PERFORMANCE AND HAEMATOLOGICAL INDICES OF *Clarias gariepinus* FED GRADED LEVEL OF CRUDE ETHANOLIC EXTRACT OF *Gmelina arborea* LEAF MEAL DIET.

[1]Fatokun, B.O.,[3]Osaguona , P.O., [2]Zaccheaus, O.S., [2]Omole E.B ., and [3] Odua, S.O

[1]Department of Animal Production and Health Technology, Federal College of Wildlife Management, Forestry Research Institute of Nigeria, P.M.B.268,New Bussa, Niger State Nigeria.

[2]Department of Basic Science, Federal College of Wildlife Management, Forestry Research Institute of Nigeria, P.M.B.268,New Bussa, Niger State Nigeria.

[3]Department of Wild life and Ecotourism, Federal College of Wildlife Management, Forestry Research Institute of Nigeria, P.M.B.268,New Bussa, Niger State Nigeria.

Corresponding Author: giftwumi4real@yahoo.com **Tel: (+234)806-115-3736 and (+234)811-492-9277**

ABSTRAC T

A twelve week trial was conducted to investigate the performance and haematological indices of juvenile Clarias gariepinus fed graded level of crude ethanolic extract of Gmelina arborea leaf. The proximate analysis of the experimental diets were determined using A.O.A.C (2000) method and the data for each parameter were subjected to analysis of variance (ANOVA) while the means of various results were compared at 5% level of significance. The ethanolic extract of the leaf was added to the experimental diet at 0%, 5%, 10%, 15% and 20% ratio respectively and were labelled T_1, T_2, T_3, T_4 and T_5. The result shows that fishes fed 15% ethanolic extract of Gmelina arborea Leaf meal (GALM) had the highest Red Blood Cell (RBC) count, Packed Cell Volume (PCV),Haemoglobin Concentration(Hb),White Blood Cell (WBC) count, globulin concentration Albumin concentration, Total protein and Total lipid concentration. This result indicated that inclusion of 15% ethanolic extract of Gmelina arborea leaf in the diets of fish can improve the growth performance and haematological indices of Clarias gariepinus without any adverse effect on their physiological status.

INTRODUCTION

The major factor militating against fish farming in Nigeria has been the high cost of fish feed ingredients particularly fishmeal and this has resulted in the dependence of fish farmers in Nigeria on imported quality fish feeds which are usually expensive (Omitoyin, 2007). An estimated 4,000 tons of quality fish feed are imported into the country each year (AIFP, 2000) and utilization of commercially formulated feeds increase the cost of production thereby reducing the profit margin of fish farmers. This ultimately translates to high cost of fish production. For instance, feed represents a high proportion (50-80%) of variable cost of production (Helfrich and Craig, 2002). In order to reduce this high cost of fish production, there is therefore the need to search for cheap alternative feed stuffs from unconventional sources. *Gmelina arborea* is one of such novel feed stuffs. Analysis of the leaf showed that it contains 11.9% crude protein and some anti-nutritive factors such as Tannin, Saponin, Alkaloids and Terpenoids (Wikipedia, 2014). The objective of the current study therefore was to assess the bioactivity of the crude ethanolic extract of *Gmelina arborea* leaf on the growth performance and haematological parameters of Africa Cat-Fish (*Clarias gariepinus).*

MATERIALS AND METHODS

Experimental site and leaf ethanolic extract preparation.

Gmelina arborea leaves were collected from the estate of Federal College of Wildlife Management, New Bussa, Niger-State Nigeria and were taken to the herbarium of Forestry Research Institute of Nigeria for authentication. The collected leaves were washed in sterile distilled water and were air dried for ten days until constant weight was obtained. 100g of the powered dried leaves was added into a conical flask containing one litre (1litre) of 99.5% ethanol and the conical flask was covered and left for another seven days (7 days) at room temperature with daily agitation. After seven days, the supernatant was removed and

the ethanol was evaporated using a rotary evaporator; the residue was then freeze – dried using an instrument manufactured by Labconco USA and then stored in a sterile bottle as described by Harikrishnan et al, 2009.

Experimental diet formulation and processing

Five different diets were prepared using Pearson's method of fish feed formulation to contain 40% crude protein. The ethanolic extract of *Gmelina arborea* leaf was incorporated into the experimental diet at 0% (control), 5%, 10%, 15% and 20% to replace equal weight of fish meal. The feed ingredients were grounded individually to a fine powder by using hammer mill and were individually weighed before incorporation into the experimental diets. The pelleted feed were air dried for three days to remove moisture content and to maintain the proximate composition as described by Eyo. A.A. (1994). Samples of experimental diets were analyzed for their proximate composition as described by A.O.A.C (2000).

Experimental design and feeding trials.

Two hundred African Catfish Juveniles (*Clarias gariepinus*) of average weight 32.69 ± 0.91g were purchased from the hatchery unit of National Institute for Freshwater Fisheries Research, New-Bussa, Niger State, Nigeria and were fed on commercial diet for three days to allow for acclimatization (Okoye and Sule 2001). Prior to the commencement of the experiment all the fish were starved for 24hours, this in order to eliminate variation in weight due to residual food in the gut and also to prepare the Gastrointestinal tract for the experimental diets while at the same time to increase the appetite of the fish. The initial weight of the fish and the initial mean length were measured with graduated rule and recorded. The experimental fishes were randomly allocated into five treatment diets (T_1, T_2, T_3, T_4 and T_5) in quadruplicate in 20 concrete tank at a stocking density of 10 (ten) fingerlings per tank. Subsequently total weight and standard length measurement were taken fortnightly and all the water quality parameters were fortnightly monitored.

Blood collection and haematological analysis

Blood samples were collected in triplicate samples following the procedure of Klontz and Smith (1968) and Wedemeyer and Yasutake (1977) into sterile sample bottles containing Ethylene Diamine Tetra-Acetic acid (EDTA) as anticoagulants and the collected blood samples were taken to National Institute of Science Laboratory Technology (NISLT) Samonda, Ibadan, Oyo State, Nigeria for haematological analysis.

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) and the means were separated by Duncan's Multiple Range Test at a significant level of 0.05. All computation were performed using SPSS 15.0 (SPSS Inc., Chicago II U.S.A)

RESULTS

TABLE 1: Percentage composition of the experimental diet

Ingredients	T_1(0%GALM)	T_2(5% GALM)	T_3(10% GALM)	T_4(15% GALM)	T_5(20% GALM)
Fish meal	30.00	25.00	20.00	15.00	10.00
Soya beans meal	19.00	19.00	19.00	19.00	19.00
Groundnut cake	20.00	20.00	20.00	20.00	20.00
Maize	29.00	29.00	29.00	29.00	29.00
GALM	0.00	5.00	10.00	15.00	20.00
D.C.P	1.00	1.00	1.00	1.00	1.00
Vitamins premix[1]	0.50	0.50	0.50	0.50	0.50
Lysine	0.3	0.30	0.30	0.30	0.30
Methionine	0.2	0.20	0.20	0.20	0.20
Moisture content (%)	9.85	8.06	8.12	7.97	7.31

Crude Lipid (%)	5.05	5.03	4.74	4.61	4.47
Crude protein (%)	40.85	39.71	38.76	37.63	37.085
Total Ash (%)	4.97	4.84	5.05	5.37	5.70
NFE	35.31	36.65	36.72	35.91	35.15
Crude fibre	3.97	5.36	6.25	6.97	7.21

[1]Content per Kg of premix :Dibasic Calcium Phosphate (DCP), 50g; Calcium Carbonate, 215g; Sodium Chloride, 40g; Potassium Chloride, 90g; Magnesium hydroxide, 124g; Iron Sulphate, 250g; Zinc Sulphate, 4g; Magnesium Sulphate, 3g; Cobalt Sulphate, 0.02g; Potassium Iodide, 0.04g Sodium Selenite, 0.03g and Sodium Fluoride 1g.

Table 1 above shows the percentage composition of the various ingredients used in the formulation of the experimental diets. T_1 contains 0% *Gmelina arborea* leaf meal (GALM), T_2, T_3, T_4 and T_5 contained 5%, 10%, 15% and 20% GALM respectively.

Table: 2 Growth response, Nutrient utilization and Survival rate parameters of Clarias *gariepinus* Juvenile fed crude Ethanolic extract of GALM.

Parameters	T1(0% GALM)	T2(5% GALM)	T3(10% GALM)	T4(15% GALM)	T5(20% GALM)
Initial mean weight (g)	$39.00^{a} \pm 0.53$	$39.36^{a} \pm 0.64$	$35.57^{b} \pm 0.07$	$24.17^{c} \pm 0.17$	$20.04^{d} \pm 0.54$
Mean final weight (g)	$60.69^{a} \pm 0.17$	$60.39^{a} \pm 0.34$	$60.10^{b} \pm 0.79$	$49.45^{c} \pm 2.18$	$29.40^{d} \pm 0.87$
Mean weight gained (g)	$21.69^{b} \pm 1.63$	$21.03^{b} \pm 0.03$	$24.53^{a} \pm 0.13$	$25.28^{a} \pm 0.39$	$9.36^{c} \pm 0.05$
Mean daily weight gain (g)	$0.26^{b} \pm 0.02$	$0.25^{b} \pm 0.02$	$0.29^{a} \pm 0.04$	$0.3^{a} \pm 0.05$	$0.11^{c} \pm 0.01$
Feed intake (g)/fish	$43.35^{c} \pm 1.30$	$43.20^{c} \pm 0.05$	$43.61^{b} \pm 1.02$	$45.33^{a} \pm 0.02$	$40.02^{d} \pm 0.05$
Specific growth rate (SGR)	$0.34^{a} \pm 0.02$	$0.29^{b} \pm 0.01$	$0.31^{b} \pm 0.02$	$0.33^{a} \pm 0.02$	$0.26^{c} \pm 0.02$
Food conversion ratio (FCR)	$2.00^{b} \pm 0.02$	$2.05^{b} \pm 0.25$	$1.78^{c} \pm 0.05$	$1.79^{c} \pm 0.01$	$4.28^{a} \pm 0.07$
Protein efficiency ratio (PER)	$1.31^{c} \pm 0.04$	$1.28^{c} \pm 0.05$	$1.496^{b} \pm 0.06$	$1.68^{a} \pm 0.03$	$0.66^{d} \pm 0.02$
Protein Intake (PI)	$16.54^{a} \pm 1.04$	$16.48^{a} \pm 0.20$	$16.40^{b} \pm 0.44$	$15.05^{c} \pm 0.88$	$14.21^{d} \pm 0.33$
Standard length (SL)	$15.50^{c} \pm 1.00$	$15.00^{c} \pm 0.50$	$15.65^{b} \pm 0.75$	$15.90^{a} \pm 0.005$	$10.50^{d} \pm 0.33$
Condition Factor (K)	$1.63^{c} \pm 0.15$	$1.79^{b} \pm 0.13$	$1.57^{c} \pm 0.15$	$1.23^{d} \pm 0.10$	$2.54^{a} \pm 0.40$
Survival Rate (SR %)	96.67	93.33	90.02	94.09	92.18

***Means within the same row with different superscript are significantly different (P< 0.05)*

The results shown on Table 2 showed the growth performance of the experimental fishes The fishes fed 0% and 5% GALM diet did not show statistical significant in their weight gain. The results for Specific Growth Rate (SGR) showed that fishes fed 15% GALM diet had the highest value of 0.33 and lowest value of 0.26 was recorded in fishes fed with 20% GALM diet. The Specific Growth Rate (SGR), Mean Weight Gain (MWG) and Protein Efficiency Ratio (PER) showed a negative correlation with increase in GALM diet. The highest value of 1.68 recorded for protein efficiency ratio was observed in fishes fed 15% GALM diet and the lowest value of 0.66 was recorded in fishes fed diet containing 20% GALM. Fish growth exhibited significant inverse correlation with condition factor (K). The correlation coefficient (r) was recorded as -0.85.

Table 3: Haematological parameters of Clarias *gariepinus* juvenile fed graded level of GALM diet.

Blood parameter	Initial Values	T1(0% GALM)	T2(5% GALM)	T3(10% GALM)	T4(15% GALM)	T5(20% GALM)
PCV (%)	$29.60^{d} \pm 0.90$	$33.80^{a} \pm 1.80$	$32.88^{a} \pm 0.80$	$31.96.^{b} \pm 1.63$	$30.59^{b} \pm 2.00$	$26.80^{c} \pm 2.43$
WBC (10^3MM^3)	$8.40^{c} \pm 0.4$	$8.65^{bc} \pm 0.2$	$8.70^{bc} \pm 0.2$	$8.80^{b} \pm 0.2$	$8.93^{b} \pm 0.2$	$9.20^{a} \pm 0.22$
RBC (10^6MM^3)	$3.80^{c} 0.02$	$4.90^{a} \pm 0.20$	$4.79^{a} \pm 0.4$	$4.65^{b} \pm 0.19$	$4.37^{b} \pm 0.10$	$3.44^{d} \pm 0.22$
Hb (g/100ml)	$8.50^{b} \pm 0.40$	$9.32^{a} \pm 0.3$	$9.50^{a} \pm 0.10$	$9.69^{b} \pm 0.24$	$10.50^{b} \pm 1.21$	$7.50^{c} \pm 0.4$
Lymphocytes (%)	$63.00^{c} \pm 9.00$	$66.00^{b} \pm 1.90$	$66.02^{b} \pm 2.00$	$68.00^{ab} \pm 1.9$	$72.00^{a} \pm 1.9$	$73.51^{a} \pm 2.00$
MCHC (%)	$32.78^{ab} \pm 2.01$	$32.75^{ab} \pm 1.00$	$34.00^{a} \pm 2.00$	$34.00^{a} \pm 0.47$	$34.77^{a} \pm 0.99$	$33.17^{a} \pm 0.5$

MCH (pg)	32.57^{cd}±0.4	29.54^{d}±1.43	32.13^{cd}±0.29	44.40^{b}±2.6	46.17^{b}±0.42	40.84^{c}±0.4
MCV (fl)	98.96^{b}±1.93	86.74^{c}±2.10	$91.62b^{c}$±1.68	133.62^{a}±2.7	134.46^{a}±0.8	131.21^{ab}±0.3

Means within the same row with different superscript are significantly different (P< 0.05)

Table 3 revealed the haematological indices of fishes fed GALM based diet during the experiment. White Blood Cell (WBC) results showed that fishes fed 10% to 20% GALM diets had higher values than fishes fed 0% and 5% GALM diets. Haemoglobin concentration decreased in fishes fed diet containing 10% to 20% GALM. Lymphocyte count showed an increase as the level of GALM increases in the diet. The highest value of 73.51% was recorded in fish fed diet containing 20% GALM diet and the least value of 66.00% was recorded in fish fed the control diet (0% GALM). The results obtained for MCH and MCV showed that the fishes fed diets containing 15% GALM had the highest values of 46.17pg and 134.46fl for MCH and MCV respectively and the least values of 29.54pg and 86.74fl were recorded for MCH and MCV in fishes fed 0% GALM based diets. Conclusively, the PCV, RBC and Hb concentration showed a significant (P<0.01) inverse correlation with increase in GALM in the diets. The correlation coefficient (r) of -0.70, -0.92 and -0.65 were recorded for PCV, RBC and Hb concentration respectively.

DISCUSSION

Growth and nutrient utilization by the experimental fishes decreased as the level of GALM increases in the experimental diet. This observation may be as a result of persistence increase in the substitution level of fish meal with GALM in the diets which cause growth retardation. The decrease in the growth rate could also be due to reduction in the level of protein and essential amino acids in the diet having higher substitution level of fish meal with GALM (Russel., *et al* 1983). The result of the specific growth rate (SGR) could be due to differences in the GALM substitution level which decreased considerably from 15% to 20% substitution level in the diets. The consumption of anti-nutritional factors contained in GALM (Phenols, Tannin and Phytates) is probably responsible for the reduction in the palatability of the diets resulting in reduced feed intake leading to retarded growth response in the experimental fishes. Protein Efficiency Ratio (PER) was highest in fishes fed 15% GALM in their diet and it shows statistical significant difference (P<0.05) from fishes fed with other inclusion level of GALM. Haematological components of blood are valuable in monitoring feed toxicity especially with feed constituents that affects the formation of blood (Oyawoye and Ogunkunle 1988). All the haematological parameters measured in this study were within the recommended physiological ranges reported for *Clarias gariepinus.* However, a decrease in the haematological values of fishes at significant level (P<0.05) from fishes fed with 20% GALM diets could be as a result of the presence of higher concentration of anti-nutritional factors in the diets. Adeyemo (2005) reported that the reduction in values of |PCV, Hb and RBC count were due to the presence of toxic substances in the diets of the experimental fishes. An increase in the WBC and lymphocyte count is usually associated with microbial infection or the presence of foreign body or antigen in the circulatory system (Oyawoye and Ogunkunle 1998). Conclusively, the results obtained from this study showed that GALM could be substituted with fish meal up to 15% level in *Clarias gariepinus* diets without any negative effect on the growth, feed efficiency and Haematological status of the fishes.

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