

Growth Performance and Hematology of the African Catfish (*Clarias gariepinus* Burchell, 1822) Juveniles Fed Graded Level of *Anisophyllea Laurina* R. Br. Ex. Sabine (Monkey Plum) Seed Meal

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ABSTRACT---- *Anisophyllealaurina* seed meal (ASM) in this study partially replaced fish meal (FM). Isonitrogenous and isocaloric diets (40% crude protein) containing 0%, 25%, 50%, 75%, 100% ASM were formulated. *Clarias gariepinus* juveniles (32.72 ± 0.32) stocked at 10 fish/50L troughs fed twice daily for 98 days utilized effectively the formulated diets for growth. Mean weight gained (MWG) was highest at 0% and 25% but decreased as ASM increased. Specific growth rate (SGR), Protein efficiency ratio (PER), Protein intake (PI) were significant ($p < 0.05$) at 0% and 25% inclusion. Survival rate (%SR) was unrelated to treatment - highest at 0% and 100% (97%SR) and least at 75% (90%SR). Carcass protein, packed cell volume and haemoglobin counts were statistically the same at 25%, 50%, 75% and 100% inclusion. Since *C. gariepinus* perform better at 25% ASM inclusion it is recommended as the level of replacement for FM in fish diets.

Keywords--- Monkey plum, haematology, *Clarias gariepinus*, replacement

1. INTRODUCTION

The rise in aquaculture production is increasing intensive feeding of fish. Fish require food to supply the energy needed for movement and all the other activities in which they engage, and the building blocks for growth [1]. Consequently, fish nutrition experts world over have considered the recruitment of alternative protein feed ingredients necessary for inclusion in fish diet. Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity [2, 3].

Plant proteins, especially those that are not suitable for human consumption appear to be the most appropriate alternatives to fish meal in fish diets. Partial replacement of fish meal by plant proteins has been accomplished in many carnivorous cultured fish [4, 5, 6, 7, 8, 9], but total replacement according to [5, 10] has met with success in only a few cases. Some studies have also stressed that a mixture of plant protein sources is more appropriate than the incorporation of a single plant protein source because of improved IAA profiles [10, 11, 12].

The use of plant derived materials as fish feed ingredients is limited by the presence of a wide variety of anti-nutritional factors (ANFs). It has also been reported that, excessive consumption of plant protein sources by fish could cause slower growth rates and poor performance which may result in mortalities if condition persists [13, 14]. Reduction of ANFs in fish feed ingredients have however been accomplished through a wide range of processing techniques such as cooking, dehulling, germination, roasting, extrusion, soaking and recently extrusion cooking [15, 16, 17, 18, 19, 20, 21, 22].

Anisophyllealaurina is highly exploited in Sierra Leone but information on its use as a protein source for fish feed formulation is rare. *A. laurina* R. Br. Ex Sabine (Anisophylleaceae) is a tropical woody dicotyledonous plant that occurs in wet lowland primary forests. The species *A. laurina* is wide spread in certain area of Sierra Leone especially Moyamba district where it is found in all ages of farm bush. In the forest it serves as habitat for arboreal species and in degraded land it is an indicator of erosion. This useful plant *A. laurina* is a source of conflict between local land owners, forest managers and surrounding communities, which is attributed to its numerous uses. Pounded kernel of *A. laurina* is used as vomitive while the twigs are used as tooth-sticks. The young leaves when ground and mixed with oil are applied to circumcision wound and other sores. Decoction of the leaves is also used as mouth rinse for tooth ache. The tree is also an occasional host to the silkworm – *Anapheambrizia* [23].

The aim of this present study is to evaluate the suitability of *A. laurina* as an alternative plant protein source and to determine the inclusion level at which it perform best as a partial replacement for fish feed in fish diet. The study also

aims at investigating the implication of the diet on the haematological parameters of the cultured fish – *Clariasgariepinus* (Burchell 1822).

2. MATERIALS AND METHODS

2.1 Collection and processing of *Anisophyllalaurina*

Large collection of *A. laurina* fruits was obtained towards the end of the dry season (November) within Njala University Campus. Samples were first sun dried and the shells were thereafter removed by breaking the nut using stones and Hammers. The seeds inside the nuts were gathered and sun-dried for about a week. Shelled and sun dried *A. laurina* seeds were roasted using hot sand in order to destroy/reduce any anti-nutritional factor that may be present in the seed. The roasted *A. laurina* was further dried and thereafter finely grinded before adding to other feed ingredient at the desired inclusion level.

2.2 Collection and preparation of feed stuff

All ingredients used in the formulation were ensured constant except FM and ASM that were made substitute for one another on percentage basis. Slight variations however occurred in the crude protein content of the formulated diets when chemically analyzed and this may be due to differences in their compositions. The ingredients used along with *A. laurina* in the study include fish meal (65% CP), yellow maize, soybeans, vitamin premix, vegetable oil, salt and starch as binders. These feed stuffs were bought at a nearby market. The soybeans were processed by roasting using hot sand and later grinded into fine powder with the aid of a hammer mill. The yellow maize was also grinded into powder. The vitamin premix (methionine) was added to supply vitamins and minerals, which the feed stuffs could not supply.

2.3 Experimental fish and feeding methods

Clariasgariepinus juveniles (32.72 ± 0.32) used for this study were obtained from River Tia and Bo fish farm. They were acclimatized in an open tank for a period of three days before distributing them into the rearing trough. Ten juveniles of *Clariasgariepinus* were randomly selected and distributed into each of the experimental trough of 50 litre capacity. The experiment was completely randomized (CRD) comprising 5 (five) treatments and 15 replicates. Water inflow rate of 2.5L/min was maintained throughout the study to ensure constant oxygen supply to the system. Three set of tanks were assigned to each of the experimental diets containing different levels ASM in the proportion of 0%, 25%, 50%, 75% and 100%. The 0% inclusion (fish meal only) was used as control. Fish were fed twice daily between the hours of 08:00h - 09:30h am and 16:00h - 17:00h at 5% body weight of fish. At the early stage of the experiment, feeds were crushed for easy picking and digestion by the fish. Solid wastes were siphoned out of troughs every day before feeding and total cleaning of fish troughs and replacement with clean fresh water was done every three days. The feeding process lasted for 98 days. Fish were weighed every one week throughout the period of the experiment to record changes in weight and to adjust feeding. Chemical analysis of experimental feeds and fish were done before feeding trials using the method of [24] while that of the experimental fish alone was repeated at the end of the experiment. At the onset of the feeding trials, the *Clariasgariepinus* juveniles were weighed with Soehnle Electronic Kitchen balance Model Art – Nr. 65055. Mortality in each tank were recorded and estimated at the end of the experiment.

2.4 Feed utilization, haematology and data analysis

Data were collected weekly on fish growth performance and nutrient utilization by determining mean weight gain (MWG), feed intake (FI), specific growth rate (SGR), percentage survival rate (%SR), protein efficiency ratio (PER), feed conversion ratio (FCR) and protein intake (PI). Mean weight gain (MWG) of individual fish in each trough was estimated by subtracting the initial mean weight from the final mean weight at harvest.

$$MWG = W_2 - W_1$$

Where W_2 = Final mean weight of fish at 98 days

W_1 = Initial mean weight of fish at stocking.

Specific growth rate (SGR) was calculated after Brown (1975) as follows:

$$SGR = \frac{(\text{Loge final weight} - \text{Loge initial weight}) \times 100}{\text{Cultured period}}$$

Survival Rate expressed in percentage was calculated from the relationship;

$$\% SR = \frac{\text{Initial number of fish} - \text{Mortality}}{\text{Initial number of fish stocked}} \times 100$$

Feed intake was calculated by the addition of daily mean feed intake of fish in each treatment throughout the experimental period.

Feed conversion ratio (FCR) was calculated with the equation below.

$$\text{FCR} = \frac{\text{Total feed fed}}{\text{Total wet weight gained}}$$

Protein Efficiency Ratio (PER) was calculated with the equation

$$\text{PER} = \frac{\text{Wet weight gained}}{\text{Amount of Protein fed}}$$

Protein Intake was determined from the proportion of protein content in total feed fed to the fish.

$$\text{PI} = \text{Total Feed fed} \times \text{percentage protein fed (40\%)}$$

Fish haematology was determined using standard procedures [25, 26]. Initial blood samples were collected prior to feeding trial that is 0th day while the final blood samples were collected from the fish in triplicates on the last day of the experiment (98 day). The fishes were taken out individually using a small hand net, and placed belly upward on a table. Blood samples of about 4 millimeters were collected from the caudal peduncle with the aid of a 2cm³ plastic syringe. The blood sample was dispensed into ethylene diamine tetra acetic acid (EDTA) an anti-coagulant placed in a plastic sample bottles. The use of plastic syringe is a necessary precaution with fish blood because contact with glass result in decreased coagulation time. The haematological indices of mean cell haemoglobin concentration (MCHC), were calculated using the total red blood cell count (RBC), Haemoglobin concentration (Hb) and haematocrit (HCT)

$$\begin{aligned}\text{MCHC (\%)} &= (\text{Hb/PCV}) \times 10 \\ \text{MCH (pg)} &= (\text{Hb/RBC}) \times 10 \\ \text{MCV (fl)} &= \text{PCV/RBC} \times 100\end{aligned}$$

Data collected during the experiment were subjected to analysis of variance (ANOVA) and correlation analysis using the SPSS package version 10 and significant mean differences were separated at 0.05 probability levels according to [27].

3. RESULTS

Diet formulation and proximate composition of *A. laurina* based diets is presented in Table 1. Values for crude protein content ranged from 15.8% to 19.7%. Growth performance, feed and protein utilization is presented in Table 2. Highest mean weight gain (MWG) was recorded for fish fed 0% and 25%. MWG of fish continue to decrease with increase in the level of ASM. Figure 1 gives the relative weekly mean weight gain of the experimental fish. Specific growth rate (SGR), protein efficiency ratio (PER) and protein intake (PI) decreases with increase ASM. SGR, PER and PI were significantly higher ($p < 0.05$) in fish fed 0% ASM followed by 25% inclusion rate. Survival percentage does not show any relationship with ASM inclusion. Order of percentage survival was 0% > 100% > 25% > 50% > 75%. Correlation coefficient of growth performance is shown in Table 3. FMWG correlate negatively with PER (-0.0115*). Carcass and Haematological composition of experimental fish fed ASM is shown in Table 4. Haematological parameters determined include PCV, Hb, Rbc, Wbc, MCV and MCHC. Carcass crude protein of 0% was significantly different ($p < 0.05$) when compared to other levels of inclusions of ASM. Fish carcass protein increased in all the diets. Initial carcass protein value was 49.1% while 0% produced 59.38% CP and 75% ASM produced the least 52.37% CP. There were no significant differences ($p > 0.05$) in the dry matter content of the carcass. Crude fat was significantly different ($p < 0.05$) at 0% and 25% ASM inclusion while crude fibre was significantly different ($p < 0.05$) at 50% and 25% respectively. Fish PCV increased from initial 08% to 30% in 0% ASM inclusion and subsequently decreased marginally to 28.3% in 25% ASM inclusion. The least PCV value was recorded for 75% ASM inclusion from 21.7% which was not significantly different ($p > 0.05$) from other levels of inclusions. Result of Hb increased from the initial value but was least in 75% inclusion. However, there was no significant difference at all levels of ASM inclusion. Rbc increased in value from the initial 2.02 ($\times 10^6/\text{ml}$) to 9.40 ($\times 10^6/\text{ml}$) in 25% ASM inclusion. The highest value of MCV was found in 75% ASM but show no significant variation with other levels of inclusion. Figure 2 represents the proximate composition (%) of processed *A. laurina*. Chemical analysis showed that *A. laurina* contains 5.70% crude protein, 30% crude fibre but low in dry matter (2.23%).

4. DISCUSSION

The growth and nutrient parameters revealed that *Clarias gariepinus* responded well to the feeding trials. However MWG by fish decreased as level of ASM inclusion increases in the diets. Growth and nutrient utilization by fish was highest at 25% and 50% inclusion level of ASM respectively and this compared favourably with what was obtained at the 0% inclusion level (control diet). The disparity in growth and nutrient utilization may be due to the differences in the protein content of the diet. Protein efficiency ratio (PER) was highest in fish fed 0% ASM but did not differ statistically

when compared with 25%, 50%, 75% and 100% ASM inclusion. These results seem to have direct relationship with feed intake.

The importance of feed intake by fish as a determinant of fish performance has been strongly emphasized [28, 29, 30] while other studies [31, 32] pointed out the possibility of protein sparing effects by other nutrients in a feed, that is as more energy was supplied for metabolism through other nutrients, more protein is available for fish growth and tissue development. All diets produced higher values of fish carcass protein than initial values but with marginal differences indicating different degree of utilization of the diets. These relatively high values of crude protein could be view alongside the work of [33] who reported that effective utilization of bambara groundnut at varying inclusion was responsible for variations in *Heteroclinus* carcass protein and lipid. The low level of crude fibre in the proximate composition of the fish carcass was the same in all treatments and the result agreed with the findings of [34] in research on growth performance and haematology of *Clarias gariepinus* fed varying inclusions of *Leucaena leucocephala* seed meal based diets.

Hematological parameters of the experimental fish decreased with increased level of *A. laurina* seed meal but were not statistically different. Hemoglobin and packed cell volume (PCV) according to [35] could be used on routine basis in fish hatchery as a check on fish health status. There were marked difference between the values of the initial and final hematological parameters and this corroborates the findings of [36] that survival of fish can be linked with increase in antibody production which helps in the survival and recovery of fish. Hematological characteristics have been widely used in clinical diagnosis of diseases and pathologies of human and domestic animals. The applications of hematological techniques have proved valuable for fishery biologist in assessing the health of the fish [37] and monitoring stress response [38]. Low values recorded in some of the treatment may be due to the condition under which the fishes were kept; the small sizes of the plastic troughs, fluctuating water supply especially at the peak of dry season at Njala where the experiment was conducted.

In a stress situation, erythrocyte count is one of the first parameters that are affected. Increase in WBC (leucopomia) as observed in the fish fed *A. laurina* seed meal diet may be attributed to increase in production of leucocytes in the hematopoietic tissue of the kidney and perhaps the spleen [39].

Lymphocytes are the most numerous cells comprising the leucocytes, which function in the production of antibodies and chemical substances serving as defense against infection. The primary consequences of observed changes in leucocyte count in stressed fish are the suppression of the immune system and increased susceptibility to disease [39].

In conclusion this present study has clearly revealed that utilization of processed ASM by fish perform better at 25% inclusion level than at higher inclusion. Since weight gain of fish at a reduced cost of production is what would translate into income for the fish farmer at the end of the production cycle, 25% inclusion level of ASM in catfish diet is recommended. Although ASM did not show any negative effect on the growth performance of the experimental fish it is not recommended to be used singly as a replacement for fishmeal in fish diet because of its low amino acids profile.

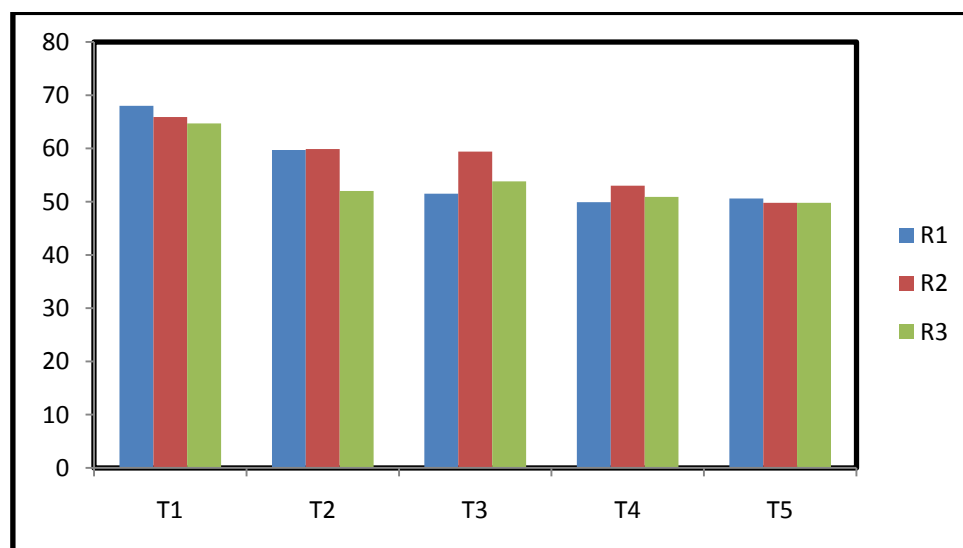


Figure 1: Weekly Fish Mean Weight Gain

T = Treatment; R = Replicate

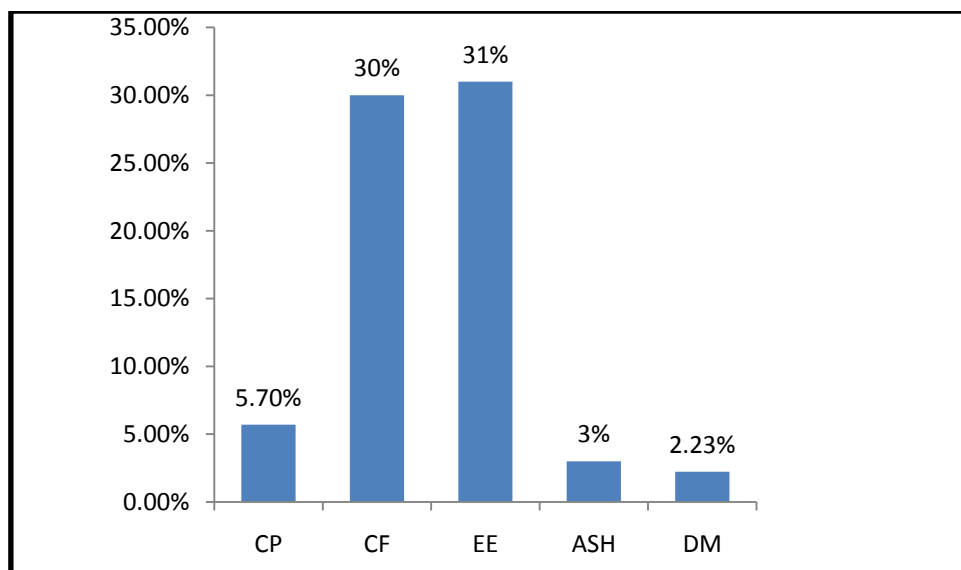


Figure 2: Proximate composition (%) of processed ASM
CP = Crude protein; CF = Crude fiber; EE = Ether extract; DM = Dry matter

Table1: Diet formulation and proximate composition of ASM based diets in partial replacement of Fish Meal (0 ~ 100%)

Gross composition ingredient (g/100g/DM)	Final values at different ASM inclusion rates (%)				
	Diet 1 0%	Diet 2 25%	Diet 3 50%	Diet 4 75%	Diet 5 100%
Fish meal	24.4	18.3	12.2	6.10	0
<i>Anisophyllealaurina</i>	0	6.10	12.2	18.3	24.4
Yellow maize	26.8	26.8	26.8	26.8	26.8
Soybean meal	45.8	45.8	45.8	45.8	45.8
Vitamin premix	0.5	0.5	0.5	0.5	0.5
Vegetable oil	2.0	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5
Chemical composition (%) on a dry matter basis					
Crude protein	16.4	15.8	18.6	19.7	16.2
Crude fiber	2.0	2.0	2.0	2.0	1.0
Ether Extract	26.0	24.0	24.0	22.0	23.0
Ash	2.0	2.0	3.0	2.0	2.0
Dry Matter	2.13	2.13	2.10	2.19	2.17

Vitamin premix used – methionine.

Table 2: Growth and nutrient utilization of *Clariasgariepinus* fed different inclusions of ASM based Diet

Parameter	Treatment ASM inclusion rates (%)				
	T1 0%	T2 25%	T3 50%	T4 75%	T5 100%
Culture period (days)	98	98	98	98	98
Initial number of fish	30	30	30	30	30
Final number of fish	29	28	28	27	29
Initial mean weight (g)	38.5±0.42	37.4±0.59	36.3±0.20	36.2±0.24	35.5±0.16
Final mean weight (g)	66.2±1.36	57.2±3.68	54.9±3.32	51.3±2.24	49.7±0.91
MWG (g)	27.7	19.8	18.6	15.1	14.2
FI (g)	191.08	172	167.6	151.8	148.4
SGR	0.24	0.19	0.18	0.15	0.15
FCR	6.90	8.70	9.01	10.05	10.45
PER	0.36	0.29	0.28	0.25	0.24
PI	76.43	68.8	67.04	60.72	59.36
Survival Rate (%)	97	93	93	90	97

Table 3: Pearson correlation coefficient for growth parameters

VARIABLES	FI	PI	FMWG	FCR	PER
FI	1.00				
PI	1.00	1.00			
FMWG	0.9974	0.9974	1.00		
FCR	0.1249	0.1249	0.0902	1.00	
PER	-0.074	-0.0737	-0.0115*	-0.2410	1.00

FI = Feed intake; PI = Protein intake; FMWG = Fish mean weight gain; FCR = Feed conversion ratio; PER = Protein efficiency ratio; * = Correlate significantly.

Table 4: Carcass and haematological composition of *Clariasgariepinus* fed *Anisophyllalaurina* Seed Meal (ASM) for 98 Days

Parameters (%)	Initial Value	Final values at different ASM inclusion rates (%)					SE Mean
		0%	25%	50%	75%	100%	
Crude protein	49.1	59.38 ^a	57.24 ^{ab}	54.43 ^{ab}	52.87 ^b	56.34 ^{ab}	3.210
Crude fibre	3.0	0.25 ^{ab}	0.09 ^b	0.31 ^a	0.26 ^{ab}	0.22 ^{ab}	0.099
Crude fat	ND	7.16 ^a	2.67 ^b	6.07 ^{ab}	5.80 ^{ab}	6.42 ^{ab}	1.932
Ether Extract	21.0	ND	ND	ND	ND	ND	ND
Ash	21.0	13.95 ^b	17.34 ^a	13.12 ^b	12.94 ^b	13.44 ^b	1.217
Dry matter	2.06	7.10 ^a	7.21 ^a	7.11 ^a	7.20 ^a	7.42 ^a	0.515
CHO	ND	12.17 ^b	51.73 ^a	18.63 ^{ab}	20.27 ^{ab}	15.17 ^b	17.329
PCV (%)	08	30 ^a	28.3 ^a	21.7 ^a	23.67 ^a	26.67 ^a	7.247
Hb(gm/100ml)	2.7	9.9 ^a	9.3 ^a	7.2 ^a	7.8 ^a	9.5 ^a	2.462
Rbc(x106/ml)	2.02	8.6 ^a	9.4 ^a	5.67 ^a	6.67 ^a	7.04 ^a	3.043
Wbc (x103/ml)	0.8	10 ^a	9.1 ^a	6.8 ^a	5.8 ^a	9.07 ^a	2.925
Platelet	06	9.3 ^a	8.3 ^a	5.3 ^a	06 ^a	6.67 ^a	3.615
MCV(fl)	39	38 ^a	29.7 ^a	42 ^a	36.7 ^a	34.67 ^a	8.265
MCH(PG)	13	13 ^a	10 ^a	14 ^a	12 ^a	11.33 ^a	2.823
MCHC (%)	03	33 ^a	33 ^b	33 ^c	33 ^d	33 ^e	0
Lym(%)	40	65 ^a	62.3 ^a	63.7 ^{ab}	63.3 ^{ab}	64.3 ^a	0.847
Neut (%)	59	35 ^b	37 ^a	35.3 ^{ab}	35.3 ^{ab}	35 ^b	0.992

ND = Not Detected; Means with the same superscript in the same row are not significantly different (p<0.05)

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