Growth Performance and Haematological Parameters of Clarias gariepinus Fingerlings Fed with Bitter Kola (Garcinia kola) Seed Meal

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Abstract

The effect of varied levels of inclusion of Garcinia kola seed meal in diets 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 kola 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 (1.34±0.02% day-1) and best feed conversion ratio (16.28±0.05) were obtained in 100 g kg-1inclusion level of Garcinia kola seed. Protein efficiency ratio and feed conversion efficiency 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. Protein and lipid content increased (p<0.05) in fish supplemented with seed meal. Also there was an improvement in blood parameters (WBC, HGB, RBC, and PCV) in fish fed with Garcinia seed meal compared to the control. The result suggests that dietary supplementation with Garcinia kola seed meal improved growth rate and feed utilization was the best at 100 g kg⁻¹ for Clarias gariepinus fingerlings.

Keywords: Bitter kola, medicinal plants, bioflavonoid, blood indices, growth promoters

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Introduction

The need to intensify 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 inputs 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 has been reported in *Garcinia kola* seeds (Braid 1991). Dietary trials of *G. kola* on rats and poultry have been reported to promote growth (Braid et al., 2003, Akpantah et al., 2005, Oluyemi et al., 2007). *G. kola* (bitter kola) is a highly valued ingredient in African ethnomedicine because of its varied uses which are social and medicinal; thus making the plant an essential ingredient in folk medicine. Medicinal plants such as *G. 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 for monitoring physiological and pathological changes in fishes. (Xiaoyun 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

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stress status before they are present in a clinical setting (Bahmani et al., 2001). Exogenous factors, such as management, diseases and stress 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 biochemical parameters (Coz-Rakovac et al., 2005). This study therefore seeks to investigate growth response of African catfish (*C. gariepinus*) and the hematological changes that occur due to dietary supplementation using *G. kola*.

Materials and Methods

The study was carried out at the Departmental fish farm of the University of Agriculture, Makurdi, Nigeria and lasted for 56 days. The feed ingredients used for diet preparation (fish meal, yellow maize, soybean, vitamin and mineral premix and G. kola seed) were purchased from North Bank market, Makurdi, Benue State. G. kola seeds were prepared into a meal by removing outer coats, sun-drying and milling to a fine powder. Isonitrogenous and isocaloric diets (40% crude protein and 140 K cal-kg) were formulated using Pearson square method. The various inclusions of feed were weighed into a bowl, dry mixed and pelleted using a 3 mm pelletizer. Water of 60°C was added to the mixture to form a tough dough which was then passed through a 3 mm dice. Diets were formulated by including G. kola meal at 0, 50, 100, 150 and 200 g kg-1 levels and designated as diets D₁, D₂, D₃, D₄ and D₅ respec-

Two hundred and fifty (250) fingerlings of *C. gariepinus* from a homogenous source produced by induced breeding with mean weight of 5.75 ± 0.03 g were purchased from a research farm and acclimatized in plastic bowls for two weeks before the start of the experiment. Twenty fish were weighed and stocked randomly in duplicate hapas of 1 m³partially submerged in 48 m³ earthen pond. Fish were handfed twice a day (08:00 am and 6:00 pm) at a rate of 5% of their body weight. Feeding rates were adjusted weekly for 8 weeks based on the weight gain of each group of fish. Diets formulated as well as initial and final carcass of *C. gariepinus* fingerlings were analyzed for proximate composition according to standard methods (AOAC, 2002).

Blood collected from caudal fin using a syringe and blood from four to five fish was 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

Weight gained = Final weight - initial weight

Growth rate = Weight gained

Duration of the Experiment

Specific growth rate (SGR) =

Final Weight – Initial Weight

Duration of the Experiment (Days)

Feed conversion ratio (FCR) = $\frac{\text{Feed Intake}}{\text{Body Weight Gain}}$

Feed conversion efficiency = $\frac{\text{Weight Gained x 100}}{\text{Feed Intake}}$

Apparent Net Protein Utilization (ANPU) = $\frac{\text{Protein gain x 100}}{\text{Protein fed}}$

Percentage Survival = $\frac{N_t \times 100}{N_0}$

Where N_t and N_0 are the number of fish at the end of the experiment and the at the start of the experiment respectively.

Temperature, dissolved oxygen and pH of water in the experiental tanks were monitored biweekly as per APHA (1988). Each experimental diet was fed to two groups of fish in a completely randomized design. Statistical analyses included descriptive statistics as well as analysis of variance using a computer software GENSTAT Discovery edition 3 from Lawes Agricultural Trust, Rothamsted.

Results and Discussion

The inclusion levels of various ingradients in the experimental diets and their respective proximate composition are depicted in Table 1. It shows

inclusion level of *G. kola* at 0, 50, 100, 150 and 200 g kg⁻¹. Mean water quality parameters monitored during the experiment shown in Table 2 reveals uniformity all through the eight weeks of the study. Water quality parameters were within the recommended ranges for the culture of *C. gariepinus* (Viveen et al., 1986). Table 3 shows proximate composition of experimental fish fed at various levels of *G. kola* meal. The result shows that the fish fed with *G. kola* had a significantly higher protein and lipid content than fish fed with the control diet.

Growth improvements were observed in fish fed with *G. kola* meal compared to the control. However, the highest growth response was observed in the fish fed with D3 diet (100 g kg⁻¹ of *G. kola* seed powder) while the lowest growth was obtained in the fish fed with the control diet with 0% *G. kola* seed meal (Table 4). Growth curves of experimental fish in different treatments (Fig. 1) also indicate that fish fed with D3 had better growth compared to other dietary treatments and the control. Higher values were obtained for feed conversion ratio (FCR) in fish fed with *G. kola* meal than the control fish with the highest value recorded in D3. The feed

conversion ratios (FCRs) were 17.58, 16.28, 16.66 and 16.97 for diets D2, D3, D4 and D5 respectively. PER obtained in the present study were 0.14, 0.15, 0.15 and 0.15 for the fish fed with diets D2, D3, D5 and D5 respectively. The results of feed efficiency followed the same trend as FCR and PER which was found to be 0.05 for fish fed on the control diet and 0.06 for fish fed on *G. kola* seed meal.

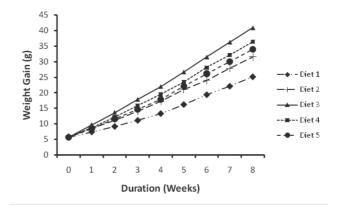


Fig. 1. Growth curves of experimental fish fed with different diets.

Table 1. Inclusion levels of various feed stuffs used for the experimental diets

Ingredients	Di	ets					
	D1	D2	D3	D4	D5		
Fish meal	368.8	343.8	318.8	293.8	268.8		
Soybean meal (g kg ⁻¹)	244.1	244.1	244.1	244.1	244.1		
Yellow maize (g kg ⁻¹)	337.1	337.1	337.1	337.1	337.1		
Mineral premix (g kg ⁻¹)	25	25	25	25	25		
Vitamin Premix (g kg ⁻¹)	25	25	25	25	25		
Garcinia kola seed meal (g kg ⁻¹)	0	25	50	75	100		
	Proximate Composition of experimental diet						
	D ₁	D_2	D_3	D_4	D_5		
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		

 $D_{1,}D_{2,}D_{3}$, D_{4} and D_{5} are experimental diets with *Garcinia kola* seed meal incorporated at 0, 50, 100, 150 and 200 g kg⁻¹ levels respectively

Table 2. Water quality parameters monitored

Weeks	pН	Dissolved Oxygen (ppm)	Temperature (°C)
Week 1	7.05±0.15	4.05±0.25	22.26±049
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

Table 5 shows haematological parameters of the fish fed at varied levels of bitter kola. The result obtained reveals that fish fed with *G. kola* seed meal supplemented diets had a significantly higher (p<0.05) white blood cell (WBC), red blood cell (RBC) and packed cell volume (PCV) compared to those fed with the controlled diet. However, there were no significant differences (p>0.05) in haemoglobin (HGB) and platelet crit in all the treatments while red blood cell distribution width-sedimentation and mean cell haemoglobin (MCH) reduced across all treatments

The results suggest that dietary *G. kola* seed meal at all levels of inclusion promoted the growth of *C. gariepinus* juveniles. These results showed that the *G. 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. Growth increased as level of inclusion increased upto Diet 3 and thereafter decreased, this is similar to results obtained for dietary inclusions of ethanolic extract of G. kola seed in C. gariepinus brood stocks by Dada & Ikuenerowo (2009) in which the authors found that the weight gain increased as amount of extract increased up to 1.0 g kg-1 and decreased thereafter. Red clover, Trifolium pretense was 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 C. 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 are largely due to the presence of bioflavonoids in G. 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, Braid et al. (2003), Akpantah et al. (2005) and Oluyemi et al. (2007) had reported growth improvement of rats at inclusion levels of 200 mg kg⁻¹ body weight and at 7.5 g 100 g⁻¹ in poultry.

Body composition values obtained in this study were similar to those reported by Diab et al. (2002),

Table 3. Proximate composition of fish fed with different experimental Diet (% dry weight)

Parameter	Initial	D1	D2	D3	D4	D5
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±0.01a	3.08 ± 0.01^{cd}	3.06±0.01 ^d	3.02±0.01e	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±0.00a	13.55±0.03°	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)

 D_1 , D_2 , D_3 , D_4 , and D_5 are experimental diets with *Garcinia kola* seed meal incorporated at 0, 50, 100, 150 and 200 g kg⁻¹ levels respectively

Table 4. Asses	sment of	teed	utilization	bv	the	experimental	fish
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Parameters	D1	D2	D3	D4	D5
MIW (g)	5.73±0.01a	5.77±0.01a	5.76±0.04a	5.77±0.02a	5.70±0.01e
MFW (g)	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 (g)	19.40±0.50e	25.76±1.14 ^d	35.19±0.46a	30.58±0.37 ^{bc}	28.25±0.63°
MWG (g)	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	0.10 ± 000^{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±0.01a	17.58±0.31 ^b	16.28±0.05 ^e	16.66±0.70 ^{de}	16.97±0.05 ^{cd}
FCE	53.1±0.01 ^d	56.9±0.10 ^c	61.4±0.02a	60.1±0.03ab	59.0±0.02 ^b
Feed intake (g)	365.69±9.24 ^e	452.5±12.1 ^d	575.79±9.17 ^a	509.30±4.25 ^{bc}	479.17±9.29°
ANPU	2.09 ± 0.04^{d}	1.12±0.04 ^e	4.81±0.08a	$4.27 \pm 0.04^{\rm b}$	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, ANPU = Apparent Net Protein Utilization

 D_1 , D_2 , D_3 , $D_{4'}$ and D_5 are experimental diets with *Garcinia kola* seed meal incorporated at 0, 50, 100, 150 and 200 g kg^{-1'} levels respectively

Table 5. Haematological characteristics of Clarias gariepinus fed with the experimental diets

			Treatments			
Blood Parameters	Initial	D1	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±0.70a	146.00±0.20a
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±0.03ab
MCV (f L)	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±1.00ab
MCH (Pg)	57.70±0.60a	53.10±0.30 ^{bc}	$51.40\pm0.70^{\rm cd}$	50.66±0.70 ^{fd}	50.65±0.65 ^d	50.25±0.95 ^{ed}
MCHC (g dL-1)	47.05±0.95a	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	$10.10 \pm 0.40^{\rm cde}$
RDW-SD (FL)	76.85±0.45 ^a	64.60 ± 0.40^{d}	$57.30\pm0.40^{\rm f}$	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±0.30a	7.15 ± 0.25^{abc}	6.60±0.30°	7.25 ± 0.35^{abc}
PDW	0.00 ± 0.00^{a}	$17.20 \pm 0.30^{\mathrm{bd}}$	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

 D_1 , D_2 , D_3 , D_4 and D_5 are experimental diets with *Garcinia kola* seed meal incorporated at 0, 50, 100, 150 and 200 g kg^{-1'} levels respectively

Lara-flores et al. (2003) and Hamid & Mohammed (2008). Though fish fed dietary inclusions of G. 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 Heteroclaria carcass protein and lipid. This characteristic feed utilization efficiencies and consequent growth rates has been attributed to dietary protein quality (Cho et al.,1974; Sotolu & Faturoti, 2008) and the presence of bioflavonoids is attributed to be the cause of difference since G. kola is very low in protein. Low level of fiber in fish carcass fed with dietary inclusion of bitter kola compared to values obtained for initial and control group is an evidence of effective feed utilization. Sotolu (2008) reported that in experiments with water hyacinth, crude fiber was not detected in any of the 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 G. kola seed meal. The haematological values obtained from the study of Dada & Ikuenerowo's (2009) reveal statistical similarities for all levels of inclusion of extract of G. kola as a growth promoting agent in C. gariepinus brood stocks. Differences observed in this study are 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 G. kola seed meal inclusion at 50-200 g kg-1 enhances growth performance and feed utilization of cultured C. gariepinus better than control. However, growth according to this study was maximum at an inclusion level of 100 g kg⁻¹. It is concluded that G. kola can be used as a growth promoting agent in the culture of *C. gariepinus* with better haematological parameters.

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