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Full Length Research Paper

Effects of ethanolic extracts of *Garcinia kola* seeds on growth and haematology of catfish (*Clarias gariepinus*) broodstock

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A 56 day study was undertaken to evaluate the effects of dietary ethanolic extracts of *Garcinia kola* (Bitter kola) in African catfish, *Clarias gariepinus* broodstock on growth performance and basic haematological indices. Catfish broodstock (mean weight, 245.20 – 255.00 g) were randomly distributed into concrete tanks (2 x 2 x 1.2 m) at 10 fish/tank in triplicate treatments. 5 diets (40% crude protein) containing varying levels of ethanolic extracts of *G. kola* at 0, 0.25, 0.5, 1.0 and 2.0 g/kg diets were prepared (representing diets 1 - 5, respectively) and fed to mixed sex *C. gariepinus* brodstock twice daily (0900 - 0930 h and 1700 - 1730 h) for 56 days to evaluate the effects on growth parameters, feed utilization and haematological parameters. There were significant variations (p > 0.05) in the growth parameters and food conversion ratio. Fish fed 1.0 g/kg diets of ethanolic extracts of *G. kola* had the best weight gain than fish fed ethanolic extracts of *G. kola* seeds of 0, 0.25, 0.5 and 2.0 g/kg feed with significant differences (P < 0.05) among the treatments. Similarly the specific growth rate (SGR) was higher in fish fed 1.0 g/kg diet of ethanolic extracts of *G. kola* seeds with significant different found between the treatments, the weight gain increased as the inclusion levels of ethanolic extracts of *G. kola* seeds in the diets increased up to 1.0 g/kg diets and decreased as the inclusion levels increased up to 2.0 g/kg feed. This support the probiotic effects of *G kola* seeds as growth promoter, haematological parameters such as pack cell volume (PCV), haemoglobin concentration (Hb) and red blood cells (RBC) for fish fed on diets D2 to D5 were not significantly different from that of the control (D1). However there was a significant (P < 0.05) proliferation of the white blood cells (WBC) in this study. This probably explains the antimicrobial effect of ethanolic extracts of *G. kola* seeds in view of the major role that the white blood cells assume in the immunity defence mechanism of the body in both man and animals.

Key words: *Garcinia kola*, growth, haematology, *Clarias gariepinus.*

INTRODUCTION

With the shifting of attention from synthetic drugs to natural plant products, the use of plant extracts for enhancing growth performance in animals is now in the increase. Plants that were once considered of no value are now being investigated, evaluated and developed into drugs with little or no side effects. One of such plant is *Garcinia kola* (bitter kola) a plant found in the moist forest areas. It is a medium-sized tree growing up to 12 m tall and 1.5 m wide and usually found in the rain forest of Nigeria (Iwu, 1993). It is used as food and herbal medicine and produces reddish, yellowish or orange colour fruits containing 2 to 4 seeds (Adesanya et al., 2007). The seeds when chewed have a bitter astringent taste. *Garcinia spp* are known to elaborate a complex mixture of phenotic compounds including biflavonoids, xanthones and benzophenones (Iwu et al., 1990). The phenolic compounds possess anti-inflammatory, anti-microbial, anti-diabetic and antiviral properties (Adedeji et al., 2006a). The seed extract and dry powdered seed of *G. kola* plants have been made into various forms including tablets, cream, and toothpaste (Iwu, 1985).

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The presence of biflavonoids and xanthone in *G. kola* seeds have been confirmed (Farombi et al., 2002). These compounds are potent antioxidants (Oluyemi et al., 2007). Administration of *G. kola* seed extracts caused an increase in testosterone production in spraque –dawley rats (Braide et al., 2003; Akpantah et al., 2005) and improved growth performance in poultry (Adedeji et al., 2006b).

Clarias gariepinus (Burchell, 1822) is the most important cultivated fish in Nigeria. This species has shown considerable potential as a fish suitable for use in intensive aquaculture. In spite of the remarkable aquaculture potential of *C. gariepinus*, the size obtained under culture is still poor compared to its hybrid and *Heterobranchus spp.* Previous studies on rats (Oluyemi et al., 2007) and poultry (Adedeji et al., 2006b) showed that inclusion of *G. kola* seeds in powder and methanolic extracts forms improved their growth performance. However, the potential of *G. kola* seeds as growth promoter has not been investigated in fish. The objective of this study was to investigate the effects of varying dietary supplementation of ethanolic extracts of *G. kola* seeds on growth performance, feed utilization and basic haematological profile in African catfish, *C. gariepinus* broodstock for 56 days.

MATERIALS AND METHODS

The feedstuffs were purchased from a market in Akure, Ondo State, Nigeria. *G. kola* seeds were also obtained from a market in Akure, Ondo state. The outer coats were removed and the seeds air-dried at ambient temperature for 72 h. The dried seeds were grounded into fine powder and the crude ethanolic extraction was done using 70% alcohol in a soxhlet extraction. The filtrate was evaporated using soxhlet extractor and was poured into a sample bottle and left opened for 2 days to allow the residual ethanol to escape. A basal diet (D1) was formulated to provide 40% crude protein according to Fagbenro and Adebayo (2005) (Table 1). Graded levels of ethanolic extracts of *G. kola* seeds were added to the basal diet (D1) at 0.25, 0.5, 1.0 and 2.0 g/kg and designated as treatments D2, D3, D4 and D5 respectively. The feedstuffs were thoroughly mixed in a Hobbart A- 200T pelleting/mixing machine. Hot water was added at intervals to gelatinize starch. All 5 diets were pelletized using a die of 8 mm diameter. The diets were air-dried at ambient temperature for 72 h, broken, sieved into small pellet sizes, packed in air-tight containers, labeled and stored.

150 healthy matured *C. gariepinus* broodstock (mean weight, 245.20 - 255.00 g) were obtained from a fish farm and acclimated for 1 week in outdoor concrete tanks during which they were fed with a commercial diet (40% crude protein). After acclimation, 5 male and 5 female were stocked in each of 15 concrete tanks (2 x 2 x1.25 m) supplied with 500 l of fresh water (water temperature, 26℃, pH 9.6, dissolved oxygen, 6.10 - 6.60 mg/L). The diet treatments were replicated thrice and fish were fed at 3% body weight / day in 2 instalments at 0900 - 0930 h and 1700 - 1730 h for 56 days. All fish were removed from each concrete tank every week and batch-weighed. The growth performance and feed utilization of the fish were determined at the end of the experiment as described by Steffens (1981). 4 fish (2 male and 2 female) were removed from each tank for blood analysis at the end of the experiment and blood analysis followed the methods described by Svobodova et al. (1991). Red and white blood cell counts were counted under light microscope with an improved Neubauer haemocytometer. Haemoglobinometer was used for haemoglobin estimation while packed cell volume (PCV) was determined by drawing blood of capillary action into microhaematocrit tube. The PCV was measured using microhaemotocrit reader and expressed as %.

Temperature, pH and dissolved oxygen concentration were monitored weekly throughout the study using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607 model).

Statistical analysis

The values were recorded as mean ± standard deviation. The statistical significance of difference in the mean and standard deviation ($P < 0.05$) was analyzed by one-way ANOVA test comparison of each of the test groups and the control using the SPSS 15. Duncan's multiple range was used to compare differences among individual means (Zar, 1984). Differences were considered significant at p levels < 0.05.

RESULTS

Growth performance and feed conversion by *C. gariepinus* fed varying dietary ethanolic extracts of *G. kola* levels

Data on fish growth performance are presented in Table 2. There were variations in weight gain, percentage weight gain (%WG), mean daily weight gain (MDWG), specific growth rate (SGR), feed conversion ratio (FCR) and total feed intake with significant differences among the treatment ($P<0.05$). The best overall growth response was obtained in fish fed with diet D3, and weight gain and average weight gain was lower in fish fed with the control diet (D1). A similar trend was observed with the specific growth rate (SGR), as the values increased with increasing dietary ethanolic extracts of *G kola* levels up to 1.0 g/kg diet. Survival of fish during the study was high, ranging from 80% to 100% and was not related to the inclusion levels of ethanolic extracts of *G. kola* seeds in the diets.

Haematological indices of *C. gariepinus* fed varying dietary ethanolic extracts of G. kola levels

Table 3 shows the initial haematological parameters of the fish with the groups fed with the experimental diets. The results show no significant difference ($P \geq 0.05$) in red blood cell, haemaglobin (Hb) and Pack cell volume in all the treatments. Although, the final values reported in this study highly varied but no significant different and this signifies that ethanolic extracts of *G. kola* seeds has a positive influence on the haematological indices. The highest mean values of pack cell volume (PCV) were obtained in fish fed with diet D4 while the control (D1) had the lowest PCV. Fish fed with diet D4 had the highest mean Hb concentration while the least mean Hb concentration was obtained in fish fed with diet D2. The same variation was recorded for White blood cell with the least

Table 1. Ingredient and proximate compositions (g/100g dry matter) of the experimental diets.

Ingredients	Treatments				
	A	B	C	D	E
Fish meal (65% CP)	250	250	250	250	250
Soybean meal (45% CP)	350	350	350	350	350
Yellow maize	150	150	150	150	150
Blood meal (85% CP)	100	100	100	100	100
Fish oil	60	60	60	60	60
Vegetable oil	40	40	40	40	40
Vitamin/Mineral premix	30	30	30	30	30
Binder (cassava starch)	20	20	20	20	20
Ethanolic extracts of *G. kola* seeds mg/kg	0.0	250.0	500.0	1000.0	2000.0
Moisture	9.42	9.39	9.40	9.36	9.34
Crude protein	9.42	40.22	40.16	40.25	40.34
Crude lipid	9.18	9.14	9.25	9.13	9.08
Crude fibre	9.55	9.46	9.43	9.52	9.48
Ash	11.70	11.65	11.58	11.62	11.73

Vitamin/mineral premix: Vitamin A, 4500, I. U. Vitamin D, 11252.U; vitamin E. 71.U; vitamin K_3, 2mg; Vitamin B_{12} 0.015mg, panthothenic acid; 5mg, nicotinic acid, 14mg; folic acid, 0.4mg; biotin, 0.04mg; choline, 150mg, cobalt 0.2mg; copper 4.5mg; iron 21mg; manganese 20mg; iodine, 0.6mg; selenium 2.2mg; zinc 20mg, antioxidant, 2mg.

Table 2. Growth performance and feed conversion by *C. gariepinus* broodstock fed on ethanolic extracts of *G. kola* seeds diets.

	Dietary ethanolic extracts of *G. kola* seeds level				
	0 g/kg	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Initial weight(g)	250.00 ± 7.07	251.50 ± 0.71	255.00 ± 7.07	245.20 ± 6.51	250.75 ± 3.89
Final weight(g)	400.00 ± 0.00^{a}	432.50 ± 17.68^{b}	506.67 ± 23.09^{c}	541.68 ± 1.87^{ab}	486.50 ± 9.78
Weight gain(g)	150.00 ± 7.07^{a}	181.00 ± 0.71^{a}	251.67 ± 24.75^{c}	296.48 ± 8.41^{b}	235.75 ± 5.75
%weight gain[1]	60.07 ± 4.53^{a}	71.97 ± 0.39^{a}	98.69 ± 10.33^{c}	120.91 ± 6.53^{b}	94.02 ± 1.00
ADG[2]	2.68 ± 0.14^{a}	3.23 ± 0.02^{a}	4.49 ± 0.50^{c}	5.29 ± 0.17^{b}	4.21± 0.01
SGR[3]	0.84 ± 0.06^{a}	0.97 ± 0.00^{a}	1.23 ± 0.14^{a}	1.42 ± 0.06^{b}	1.18 ±0.02
FCR[4]	4.52 ±2.30^{a}	7.38 ± 5.10^{d}	5.93 ± 0.28^{c}	3.14 ± 0.68^{b}	5.94 ± 2.56

[1]% weight gain (%. fish^{-1}) = final wt. – initial wt./initial wt. × 100
[2]average daily growth (g) = final wt. – initial wt./no. of days
[3]specific growth rate (%. day –1) = (In final wt. – In initial wt./ no. of days) × 100
[4]feed conversion ratio = feed intake (g)/ body weight gain (g)
values in each row having different superscripts are significantly different (P < 0.05g).

level recorded in fish fed with diet D1 (control) and highest in fish fed with diet D4. A Similar trend was observed with the red blood cell (RBC), as the values increased with increasing dietary ethanolic extracts *G. kola* levels up to 1.0 g/kg and decreased as the inclusion levels increased up to 2.0 g/kg feed.

DISCUSSION

Dietary inclusion of ethanolic extracts of *G. kola* improved the growth of *C. gariepinus* broodstock. Similar results were obtained in pullet chicks when fed on different inclusion levels of *G. kola* dry seeds powder (Adedeji et al., 2006b). Fish fed with diets containing ethanolic extracts of *G. kola* seeds showed a significant increased in specific growth rate (SGR) compared to the control. The best growth performance was achieved by feeding *C. gariepinus* broodstock at 1.0 g/kg dietary level attaining a total average weight gain of 296.45 g within eight weeks. Increasing the levels of ethanolic extracts of *G. kola* in diets up to 1.0 g/kg dietary level significantly resulted in increased specific growth rate (SGR). The study demonstrated that ethanolic extracts of *G. kola* is a good growth promoter in *C. gariepinus* broodstock. This study therefore infers that ethanolic extracts of *G. kola* can be included in *C. gariepinus* broodstock diets at 1.0 g/kg dietary level. This would ensure that the fish grows to acceptable size for fish seeds production within a short period.

Table 3. Some haematological characteristics of *Clarias gariepinus* fed the experimental diets.

Blood parameters	Dietary Ethanolic extracts of *G. kola* seeds level					
	Initial	**0 g/kg**	**0.25 g/kg**	**0.5 g/kg**	**1.0 g/kg**	**2.0 g/kg**
PCV (%)	30.50 ± 2.83[a]	31.50 ± 3.54[a]	29.50 ± 2.12[a]	31.50 ± 2.12[a]	33.00 ± 1.41[a]	30.50 ± 2.12[a]
Hb (g/100ml)	9.24 ± 0.65[a]	10.50 ± 1.13[a]	9.85 ± 0.78[a]	10.55 ± 0.64[a]	11.05 ± 0.49[a]	10.53 ± 0.09[a]
WBC ($\times 10^3/\mu L$)	6223.00 ± 625[c]	6350.00±636.40[c]	7150.00± 636.40[a]	9500.00±424.26[b]	10,125.00±247.49[b]	8281.25 ± 1572.75[a]
RBC ($\times 10^6/\mu L$)	3.48 ± 0.35[a]	3.55 ± 0.49[a]	3.20 ± 0.28[a]	3.50 ± 0.28[a]	3.75±0.35[a]	3.50 ± 0.2[a]

Figures in each row having the same superscripts are not significantly difference ($p > 0.05$).

Table 3 shows the initial haematological values and haemogram of the fish to ethanolic extracts *G. kola* seeds dietary feeding. The values obtained in fish fed diets D1 – D5 were within the normal ranges for fish (Svobodova et al., 1991). This suggests that inclusion of ethanolic extracts of *G. kola* in the diets can be beneficial to fish. White blood cells (WBC) were proliferated and significantly ($P < 0.05$) higher in fish fed diets D2, D3, D4 and D5.

Blood composition is usually altered during diseases or malnutrition conditions (Feist et al., 2000). Aletor and Egberongbe (1998) reported that red blood cell counts and packed cell volume (PVC) are mostly affected by dietary treatment. Under normal conditions the composition of blood is reasonably constant for any particular species with changes falling with fairly narrow limits Banerjee et al. (2002). Differences in blood parameters of fish in this study could therefore be ascribed to differences in the dietary inclusions of ethanolic extracts of *G. kola* in the diets. The differences in water quality parameters were not significantly different among the treatments and were within the recommended ranges for *C. gariepinus* (Viveen et al., 1986). Based on the results and the foregoing, this study established the efficacy of ethanolic extracts of *G. kola* seeds as growth promoter in *C. gariepinus* broodstock and should be encouraged as it will minimize the dependence on synthetic hormones as growth promoter.

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