but where evidently lower in all other minerals across all the other treatment groups (1.00g/100g, 2.00g/100g and 3.00g/100g). While P, Mg. Na and Fe content were higher in fish fed diet containing 1.00g/100g, the diet containing 2g/100g had higher potassium content compared to other treatments (Table 8). Whereas, all mineral contents in carcass of *H. bidorsalis* broodstock fed ethanolic extracted MOL were not significantly different ($P>0.05$) from the control diet to the inclusion levels except the calcium content which were higher (162.18±0.35) in the control (0.00g/100g) and lower (158.05±0.40) in the diet containing 3.00g/100g MOL extract (Table 10). There was no significant difference in the crude protein, ash, ether extract and Nitrogen Free Extract (NFE) between the fish fed aqueous extracted and ethanolic extracted MOL based diet. The crude fiber and moisture content of fish fed ethanolic extract based diet was significantly higher ($p < 0.05$).

Mineral Composition of *Moringa oleifera* Leave Subjected to different Extraction Methods (mg/g): All mineral contents were significant except Manganese whose values ranged from to 0.01±0.00 - 0.06±0.01mg/g. Aqueous extraction method had the highest significant ($P<0.05$) retention of mineral concentrations: Ca (21.04), P (14.89), Mg (16.40), Na (23.33), K (65.57), Fe (10.67), Cu (6.48) and Zn (5.55) compare to ethanolic extraction method: Ca (2.87), P (12.65), Mg (7.65), Na (19.24), K (13.76), Fe (9.07), Cu (2.40) and Zn (2.32) and Methanolic extraction method: Ca (4.37), P (13.32), Mg (7.24), Na (16.30), K (22.44), Fe (8.35), Cu (0.94) and Zn (1.35). For the mineral Composition of *H. bidorsalis* broodstock carcass fed inclusion levels of Aqueous and ethanolic *Moringa oleifera* Leave Extract (AMOLE and EMOLE) diet (mg/g), Only Manganese, Iron and Copper compositions were significant ($P<0.05$) among all treatment groups and the control. Manganese composition was higher in the fish fed 3.0/100g of AMOLE (0.47) followed by 1.0/100g of AMOLE (0.44) and least in control diet (0.05). While the Iron and Cupper were higher in fish fed 1.0/100g of AMOLE (15.13 and 0.22) and lower in the control (0/100g) (11.16 and 0.14) respectively. There were no significant difference ($P>0.05$) in the calcium, phosphorous, magnesium, sodium, potassium and zinc compositions in *H. bidorsalis* fed inclusion levels of aqueous MOLE diet. However**,** all mineral contents in *H. bidorsalis* broodstock carcass fed ethanolic extracted MOL were not significantly different ($P>0.05$) from the control diet to the inclusion levels except the calcium content which were higher (162.18±0.35) in the control (0.00g/100g) and lower (158.05±0.40) in the diet containing 3.00g/100g MOL extract as well as the manganese content which was (0.63±0.05) higher in the diets containing 1.00g/100g MOLE and 2.00g/100g MOLE than the control (0.05±0.00). The higher mineral content (calcium) found in Osteichthyes especially during the breeding season has been suggested to be due to increase in protein bound calcium during the breeding period (Ugwu *et al.*, 2007).

The significantly higher values of mineral composition in the treatments could be attributable to the effect of the solvent used for the extraction of *Moringa oleifera* leave extract as well as the quality of the formulated feed constituent diets. *Moringa oleifera* leave even as an additive is rich in calcium, phosphorus, iron, zinc, iodine and potassium. Notably, calcium was found to be significant in fish fed ethanolic *Moringa oleifera* leave extract diet (EMOLE), indicating that the fish made utmost use of these elements for its physiological activities, especially for bone and scale formation; hence their absorption rate must be very high. The range value for Cu, P, Mg, Cu and Fe fell within the ranges obtained by Sotolu (2010) in their study. These high figures might probably be due to high requirement of *C. gariepinus*, which is a carnivorous fish. Hence its mineral absorption will be high, a fact attested to by Mohammed *el at.*, (2007), as reported from their study that, *C. gariepinus* has better mineral uptake than *O. niloticus*. This is further supported by report from Nwanna *et al.*, (2003) study that, *C. gariepinus* exhibited higher calcium phosphorous uptake when fed with phytase enzyme supplemented diets. Deficiency of dietary minerals, have however been noted for reduction in growth rate, poor appetite and skeletal deformities. Minerals perform a wide variety of structural, biochemical and physiological functions in fish (DeSilva and Anderson, 1995). Six (6) major elements (Fe, Zn, Mn, Ni, I, Mb and Co) have been identified as essential for animal life (Underwood, 1977). Although most of these elements might be required by fish, only 6 dietary minerals have been shown to be required or utilized by salmonids (DeSilva and Anderson, 1995). Most fish species derive their minerals from food or water in which they live. Sea fish therefore contain more minerals than freshwater fish (Laglar *et al.*, 1977). The higher mineral content (calcium) in female Osteichthyes than in males especially during the breeding season has been suggested to be due to increase in protein bound calcium during the breeding period (Urist and Schyeide, 1961).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAY1640

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