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Growth Performance and Hematological Parameters of *Clarias gariepinus* Fingerlings Fed Varied Levels of Bitter Cola (*Garcinia kola* Seed) Meal

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Abstract: The effect of varied levels of inclusion of *Garcinia kola* on growth performance and haematological parameters was investigated in *Clarias gariepinus* fingerlings. Fish were fed diet supplemented at 0, 50, 100, 150 and 200 g kg^{-1} inclusion levels of *Garcinia kola* seed meal for eight weeks. Fish feed supplemented with Garcinia cola meal showed significantly improved growth performance and feed utilization over the control (0 kg^{-1} Garcinia kola seed meal) treatment. The highest Specific Growth Rate (SGR) (1.34±0.02% day^{-1}) and best Feed Conversion Ratio (FCR) (16.28±0.05) were obtain in 100 g kg^{-1} inclusion level of *Garcinia kola* seed meal. Protein Efficiency Ratio (PER) and Feed Conversion Efficiency (FCE) were also higher in fish fed with *Garcinia kola* seed meal and lowest in the control generally growth decreased beyond 100 g kg^{-1} level of inclusion. Higher values were obtained in fish carcass fed dietary inclusion for protein and lipid ($p<0.05$), blood parameters (WBC, HGB, RBC and PCV) reveals better condition for fish fed included *Garcinia kola* seed meal compared to the control. The result suggests that dietary supplementation with *Garcinia kola* seed meal improved growth rate and feed utilization best at 100 g kg^{-1} for *Clarias gariepinus* fingerlings.

Key words: Bitter kola, medicinal plants, bioflavonoid, blood indices, growth promoters

INTRODUCTION

The need to intensify the culture of fish to meet the ever increasing demand has made it essential to develop suitable diets either in supplementary forms for ponds or as a complete feed in tanks and raceways. Feed is one of the major input in aquaculture and constitute over 60% of running cost (Gabriel *et al*., 2007). The use of antibiotic growth promoters as feed additives in the aquaculture industry has been criticized by government policies and consumers because of possible development of microbial resistance to these products and their potential harmful effects on human health (Baruah *et al*., 2008). With the shift away from synthetic drugs, the use of plants for enhancing growth performance in animal is becoming acceptable (Adedeji *et al*., 2008).

Bioflavonoid, a plant growth promoter have been reported in *Garcinia kola* seeds (Braid 1991), dietary trials of *Garcinia kola* on rats and poultry have been reported to promote growth (Braid *et al*., 2003; Akpantah *et al*., 2005; Oluyemi *et al*, 2007). *Garcinia kola* (Bitter kola) is a highly valued ingredient in African ethnomedicine because of its varied and numerous uses which are social and medicinal thus, making the plant an essential ingredient in folk medicine. Medicinal plants such as *Garcinia kola* are believed to be an important source of new chemical substances with potential therapeutic benefits (Eisner, 1990).

Knowledge of haematological characteristics is an important tool used as an effective and sensitive index of monitoring physiological and pathological changes in fishes (Zhou *et al*., 2009). The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al*., 2001). Exogenous factors, such as management, diseases and stress, always induce major changes in blood composition (Svobodova *et al*., 2008; Chen *et al*., 2005; Cnaani *et al*., 2004). Basic ecological factors, such as feeding regime and stocking density, also have a direct influence on certain biochemistry parameters (Coz-Rakovac *et al*., 2005). This study therefore seek to investigate growth response of African catfish to different dietary inclusions of *Garcinia kola* and the hematological changes accrued to difference in dietary inclusions of *Garcinia kola* in the diet of *Clarias gariepinus*.

MATERIALS AND METHODS

This present study was carried out at the Departmental Fish Farm of the University of Agriculture, Makurdi and lasted for 56 days. The feed stuffs used for diets preparation (fish meal, yellow maize, soybean, vitamin and mineral premix and *Garcinia kola* seed) were

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all purchased in north bank market Makurdi, Benue State. *Garcinia kola* seeds were prepared into a meal by removing its outer coats, it was then sun-dried and milled to a fine powder. All other dietary ingredients were milled packaged and stored for use. An isonitrogenous and isocaloric diets (40% Crude protein and 140 $Kcal^{-kg}$) was formulated using Pearson square method. The various inclusions of feedstuffs were weighed into a bowl, dry mixed and pellected using a 3 mm pelletizer. Water of 60°C was added to the mixture to form a tough dough which was then passed through the 3 mm dice.

About 250 Fingerlings of *Clarias gariepinus* from an homogenous source by induced breeding with mean weight of 5.75g±0.03 were purchased from the research farm and acclimatized in plastic bowls for 2 weeks before the start of the experiment. About 20 fish were weighed and stocked randomly in duplicate hapas of 1 m^3 (i.e., 1×1×1 m) partially submerged in 48 m^3 earthen pond. Fish were hand-fed twice a day (08:00 a.m. and 6:00 p.m.) at a rate of 5% of their body weight daily. Feeding rates were adjusted weekly for 8 weeks based on the weight gain of each group of fish per week. Diets formulated as well as initial and final carcass of *Clarias gariepinus* fingerlings were analyzed for proximate composition according to standard methods (AOAC, 1997).

Blood was collected by cutting the fish at the caudal fin then hyperdemic needle and syringe was used to collect the blood. Blood from 4-5 fish were pooled to get enough blood for hematological analysis. Hematological parameters were determined using the methods described by Svobodova *et al*. (1991).

Performance in growth and feed utilization were determined as:

$$\text{Weight gain} = \text{Final weight} - \text{initial weight}$$

Growth rate was determined by calculating the value of:

$$\frac{\text{Weight gained}}{\text{Duration of the experiment}}$$

Specific Growth Rate (SGR) was calculated as:

$$\text{SGR} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{Duration of the experiment (days)}}$$

Feed Conversion Ratio (FCR) was measured by:

$$\text{FCR} = \frac{\text{Feed intake}}{\text{Body weight gain}}$$

Feed Conversion Ef iciency (FCE) was measured by:

$$\text{FCE} = \frac{\text{Weight gained} \times 100}{\text{Feed intake}}$$

Percentage survival:

$$\frac{N_t \times 100}{N_0}$$

Where:

N_t = Number of fish at the end of the experiment

N_0 = The initial number of fish stocked at the start of the experiment

Each experimental diet was fed to 2 groups of fish in a completely randomized design. Statistical analyses in the present study included descriptive statistics as well as analysis of variance using a computer software GENSTAT Discovery edition 3 from Lawes Agricultural Trust Rothamsted.

RESULTS

Mean water quality parameter monitored during the experiment is shown in Table 1 reveals statistical uniformity all through the 8 weeks of the study. Table 2 shows the inclusion levels of various ingredients in the experimental diets and their respective proximate composition. Table 1 shows inclusion level of *Garcinia kola* at 0, 50, 100, 150 and 200 g kg^{-1}. Table 3 shows the proximate composition of experimental fish fed various levels of *Garcinia kola* meal. The result shows that the fish fed *Garcinia kola* had a significantly higher protein and lipid content than fish fed the control diet.

Table 1: Water quality parameters monitored

Weeks	pH	Dissolved oxygen	Temperature
Week 1	7.05±0.15	4.05±0.25	22.26±0.49
Week 2	6.55±0.25	4.25±0.25	23.71±0.63
Week 3	7.50±0.10	4.45±0.35	23.17±0.58
Week 4	6.65±0.25	5.05±0.15	23.17±0.45
Week 5	6.40±0.30	3.60±0.60	22.99±0.55
Week 6	7.05±0.15	5.00±0.10	23.76±0.60
Week 7	6.95±0.25	3.90±0.80	24.06±0.62
Week 8	7.60±0.20	5.00±0.20	23.21±0.64

Mean in the same column followed by different superscript differ significantly; $p<0.05$

Table 2: Inclusion levels of various feed stuffs used for the experimental diets

	Diets				
Ingredients	D1	D2	D3	D4	D5
Fish meal	737.6	737.6	737.6	737.6	737.6
Soybean meal	488.2	488.2	488.2	488.2	488.2
Yellow maize	674.2	674.2	674.2	674.2	674.2
Mineral premix	50	50	50	50	50
Vitamin premix	50	50	50	50	50
Garcinia kola seed meal (g kg^{-1})	0	50	100	150	200
Proximate composition of experimental diet					
Moisture	8.02.±0.01	6.85±0.01	7.71±0.10	7.51±0.01	7.10±0.01
Ash	7.20±0.01	7.03±0.01	7.71±0.10	7.01±0.01	6.90±0.01
Lipid	8.24±0.00	7.60±0.01	8.12±0.01	7.82±0.01	8.06±0.01
Fibre	5.15±0.00	5.11±0.00	4.98±0.01	5.02±0.00	4.63±0.01
Protein	40.57±0.01	39.42±0.00	40.10±0.01	40.16±0.01	40.12±0.00
NFE	30.83±0.01	34.01±0.01	32.06±0.02	32.50±0.00	33.21±0.01

Table 3: Proximate composition of experimental diets

Parameters	Initial	D1	D2	D3	D4	D5
Moisture	-	8.02±0.01	6.85±0.01	7.71±0.10	7.51±0.01	7.10±0.01
Ash	-	7.20±0.01	7.03±0.01	7.71±0.10	7.01±0.01	6.90±0.01
Lipid	-	8.24±0.00	7.60±0.01	8.12±0.01	7.82±0.01	8.06±0.01
Fibre	-	5.15±0.00	5.11±0.00	4.98±0.01	5.02±0.00	4.63±0.01
Protein	-	40.57±0.01	39.42±0.00	40.10±0.01	40.16±0.01	40.12±0.00
NFE	-	30.83±0.01	34.01±0.01	32.06±0.02	32.50±0.00	33.21±0.01
Proximate composition of fish fed experimental diet (dry weight %)						
Moisture	4.10±0.02	6.58±0.01	6.56±0.01	7.23±0.01	6.10±0.00	6.72±0.01
Ash	12.10±0.01	12.32±0.05	12.34±0.01	12.21±0.00	11.86±0.00	12.08±0.01
Lipid	5.22±0.01^{f}	6.25±0.01^{d}	6.16±0.01^{e}	7.18±0.00^{a}	7.02±0.01^{b}	6.31±0.01^{c}
Fibre	5.87±0.01^{a}	3.08±0.01^{cd}	3.06±0.01^{d}	3.02±0.01^{e}	3.12±0.01^{b}	2.98±0.00^{f}
Protein	53.13±0.00^{f}	58.19±0.02^{d}	57.14±0.01^{e}	66.15±0.01^{a}	63.81±0.00^{b}	61.01±0.01^{c}
NFE	19.59±0.00^{a}	13.55±0.03^{c}	14.76±0.01^{b}	4.23±0.02^{f}	7.33±0.02^{e}	10.92±0.01^{d}

Means in the same column followed by different superscripts differ significantly; $p<0.05$

Table 4: Assessment of feed utilization by the experimental fish

Parameters	D1	D2	D3	D4	D5
MIW	5.73±0.01^{a}	5.77±0.01^{a}	5.76±0.04^{a}	5.77±0.02^{a}	5.70±0.01^{e}
MFW	25.13±0.05^{e}	31.52±1.15^{cd}	40.95±0.42^{a}	36.35±0.40^{b}	33.95±0.65^{cb}
WG	19.40±0.50^{e}	25.76±1.14^{d}	35.19±0.46^{a}	30.58±0.37^{bc}	28.25±0.63^{c}
MWG	0.35±0.01^{e}	0.46±0.02^{d}	0.63±0.01^{a}	0.55±0.01^{b}	0.50±0.01^{cd}
SGR	0.100..00^{e}	1.20±0.01^{d}	1.34±0.02^{a}	1.28±0.01^{bc}	1.25±0.00^{c}
PER	0.13±0.00^{a}	0.14±0.00^{a}	0.15±0.00^{a}	0.15±0.00^{a}	0.15±0.00^{a}
FCR	18.85±0.01^{a}	17.58±0.31^{b}	16.28±0.05^{e}	16.66±0.70^{de}	16.97±0.05^{cd}
FCE	5.31±0.01^{d}	5.69±0.10^{c}	6.14±0.02^{a}	6.01±0.03^{ab}	5.90±0.02^{b}
FE	0.05±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}
Feed intake	365.69±9.24^{e}	452.5±12.1^{d}	575.79±9.17^{a}	509.30±4.25^{bc}	479.17±9.29^{c}
ANPU	2.09±0.04^{d}	1.12±0.04^{e}	4.81±0.08^{a}	4.27±0.04^{b}	3.07±0.06^{c}

Means in the same column followed by different superscripts differ significantly; $p<0.05$; MIW = Mean Initial Weight; MFW = Mean Final Weight; WG = Weight Gain; MWG = Mean Weight Gain; SGR = Specific Growth Rate; PER = Protein Efficiency Ratio; FCR = Feed Conversion Ratio; FCE = Feed Conversion Efficiency; FE = Feed Efficiency; ANPU = Apparent Net Protein Utilization

Growth improvements were observed in fish fed *Garcinia kola* meal compared to the control, however the highest growth response was observed in the fish fed D3 (100 g kg^{-1} of *Garcinia kola* seed powder) while the lowest growth was obtained in the fish fed the control diet with 0% *Garcinia kola* seed meal (Table 4). Growth curves of experimental fish in the different treatments (Fig. 1) also indicate D3 to growth better compared to other dietary treatments and the control. There were greater values obtained for Feed Conversion Ratio (FCR) in fish fed *Garcinia kola* meal than the control fish with highest value recorded in D3. The Feed Conversion ratios (FCRs) were 17.58, 16.28, 16.66 and 16.97 for diets D2-D5, respectively. The Protein Efficiency Ratios (PER) obtained in the present study were 0.14, 0.15,

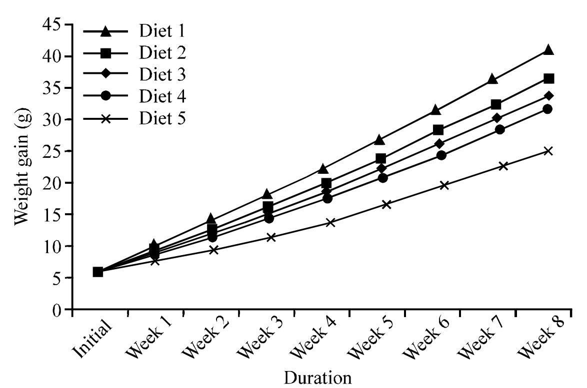

Fig. 1: Growth curves of experimental fish in the various treatments

Table 5: Haematological Characteristics of *Clarias gariepinus* ed the experimental diets

		Treatments				
Blood parameters	Initial	D1 (control)	D2	D3	D4	D5
WBC ($X10^9$ L^{-1})	11.10 ± 0.20^e	123.85 ± 1.35^d	136.55 ± 0.35^b	136.60 ± 0.80^{cb}	144.10 ± 0.70^a	146.00 ± 0.20^a
HGB (g dL^{-1})	1.05 ± 0.15^b	6.85 ± 0.25^a	7.10 ± 0.30^a	6.65 ± 0.35^a	7.20 ± 0.30^a	7.40 ± 0.20^a
RBC ($X10^{12}$ L^{-1})	0.23 ± 0.02^d	1.28 ± 0.03^c	1.40 ± 0.06^{bc}	1.42 ± 0.03^{abc}	1.45 ± 0.15^a	1.49 ± 0.03^{abc}
PCV (%)	2.85 ± 0.025^e	18.05 ± 0.25^{cd}	19.00 ± 0.40^c	20.20 ± 0.40^b	21.05 ± 0.25^a	21.20 ± 0.03^{ab}
MCV (fL)	128.30 ± 1.10^e	146.10 ± 0.70^b	139.70 ± 0.40^d	149.35 ± 0.85^a	142.30 ± 0.80^{cd}	146.80 ± 1.00^{ab}
MCH (Pg)	57.70 ± 0.60^a	53.10 ± 0.30^{bc}	51.40 ± 0.70^{cd}	50.66 ± 0.70^{fd}	50.65 ± 0.65^d	50.25 ± 0.95^{ed}
MCHC (g dL^{-1})	47.05 ± 0.95^a	35.40 ± 0.60^b	36.20 ± 0.30^b	33.30 ± 0.60^c	35.75 ± 0.45^b	33.15 ± 0.25^{cd}
RDW-CV (%)	13.05 ± 0.35^a	12.35 ± 0.25^{ab}	11.00 ± 0.20^c	11.75 ± 0.25^{bc}	10.15 ± 0.35^e	10.10 ± 0.40^{cde}
RDW-SD (FL)	76.85 ± 0.45^a	64.60 ± 0.40^d	57.30 ± 0.40^f	57.90 ± 0.30^b	57.60 ± 0.80^{ef}	66.85 ± 1.05^c
PLT ($X10^9$ L^{-1})	11.00 ± 1.00^f	30.50 ± 0.50^a	18.00 ± 1.00^{de}	17.50 ± 0.50^e	22.50 ± 1.50^c	26.00 ± 1.00^b
MPV (FL)	0.00 ± 0.00^a	7.00 ± 0.20^b	7.90 ± 0.30^a	7.15 ± 0.25^{abc}	6.60 ± 0.30^c	7.25 ± 0.35^{abc}
PDW	0.00 ± 0.00^a	17.20 ± 0.30^{bd}	18.00 ± 0.10^a	16.95 ± 0.25^d	17.15 ± 0.25^{cbd}	17.55 ± 0.25^{abcd}
PCT	0.00 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00

Means in the same column with different superscript differ significantly; $p<0.05$; WBC = White Blood Cell Count; HGB = Haemoglobin; RBC = Red Blood Cell Count; PCV = Pack Cell Volume; MCV = Mean Cell Volume; MCH = Mean Cell Haemoglobin; MCHC = Mean Haemoglobin Concentration; RDW-CV = Red Blood Cell Distribution Width-Cell Volume; RDW-SD = Red Blood Cell Distribution Width-Sedimentation; PLT = Platelet count; MPV = Mean Platelet Volume; PDW = Platelet Distribution Width; PCT = Platelet Crit

0.15 and 0.15 for the fish fed diets D2, D3, D5 and D5, respectively. The results of feed efficiency followed the same trends as Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) which was found to be 0.05 for fish fed on the control diet and 0.06 for fish fed on *Garcinia kola* seed meal.

Table 5 shows haematological parameters of the fish fed varied levels of bitter kola. The result obtained reveals that fish fed diets supplemented with *Garcinia kola* seed meal had a significantly higher ($p<0.05$) White Blood Cell (WBC), Red Blood Cell (RBC) and Packed Cell Volume (PCV) compared to those fed the controlled diet. However, there were no significant differences ($p\geq0.05$) in Haemoglobin (HGB) and platelet crit in all the treatments while red blood cell distribution width-cell volume, red blood cell distribution width-sedimentation and Mean Cell Haemoglobin (MCH) reduced across all treatment.

DISCUSSION

Water quality parameters were not significantly different between treatments and were within the recommended ranges for the culture of *Clarias gariepinus* (Viveen *et al*., 1986). The results suggest that dietary *Garcinia kola* seed meal at all levels of inclusion promoted the growth of *Clarias gariepinus* juveniles. These results showed that the *Garcinia kola* seed meal treatment enhances nutrient utilization which is reflected in improved weight gain, feed conversion ratio, protein efficiency ratio, feed conversion efficiency and specific growth rate. Generally, high feed conversion ratio values were obtained in treatments with included levels of *Gracinia kola* compared to the control diet, growth increased as level of inclusion increase to Diet 3 and there after decreased, this is similar to the result obtained for dietary nclusions of ethanolic extract of *Garcinia kola* seed in *Clarias gariepinus* broodstocks by Dada and Ikuerowo (2009), the reearchers found that the weight gain increased as amount of extract increased up to 1.0 g kg^{-1} and decreased thereafter, also, medical hero red clover *Trifolium pretense* were reported as a growth promoting agent for Tilapia *Oreochromis aureus* (Turan, 2006). Diab *et al.* (2008) reported that Nile Tilapia, *Oreochromis niloticus* fingerlings fed on diets supplemented by medicinal plants exhibited faster growth than those fed with the control diet. Similar results were reported by using medicinal plants as growth promoting agents for common Carp *Cyprinus carpio* (Yilmaz *et al*., 2006); Guppy poecilia reticulata (Cek *et al*., 2007a), the cichlid, *Cryptoheros nigrofasciatus* (Cek *et al*., 2007b), African catfish *Clarias gariepinus* (Turan *et al*., 2007) and Tilapia *Oreochromis niloticus* (Metwally 2009). Kim *et al.* (1998), suggested that unknown factors in various medicinal herbs led to favourable results in fish growth trials, however the findings of the present study is largely due to the presence of bioflavonoids in *Garcinia kola* which stimulates growth in fish (Braid *et al*., 1991). Kocour *et al.* (2005) had reported bioflavonoids as plant chemicals with estrogenic activity; dietary trials in common carp have shown that estrogen promotes growth (Kocour *et al*., 2005). Earlier also, Braid *et al.* (2003), Akpantah *et al.* (2005) and Oluyemi *et al.* (2007) had reported growth improvement of rats at an inclusions level of 200 mg kg^{-1} body weight and 7.5 g 100 g^{-1} in poultry have been reported to promote growth.

The body composition values obtained in this study were similar to those reported by Diab *et al.* (2002), Lara-flores *et al.* (2003) and Harnid and Mohamed (2008). Though fish fed dietary inclusions of *Garcinia kola* produced higher values of fish carcass protein and lipid than initial values and control, yet significant difference were obtained among them indicating different utilization levels of the diets. These relatively high values of crude protein could be viewed alongside the work of Alegbeleye *et al.* (2001) who reported that effective utilization of bambara groundnut at varying rates was responsible for variations in *Heteroclarias* carcass protein and lipid. This characteristic feed utilization efficiencies and consequents growth rates has been attributed to dietary protein quality, however the presence of bioflavonoids is attributed to be the cause of difference since *Garcinia kola* is very low in protein (Cho *et al.*, 1974; Sotolu and Faturoti, 2008). Low level of fiber in fish carcass fed with dietary inclusion of Bitter kola compared to values obtained for initial and the control is an evidence of effective feed utilization, in Sotolu (2008) experiment with water hyacinth, crude fiber was not detected in all dietary treated fish and was said to be associated with effective utilization of diets.

White blood cell counts were significantly higher ($p<0.05$) in fish fed diets including of *Garcinia kola* seed meal. The haematological values obtained in Dada and Ikuerowo (2009)'s experiment reveals statistical similarities for all levels of inclusion of extract of *Garcinia kola* as a growth promoting agent in *Clarias gariepinus* brood stocks. Differences observed in these study is likely due to developmental stage which may affect tolerance level of chemical inclusion in feed, however marginal differences follow the trend reported in the present study. Dietary *Garcinia kola* seed meal inclusion at 50-200 g kg^{-1} which enhances growth performance and feed utilization of cultured *Clarias gariepinus* better than control. However, growth according to this study was maximized at an inclusion level of 100 g kg^{-1}. It is concluded that *Garcinia kola* can be used as a growth promoting agent in the culture of *Clarias gariepinus* with better haematological parameters.

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