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Effects of Moringa leaf meal (MLM) inclusion as premix on the haematological parameters of *Clarias gariepinus*

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Abstract

This study was conducted to determine the haematological parameters of *Clarias gariepinus* juveniles, fed diets with *Moringa oleifera* leaf meal (MLM) as premix for 8 weeks. Fish mean weight of 12.5±0.04g was used. The experimental diets were formulated at 40% crude protein into three treatments of the same composition, but different inclusion levels of moringa, before processing into pelleted sinking feed. The treatments based on % mLM were 0, 0.5, 1.0 and 1.5. Both floating and pelleted sinking feeds were used. Each diet was fed to a group of thirty (30) juveniles of *Clarias gariepinus* per treatment at 5% body weight. Each treatment was replicated three times. The results showed that Treatment IV had the best haematological indices; and was not significantly different (P>0.05) from other treatments with respect to packed cell volume (PCV) 27.83±1.23; Haemoglobin (Hb): 8.73±0.81 and Red Blood Cell (RBC): 2.66±0.54. Treatment I equally had good haemotological Parameters: PCV (24.37±2.85); Hb (8.34±0.79); RBC (2.83±0.53), Platelet (123.45±7.81). Treatments II and III had higher levels of White Blood Cell (WBC) which ranged from 17.43±1.235 to 17.61±1.33. Haematological parameters were within the recommended range for fish health. This study revealed that moringa leaf meal (MLM) was best as replacement of premix; for improved performance for the tested parameters of *Clariasgariepinus*. Based on the findings of the study, it is recommended that 1.5%mLM inclusionrate as premix for enhancement of haematological parameters and better fish health of *Clarias gariepinus*.

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Introduction

Catfish (*Clarias gariepinus*) is a freshwater fish which is extremely nutritional, containing a high concentration of unsaturated fatty acids, vitamins, proteins and minerals (Nelson *et al.*2016). Abdul-Mebdy *et al.* (2021) analyzed the nutritive content of catfish meat in mg/100g and reported that it had 304.82 Calcium, 279.45 Phosphorus, and 17.03 Iron, 41.81g/100g essential amino acids, and high content of Oleic acid which links it lower risk of cardiovascular disease. As a nutritive, tasty and acceptable fish, the production of catfish aims at satisfying the total protein requirement of the average Nigerian which has been estimated to be about 41% (Atanda, 2012), while the entire country requires about 2.66 million metric tonnes of fish to feed her teeming population. FDF (2008) however reported that since local production generates only 0.62 metric tonnes, about $594.4 million is spent annually on importation of fish and fishery products in Nigeria (Falaye, 2013).

Inclusion of mLM into fish nutrition is a way of improving its balanced diet with a view to enhancing optimal growth and health of the fish. This is a local alternative means of sourcing ingredients that are common, cheaper and readily available to resource-poor farmers. Mohamed *et al.* (2018) reported that Moringa, a fast growing tropical plant, has high biological and nutritional values, and can be used as animal as well as fish feed, green, fertilizer, medicine, bio-pesticide and in seed production. The report further indicated that Moringa has been characterized as a potentially useful animal feed owing to its high contents of protein, carotenoids, several minerals and vitamins (such as iron and ascorbic acid) and certain phyto-chemical, including; kaempferitin, isoquercitrin, rhamnetin, kaempferol and quercetin. Dienye and Olumuji (2014) studied the growth performance and haematological response of African mud Catfish (*Clarias gariepinus*) fed dietary levels of *Moringa Oleifera* leaf meal, and indicated that 10% substitution rate of mLM in catfish diet would not have any adverse effect on the blood and serum enzyme. Faleye A.E *et al.* (2018) showed that premix in fish feed could be replaced with *Moringa oleifera* leaf meal up to 1.5% level in diets of *Clarias gariepinus* on the haematological parameters, health and feed efficiency. The nutritive values and huge economic importance of the African Catfish notwithstanding, there are no recommended standards with respect to the level of inclusion of Moringa leaf meal as premix in the diet of the fish optimum performance. The objective of this study therefore was to determine the effect of four different levels of mLM on haematological parameters of *Clarias gariepinus* with a view to ascertaining the most appropriate level on its inclusion as premix for improved performance of the tested parameters.

Materials and methods

Experimental location

The study was carried out in 2021 at the Department of Aquaculture and Fisheries, Delta State University Abraka, Nigeria. The juveniles were sourced from a commercial fish farm within Abraka. The fish was cultured for eight (8) weeks. The experimental fishes were first acclimatized to laboratory conditions for two weeks (14days) at a temperature of 26.5±0.61^{o}C, and were fed with commercial fish feed (45% C.P) twice daily 7am&6pm on 5% of the biomass total.

Experimental treatments

The experiments were made up of four (4) treatments. Treatment I: fed commercial fish feed (0% Moringa Leaf Meal (MLM) inclusion, as control, Treatment II: fed 0.5% mLM inclusion, pelleted sinking feed. Treatment III: fed 1.0% mLM inclusion, pelleted sinking feed .Treatment IV: fed 1.5% mLM inclusion, pelleted sinking feed. A total of 120 *Clarias gariepinus* juveniles of a mean initial weight of 12.5± 0.04g were randomly distributed into 12tanks with 75-L capacity of water. The fish were fed with commercial fish feed , and three other formulated diets for 8 weeks during the experiment.

Processing of moringa leaves to meal

Moringa plant was cultivated for the purpose of this research, and was harvested from the same source at maturity. The leaves were thoroughly washed with water to remove dirt, drained properly and later sun-dried for 4 days.

Therefore, the leaves were blended into fine powder and analyzed for proximate composition according to AOAC 2000. The parameters of importance were: crude protein, crude fat, crude fibre, moisture content and total ash, vitamins and mineral were analyzed.

Collection and evaluation of blood samples of experimental fish

Prior to the commencement of the experiment baseline blood samples were collected from four fish randomly selected from all the fish for haematological analysis, and 2ml of blood was also collected at the end of trial (week 8) from the caudal peduncle of fish in each experimental unit according to standard methods of Joshi *et al.* (2002). Blood collected were dispensed into tubes (Lithium heparin) containing EDTA an anticoagulant and taken to the haematology unit Microbiology Department, Delta State University Abraka for determination of packed cell volume (PCV), platelet (PLT) lymphocyte (LYMP) mean cell volume (MCV), haemoglobin (Hb), white blood cells (WBC) and red blood cells (RBC) using the method of Blaxhall and Daisley (1973). The mean corpsular haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated using the method described by (Merghani, 2010).

Haemoglobin estimation

The haemoglobin concentration estimate was determined using the cyanomethaemoglobin method. 20µl of blood sample was taken from the lithium heparinized tube with the aid of a pipette. Mixing was achieved by slow inversion of the tube, for about 20times with 4.0ml of Drabkin's solution. After which the text tube was taken into a calorimeter for reading. The final haemoglobin result was calculated from:

$$\text{Hb} = \frac{\text{T x C x DF G/100ml}}{\text{A x 1000}}$$

Legend: T = the test absorbance, A = the standard absorbance, C = the concentration of cyanmethaemoglobin, DF = the dilution factor

Red Blood Cell (Erythrocyte)

Commonly available diluents were used for the red blood cell count (RBCC) i.e. a solution of formal-citrate. The blood sample diluted by washing 20Nl of blood into a shellback pipette, and into 4.0ml of modified Drabkin's fluid to give a final solution of 1 in 20litres. The diluted sample were then mixed and loaded into the haemocytometer.

After the cells sedimentation, the number lying on 5 of the 0.04mm² area are counted, by charging the Neuberger's chamber and placed under a microscope and count. The final value is express as the number of the cell per litre.

$$\text{RBC} = \frac{\text{N x DF x } 10^6 \text{ per liter}}{\text{A x D}}$$

Legend: N = the number of cell counted, DF = the dilution factor, A = the area chamber counted, D = the depth of chamber

White Blood Cell (Leucocytes)

The WBC is determined by drawing the blood sample with the aid of pipette about 20µL and put into test tube and mixed with diluents solution at 0.38ml. This prepared diluents will react, the red blood cells and this make the white cells more readily visible. It is spread on blood film and counted until a minimum of 200 cells have been enumerated.

Packed Cell Volume (PCV)

The PCV (hematocrits) was determined with Wintrobe hematocrits, filled to 10 mark, taking care to avoid bubbles, as according to Wintrobe's and Wintergreens' methods as described by Blaxhall and Daisley (1973);With commercially available heparins capillary tubes of 75mm.This is done by centrifuging the hematocrits at 3000 r.p.pm for 30minutes and take a further reading. The readings are not the same, repeat centrifugation until two identical consecutive readings are obtained. This is the time required to pack cell on this particular centrifuge, remove from centrifuge and note the height of the red cell column i.e. to the button of the buffy layer. The tube is divided into 100 divisions; the height of the column of red cells is read off and is expressed as a fraction of the whole blood.

$$\text{PCV} = \frac{\text{Column reading}}{\text{100 divisions}}$$

Observation

Experimental fish in each tank were thoroughly observed daily for any behavioral and morphological changes, injuries and general well-being. All observation and mortalities were recorded. Feed acceptability was low for treatment II III and IV for the first few weeks, and they later pick-up on week 3-8.

Statistical analysis

Data collection from the experiment were subjected to analysis of variance (ANOVA) test using Duncan Multiple Range Test (DMRT, 1955) was used to compare differences among individual means. The data was analyzed in SPSS 15.0 window version. Differences were considered significant at 0.05 level of significance ($P<0.05$).

Results and discussion

Proximate analysis of experimental diets

The result of proximate analysis of the experimental diets is shown in Table 2, due to the inclusion of moringa leaf meal (MLM) as premix, in the following proportions 0.5, 1.0 and 1.5 respectively.

The crude protein showed higher values for diets 2,3 and 4 on pelleted sinking feed after analysis,(D_2 = 40.25%, D_3 = 41.30% and D_4 = 41.50%).

Note that the crude protein of D_1 = 45% with no moringa leaf meal inclusion, for the floating pelleted feed was analyzed too and results are shown in table 2 and 3 below.

Table 1. Proportions of different ingredients in the formulated Diets with Moringa Leaf Meal (MLM) inclusion.

	Diets based on % mLM			
Ingredients	0	0.5	1.0	1.5
Maize	19.74	30	30	30
Soybean meal	18.34	22	22	22
Groundnut cake	18.34	22		
Rice bran	19.74	-	-	-
Vitamin premix	2.5	-	-	-
Fish meal	18.34	21	21	21
Moringa leaf meal	0.00	5	1.0	1.5
Toxin binder (BHT)	-	0.5	1.0	1.5
Bone meal	1.5	-	-	-
Salt	1.50	0.4	0.4	0.4
Palm oil	-	3.2	3.2	3.2
Total (%)	100	100	100	100

Table 2. Proximate analysis of commercial feed and formulated diets with moringa leaf meal inclusion as premix (Mean ± SD).

Parameters	D_1 (CT)	D_2	D_3	D_4
Crude protein (%)	45.00 ± 0.52^a	40.10 ± 0.15^b	40.593 ± 0.05^b	40.60 ± 0.03^b
Crude fibre (%)	2.9 ± 0.14^b	5.00 ± 0.04^a	5.10 ± 0.02^a	5.15 ± 0.01^a
Crude fat (%)	$13,49\pm0.10^a$	3.52 ± 0.12^b	3.53 ± 0.02^b	3.51 ± 0.52^b
Ash (%)	8.4 ± 0.30^a	8.34 ± 0.02^a	8.42 ± 0.03^a	8.56 ± 0.07^a
Dry matter (%)	60.05 ± 0.10^b	85.89 ± 0.04^a	85.81 ± 0.16^a	85.07 ± 0.15^a

Means with same superscripts along the same row are not significantly different ($P>0.05$)

Legend: CT: Control

Table 3. Proximate analysis of Moringa Leaf Meal Component.

Sample	%CP	%ASH	%EE	%CF	%DM
1	26.40	4.90	4.08	6.92	91.03

Legend: C.P = Crude protein, Ash = ash, EE = Ether attract, CF = Crude fibre, DM = Dry matter

Haematological report

From the results obtained in this study; the packed cell volume (PCV) values for treatment II is significantly ($P < 0.05$) different but those of treatments III and IV are not significantly different and the values range from 25.43±2.98 to 27.83±1.23.

There was no significant difference in the Haemoglobin in respect to treatments I and IV and the values ranged from 8.34±0.79 to 8.73±0.81.Red Blood Cell (RBC) across the treatments (II, III & IV) showed no significant difference ($P>0.05$), with the highest value obtained in treatment IV (2.66±0.54) and the lowest value in treatment II (2.35±0.78). White Blood Cell (WBC) were not significantly ($P > 0.05$) different in treatments II and III, ($P>0.05$). Higher values were obtained in treatments II and III (17.43±1.25, 17.61±1.33), and lowest in treatment I (15.68±0.76), which was significantly different

(P<0.05). Platelet (PLT) content was not significantly (P > 0.05) different in treatments II, III and IV, compared to treatment I (control). Blood platelets in the control experiment (Treatment I) was higher compared to other treatments. There is significant (P < 0.05) difference in lymphocyte (LYMP) for treatments I, II and IV, but there is significant difference in treatments III (P<0.05). The highest value is from treatment III (73.57±2.87), mean corpsular haemoglobin (MCH) of treatments I and IV did not show significant difference (P>0.05). The other treatments showed significant (P < 0.05) difference onmc H and ranges were between 3.57±0.87 (treatment III) and 2.87±1.04 (treatment II), mean corpsular volume (MCV) was highest in treatment II (102.30±18.43) and lowest in treatment IV (97.47±6.75).

There was no significant (P > 0.05) difference in mean corpuscular haemoglobin concentration (MCHC) among the various treatments (P>0.05).

Table 4. Vitamin composition of Moringa Leaf Meal.

Vitamins	A(ug/100g)	B_1(mg/100g)	B_2(mg/100g)	C(mg/100g)
Amount	5382.45	57.24	5.73	23.3

Table 5. Mineral composition of moringa leaf meal.

Minerals	Ca	Mg	K	Na	Mn	Fe	Cu	Zn	P	N(%)
Amount (mg/kg)	30,100	2000	10,900	100	10	116	108	24	10	2.16

LEGEND: Ca = Calcium, Mn = Manganese,mg = Magnesium, Fe = Iron, K =Potassium Cu = Copper, Na = Sodium Zn = Zinc, N = Nitrogen P = Phosphorus

Table 6. Mean value of haematological parameters of *Clarias gariepinus* (Juveniles) fed experimental diets for eight weeks (Mean±SD).

Parameters	Treatment I	Treatment II	Treatment III	Treatment VI
PCV(%)	24.37±2.85^{a}	25.43±2.98^{c}	26.45±0.86^{B}	27.83±1.23^{a}
Hb (g/100ml)	8.34±0.79^{a}	7.75±0.71^{b}	7.76±0.80^{B}	8.73±0.81^{a}
RBC (x10^{6}/m^{-3})	2.83±0.53^{a}	2.35±0.78^{a}	2.57±0.11^{a}	2.66±0.54^{a}
WBC (10^{3} m^{-3})	15.68±0.76^{c}	17.43±1.25^{a}	17.61±1.33^{a}	16.83±1.88^{b}
PLT 9x10^{3}/mm^{3})	123.45±8.18^{a}	111.33±32^{c}	120.47±8.30^{ab}	119.76±5.98^{b}
LYMP (%)	67.83±5.23^{a}	66.70±2.30^{a}	73.05±2.59^{b}	70.34±1.73^{a}
MCH (pg)	1.93±1.43^{c}	2.87±1.04^{b}	3.57±0.87^{a}	1.93±0.59^{c}
MCV (fl)	93.17±12.28^{d}	102.30±18.43^{a}	97.47±6.75^{b}	93.20±14.07^{c}
MCHC (%)	35.43±1.01^{a}	33.22±1.24^{a}	33.22±2.17^{a}	33.63±0.59^{a}

Means with the same superscript along the same row are not significantly different (P>0.05)

LEGEND: PCV = Packed cell volume, Hb = Haemoglobin, RBC = Red Blood Cell, WBC = White Blood Cell, PLT = Platelet, LYMP = Lymphocyte,mcH = Meancorpsular Haemoglobin,mcV = Mean corpsular volume,mcHC = Mean corpsular Haemoglobin concentration

Table 7. Mean values of water quality parameters of the experimental tanks *(mean ± SD).*

Treatments	Temperature	pH	Do (mg/l)
I	26.04±0.37^{b}	7.6±0.06^{a}	7.6±0.04^{a}
II	26.9±0.43^{b}	7.8±0.06^{a}	7.6±0.07^{a}
III	27.5±0.64^{b}	6.9±0.09^{a}	7.6±0.06^{a}
IV	27.2±0.51^{a}	5.4±0.28^{a}	7.4±0.03^{a}

Water quality analysis

Water quality parameters monitored included temperature (°C), pH, and dissolved oxygen. The mean values recorded for each parameter is presented in Table 7. Mean temperature value was within the range of 25.04±0.37 to 27.7+0.35 throughout the period of the experiment. Mean pH levels varied between a range of 7.2±0.02 to 7.5±0.06. Mean dissolved oxygen levels for all reading tanks were measured to be within the range 5.8±0.15 to 7.9±0.06.

Proximate analysis of experimental diets

The feeding trials revealed that *Clarias gariepinus* responded to all the diets, irrespective of their moringa leaf meal inclusion. The haematological parameters of fish are reported to be affected by a range of factors which include species, size, age, physiological status, environmental conditions and dietary regime e.g. quality and quantity of food, dietary ingredients, protein sources, vitamins, etc. However, moringa leaf meal inclusion has been used as premix to affect the haematological parameters, which are important and in checkmating the health

status of fish. Faleye A.E *et., al* (2018) showed that premix in fish feed could be replaced with moringa oleifera leaf meal up to 1.5% level in diets of *Clarias gariepinus* on the haematological parameters, health and feed efficiency. The high crude protein of the pelleted diets is due to the various inclusion levels of moringa leaf meal, which has been shown to reduce amino acid availability (Singh *et al*., 2007). The crude fibre, ash, and dry matter had higher values in the formulated feeds as compared to the floating feed used for the control experiments.

Haematological report

The haematological indices of blood are useful in monitoring feed toxicity especially with feed constituents that affect the formation of blood. This study is within the recommended physiological ranges reported for *Clarias gariepinus* by (Ozovehe, 2013). The Packed cell volume (PCV), Haemoglobin (Hb) and Red blood cell (RBC) was low in fish fed experimental diets 0.5% and 1% mLM inclusion (treatment II and III). However, a decrease in the haematological parameters of fish at significant level ($P<0.05$) was due to the level of inclusion in their diets. This observation supports the works of Osuigwe *et al.* (2006); Sotolu and Faturoti (1989) who reported the reduction in value of packed cell volume (PCV), Haemoglobin (Hb) and Red blood cell (RBC) diet of fish. Increase haemoglobin (Hb) in the diets with highest level of moringa inclusion, may be attributed to increase in the iron content of the feed, as iron is a major source of haemoglobin in fish fed diets containing 20-50% leaf meal inclusion as protein source ingredients. Moringa leaf meal is used as animal feed and most recently consumed as supplement in human diet, and could have led to an increase in the various haemotological parameters. Moringa leaf meal inclusion at 1.5% as premix is better. This is similar to the findings of Falaye *et al.* (2018) which recommended 1.5% inclusion rate ofmLM for increases in haematological parameters of *Clarias gariepinus*. It is also synonymous tothe report of Mohammed *et al.* (2018) which characterized mLM to possess high contents of protein, carotenoids, minerals and vitamins that enhance the haematological parameters of *Clarias gariepinus*. This is consistent with the findings of Dienye and Olumuji (2014) who reported that the growth performance and haematological response of African mud Catfish (*Clarias gariepinus*) fed dietary levels of *Moringa Oleifera* leaf meal, and indicated that 10% substitution rate ofmLM in catfish diet would not have any adverse effect on the blood and serum enzyme.

Conclusion and recommendation

The study has shown that *Moringa oleifera* leaf meal could be used as premix up to 1.5% level in the diet of *Clarias gariepinus* for improved performance with respect to haematological parameters and feed efficiency. The toxicology test showed that percentage replacement of premix in the diet will be compatible with the health of the fish. Based on the findings of this study, it is recommended that 1.5% ofmLM be included as premix for enhancement of haematological parameters and better fish health of *Clarias gariepinus*

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