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NUTRIENT UTILIZATION, GROWTH PERFORMANCE AND HEAMATOLOGY OF *CLARIAS GARIEPINUS* FED DIETARY *GARCINIA KOLA* FOR QUALITY FISH PRODUCTION

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Abstract

One hundred and fifty African catfish (mean weight, 673.30-813.30g) were divided equally among five groups in triplicate. Each group is a treatment of graded bitter kola (Garcinia kola) inclusion level for ensuring healthy fish production. Five diets (42% crude protein) containing varying levels of powdered whole seed of G. kola at 0.00, 0.25, 0.50, 0.75 and 1.00g/kg were prepared as treatments T1- T5 respectively. All groups were fed respective treatment-based-diets for forty-two (42) days during which data on body weight and blood parameters were recorded. There were significant differences (P < 0.05) in the growth performance and nutrient utilization efficiency. Fish fed 0.25g/kg G. kola inclusion (T2) had the best weight gain and specific growth rate with relative inferior feed conversion ratio and protein efficiency ratio to T4 and 5 (p>0.05). Values of Pack Cell Volume (PCV), Haemoglobin Concentration (Hb) and Red Blood Cell (RBC) count were highest in T2 but they all decreased with increasing levels of G. kola inclusion. White Blood Cells (WBC) count generally decreased from T1 to T5. The bacterial load of the culture medium decreased accordingly with increasing G. kola inclusion in tanks which may be as a result of the antimicrobial effect of G. kola whole seeds. Inclusion of G. kola whole seeds at 0.25g/kg in catfish diet is recommended to ensure successful fish production.

Key words: Catfish, *Garcinia kola*, Antimicrobial, Successful Fish Production, Feed additive

INTRODUCTION

Varieties of substances including synthetic, probiotic bacterial, animal and plant products have been reported useful as feed additives for improved fish performance (Afolabi *et al.*, 2006). Some plants are rich sources of compounds that enhance growth rates and buildup of immunity. These natural plant products have activities like anti-stress, appetizer, tonic, antimicrobials and immunostimulants (Citarasu *et al.*, 2002). Bitter kola (*Garcinia kola*) is known to contain a complex mixture of phenolic compounds like biflavonoids, xanthones and benzophenones (Iwu and Igboko, 1982). Afolabi *et al.* (2006) reported that bitter kola hull which hitherto is being discarded is a potential source of nutritionally valuable substance and industrial raw material and suggests the use of both the seed and hull in food and feed formulation. Besides, a good fish in terms of size and quality is mostly preferred by consumers. Considering these facts, whole bitter kola seed was used as feed additive towards ensuring sustainable quality fish production.

Materials and Methods

Preparation of test diets

Whole seeds (seeds and hulls) of *G. kola* were purchased from a local market in Lafia and were loaded into a conventional laboratory oven at 50^0C for 48 hours, milled, packaged in a sterile polyethylene sachet, labeled and stored in a refrigerator (3 ± 1^0C) until used. The experimental diets were prepared by incorporating the whole *Garcinia kola* seed (test ingredient) in each treatment group at 0.00, 0.25, 0.50, 0.75 and 1.00g/kg of feed, thus making up five (5) treatments. The test ingredient was mixed thoroughly in starch solution prepared at 9g/l of water, which enabled effective and uniform coating of the feed pellets. Proximate composition of the experimental diets is shown in Table 1.

Table 1: Proximate Compositions of experimental diets supplemented with *G. kola*

	Diets				
Proximate composition (%)	**1**	**2**	**3**	**4**	**5**
G. kola whole seed powder (g/kg)	0.00	0.25	0.50	0.75	1.00
Protein	42	42	42	42	42
Fat	12	12	12	12	12
Ca	1.2	1.2	1.2	1.2	1.2
P	1.0	1.0	1.0	1.0	1.0
Ash	7.5	7.5	7.5	7.5	7.5
Fiber	2.5	2.5	2.5	2.5	2.5
Mn	30ppm	30ppm	30ppm	30ppm	30ppm
Vitamin A (Iu/kg)	10000	10000	10000	10000	10000
Vitamin D3 (Iu/kg)	2000	2000	2000	2000	2000
Vitamin E (mg/kg)	200	200	200	200	200
Vitamin C (mg/kg)	100	100	100	100	100

Experimental Design, Feeding Trial, Sampling and statistical Analysis

After acclimation, fish (650-850g) were divided randomly into 5 treatment groups in triplicates ($T_1 - T_5$) with 3 fish in each group, maintained in 1.5x1x1.5m^3 concrete tanks. Data were collected on feed intake and weight gain forthnightly. Initial and final fish blood samples were analysed for PCV, Hb, RBC, WBC, MCV, MCH and MCHC. Water in the culture tanks was analysed for total bacterial count. Data were analyzed by a one-way Analysis of Variance (ANOVA) and significant mean differences were separated at 0.05 probability level as described by Steel *et al.* (1997).

Results

The results obtained on the effect of dietary *G. kola* whole seed on the average performance of *Clarias gariepinus* are presented in Table 2 while forthnight weight changes of fish are shown in Fig. 1.

Table 2: **Effect of dietary *G. kola* whole seed on the performance of *Clarias gariepinus***

Performance indices	Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	**SEM**
Initial weight (g)	673.30^b	770.00^a	740.00^{ab}	813.30^a	753.33^a	0.43
Final weight (g)	776.70^b	1123.30^a	886.70^b	1153.30^a	1050.00^a	6.10
Weight gain (g)	103.40^d	353.30^a	146.70^c	340.00^a	296.67^b	4.12
% weight gain	15.36^c	45.88^a	19.82^c	41.81^a	39.38^b	0.03
SGR (%/day)	0.36^c	0.88^a	0.43^b	0.83^a	0.81^a	0.02
Feed intake (g)	433.33^{ab}	533.33^a	340.00^c	476.67^a	436.67^{ab}	0.10
PER	0.57^c	1.58^a	1.03^b	1.70^a	1.62^a	0.31
FCR	4.19^a	1.51^c	2.31^b	1.40^c	1.47^c	0.11

Mean values with the same superscripts in a row are not significantly different P>0.05

The values of haematological parameters obtained were within the normal range for catfish (Table 3). The highest value of Pack Cell Volume (PCV) was obtained in fish fed diet T2 with a value of 25% as against 23.50% recorded for the initial fish, while the lowest value of PCV was obtained in fish fed diets T4 and T5 which had 21%. The results obtained on the effect of dietary *G. kola* on the bacterial load of culture water in cfu/ml are shown in Table 4. The values obtained decreased accordingly with increase in the levels of dietary *G. kola* whole seed inclusion for both 24h/37^{o}C and 22h/22^{0}C incubation periods. The total bacterial counts for fresh water obtainable during the experiment were 4.5 and 4.5 for 1:10 dilution and undiluted state both within 24h at 37^{o}C incubation.

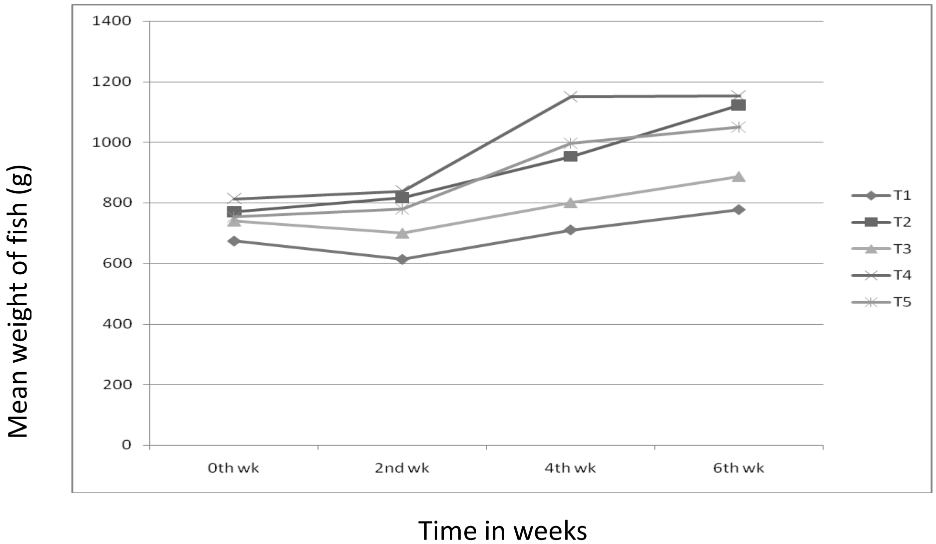

Figure 1: Fortnight mean weight change of *C. gariepinus* fed graded levels of dietary *G. kola* whole seed

Table 3: Effect of Dietary *G. kola* whole seed on the Haematology of *Clarias gariepinus*

Parameter	Initial	Final				
		T1	T2	T3	T4	T5
PCV (%)	23.50	24.00	25.00	23.00	21.00	21.00
Hb (g/dl)	6.95	7.70	7.90	7.70	6.60	6.50
RBC (x 10^{12}/l)	1.67	1.88	2.00	1.88	1.60	1.57
WBC (x 10^{9}/l)	152.50	153.40	139.40	145.00	149.90	134.70
MCV (fl)	140.72	127.66	125.00	122.34	131.25	133.76
MCH (Pg)	41.62	40.96	39.50	40.96	41.25	41.40
MCHC (g/dl)	29.58	32.08	31.60	33.48	31.43	30.95

Table 4: Bacterial Load of Fresh Water and Water from Culture Tanks (cfu/ml) at the end of the experiment

	Incubation for 24h at 37°C		Incubation for 22h at 22°C	
	1:10 dilution (cfu/ml)	Undiluted (cfu/ml)	1:100 dilution (cfu/ml)	Undiluted (cfu/ml)
T0	4.5	4.5	0	0
T1	37.5	143.5	83.5	187
T2	19	26.5	32	126
T3	17	24	24	29
T4	13.5	20.5	20	25.5
T5	5.5	15	2.5	10

Keys: T0: Fresh water used during the experiment; T1: 0.00 g/kg *G. kola* inclusion
T2: 0.25 g/kg *G. kola* inclusion; T3: 0.50 g/kg *G. kola* inclusion
T4: 0.75 g/kg *G. kola* inclusion; T5: 1.00 g/kg *G. kola* inclusion

DISCUSSION

The significant differences among treatments 2 to 5 when compared with the control (T1) in terms of weight gain and specific growth rate could be attributed to the fact that *G. kola* is a good growth promoter as established by Afolabi (2006). Their study inferred that ethanolic extracts of *G. kola* seed can be included in *C. gariepinus* broodstock diets at 1.0g/kg dietary level. The variations observed among treatments in the present study could be as a result of the high crude protein and amino acid composition in the hull of *G. kola* (Afolabi *et al.*, 2006), which is necessary for tissue deposition and cellular metabolism. Similar results of improved growth were obtained in pullet chicks when fed different inclusion levels of *G. kola* dry seeds powder by Adedeji *et al.* (2006). In a related study, Heidarieh *et al*. (2013) revealed that the inclusion of Aloe vera in the diets of rainbow enhanced growth rate in fish as well as immunostimulants which is similar to the observation in the present study. Generally, the test diets contain more essential nutrients including minerals such as Na, maintain K, Ca, Mg and P. Specifically Na and K are requiredfor osmotic balance maintenance of body fluid, pH of the body, regulation of muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth (NRC, 1989). Hence, the variations observed among the treatments in terms of feed utilization may be supported. Protein Efficiency Ratio indicates the utilization of dietary protein by means of the gain in biomass. The results on PER seemed to have direct link with feed intake probably because protein intake depends on amount of feed consumed by fish, which is in line with the reports of Sotolu and Faturoti (2010). However, excess protein in a diet may not significantly improve the growth of fish because the excess amino acids are metabolized by oxidative deamination and used to generate energy (Kim and Lee, 2009). Also, results of FCR showed low values which are required for good growth performance at higher inclusions compared to control. This is evident by the fact that Feed conversion efficiency is determined by the relationship between feed intake and growth rate of fish which is fed at the level required to satisfy its maintenance requirements (Millward, 1989). The variations in the FCR obtained in the present study could therefore be related to the dietary *G. kola* inclusion. This is in agreement with Guillaume *et al.* (1999) whose results implied that a high portion of the dietary protein in the low-protein test diet was metabolized to maintain energy. Table 3 show the initial and final haematological values of fish fed graded levels of dietary *G.kola* whole seed. The values obtained in all fish sampled were within the normal ranges for fish (Rey-V'asquez and Guerrero, 2007). The PCV, Hb and RBC values were highest in fish fed diet T2 and least in fish fed diet T5. Aletor and Egberonge (1998) reported that Red Blood Cell count and Pack Cell Volume are mostly affected by dietary treatments. Under normal conditions, the composition of blood is reasonably constant for any particular species (Banerjee *et al.*, 2002). Differences in blood parameters of fish in this study could therefore be ascribed to differences in the dietary inclusions of *G. kola* whole seed powder in the feed. A decrease in the percent of PCV and the erythrocyte count indicates the worsening of a fish state and development of anaemia (Adeyemo, 2007). In the light of the present study, a decrease in these parameters translated into a decrease in haemoglobin concentrations. That is, *G. kola* inclusion increased but did not lead to proliferation of white blood cell. WBC decreased along with increasing dietary *G. kola* inclusion levels. This could be inferred from the non allergic reactions to the test ingredient and microbial load in the fish following reduced level of bacteria in the water (Table 4). This means that, although, more blood cells (leucocytes) might have been produced through Leucocytosis in the bone marrow, it does not have to travel to the blood stream to combat the cause of an infection (Lindsay, 2011). Erythrocyte indices (MCV, MCH and MCHC) are dependent on the values of haematocrit (PCV), erythrocyte count (RBC) and haemoglobin concentrations (Baker and Silverton, 1982). The values of the erythrocyte indices obtained in this study did not show wide variations; however the MCV and MCH showed a decreasing trend from the initial along with the values of PCV, Hb and RBC count, while the

MCHC increased. These values agreed with those set as reference intervals (MCV: 70.14-198, MCH: 14.51-40.59 and MCHC: 17.43-30.31) for *Cichlasoma dimerus* by Rey-V'asquez and Guerrero (2007). The effect of dietary *G. kola* whole seed has been reported as an antimicrobial substance (Anegbeh *et al.*, 2006). Hence, its direct presence in the feed and indirectly within the culture medium (water) provides a non specific immune defense for the fish under culture (Anderson, 1992). The first place where a pathogen (bacteria) can be effectively blocked is the mucosal surface of the skin (Zapata *et al.*, 2006). Thus, because there is presence of lysozyme (immune-reactive molecule) widely distributed in the serum, tissues and mucus of fish which is effective in lysing both Gram-positive and Gram-negative microbes (Yano, 1996), bacterial activity could be significantly few in numbers. This may be responsible for the corresponding decrease in the count of bacteria in the culture medium with increase levels of dietary *G. kola* whole seed showing its antimicrobial effects. This finding is in agreement with that of Garcia *et al*. (2003), whose work revealed that addition of mango leaves as natural antimicrobial substance to aquaculture tank reduced the bacterial load in water. It is important to note that samples containing high levels of coliform density and high viable bacterial counts are ill-fit for human consumption. The feed utilization, growth performance and haematological status of fish were best at 0.25 g/kg inclusion of dietary *Garcinia kola* whole seed powder compared to higher rates of inclusion adopted in this study. The use of *G. kola* whole seeds is therefore recommended for aquaculturists as growth promoter as well as antimicrobial when attempting to produce healthy fish for improved marketability on sustainable basis. However, further studies may be carried out on the use of *G. kola* as an immunostimulants that is transferable for successful hatchery operations.

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