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Effect of graded levels of *Moringa oleifera* leaf meal on growth, haematology and serum biochemistry of African catfish *Clarias Gariepinus* juveniles

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ABSTRACT

The effects of the leaf meal of *Moringa oleifera* (MLM) as a source of protein in the diet of *Clarias gariepinus* using growth, haematology and serum biochemistry as indicators were investigated. Four experimental diets MLM1, MLM2, MLM3 and MLM4 were formulated, with *M. oleifera* leaf meal replacing fish meal at 0%, 10%, 20% and 30% levels of inclusion. The four diets were allotted to triplicate groups of one hundred and eighty (180) *C. gariepinus* (12.5±0.03g mean weight) randomly distributed into 12 plastic aquaria for 84 days at 3% body weight daily. Growth variables including Mean weight gain (MWG), Specific growth rates (SGR), Feed conversion ratio (FCR) and Survival were determined using standard procedures. Samples of blood were collected from randomly selected fish samples before and after the feeding trial for haematological and serum biochemical analysis. Results show that MLM has fat, crude protein, crude fibre and ash contents of 5.34%, 26.62%, 18.97% and 12.01% respectively. Mean weight gain was significantly higher in MLM1 and MLM2 groups (16.75±0.35g and 15.49±0.22g respectively), with these groups having superior FCR values. Survival rate was 95% across treatment. PCV and blood platelets were significantly higher ($P<0.05$) in fish fed MLM diets. Creatinine and total protein showed significantly higher values in the control group with values ranging from 0.40 (MLM3) to 0.67 (MLM1) and 3.16 (MLM3) to 4.70 (MLM1) and respectively. The results indicate that the leaf meal of *Moringa oleifera* can only replace up to 10% fish meal in *Clarias gariepinus* diets when growth and haematological parameters are considered.

Introduction

The achievement of aquaculture production on a sustainable level in the Sub-Saharan Africa requires a reduction in input costs, especially the feed component which accounts for 50-60% of total investment. The high cost of feed is mainly accounted for by the protein fraction mostly supplied by fishmeal. Fishmeal is not only expensive, it is also scarce, due to the large demand from animal and fish feed industries. According to FAO (2022), fishmeal production only increased by 3.6% with a total estimated at 2.443 million metric ton in 2021 as against the 2.359 million metric ton reported in 2020. The production figures are negatively affected in any El Nino year and the extent depends on severity (Ubilava, 2014). With the expansion of aquaculture, pressure is being mounted on fishmeal requirements, even as the production of this protein source remains unsustainable (Orisasona, 2018).

There is therefore a pressing need for cheaper, readily available and accessible alternative protein sources for fish feed production. The alternatives must also have nutritional values closer to the conventional feed ingredients. Fish farmers must supply fish with rations containing quality dietary protein that ranges between 24-70% (Zahidul *et al.*, 2021), depending on species, age and production goal. Although fishmeal is superior in terms of quality of protein, amino acids profile and mineral contents,

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many plants show potentials in fish nutrition (Macusi *et al*., 2023)

Moringa belongs to the flowering Moringacea family as the only genus. There are thirteen species found in subtropical and tropical climates (Devkota *et al*, 2020). The species *Moringa oleifera* is fast growing and highly abundant in Nigeria with its beneficial properties being reported to be from all parts of the tree. The leaf meal can serve as a protein source in animal nutrition, and also provide oxycarotenoids, minerals and some vitamins (Dhakad, *et al*., 2019). *Moringa Olifera* leaf is reported to contain (Chukwebuka, 2015). The leaf has abundance of essential amino acids (Devkota *et al*., 2020) making it suitable for fish diet. Puycha *et al.* (2017) reported that *Pangasius bocourti* fed diet containing 10% Moringa leaf meal exhibited better growth than other treatments, but did not vary significantly. Similar result of improved growth at 10% inclusion level and better nutrient digestibility were reported in Hussain *et al.* (2018) when *Labeo rohita* were fed moringa leaf meal.

African catfish *Clarias gariepinus* is the most popular cultured species worldwide especially in Africa including Nigeria (Marimuthu *et al*., 2019; Adeniyi *et al.,* 2021; Ajiboye *et al*., 2022). In 2015, of the 1027058 Metric Tonnes of fish produced, Catfish accounted for 122330 Metric Tonnes (NBS, 2017). This study therefore evaluates the nutritive value of the leaf meal of *Moringa oleifera* using growth, hematology and serum biochemistry in *Clarias gariepinus*.

Materials and Methods

Experimental diets preparation

Moringa oleifera leaves collected from the demonstration farm of the Department of Forestry Management, University of Ibadan were identified at the herbarium Laboratory of the Department of Botany. Leaves were air-dried and reduced to fine particles in a mill and analyzed for proximate composition. *Moringa oleifera* leaf meal (MLM) was used to replace fishmeal at 0% (MLM1), 10% (MLM2), 20% (MLM3) and 30% (MLM4) producing four 30% crude protein diets (Table 1). MLM and other feedstuffs were thoroughly mixed and the homogenous mass pelletized using a 2 mm die size (Hobart A200 pelletizing machine, Troy-Ohio USA). Pellets were air-dried, packed into well labelled polythene bags and stored in refrigerators until ready for use.

Experimental fish and design

Two hundred (200) juveniles of *Clarias gariepinus* from a reputable fish hatchery in Ibadan, Nigeria were procured and transported to the Wet Lab of the Aquaculture and Fisheries Management Department, University of Ibadan. Fish were fed commercial diets twice daily during a 14 days period of acclimatization. After acclimatization, fish (average weight 12.50± 0.03g) were allotted randomly into 12 plastic aquaria at 15 fish/tank. The four experimental diets were each fed to three groups of randomly distributed fish. Diets were fed to apparent satiation twice daily (0700-0730hrs and 1600-1630hrs for 12 weeks, with feed supplied at each feeding recorded. A 2mm diameter hose was used to siphon uneaten feed to allow the calculation of daily feed intake (Omitoyin *et al*., 2019). Water in each aquarium was replaced with fresh water every 48 hours. The pooled mean weekly dissolved oxygen, pH, temperature and Nitrite were 4.9mg/l, 6.6, 28^{0}C and 0.01mg/l respectively. Fish in each tank were weighed biweekly to determine growth and nutrient utilization parameters.

Table 1. Gross composition of experimental diets

Ingredients	MLM1 (0%)	MLM2 (10%)	MLM3 (20%)	MLM4 (30%)
Fish meal (72%)	35.00	31.50	28.00	24.50
Moringa oleifera leaf meal	-	9.50	19.00	28.51
Groundnut cake	31.50	31.50	31.50	31.50
Filler	25.65	19.65	13.65	7.65
Vegetable oil	4.00	4.00	4.00	4.00
*Premix	0.50	0.50	0.50	0.50
Vitamin C	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
Dicalcium phosphate	3.00	3.00	3.00	3.00
Total	100	100	100	100
Proximate composition (%)				
Crude protein	30.84	28.98	29.97	30.09
Crude fibre	4.87	5.03	5.16	6.11
Ether extract	11.26	4.28	3.63	3.76
Ash	7.84	10.54	11.31	10.87

Premix* vitamin A, 4,000,000 IU; vitamin B1, 4,000 mg; vitamin B2, 3,000 mg; vitamin B6, 3,800 mg; vitamin B12, 3 mcg; vitamin D3, 8,00,000 IU; vitamin E, 40, 000 IU; vitamin K3, 1,600 mg; Nicotinic acid 18000 mg; Folic acid, 800 mg; Biotin, 100 mcg; Pantothenic acid, 8000 mg; Choline chloride 120,00 per kg

Measured parameters

Feed intake (g)

Sum of Feed given – uneaten feed during experimental period.

Weight gain (g)

$$\text{Weight gain (g)} = W2 - W1$$

Average Daily Weight Gain (g)

$$\text{DWG (g)} = \text{Weight gain} \div \text{days of feeding}$$

Specific Growth Rate (SGR)

$$\text{SGR (\%/day)} = \{[\text{Log}_e W2 - \text{Log}_e W1] \div [T2 - T1]\} \times 100$$

Where, W2 = final weight, W1 = initial weight, Log_e= Natural logarithm, T2 – T1 = Duration of feeding trial (days)

Feed Conversion Ratio (FCR)

$$\text{FCR} = \text{Feed Intake (g)} \div \text{Weight gain(g)}$$

Protein Efficiency Ratio (PER)

$$\text{PER} = \text{Mean weight gain} \div \text{PI}$$

$$PI\ (Protein\ Intake) = \%\text{Protein in diets} \times \text{Feed intake}$$

Survival rate (%)

SR (%)= No. of fish at the end of experiment/ No. of fish at start of experiment) x 100

Analytical procedures

The proximate composition of fish (before and after feeding trial) and experimental feeds were determined as described by A.O.A.C. (2005).

After 12 weeks of feeding, 5 fish randomly selected per treatment were serially bled according to Omitoyin *et al* (2019). Collected blood samples per treatment were separated into two. The first group had sodium heparinate (20 U/L) as anticoagulant for haemoglobin (Hb) determination as described by Kelly (1979), white blood cells (WBC), red blood cells (RBC) and packed cell volume (PCV) as described by Jain (1986), and the other group had no anticoagulant. This second group for serum biochemical analysis were centrifuged at room temperature for 10 min at 5,000 revolutions/min using methods of Schalm *et al.,* (1975). Determination of serum protein and albumin were done using the biuret reaction and bromocresol green binding methods. Transaminases were determined using a solution of phosphate buffer, sodium pyruvate, alpha-ketoglutarate, d1- aspartate (for SGOT), or dl- alanine (for SGPT) and sodium hydroxide. Serum urea and creatinine were determined using direct reaction between diacetyl monoxime and urea which forms a pink chromogen, while sodium and potassium were measured using the flame photometry method.

The pH, nitrate, nitrite and ammonia contents were detected using the Interpret Water Test Kit (1247) while the methods of Boyd (1979) were used to determine dissolved oxygen and total alkalinity.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze data resulting from the experiment at 95% confidence interval. Means were separated using Duncan multiple range test.

Results

Proximate composition of *Moringa oleifera* leaf meal

A comparison between the result of the proximate analysis of *M. oleifera* leaf meal and fishmeal was presented in Table 2. *M. oleifera* leaf meal has 26.62% crude protein, 5.34% fat, 18.97% crude fibre and 12.01% ash. The crude protein of the experimental fish increased from 74.25% to 79.6%, 83.45% and 87.50% for the control, treatments 2,3 and 4 respectively as presented in Table 3.

Table 2. Proximate composition of *Moringa oleifera* leaf meal in comparison to fishmeal

Comp.	CP	CL	CR	Ash	Moist
Moringa Oleifera leaf	26.62	5.34	18.97	12.01	10.32
Fishmeal*	72.0	8-10	-	14.0	7.00

CP= Crude protein, CL= Crude lipid CR= Crude fiber

Table 3. Proximate analysis of fish sample before and after the experiment

Experimental Fish	DM	CP (%)	EE (%)	Ash (%)	CF (%)	NFE (%)
Initial	11.6	74.3	4.18	7.45	0.75	1.75
MLM1	24.4	79.6	10.60	7.00	-	2.80
MLM2	24.6	79.6	10.60	7.00	-	2.80
MLM3	23.6	83.5	9.50	7.00	-	0.05
MLM4	22.0	87.5	5.50	6.00	-	6.00

DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen free extract.

Growth and nutrient utilization

Mean weight gain ranged from 10.41g (MLM4) to 28.81g (MLM1), however results in MLM1 and MLM2 did not vary significantly ($p>0.05$). The MLM4 group had a significantly different feed intake when compared to others. Feed conversion ratio was superior in MLM1 (2.04) and MLM2 (2.00), with SGR and PER following this same trend (Table 4). Fish survival did not vary significantly ($p>0.05$) across treatment.

Table 4. The growth and utilization of nutrient in *Clarias gariepinus* fed varying levels *M. oleifera* leaf meal diets

Parameter	Treatment			
	MLM1	MLM2	MLM3	MLM4
Initial weight (g)	12.06±0.01	12.14±0.09	12.05±0.03	12.10±0.06
Final weight (g)	28.81±0.35^{b}	27.63±0.30^{b}	24.04±0.55^{a}	22.51±0.92^{a}
Weight Gain (g)	16.75±0.35^{b}	15.49±0.22^{b}	11.98±0.58^{a}	10.41±0.90^{a}
Feed intake (g)	34.14±0.58^{b}	31.03±0.56^{a} ^{b}	30.37±0.33^{a} ^{b}	28.48±2.09^{a}
FCR	2.04±0.07^{a}	2.00±0.04^{a}	2.54±0.1^{5b}	2.77±0.33^{b}
SGR (%/day)	1.03±0.01^{b}	0.97±0.00^{b}	0.82±0.03^{a}	0.73±0.04^{a}
PER	1.63±0.05^{b}	1.66±0.03^{b}	1.31±0.07^{a}	1.23±0.10^{a}
Survival rate (%)	95.33±3.00	95.00±0.81	95.00±0.10	95.33±0.51

Means with the same superscripts along rows are not different significantly at 95% confidence interval. FCR, feed conversion ratio; SGR, specific growth rate; PER, protein efficiency ratio. MLM1, 0% Moringa leaf meal; MLM2, 0% Moringa leaf meal; MLM3, 20% Moringa leaf meal and MLM4, 30% Moringa leaf meal

Blood parameters

The PCV values showed significant increment in all fish fed experimental diets when compared to the initial value. PCV value ranged from 25.16% in MLM1 to 38.00% in MLM2 and was significantly highest. Similarly platelets, Hb, MCHC and MCH values were significantly higher in all fish compared to the initial values. Among treatments, there is a dose related significant increase in the platelets value as the inclusion levels of Moringa leaf meal increased (Table 5). Red blood cell count was significantly highest in MLM2 (2.421 x$10^{6}$$mm^{-3}$). This same trend was observed in Hb with the highest value of 10.70m/l recorded in MLM2. For differential WBC, eosinophils was not recorded in all experimental fish, while percentages for lymphocytes and heterophils ranged from 54.33 to 75.50% and 25.03 to 45.96% respectively.

Table 5. Haematological parameter values of experimental fish before and after feeding trial

Parameters	Initial	MLM1	MLM2	MLM3	MLM4
PCV (%)	20.66±0.33^{d}	25.16±0.16^{c}	38.00±0.00^{a}	30.66±0.33^{b}	30.50±0.28^{b}
Platelets (x 10^{3}/µl)	88.00±6.50^{e}	200.01±5.77^{d}	210.07±69.83^{c}	244.03±33.16^{a}	236.03±33.33^{b}
RBC(cell x$10^{6}$$mm^{-3}$)	2.12±0.00^{c}	1.51±0.00^{d}	2.42±0.00^{a}	1.32±0.00^{e}	2.31±0.00^{b}
Hb (m/l)	5.30±0.00^{e}	7.51±0.00^{c}	10.70±0.30^{a}	5.79±0.05^{d}	9.53±0.03^{b}
WBC (cell x $10^{3}$$mm^{-3}$)	16.50±3.17^{b}	15.20±1.15^{d}	15.90±0.57^{c}	13.20±0.33^{e}	16.70±0.33^{a}
Lymphocytes (%)	51.33±0.33^{e}	74.33±0.33^{b}	64.16±0.16^{c}	75.50±0.28^{a}	54.33±0.33^{d}
Heterophils (%)	42.96±0.03^{b}	26.10±0.05^{d}	36.10±0.05^{c}	25.03±0.03^{e}	45.96±0.03^{a}
Eosinophils (%)	6.96±0.03	0	0	0	0
MCV (fl)	150.33±0.33^{c}	166.33±0.33^{a}	158. 33±0.33^{b}	145. 33±0.33^{d}	129. 33±0.33^{e}
MCHC	18.00±0.00^{d}	30. 33±0.33^{b}	29. 33±0.33^{c}	29.66±0.33^{bc}	31.66±0.33^{a}
MCH	25.00±0.57^{d}	49.00±0.57^{a}	45.66±0.33^{b}	42.33±0.33^{c}	41. 33±0.33^{c}

PVC, pack cell volume; RBC, red blood cell; WBC, white blood cell MCH, mean cell haemoglo- bin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume. MLM1, 0% Moringa leaf meal; MLM2, 0% Moringa leaf meal; MLM3, 20% Moringa leaf meal and MLM4, 30% Moringa leaf meal

Serum Biochemistry

Significantly higher ($p<0.05$) sodium ion in serum were observed in fish fed MLM based diets with values ranging from 134.33mm/l in MLM1 to 139.66mm/l in MLM2, while potassium ranged from 3.03mm/l in MLM4 to 4.46mm/l in MLM3 (Table 6). Urea was significantly highest in the MLM1 groups while values for MLM2, MLM3 and MLM4 were not varied significantly ($p>0.05$). This same trend was recorded for the creatinine value which was significantly highest in MLM1. The least value of serum glutamate oxaloacetate transaminase (SGOT) was recorded in MLM2 (241.33iu/l), while total protein (TP) values ranged from 3.16g/l (MLM3) to 4.70g/l (MLM1). Albumin values were lower in the control group compared to fish fed MLM diets.

Table 6. Serum parameters of experimental fish before and at the end of experimental period

Parameter	MLM1	MLM2	MLM3	MLM4
Na^{+}	134.33±0.33^{c}	139.66±0.33^{a}	137.66±0.88^{b}	136.33±0.33^{b}
K^{+}	3.66±0.03^{b}	3.30±0.05^{c}	4.46±0.03^{a}	3.03±0.08^{d}
Urea	4.66±0.88^{a}	3.0±0.00^{b}	2.0±0.00^{b}	2.0±0.00^{b}
Creatinine	0.67±0.06^{a}	0.50±0.05^{b}	0.40±0.00^{b}	0.50±0.00^{b}
SGOT	366.66±1.20^{a}	241.33±0.33^{c}	348.33±0.33^{b}	365.00±0.57^{a}
SGTP	108.00±0.00^{b}	58.66±0.66^{c}	112.00±0.00^{a}	26.00±1.15^{d}
TP	4.70±0.05^{a}	3.80±0.05^{b}	3.16±0.03^{c}	3.30±0.05^{c}
ALB	1.30±0.00^{c}	1.40±0.05^{c}	1.60±0.11^{b}	1.80±0.00^{a}

SGOT: Serum glutamate oxaloacetate transaminase, SGTP: Serum glutamic pyruvic transaminase, TP: Total protein, ALB: Albumin. MLM1, 0% Moringa leaf meal; MLM2, 0% Moringa leaf meal; MLM3, 20% Moringa leaf meal and MLM4, 30% Moringa leaf meal

Dicussion

There is renewed interest in the evaluation of non-conventional sources of protein mostly from plant leaves and seeds (Abo-State *et al*., 2014). These protein sources are expected to be low in fibers, starch and anti-nutrients. The crude protein level recorded for the leaf meal of *Moringa oleifera* in this study is similar to the 26.8% to 29.6% range reported in Chikara (2020). Higher crude fibre content is recorded in this present study compared to the 6.5% reported in Etalem *et al* (2013) and this may be attributed to the differences in plant age during leaf harvesting. The crude fibre recorded in our study is higher than 5.44% reported for soya bean flour in Ogbemudia *et al* (2018) and 6.5% in leaf reported in Etalem *et al* (2013). Growth was significantly reduced at MLM inclusion above 10%. This is in agreement

with Bello and Nzeh (2013) where growth in *Clarias gariepinus* was depressed when fed diets with more than 10% moringa leaf meal inclusion level. Similar result was reported in Abo-State *et al* (2014) for Nile tilapia. Feed intake and weight gain exhibited a direct relationship in this present study. The interaction effect with high plant protein usually results in lower feed intake and growth reduction in fish (Brezas and Hardy, 2020). According to the authors, reduced feed intake may be attributed to differences in the biologically significant components such as saponins, tannins, and amino acid balance which may affect palatability. Similarly, the high fiber content in MLM including Neutral Detergent fibre and Acid Detergent fibre negatively affects the utilization of feedstuff by fish. This assertion is supported by Chuang *et al* (2021) which reported that high-fibre feedstuffs cause reduced nutrient utilization and reduced growth in monogastric animals. The reducing trend of growth as the inclusion level of leaf meal increases suggests that *Clarias gariepinus* utilizes *M. oleifera* leaf poorly at higher levels of inclusion in feed.

Hematological indices are important tools for assessing the status of fish physiology and pathology (Fazio, 2018). Increased PCV values were observed in all experimental fish when compared with the initial value and all fall within the range recommended for tropical fish (Omitoyin *et al.,* 2019). Values of Hb and RBC did not allow for any trend establishment in this present study, however, values are within recommended range for *Clarias gariepinus*.

Similarly, the values recorded for lymphocyte across treatments in this present study is not indicative of compromised immune status, even at higher levels of *M. oleifera* leaf meal. This is supported by the observed survival rates which was 95.33% in all treatments. Mean Corpuscular values are utilized to classify anaemias morphologically and represent an estimation of alterations in size and haemoglobin concentration of individual red blood cells. Erythrocytes are normocytic if the cells are of normal size, while cells smaller than normal are microcytic and those larger than normal are macrocytic. Decrease in MCV values with increasing MLM may indicate the presence of a stressor. This assertion is supported in Shin *et al.* (2016) where fish exposure to various chemical stressors resulted in MCV reduction. With respect to MCHC, all treatment recorded markedly higher values than the initial and the values recorded precludes the possibility of anaemia, and values in treated fish were similar to results in Falaye *et al.* (2018), when *C. gariepinus* were fed moringa leaf meal.

Higher values of serum sodium were observed in fish fed MLM diets. According to Weiner *et al.* (2015), the determination of the most abundant non-protein nitrogen constituents in the body (creatinine and urea) is used to test the ability of kidney to excrete metabolic wastes. According to Ajeniyi and Solomon (2014) the liver synthesizes more than 99% of urea from dietary protein. Substrate delivery and liver efficiency dictates the amount of urea produced. In this present study, significantly lower ($p<0.05$) values of urea and creatinine were observed in fish fed MLM diets. This may be attributed to the low protein quality (Ajeniyi and Solomon, 2014) in MLM compared to fishmeal which has an excellent protein quality. Result from this present study contradicts the report of Adeshina *et al* (2018) where Moringa leaf meal resulted in increased urea and creatinine at even lower inclusion levels. Increase in creatinine is generally associated with renal dysfunction. Although the urea:creatinine ratio in this present study ranged from 4:1 to 6.9:1, it is far below the 10:1 to 20:1 recommended in Ajeniyi and Solomon (2014). Thus, proper functioning of the fish organs like liver and kidney cannot be inferred from this present study based on values recorded. Increase in serum glutamate oxaloacetate transaminase (SGOT) may be associated with blood protein. Although the liver plays a major role in the biosynthesis of the majority of plasma proteins, and decrease in albumin is typical of liver damage, alterations in various fractions of plasma protein are not specific for liver damage. The high total protein confirms the increase in crude protein observed in the fish after the experimental period.

This study reveals that the leaf meal of *Moringa oleifera* can only replace 10% of fishmeal, as higher replacement levels resulted in reduced nutrient utilization and poor growth and dysfunction in digestive organ.

Conclusion

It is concluded that that the leaf meal of *Moringa oleifera* can only replace up to 10% fish meal in *Clarias gariepinus* diets when growth and haematological parameters are considered.

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