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Effect of partial replacement of fishmeal with *Moringa oleifera* Leaf meal on the Haematology, carcass composition and growth perfomance of *Clarias gariepinus* (Burchell 1822) Fingerlings

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Abstract
The study examined the haematology, carcass composition, growth performance and nutrient utilization of *Clarias gariepinus* fingerlings fed varying levels of *Moringa oleifera* Leaf meal diets for a period of eight (8) weeks. *Moringa oleifera* Leaf meal substituted fish meal at 0% (control), 10%, 20%, and 30% in the four different diets. *Clarias gariepinus* fingerlings (mean weight 3.45 ± 0.03 g) were randomly distributed into 12 plastic tanks at 10 fish/tank in triplicate treatments and were fed twice daily at 8.00 hrs-9.00 hrs and 17.00 hrs-18.00 hrs for 8 weeks. The mean weight gained (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) were calculated. The results obtained in the in the present study showed that fishes fed with the control diet did not show statistical significant (p>0.05) difference from fishes fed with 10%, 20% and 30% *M. oleifera* Leaf meal diet in mean weight gain (MWG). The haematological parameters results showed that the mean values of packed cell volume (PCV), red blood cell (RBC) and hemoglobin (Hb) were 18.06 ± 0.34%, 3.83 ± 0.08×106 mm-3 and 6.03 ± 0.11 g/100 ml respectively in the fishes in the experiment. These parameters increased as *M. oleifera* leaf meal increased in the diet. The Carcass lipid showed an increase in all the treatments with fishes fed 30% *M. oleifera* Leaf meal diet recording the highest value of 7.36% and the lowest value of 6.51% was recorded in fishes fed 10% *M. oleifera* Leaf meal diet. The present study showed that *M. oleifera* Leaf meal has good potential for use as fish meal substitute in *C. gariepinus* diet up to 10% level without compromising growth.

Keywords: *Moringa oleifera,* Haematology, carcass composition, *Clarias gariepinus,* leaf meal**,** Growth performance

1. Introduction
In Nigeria today, a daily dietary protein intake of 70g, comprising of 35g of plant and animal protein respectively has been recommended (FAO, 1996) [8]. However, the minimum recommended daily intake of 6.5g of animal protein per head is yet to be met in Nigeria (Smith, 2001) [18]. The inability to meet up with this standard led to so many dietary deficiency problems in man. Therefore, the need to meet the protein requirements in human diets has led to increasing demand for fish and the domestication of some animals. Feed being a major inputs in aquaculture production, high cost of fish feed has caused a lot of problem in aquaculture sector, which has actually hindering aquaculture development in Nigeria since fish feed cost account for at least 60% of the production cost (Gabriel *et al.*, 2007) [10]. Expensive feeds has actually reduced the profitability of fish farming thereby limiting the expansion of farms and reducing the yield in terms of quantity and quality (Adikwu, 1992) [2], research interest has been directed towards the evaluation and use of non-conventional sources of plant protein.
The Moringa (*Moringa oleifera*) is a fast growing plant widely available in tropics and subtropics with several economic-important industrial and medicinal uses, and is a native food in Southeast Asia. *Moringa oleifera* Leaf is said to be an excellent source of vitamins, minerals and proteins: perhaps more than any other tropical vegetable and commonly known as "The Miracle Tree," "Horseradish-tree," or "Ben oil tree") is the best known and most widely distributed species of Moringaceae family, having an impressive range of medicinal uses with high nutritional value throughout the world (*Shuaib et al.,* 2012) [17].

It is used as growth hormone, green manure and for medications. Many do use *Moringa oleifera* leaves to fight against malnutrition and its associated diseases, such as the treatment of cardiovascular diseases: as the roots, leaves, gum, flowers, and infusion of seeds have nitrite, mustard oil glycosides, and thio -carbonate glycosides as their chemical constituents which are suggested to be responsible for the diuretic, cholesterol lowering, antiulcer, hepatoprotective, and cardiovascular protective property of the tree (Shuaib *et al.,* 2012) [17].The African catfish *(Clarias gariepinus)* is appreciated by customers for the quality of its meal, is an excellent species for aquaculture. It is an omnivorous, grows fast, and tolerates relatively poor water quality (Rad *et al*., 2003) [22]. A number of plants continue to be investigated for their potential in supplementing or even replacing fish meal in the diet of fish. (Chiseva, *et al*., 2006) [5]. *(*Ayotunde, *et al*; 2010) [4]. Catfish is the most sought after species among fish farmers and consumers because it commands good commercial value in not only in Nigeria but all over Africa (de Graaf and Janssen, 1996). It can tolerate a large variety of feedstuffs and is very resistant to changing and suboptimal water conditions. It can be farmed in high densities reaching production levels of 6 to 16 MT/ha on an annual basis when raised in monocultures and fed high quality fish feed (Faturoti, 1989) [9]. The objectives of this research is to evaluate the haematological, carcass composition content of *Clarias gariepinus fingelings* fed varying inclusion levels of *Moringa oleifera* Leaf meal Mud.

2. Materials and methods

2.1 Experimental site and Procedure

The experiment was carried out in the nutrition laboratory of Department of Fisheries and Aquaculture Ebonyi State University, Abakaliki, for eight weeks (56 days). The fingerlings used were purchased at a *God's giving farm* in Abakaliki, Ebonyi State. They were acclimated for two weeks (2) in the laboratory before they were distributed to various treatments. One hundred and twenty (120) African cat fish fingerlings *(Clarias gariepinus*) was allotted to four rectangular plastic tanks of 20litres size, at the rate of ten fingerlings per tank and the treatments was replicated three times. The water used was source from Departmental farm exposed for a minimum of two (2) days for aeration. The feeding commenced after two weeks acclimation.

2.2 Processing of *Moringa Oleifera* Leaf

Moringa oleifera leaves were obtained at the Abakpa market in Abakaliki in Ebonyi State. The leaves were thoroughly wash with water to remove dirt, drained properly and air dried in a clean room for about two weeks to obtain a constant weight for easy grinding. It was grinded using mechanical grinder to obtain fine particle sizes for proper mixing with other feed ingredients.

2.3 Fish diet formulation and processing

Four different nitrogenous diets were formulated using Pearson's square method, the diet were formulated to contain 40% crude protein. The *Moringa oleifera* Leaf Meal (MLM) were incorporate into each of the diet at 0% (control), 10%, 20% and 30% to replace equal weight of fishmeal. Feed ingredients were purchased from Abakpa market. The ingredients used are maize, wheat offal, groundnut cake, soybean, fishmeal, *moringa oleifera* Leaf meal, palm oil, salt, premix, methionine and lysine. Prior to processing, the feed ingredients were grinded individually to powder form by using electrical grinding machine, then individually weigh and properly mixed together by hand with adequate water added to ensure smooth pelleting using a 2-mm pellet dice. The strands were cut into short pieces and were sun dried for 3 days to remove moisture (Eyo, 1994).

2.4 Chemical Analysis of the Test Diet

The proximate compositions of the test diet were determined on dry matter basis using the method of A.O.A.C (2000) [3].

2.5 Feeding and Management of the Fingerlings

The fingerlings were fed 5% of their total body weight daily. Feeding were done twice daily at 9.00 AM and 4.00 PM, the feeding regime were adjusted with respect to body weight gain.

2.6 Determination of fish growth and performance

The growth parameters measured are Weight Gain; Average Daily Weight Gain; Feed intake; each of these parameters was measured 2 weeks interval. Growth Performance characteristics were evaluated using the method of Olvera-Novoa *et al.*, (1990) as follows:

Mean Weight Gain (MWG) = Final mean weight (g) - Initial mean weight (g).

Average Daily Weight Gain (ADWG) = Mean weight gain (g) / length of feeding trial (days).

Percentage Weight Gain (PWG) = Mean weight gain (g) / Initial mean weight; x 100.

Specific growth rate (SGR % /day) = 100[(LogeW2 – LogeW1)/NO of days

Feed conversion ratio (FCR) = total feed fed (g) / net weight gain (g)

Protein Intake (PI) = total feed consumed X % Crude protein in feed

Feed intake (FI) = this is the amount of feed consumed throughout the period of the experiment

Protein Gain (PG) = mean protein intake (g) / length of feeding trial (days)

Protein Efficiency Ratio (PER) = Net weight gain (g) / Amount of protein fed (g)

2.7 Water quality parameters

Physico-chemical of water like Temperature, pH, Dissolved oxygen (DO), Temperature, Ammonia was monitored daily. The water temperature was measured using uniscope hand thermometer by probing the tip of the thermometer into the water. It was allowed for two minutes for the mercury level the glass to be stable before the reading was taken from at the meniscus level. Dissolved oxygen was measured using Hana water proof dissolved oxygen meter with the Hl 76407/2. The value was obtained by immersing the sensor probe of the meters in the water and the reading were taken from the screen of the meter. Water pH was determined by immersing the electrode bulb sensor into the water and taking the reading from the screen of the meter.

2.8 Blood collection and haematological analysis

At the end of the feeding trial, Blood (1-2ml) was sampled from groups of fish by inserting a syringe into the vertebral caudal blood vessel, and empty into 5ml heparinized blood bottles treated with Ethyl Diamine Tetracetic Acid (EDTA). Blood samples were centrifuged (1500g for 20 min) to obtain plasma. Plasma samples were stored -20°C until analysis of

mineral content. Plasma mineral contents (Na^+, K^+, $^-$) were measured in Lloyt Na/K analyser by ISE method. Dietary pH was measured as described by Tarakci. Dry matter (105°C, overnight), ash (550°C, overnight), crude protein (nitrogenx6.25, Gerhard Kjeldatherm, Königswinter, DE), ether extract (Velp Scientifica 148 Solvent Exractor Milan, IT), crude fiber (Ancom 220, Fiber Analyzer.

2.9 Blood cell count
System KX-2IN™ Automated Hematology Analyzer was used in blood cell count, the KX-2IN is an ideal hematology analyzer for a clinical satellite laboratory or research testing. It provide a CBN with 17reportable parameter and 3-part WBC differential,

2.10 Carcass Composition (Proximate) Analysis
The carcass composition of the experimental fish was run to determine the Crude Protein (CP), crude Lipid (CL), Crude Fiber (CF), Moisture (M), Ash and Nitrogen Free Extract (NFE), using standard methods (AOAC, 1990). Nitrogen was determined by the micro-kjedahl method (pearson, 1976) and the crude protein was taken as N% x 6.25 (constant factor) where N is equal to Nitrogen content per 100g sample. Total carbohydrate was determined using the phenol-sulphuric acid method (Adeyeye and Foluye, 2004). The crude fibre was obtained by dry ashing of the sample at 550ºC dissolved in 10% HCl(25ml) and 5% Lanthanum Chloride (2ml) boiled, filtered and made up to standard volume with distilled water.

2.11 Statistical Analysis
All data collected were subjected to analysis of variance (ANOVA). Comparisons among diets means were carried out by Duncan Multiple Range Test (Duncan, 1955) [6] at a significant level of 0.05. All computation was performed using statistical package SPSS 15.0 (SPSS Inc., Chicago, IL, U.S.A.).

3. Results
Table 1 shows the percentage composition of the various ingredients used in the feed formulation of the experimental diets. Treatment 1 contained 0% *Moringa oleifera* Leaf meal and treatment 2, 3, and 4 contained 10%, 20%, and 30%, *M. oleifera* Leaf meal respectively. Table 2 and 3 shows the proximate composition of the experimental diets. The crude protein content of the diet ranged between 36.84 and 39.32%, crude lipid 4.25 and 4.46% and crude fibre 2.95 and 3.10%. The results for the feed utilization and growth parameters are presented in table 4. Fishes fed the control diet gained 5.95 g, the fishes fed 10% *M oleifera* Leaf meal diet gained 5.14g, while the fishes fed 20% *M oleifera* Leaf meal diet gained 4.74g weight gain and 1.06g weight gain was recorded in fishes fed diet containing 30% *M. oleifera* Leaf meal. The fishes fed control diet, 10%, 20% and 30% *M. oleifera* Leaf meal diet did not show statistical significant (p>0.05) difference. Table 5 show the result of water quality test, which was normal throughout the experiment.

Table 1: Percentage Composition (%) 0f The Experimental Diets

Ingredients	0%	10%	20%	30%
Mlm	0.00	3.00	6.00	9.00
Fishmeal	31.00	28.00	25.00	22.00
Sbm (toasted)	21.00	21.00	21.00	21.00
Gnc	20.60	20.60	20.60	20.60
Maize	12.60	12.60	12.60	12.60
W/F	13.80	13.80	13.80	13.80
Vit. Premix	0.5.00	0.5.00	0.5.00	0.5.00
Lysine	0.3.00	0.3.00	0.3.00	0.3.00
Methionine	0.2.00	0.2.00	0.2.00	0.2.00

Table 2: Proximate composition (%) of the experimental diets.

Proximate components	O% MLM control	10% MLM	20% MLM	30% MLM
Moisture content (%)	9.25	9.17	9.37	9.22
Crude lipid (%)	4.37	4.25	4.46	4.43
Crude protein (%)	36.84	38.41	38.06	39.32
Crude fibre (%)	2.95	3.03	2.88	3.10
Total ash (%)	6.80	7.07	6.91	7.17
NFE	39.79	38.07	38.32	36.76

Table 3: Proximate composition of *Moringa oleifera* Leaf meal

Nutrients	Percentage Composition (%)
Moisture content (%)	11.07
Crude lipid (%)	10.76
Crude protein (%)	24.74
Crude fibre (%)	15.37
Total ash (%)	8.40
NFE	29.70

Table 4: Result Showing Growth Performance of Experimental Fish (Mean Values ± SE)

Parameters	T1 (control)	T2	T3	T4
Initial mean weight (g)	3.50±0.03^c	3.65±0.02^a	3.55±0.06^b	3.10±0.02^d
Mean final weight (g)	9.45±0.93^a	8.79±1.18^a	8.29±0.066^a	4.16±0.34^a
Mean weight gained (g)	5.95±0.95^a	5.14±1.18^a	4.74±0.66^a	1.06±0.32^a
Mean daily weight gain (g)	0.1±0.02^a	0.09±0.02^a	0.08±0.01^a	0.02±0.01^a
PWG (%)	170.00±3.31^a	140.82±1.12^b	133.52±2.38^b	34.19±2.57^c
Feed intake (g)	647.05±0.10^a	645.15±0.06^a	643.37±0.05^a	638.66±0.10^a
Specific growth rate (%/day)	0.79±0.06^a	0.68±0.08^b	0.66±0.07^c	0.23±0.06^d
Feed conversion ratio	3.62±0.98^b	4.18±0.48^b	4.52±0.29^b	20.08±0.09^a
Protein Efficiency Ratio	0.007± 0.00^a	0.006±0.00^a	0.005±0.00^a	0.001±0.00^b
Protein intake g/fish	794.58±0.90^a	826.01± 0.14^a	816.22±0.08^a	837.07±0.17^a
Protein Gain (PG)	14.19 ±0.66^a	14.75±0.14^a	14.56±0.41^a	14.95±0.32^a
Survival Rate (SR)	85.44	82.49	80.09	75.85

Figures on the same row having the same superscript are not significantly different (p>0.05)

The results for the specific growth rate (SGR) showed that fishes fed with 0% *M. oleifera* Leaf meal diet had the highest value of 0.79 and lowest value of 0.23 was recorded in fishes fed with 30% *M. oleifera* Leaf meal diet. There was no significant difference (p>0.05) in the feed conversion ratio (FCR) in fishes fed the control diet, 10% and 20% *M. oleifera* Leaf meal diet. The highest value of 0.007 recorded for protein efficiency ratio (PER) was observed in fishes fed control diet and the lowest value of 0.001 was recorded in fishes fed diet containing 30% *M. oleifera* Leaf meal diet. The protein intake values of 837.07, 826.01, 816.22 and 794.58 were obtained in fishes fed, 30%, 10%, 20% and 0% *M. oleifera* Leaf meal based diet respectively. These values did not show significant (p>0.05) difference. Survival rate (SR) was highest in fish fed with 0% *M. oleifera* Leaf meal based diet and lowest in fish fed with 30% *M. oleifera* Leaf meal based diet.

Table 5: Result of Water quality parameters.

parameters	T1	T2	T3	T4
Temperature (°c)	27.80	27.60	27.50	27.10
Dissolved oxygen (mg/l)	5.95	6.01	6.03	6.14
pH	7.90	7.87	7.85	7.82

Table 6: Result Showing Haematological parameters of the Experimental Fish (Mean Values ± SE)

Blood parameters	Initial	T1	T2	T3	T4
PCV (%)	15.30±0.15^{d}	17.00±0.58^{c}	18.33±0.33^{b}	19.33±0.33^{ab}	20.33±0.33^{a}
WBC (10^3mm^{-3})	4.47±0.09^{c}	5.13±0.09^{b}	5.37±0.09^{b}	5.40±0.12^{b}	5.70±0.06^{a}
RBC (10^6mm^{-3})	3.30±0.06^{b}	3.57±0.12^{b}	3.97±0.09^{a}	4.10±0.06^{a}	4.23±0.09^{a}
Hb (g/100ml)	5.23±0.03^{d}	5.77±0.20^{c}	6.13±0.09^{bc}	6.47±0.09^{ab}	6.57±0.12^{a}
MCHC (%)	34.21±0.34^{a}	33.92±011^{a}	33.46±0.22^{a}	33.46±0.38^{a}	32.29±0.15^{b}
MHC (pg)	15.87±0.37^{a}	16.18±0.47^{a}	15.47±0.31^{a}	15.78±0.26^{a}	15.51±0.05^{a}
MCV (fl)	46.39±0.87^{a}	47.69±1.25^{a}	46.24±0.69^{a}	47.15±0.41^{a}	48.04±0.37^{a}

Figures on the same row having the same superscript are not significantly different (p>0.05)

Table 6 revealed the haematological indices of fishes fed *Moringa oleifera* Leaf meal based diet during the experiment. The Packed cell volume (PCV) results showed that fishes fed diet containing 0% to 30% *M. oleifera* Leaf meal were statistically significant (p<0.05). The fishes fed diet containing 0% to 30% *M. oleifera* Leaf meal diet showed an increase in the PCV.

White blood cells result showed that fishes fed 0% *M. oleifera* Leaf meal diet was not statistically significant (P>0.05) from fishes fed diet containing 10% and 20% *M. oleifera* Leaf meal. The highest value of 5.70 x 10^3 mm^{-3} was recorded in fishes fed diet containing 30% *M. oleifera* Leaf meal.

The red blood cell (RBC) showed an increase as *M. oleifera* Leaf meal decreased in the diet. The fishes fed 10%, 20% and 30% *M. oleifera* leaf meal diet recorded values of 3.97×103 mm-3, 4.10×10^3 mm^{-3} and 4.23×10^3 mm^{-3} respectively and were not significantly different (P>0.05) but were significantly different from fishes fed diet containing 0% *M. oleifera* Leaf meal.

Haemoglobin increased in fishes fed diet containing 0% to 30% *M. oleifera* Leaf meal and the values showed a significant (P>0.05) difference. The highest value of 33.92% for Mean Cell Haemoglobin Concentration (MCHC) was recorded in fish fed diet containing 0% *M. oleifera* Leaf meal diet and the lowest value of 32.29% was obtained in fish fed 30% *M. oleifera* Leaf meal diet. The results obtained for Mean Cell Haemoglobin (MCH) and Mean Cell Volume (MCV) showed that the fishes fed diet containing 0% and 30% *M. oleifera* Leaf meal had the highest values of 16.18pg and 48.04fl respectively and the least values of 15.47pg and 46.24fl was recorded for MCH and MCV in fish fed 10% *M. oleifera* Leaf meal. The fishes fed 0% to 30% *M. oleifera* Leaf meal diet showed a significant (P>0.05) difference in MCH and MCV.

Table 7: Result Showing Proximate composition (%) of Experimental fish (Mean Values ± SE)

Proximate composition (%)	Initial	T1	T2	T3	T4
Crude protein	68.33±0.04^{a}	64.91±0.33^{b}	62.83±0.09^{d}	61.42±0.24^{e}	63.65±0.25^{c}
Crude fat	7.71±0.01^{a}	7.27±0.01^{c}	6.51±0.01^{e}	6.59±0.03^{d}	7.36±0.01^{b}
Crude fibre	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ash	9.28±0.01^{a}	8,81±0.02^{b}	8.51±0.02^{d}	8.27±0.01^{e}	8.69±0.02^{c}
Moisture	4.35±0.03^{e}	4.77±0.01^{d}	5.04±0.03^{b}	5.19±0.04^{a}	4.85±0.04^{c}
NFE	10.33±0.02^{e}	14.24±0.34^{d}	17.11±0.08^{b}	18.53±0.27^{a}	15.45±0.25^{c}

Figures on the same row having the same superscript are not significantly different (p>0.05)

Table 7 shows the proximate composition of the fish carcass fed *M. oleifera* Leaf meal based diet in the experiment. The highest protein value of 64.91% was recorded in fishes fed control diet and the lowest value of 61.42% was obtained in fishes fed 20% *M. oleifera* Leaf meal diet. Carcass lipid also showed an increase in all the treatments with fishes fed 30% *M. oleifera* Leaf meal diet recording the highest value of 7.36% and the lowest value of 6.51% was recorded in fishes fed 10% *M. oleifera* Leaf meal diet.

4. Discussion

The growth and nutrient utilization by fishes decreased as *M. oleifera* Leaf meal increased in diets. This observation may be as a result of persistent increase in the substitution levels of fish meal with *M. oleifera* Leaf meal in the diets which could retard growth as reported by Richter *et al*., (2003) [15]. The decrease in the growth rate could also be due to reduction in level of protein and essential amino acids in the diet having higher substitution levels of fishmeal with *M. oleifera* Leaf meal (Russel *et al*., 1983) [16]. The result of the specific growth rate (SGR) could be due to differences in the *M. oleifera* Leaf meal substitution levels which decreased considerably in the 30% substitution level in the diets.

The consumption of antimetabolites contained in *M. oleifera* Leaf meal based diets (phenol, tannins, phytate and saponins) are probably responsible for the retarded growth response in

the fishes. Protein efficiency ratio (PER) was highest in fishes fed the control diet but did not show statistical significant difference (p>0.05) from fishes fed with 10 and 20% *M. oleifera* Leaf meal diets. This result seems to have a link with palatability of the diets which caused reduced feed intake. The importance of feed intake by fishes as a determinant of its performance has been emphasized (Faturoti, 1989 and Pillay, 1990) [9, 11]. Fishes fed control diet, 10% and 20% *M. oleifera* Leaf meal based diet showed better feed conversion ratio (FCR) in all the experimental diets. However, the decreasing trend as *M. oleifera* Leaf meal increased in the diet has been reported in diets containing black gram seed meal (Ramachandran *et al*., 2007) [14] and diet with grass pea seed meal (Ramachandran *et al*., 2004) [13].

Haematological components of blood are valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood Oyawoye *et al.,* (1998) [20]. All the haematological parameters measured in this study were within the recommended physiological ranges reported for *Clarias gariepinus***.** However, an increase in the haematology of fishes from fishes fed with 0% to 30% *M. oleifera* Leaf meal diets could be as a result of the presence of higher concentration of anti metabolites in the diets. This observation was not in support of the work of Adeyemo (2005) [1] and Osuigwe *et al*., (2005) [19]. who reported that the reduction in value of Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cell (RBC) were due to the presence of toxic substances in the diet of fish. The haematological results showed that the fishes fed with lower substitution levels of *M. oleifera* Leaf meal diet had better health status than those of higher substitution levels.

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