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Survival Rate, Serum Biochemistry and Haematological Parameters of *Clarias Gariepinus* Juveniles Fed *Moringa Oleifera* Leaf Meal Diet

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Abstract

This study reports an eight-week feeding trial to assess the survival, serum biochemistryand haematological parameters of *Clariagariepinus* juveniles fed *Moringaoleifera* leaf meal.*Moringaoleifera* leaf meal wassubstituted for soya bean meal at 0(control) (T1), 25(T2), 50(T3), 75(T4) and 100% (T5) in five different feeding diets. The result obtained from the experiment showed that there is a significant difference ($p<0.05$) in the survival rate between the treatments, mean weight gain (MWG) and percentage weight gain (PWG).Fishes fed with the control diet (T1) had the highest MWG, while those served with treatment T5had the least. There was no significant difference ($p>0.05$) in the specific growth rate (SGR) between the fishes fed with the control diet (T1) and those fishes fed with (T2). The treatment with the highest feed conversion ratio(FCR) was T5, T4 and T3 while the lowest was the control diet T1 and T2. The haematological parameters showed the value of packed cell volume of the control T1, T2and T5 as 31.67±1.53, 32.67±0.58 and 30.33±0.58 respectively, T2 had the highest packed cell volume. Haemoglobin and red blood cell was high in treatment T2 but low in T4.The serum enzymes including total protein, albumin, globulin and cholesterol showed high values in T2 and T5. The present study revealed that *M. oleifera* has a good potential for use as soya bean meal substitute in *C. gariepinus* diet up to 25% inclusion level without compromising growth.

Keywords: survival rate, haematology, serum biochemistry, *Clarias gariepinus*, *Moringa oleifera* leaf meal

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1. Introduction

Fish farming (Aquaculture) is one of the fastest growing agriculture enterprises in Nigeria with a gross domestic product (GDP) of 5.4% (Federal Department of Fisheries,2005). Fish is one of the most important sources of animal protein available in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of a healthy body (Andrew, 2001). Fish is a good source of fluorine and iodine, which are needed for developing strong teeth and prevention of goitre in man (Andrew, 2001). Fish also stands out of all resources rich in protein due to its high digestibility with low calories compared to other protein sources. Nutritionally, fish is one of the cheapest and direct sources of protein and micronutrient for millions of people in Africa (Bene and Heck, 2005). The per capita protein intake recommended by FAO is 70g per day, half of which must be from plant and animal sources respectively. The level of protein from animal sources contribute only 17% of the total protein consumption in the Nigerian diet (Oyenuga, 1997). According to statistics, the total protein intake of an average Nigerian is about 62g per day, which is not in line with the 70g recommended by FAO. Therefore, there is a need to increase production in terms of good animal protein, and this can only be possible by reducing the cost of production to a bearable means. Feed is one of the major factors influencing the high cost of production in aquaculture. This is due to high reliance on imported feeds from European countries, which makes fish farming expensive as fish feed account for at least 60% of the total cost of production. Therefore, the local production of fish feed is crucial to the development and sustainability of aquaculture in Nigeria. For aquaculture to thrive, and bridge the gap between demand and supply, the role of locally produced fish feed in reducing the cost of production and making fish farming attractive to the

commercial and private investors cannot be emphasised. The development and management of fish feedstuff play an important role in fish farming growth and expansion. It is one of the many factors that determine the profitability of aquaculture venture accounting for at least 60% of the total cost of fish production in Africa (Jamiu and Ayinla, 2003). Therefore, there is a need to intensify the investigation of alternative feed ingredients (plant source) in producing fish feed in other to reduce the cost of production. Moringa (*M.oleifera*) is a multipurpose tropical tree mainly used for food and has numerous industrial, medicinal and agricultural uses including animal feeding. Rediscovered in 1990, its cultivation has since become increasingly popular in Africa and across Asia also referred to as the "miracle tree" or "tree of life" in popular media (FAQ, 2014; Radovich,2013; Orwal*et al*, 2019; Bosch, 2004).

Moringa originated from the southern of the Himalayas and was introduced to many tropical and sub-tropical areas, particularly by migrant Asian population (Radovich ,2013; Bosch, 2004) but has spread across many countries in Africa, South East Asia, the Pacific and Caribean Islands, South America and Arabia producing fruits and flowers continuously. Moringa thrives well where the average temperature is high (25-30^{o}C), also grows better where annual rainfall is about 1000-2000mm, although it can tolerate drought and survives where rainfall is as low as 400mm. Moringa can grow in pure stand or in a mixture of other tree species such as leucaena *(L. leucocephala)* and gaucima (*Gauzuma ulmifolia*), with vegetable species in alley. This study was carried out to evaluate the effect of moringa leaf meal on the survival rate, haematological parameters and serum biochemistry of *C. gariepinus* juveniles.

2. Materials and Method

Experimental Site

The experiment was conducted in the student experimental unit of the Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (FUNAAB) in fifteen (15) rectangular plastic tanks (49×33.5×33.5cm) for a period of 8weeks (56days). Each tank has a water holding capacity of 50 liters.

Moringa Processing

Moringa oleifera leaves were collected from the botanical garden of Federal University of Agriculture, Abeokuta. The leaves were air-dried indoors, milled into powdery form using a Daewoo electric blender with model number DBL- 819 and stored in a well-sealed plastic container.

Fish Diet Formulation and Processing

Five different diets were produced using Pearson square method of fish feed formulation. All other ingredients were milled using hammer mill, then the *M. oleifera* leaf was incorporated to each diet at different percentage level to replace Soya Bean Meal (SBM) at (a) 0% replacement in the first diet (T1), (b) 25% in the second diet (T2), (c) 50% replacement in the third diet (T3), (d) 75% replacement in the fourth diet (T4) and (e) 100% replacement in the fifth diet (T5). Each diet was weighed and mixed properly with adequate water to ensure smooth pelletizing using DELL 52 size manual pelletizing machine and thereafter sun dried for three (3) days to remove the moisture (Eyo, 1994). Samples from each treatment were subjected to proximate analyses following the procedure of AOAC, (2000).

Table 1: Ingredient composition of experimental feed diet

Ingredients	T1 (control)	T2	T3	T4	T5
Maize	19.59	15.70	11.36	6.51	1.02
Fish Meal	18.29	19.26	20.34	21.56	22.93
Groundnut cake	18.29	19.26	20.35	21.56	22.93

Soybean meal	35.83	28.15	19.60	10.78	-
Moringa Leaf meal	-	9.63	20.35	31.59	45.12
Vit. Premix	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5	0.5
Vegetable oil	6	6	6	6	6
Total	100	100	100	100	100

Experimental Design and Feeding Trials

Three hundred (300) African Catfish Juveniles (*C. gariepinus*) were purchased from a fish farm situated in Abeokuta, Ogun State, Nigeria. The fishes were transported very early in the morning to avoid mortality as a result of stress and temperature. The fishes upon arrival at the Department of Aquaculture and Fisheries Management were fed with commercial diet (Vital feed), and were allowed to acclimatise for one week.

The experimental design consisted of five (5) treatments replicated thrice at a stocking density of twenty (20) per tank. The design was a complete randomized design having the juveniles randomly distributed into the tanks. The fishes were initially weighed and their weights recorded using a Camry electronic sensitive kitchen scale (model EK 5350) before transferring them into the tanks.

The experimental fishes were starved for 24 hours before the commencement of the experimental diet to increase their appetite, eliminate variation in weight due to residual food in the gut and prepare their gastro intestinal tract for the experimental diet. After 24hours, all the experimental fishes were fed with the prepared diet twice daily. Fishes in each replicate were weighed weekly with a Camry electronic sensitive scale for eight weeks.

Survival Rate(S) %

The survival rate of the fishes was calculated using equation 1

$$S(\%)=\frac{N1}{No} \times 100$$

Where N_1 = Total number of fingerlings at the end of the experiment
N_0 = Total number of fingerlings at the beginning of the experiment.

Haematological Experiment

The following parameters were used to determine the effect of the dietary treatment on the haematological profile of *C.gariepinus* at the beginning and the end of the experiment. Blood samples for haematological analysis were collected from the experimental fish with a fine syringe into a heparinized bottled. Blood was drawn into haematological tube from this sample.

Leucocytes and Erythrocytes Count (White and Red Blood Cell) (×106/d)

Fish blood collected was diluted in an improved Neubauer pipette with formal citrate fluid at 1:20 with diluted fluid. The resulting mixture was introduced into a Neubauer Counter/ counting chamber where white and red blood cells were counted under the microscope (Blaxhall and Dalsey 1973).

Haemoglobin

The cyamethahaemoglobin method was used; 0.02cm^3 of blood was placed in 4cm of Drabkins reagent in a test tube and mixed together. After 30 minutes the optical density was read calorimetrically at 450/µm. Values of haemoglobin were determined by comparing with cynoglobin method (Robert, 1987).

Serum Collection and biochemical Analysis

Blood samples were collected in triplicate at the end of the 8th week of the experiment following the procedure of Klont and Smith; Wedemeyer and Yasutake. The blood was collected into a sterile plastic test tube without anticoagulant, the test tubes were kept in a slanting wooden rack at room temperature to allow the blood clot. The clotted blood was centrifuged for 15minutes at 3500 revolutions per minute (rpm). A clear fluid which is the serum was pipetted out into a clean and sterilized bottle for further analysis.

Analytical Procedure

The proximate analysis of the feed ingredient and fish were carried out to determine the crude protein, ash, crude fiber, fat and moisture contents.

Crude Protein

The crude protein was determined by using approved method of micro kjeldahl distillation. The process involves digestion and titration of the sample in a standard tetraoxosulphate (iv) acid (H_2SO_4) in the presence of a catalyst (HNO_3) Nitric acid. The value for the nitrogen content of the sample obtained was then converted to crude protein by multiplying with a conversion factor 6.25×N expressed as a percentage of the weight of the original sample. (AOAC, 1990)

Ash Content

Ash content was determined by burning weighed sample inside porcelain crucible in muffle furnace at 500^{0}C for 4hours. The residue was cooled in a dessicator and weighed as ash content.

$$\%Ash = \frac{\text{Ash weight (g)}}{\text{Sample weight (g)}} \text{ X } 100$$

Moisture Content

Moisture content of the sample was determined for both the feed and the fish by taking the known weight of the sample and oven dried at 100^{O}C for 24 hours. The sample was removed and placed in a dessicator to cool for 10 minutes.

$$\%Moisture = \frac{W_1 - W_2}{W_1 - W_0} \text{ x } 100$$

Where W_0 = Weight of empty crucible

. W_1= Weight of crucible + sample

W_2= Weight of crucible + oven dried sample

Feed Conversion Ratio (FCR)

Feed Conversion Ratio (FCR) = $\frac{\text{Feed give (g)}}{\text{Weight gained (g)}}$

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Protein Efficiency Ratio (PER)

Efficiency Ratio = $\frac{\text{Weight gained (g)}}{\text{Protein intake (g)}}$

Statistical Analysis of Experimental Data

The data describing the survival, serum and haematological parameters were analyzed statistically using one way analysis of variance (ANOVA) and Duncan Multiple Range Test was used to test for difference among means ($p>0.05$) in order to see if these parameters were significant or not.

3. Results

The result of the feed utilization and survival rate are presented in Table 2. Fishes fed the control diet, T1gained 40.22g body weight while the fishes fed T2 gained 35.17g, fishes fed with T3 gained 19.00g, fishes fed T4 gained 11.36g while 5.93g weight gain was recorded in fishes fed T5. Means of all treatment (1, 2, 3, 4 and 5) were significantly different. There was no significant difference ($p>0.05$) in the feed conversion ratio (FCR) in fishes fed T1 and T2. The highest value of 1.77 for protein efficiency (PER) was observed in fishes fed T1 and the lowest value of 0.83 was recorded in fishes fed T5.

The apparent net protein utilization (ANPU) of 78.67 and 74.00 were obtained in fishes fed T1 and T2 respectively. There was no significant difference ($p>0.05$) between these values but were significantly different ($p<0.05$) when compared with fishes that were fed with T3, T4 and T5 respectively.

Table 2. Nutrient Utilization and survival rate of *C. gariepinus* Juveniles fed Moringa leaf meal based diet.

PARAMETERS	T1	T2	T3	T4	T5
IMW	10.10±0.05	10.08±0.10	10.12±0.06	10.08±0.15	10.10±0.10
FMW	$50.32±0.83^{a}$	$45.26±4.37^{b}$	$29.08±3.09^{c}$	$21.45±0.97^{d}$	$16.15±1.44^{e}$
MWG	$40.22±0.8^{a}$	$35.17±4.3^{b}$	$19.00±3.1^{c}$	$11.36±0.8^{d}$	$5.93±1.44^{e}$
PWG	$3.98±5.81^{a}$	$3.49±39.0^{b}$	$1.88±30.20^{c}$	$1.13±6.55^{d}$	$58.67±13.^{73e}$
FI	$56.97±1.1^{a}$	$52.73±6.07^{a}$	$32.27±5.0^{b}$	$25.33±1.8^{b}$	$17.75±3.72^{c}$
FCR	$1.42±0.005^{d}$	$1.50±0.01^{d}$	$1.70±0.010^{c}$	$2.23±0.06^{b}$	$3.00±0.10^{a}$
SGR	$2.55±0.02^{a}$	$2.38±0.14^{a}$	$1.67±0.17^{b}$	$1.20±0.051^{c}$	$0.74±0.13^{d}$
SR	95.00±0.00	93.33±2.89	95.00±5.00	98.33±2.89	86.67±10.41
PER	$1.77±0.01^{a}$	$1.68±0.01^{b}$	$1.47±0.010^{c}$	$1.12±0.03^{d}$	$0.83±0.030^{e}$
ANPU	$78.67±1.15^{a}$	$74.00±1.0^{a}$	$67.67±4.6^{b}$	$60.67±1.15^{c}$	$53.33±2.89^{d}$

Means along the same row with different superscript are significantly different ($p<0.05$); FCR: Feed Conversion Ratio, SGR: Specific Growth Rate, PER: Protein Efficiency Ratio.

Survival Rate

The effect of *M. oleifera* leaf meal on the survival of *C. gariepinus* is shown in Table 2. In the course of feeding with the experimental diet, there was a significant difference ($p<0.05$) in the survival rate of *C. gariepinus* juveniles fed varying inclusion levels of moringa leaf meal diet for the 8weeks. The highest survival was obtained in T4 (98.33±2.89), followed by T1(95.00±0.00), T3(95.00±5.00), T2(93.33±2.89) and the least in T 5 (86.67±10.41).

Haematological Profile

The result of haematological indices of fishes fed with *M. oleifera* leaf meal-based diet during the experiment is shown in Table 3. The packed cell volume (PCV) result indicates that fishes fed T1,T2 and T5 had values of 31.67,32.67 and 30.33 respectively. These values were not significantly different ($p>0.05$). The fishes fed T3 and T4 showed a decrease PCV. The red blood cell (RBC) showed that fishes fed T1, T2 and T5 had the highest value of 2.67, 2.70 and 2.60 respectively with no significant difference ($p>0.05$), while the fishes fed T3 and T4 had the lowest values of 1.97 and 1.97 respectively.

Haemoglobin decreases in the fish fed diet T3, T4 and T5. The fishes fed diet T1 and T2 had 9.00 and 10.07g/100ml haemoglobin respectively. These values show significant difference ($p<0.05$) from fishes fed diets T3, T4 and T5. Lymphocyte count increased in the fishes fed T1,T2 and T3. The highest value of 69.33% was recorded in fishes fed T3 while the least value of 64.33% was recorded in fishes fed T5.

Table 3:Haematological parameters of *C. gariepinus* juveniles fed different levels of *M.oleifera* leaf meal diet.

BLOOD PARAMETERS	T 1	T 2	T 3	T 4	T 5
PCV	31.67±1.53^{a}	32.67±0.58^{a}	24.33±3.21^{b}	23.67±5.03^{b}	30.33±0.58^{a}
HB	9.00±0.53^{a}	10.07±0.12^{a}	7.17±0.86^{bc}	7.08±1.46^{c}	8.67±0.61^{ab}
RBC	2.67±0.29^{a}	2.70±0.10^{a}	1.97±0.29^{b}	1.97±0.45^{b}	2.60±0.20^{a}
WBC	13.2±1.17	13.03±2.05	13.33±1.22	12.33±0.64	10.60±0.53
HET	30.33±2.25	29.00±2.00	28.33±3.51	32.33±2.08	33.33±2.31
LYM	67.33±2.25^{a}	68.33±1.15^{a}	69.33±1.15^{a}	66.67±1.15^{ab}	64.33±0.58^{b}
EOS	0.67±0.58	0.67±0.58	0.00±0.00	0.67±0.58	0.67±0.58
BAS	0.00±0.00	0.67±0.58	1.00±1.00	0.00±0.00	0.00±0.00
MON	1.33±0.58	1.00±1.00	0.67±1.15	1.00±1.73	0.33±0.58
TRIGLY	96.33±3.51	90.00±4.36^{b}	1.01±2.31^{a}	96.00±2.65^{ab}	93.00±3.61^{b}

Figures on the same row having the same superscript are not significantly different ($p>0.05$).PCV- Packed Cell Volume, HB- Haemoglobin, RBC-Red Blood Cell, WBC- White Blood Cell, HET- Heterophyls, LYM- Lymphocytes, EOS- Eosinophil, BAS- Basophil, MON- Monocytes, TRIG- Triglyceride

Table 4 revealed the serum biochemistry of the fishes fed with *M. oleifera* leaf meal.T2 and T5 had the highest total protein of 5.20±0.20 and 5.07±0.12 respectively, while the lowest total protein was found in T1, 4.57±0.12. T2 and T5 had the highest albumin values of 3.00±0.10 and 2.83±0.15, while lowest was found in T4. T5 had the highest globulin value of 2.37±0.20 while the lowest level was found in T1. Cholesterol level was high in T2 and T5 having values of 88.00±4.00 and 85.67±7.37 while T3 had the lowest value of 77.00±3.61.

Table 4: Serum parameters of Clarias juveniles fed with different level of *Moringa oleifera* leaf meal diet.

PARAMETERS	T1	T2	T3	T4	T5
T.PRO	4.57±0.12	5.07±0.12	4.73±0.58	4.63±0.15	5.20±0.20
ALB	2.60±0.20	3.00±0.10	2.60±0.60	2.50±0.00	2.83±0.15
GLO	1.97±0.12	2.07±0.12	2.13±0.60	2.13±0.00	2.37±0.20
CHO	78.33±2.52	88.00±4.00	77.00±3.61	80.33±5.51	85.67±7.37
TRI	96.33±3.51	90.00±4.36	1.01±2.31	96.00±2.65	93.00±3.61

T.PRO – Total Protein, ALB – albumin, GLO – globulin, CHO – cholesterol, TRI – triacylglycerol

4. Discussion

The final mean weight showed a significant difference ($p<0.05$) between the treatments, as high weight gain in animals is attributed to increased feed intake. In this study, there was a decrease in final mean and body weight gain with an increased level of moringa. The weight gain value showed a significant difference ($p<0.05$) between the treatment. This was in contrast with the findings of Bundit and Masumoto (2012) who reported no significant difference ($p>0.05$) in weight gain. There is a significant difference ($p<0.05$) in the protein efficiency ratio (PER) of all treatments T1 to T5. It shows that there is a decrease in the PER as the inclusion of *M. oleifera* increases in the diet. The presence of *M. oleifera* in diets increases, and results in higher feed intake. Feed intake is important in the sense that it is the determinant of its performance. The feed conversion ratio (FCR) was better in fishes fed diets T1, T2, and T3 among all experimental diets in contrast to the finding by Ozovehe (2013) who reported that fishes show better feed conversion ratio in his control diet of 10 and 20% *M. oleifera* meal based diet.

The low survival rate in T5 diet might be due to presence of some toxic compound such as tannin and phenol in moringa plant which indicates that excessive use of *M. oleifera* leaf meal can pose a threat on the health of the fish. Although, the low survival rate may be due to environmental factors and handling of the fish throughout the experimental period.

All haematological parameters measured in this study were within the recommended physiological range reported for *C. gariepinus.* The packed cell volume (PCV) of 24.33 -32.67% observed in this study is within the range of 20-50 reported by Pielse *et al*., (1981) and rarely do values above 50% being reported (Etim*etal*., 1999; Clarks *et al*., 1976). There is a reduction in PCV value in fish fed with diet T3 and T4. Reduction in concentration of PCV in the blood usually suggest the presence of a toxic factor, an example of which is haemaglutin which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). The reduction in trend observed in the PCV of this study may be attributed to the presence of some anti –metabolite such as tannin and phenol; in *M. oleifera* leaf meal.

The haemoglobin result shows an increase with diet T1, T2, and T5. The haemoglobin range (7.08-10.07g/100ml) recorded were high and fell within the range (5.6-15.8g/100ml) reported for pike (Mulcahyl, 1970). It also compares well with 8.70g/100ml for *C. gariepinus* (Sowunmi, 2003). The values were higher than 4.40g/100ml reported for *Heterotis niloticus* (Fagbenro*etal*., 2000). The high range of haemoglobin in those studies can be related to large anaerobic metabolism capacity of *C. gariepinus* and decrease level of haemoglobin in the diet with high level of *M. oleifera* implies that the diets (T3, T4, T5) had negative effect on the blood.

The lymphocytes result recorded in this study showed an increase as the level of *M. oleifera* increased in the diets. The lymphocytes count was highest (69.33) in fish fed diet T3. Lymphocytes and white blood cell are the defense of the body. Douglas and Jane (2010) demonstrated that the amount has implication in the immune response and the ability of the animal to fight infection. The increase in WBC and lymphocytes as *M. oleifera* leaf meal increased in the diet could be as a result of feed toxicity. Reduction in red blood cell was recorded from fishes fed diet T3 and T4 which shows a decrease from the diets (T1, T2, T5). The range of RBC reported by Bhasker and Rao (1990) is 1.97×10-2.70×10mm. These decrease may be due to higher concentration of anti-metabolites especially tannin in diet containing *M .oleifera* leaf meal.

The examination of serum parameters revealed increase in total protein, albumin, and globulin in all treatment in comparison with the control (treatment1) with no specific pattern. High level of total protein, globulin, albumin may be associated with stronger response and disease resistance in fish.

5. Conclusion

The result obtained from this study shows that *M. oleifera* could be substituted with soya bean meal up to 25% in *C. gariepinus* diet without any negative effect on growth and feed efficiency. The haematological result also showed that 25% substitution rate of *M. oleifera* meal in *C. gariepinus* diet would not have adverse effect on the blood and serum enzymes. Fish feed can therefore be produced at a relatively cheaper cost and as thus profit of the fish farmer can be increased.

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