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Growth Performance and Hematological Parameters of Clarias gariepinus Fed Varied Levels of Cola nitida Meal

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Abstract: The effect of various levels of inclusion of *Cola notida* meal on growth performance and hematological parameters of *Clarias gariepinus* was investigated for 56 days. Fingerlings of *C. gariepinus* (6.57g $\pm$ 0.06) were stocked in 10 hapas at the rate of 20 fingerlings per hapas. Iso-nitrogenous and iso-caloric diets were formulated with inclusion levels of white kola meal at 0, 50, 100, 150, and 200gkg^{-1}. The result obtained showed that fish fed 200gkg^{-1} gave the highest weight gain of 17.92g followed by fish fed with 150g/kg white kola meal (16.49g). The least mean weight gain was recorded in the control diet with 0% kola inclusion. The same trend were observed for specific growth rate, Protein efficiency rates and food conversion ratio ($P<0.05$). Hence, to optimize the growth of *Clarias gariepinus, Cola notida* can be included up to 200gkg^{-1}.

Keywords: Antibiotics, growth promoters, African catfish, white kola.

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Introduction

The aquaculture industry in Nigeria is dominated by the culture of the catfish especially *Clarias gariepinus* because of the ready market for catfish and the ease of culturing this fish in captivity, therefore nutritional research aimed at production of least cost feed must target this important aquaculture sphere in Nigeria. It has been reported by many authors that fish feed accounts for at least 60% of the operating cost of an aquaculture enterprise and this has been identified as one of the problems hampering aquaculture development in Africa as it nullify profitability of fish farming thereby making the expansion of farms to higher productivity impossible (Gabriel *et al*., 2007).

The use of antibiotics has become common in the production of animal around the world. The growth-promoting effects of antibiotics are undisputed; in fish farming they are often used to promote growth of fish because of the desire to shorten production cycle by reaching table size quickly. Antibiotics increase the efficiency of animal growth by inhibiting the growth of microbes in the gastrointestinal tract which triggers immune responses in the host (Gaskins *et al.,* 2002). However the collateral and long-term effect are a cause for a heated debate and their banning in the European Union in 2005 (OMAFRA, 2005), hence the need for plant based growth promoters with no consequential side effects. Adediji *et al.,* (2008) said that the use of materials of plant origin in enhancing growth performance in animal is becoming acceptable as it eliminated the obvious effects associated with the use of synthetic drugs. *Kola nitida* nuts are eaten widely in Nigeria and contain active ingredients such as bioflavonoid, an active chemical that is reported to be a plant growth promoter. Braide *et al*., (2003) and Oluyemi *et al*., (2007) reported the level of inclusion in poultry at $7.5g/108g^{-1}$ and in rats at $200\ mg/kg^{-1}$ body weight promotes growth. Dry seed powder of *Kola nitida* may have similar effects on fish (Adedeji *et al*., 2008).

The analysis of blood parameters has proven to be a valuable approach for analysizing the health status of farmed animals as these parameters provides reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in clinical setting. Blood biochemistry parameters can also be used to detect health of fish (De pedro *et al.,* 2005; Bahmani *et al.,* 2001). Hence in the quest of producing feed using locally available growth promoters to substitute anti-biotic growth promoters (AGP), this study was also designed to investigate consequential effect of the inclusion of this plant on hematology so as not to compromise the health of the fish.

Materials and Methods

This study was carried out at the University of Agriculture, Makurdi, Nigeria Fish Farm. Hapas which was made using netting materials with dimensions of $1 \times 1 \times 1m^3$ was used to hold the fish in an earthen pond and were held stationary in the pond by kuralon ropes. The experiment lasted for 56 days. Feedstuffs used for the experimental diets including maize, soybean, fish meal, *Kola nitida*, Vitamins and mineral premixes were purchased from Makurdi, Nigeria. The *Kola nitida* was sliced using knife, sundried and milled to form the meal and stored for use. All other feedstuffs were also milled, packaged and stored for use. The feeds were formulated using Pearson square method and the aggregate feedstuffs were weighed, dry mixed and hot water at 60^0C was added to form a tough dough which was pelleted using a 3mm pelletizer. Five isonitrogenous and isocaloric diet were formulated containing 39% crude protein with kolanut meal inclusion at 0, 50, 100, 150, and $200g/kg^{-1}$.

The experimental fish, *Clarias gariepinus* with mean weight of 6.57g $\pm$ 0.01 were obtained from a homogenous breeding source. Twenty fingerlings were stocked in each hapa for a particular treatment which was triplicated. Fish were fed twice daily at 7a.m. and 5p.m. at 5% of their body weight for 56 days. Fish were weighed weekly and their feed adjusted as appropriate. Also water quality parameters were measured and were found to be at desirable levels. At the end of the experiment, evaluation of utilization of the feed was done using the following parameters:

(i) Specific growth rate (SGR) was calculated using the formula

$$\frac{In \log w2' - In \log w1 x 100}{T}$$

Where *In* = natural log, *w2* = final wt of fish, *w1*= Initial weight of fish

(ii) Feed conversion efficiency (FCE) was computed as

$$\frac{weight\ gain}{Total\ feed\ fed}$$

(iii) Protein Efficiency Ratio (PER)was computed as

$$\frac{wt\ gain}{protein\,\mathrm{int}\,ake}$$

(iv) Apparent net protein utilization was computed as at the end of the experiment as protein gain/protein fed,

Blood sample was collected from the fish in each treatment using 2mm syringe and needle by cutting the fish at caudal end. The collected blood was placed in coded 1.5mL heparinized plastic tubes, stored on ice according to the procedures established by Campbell and Murru (1990), standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (Hb) concentration was estimated as cyanmethemoglobin (Brown, 1980), Packed Cell Volume (PCV) was determined using microhaematocrit method of Snieszko (1960). The Red Blood Cell (RBC) were counted using haemocytometer (Improved Neubauer Weber Scientific Ltd), according to Wintrobe (1978). Also the total White Blood Cell Counts (WBC) was enumerated with an improved Neubauer Haemocytometer using Shaw's diluting fluid (Miale, 1982). Platelet (PLT) count was performed according to Rees and Ecker method (Seivered, 1983). The Red Blood Cell indices that include Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated using the formula mentioned by Dacie and Lewis (2001).

The gross composition of the Diet formulated and the proximate composition of the initial and final carcass of the experimental fish were determined according to methods described by AOAC (1990)

Statistical analysis was carried out using analysis of variance and where there were significant differences LSD were used to separate means. These analyses were performed using the Genstat, discovery edition 4.

Results

Table I shows the inclusion levels of the various ingredients used for the experiment and their proximate composition. Diet 1 (DT1), has no inclusion of kola meal while Diets 2 (DT2), 3 (DT3), 4 (DT4) and 5 (DT5) has 50, 100, 150 and 200 g/kg $^{-1}$kola meal. The proximate composition also shows that the crude protein, ether extract, Ash, crude fibre and moisture determined in the laboratory were not significantly different (P<0.05) across the diet. The proximate composition of the experimental fish before and after the experiment is presented in Table 2. The result showed that crude protein was higher in fish fed DT5 (58.34) compared to other experimental diet, moisture and ether extract however were observed higher in DT4 (7.81) and DT2 (6.86) respectively.

Table 3 shows the growth and nutrient utilization of the fish fed *Kola nitida* based diet. The result showed that the mean initial weight (MIW) of the experimental diets are not statistically significant (P<0.05). However the weight gained (WG) values differed significantly among the five diets (P<0.05). The best weight gain (17.92g) was in DT5 (200gkg^{-1}) compared with the control diet (DT1) which gave weight gain value of 12.98g. The feed conversion Efficiency (FCE) and Specific Growth Rate (SGR) values followed same trend as the weight gain with DT1 giving the best values of these parameters (6.37 and 2.34 respectively). However, the Protein Efficiency Ratio (PER) values for Diet 3 was significantly higher than the other diets (P<0.05) while DT1 had higher value for feed conversion ratio (FCR) (1.57).

The result of Hematological Parameters of *Clarias gariepinus* fingerlings fed diet containing varying levels of *Kola nitida* meal is presented in Table 4. The result showed that values of most blood parameters like white blood cells (WBC), Haemoglobin (Hb), Red blood, PCV, mean cell Haemglobin (MCH) before and after the experiment differed significantly (P<0.05). DT5 gave the highest WBC value of 146 x 10^9 while DT1 WBC value was 123.85 x 10^9. This trend was maintained for Hb, RBC, PCV, MCV and MCH.

Discussion

The objective of fish farming as an enterprise is centered at maximizing profit hence growth responses of fish under culture environment are very important. As at now, there are a lot of antibiotic growth promoters (AGPs) in the market which increase operating cost of aquaculture enterprise aside their effects on human health. Although, earlier studies have shown higher growth of fish fed diet supplemented with antibiotic growth promoters (Turan and Akyurt 2005), the use of white kolanut in this study has reveals the ability of the kolanut meal to replace these AGPs as the four diets with inclusion levels of kolanut meal (50 -200g/ kg^{-1}) gave better growth than the control diet with no kolanut meal inclusion. Differential growth among the control diet and other diets with various inclusion levels of white kolanut meal as observed in the study is definitely not due to protein since isonitrogenous diet was used for the study however, variation in this study is strongly linked to the presence of bioflavonoid in white kolanut meal which stimulates growth in fish as previously reported by Braide *et al*., (1991). Kocour *et al.,* (2005) revealed

Table 1: Inclusion Levels of Feedstuffs and the proximate composition of experiment.

Ingredients	DT1	DT2	DT3	DT4	DT 5
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
Kola meal (g/kg^{-1})	0	50	100	150	200
Proximate composition of diet					
Parameters	DT1	DT2	DT3	DT4	DT5
Moisture	6.87± 0.00	7.06± 0.01	7.12± 0.01	7.83±0.09	7.99±0.01
Ash	7.0±0.01	6.83±0.01	7.21±0.01	7.26±0.01	7.34±0.01
Ether Extract	8.15±0.01	7.91±0.01	8.16±0.01	8.17±0.01	8.30±0.01
Crude fibre	4.777±0.01	4.91±0.02	5.10±0.01	5.20±0.01	5.23±0.01
Crude Protein	39.66±0.01	39.57±0.01	38.99±0.01	39.56±0.01	39.87±0.01
NFE	33.55±0.00	33.72±0.00	33.42±0.01	31.98±0.00	31.27±0.0

Means in the same row with the different superscripts differ significant (P<0.05).

Table 2: Proximate Composition of Experimental Fish before and after the Study

	Initial	DT1	DT2	DT3	DT4	DT5
Moisture	4.09±0.11^{f}	6.88±0.01^{c}	6.50±0.01^{d}	7.11±0.01^{b}	7.81±0.01^{a}	4.87±0.01^{e}
Ash	12.08±0.2	12.02±0.01	11.40±0.10	12.29±0.01	11.65±0.05	11.65±0.05
Ether Extract	5.22±0.01^{e}	5.87±0.01^{d}	6.86±0.01^{a}	6.12±0.01^{c}	6.59±0.01^{b}	6.59±0.02^{b}
Crude fibre	3.87±0.02	4.22±0.01	3.31±0.01	3.48±0.01	3.15±0.02	3.15±0.0
Crude Protein	55.15±0.01^{f}	57.22±0.01^{e}	58.22±0.01^{b}	57.74±0.01^{c}	57.34±0.01^{d}	58.34±0.01^{a}

Means in the same row with the different superscripts differ significant (P<0.05).

Table 3: Assessment of Nutrient Utilization by Fish Fed Kola *Nitida* Based Diet

Parameter	DT1	DT2	DT3	DT4	DT5
MIW (g)	6.57±0.01	6.58±0.01	6.59 ±0.01	6.57±0.01	6.55±0.01
MFW(g)	19.54±0.47^{d}	21.52±1.40^{c}	21.01±0.01^{c}	23.60±0.01^{b}	24.52±0.01^{a}
WG(g)	12.98±0.46	14.94±1.50^{b}	14.42±0.01^{b}	16.49±0.01^{c}	17.92±0.00^{c}
SGR	1.95±0.04^{e}	2.12±0.14^{c}	2.06±0.01^{d}	2.25±0.00^{b}	2.34±0.00^{a}
PER	0.13±0.00^{d}	0.13±0.01^{d}	2.06±0.01^{a}	0.15±0.00^{c}	1.16±0.00^{b}
FCR	1.98±0.19^{a}	1.65±0.47^{c}	1.84±0.15^{b}	1.68±0.01^{c}	1.57±0.19^{d}
FCE	5.07±0.05^{e}	5.51±0.39^{c}	5.44±0.05^{d}	5.95±0.00^{b}	6.37±0.00^{a}

Means in the same row with the different superscripts differ significant (P<0.05).

MIW= Mean Initial Weight
PER = Protein Efficiency Ratio
MFW = Mean Final weight
FCR = Food Conversion Ratio
WG = Weight gain
FCE = Food Conversion Efficiency
SGR = Specific Growth Rate.

that bioflavonoid is plant chemicals with estrogenic activity which promotes growth in fishes. Oluyemi *et al*., (2007) also reported growth improvement in rats at an inclusion level of 200 mgkg^{-1} body weight of *Kola nitida*. The best weight gain was in diets with the highest inclusion level of white kola meal (200 gkg^{-1}). The feed conversion efficiency (FCE) and specific growth rate (SGR) values still follows the same trend as stated above. This result confirms the hypothesis of Sarmah *et al*., (2006) that antibiotics improve feeds nutritional efficiency and according to FAO (2005), this nutritional efficiency is achieved by the action of antibiotics preventing irritation of intestinal lining thereby enhancing the uptake of nutrients from the intestine by thinning of the mucosal layer. Specific growth rate (SGR) of the experimental diet shows that lesser kilogram of feed (range of1.58-1.84) is needed to produce 1kg of flesh as the inclusion of white kola meal increase in the diet compared to the control (1.97). FCR in this study is higher than the reported value observed for *C. gariepinus* fed diet supplemented Aqua booster® and Aquapro® in Dada and Olugbemi's (2013) experiment, however, growth promoting effect of white kola was better than the mixture of *Lactobacillus* and *Bifidobacterium* species used to feed *C. gariepinus* in Ayoola *et al*., (2013) experiment. The difference in the FCRs may be due to difference especially in the type and origin of the antibiotic growth promoter, level of inclusions of the growth promoter, age and developmental stage of the experimental fish and anti-nutritional composition of other feed stuff used to formulate the different diet.

WHO (2006), stated that antibiotics inhibit or kill beneficial microbiota in the gastrointestinal (GI) ecosystem hence at higher level of inclusion tend to cause decrease in growth, similarly Lawal *et al.,* (2012) reported that at minimal concentration of Oxytetraxycline (OTC) inclusion in the diet of *C. gariepinus* (0.2%/100g feed), the average final weight gain of the fish was greater than the weight at higher concentration and explained it could be due to the fact that at higher concentration, antibiotics that eliminate disease-causing bacteria could also eliminate the beneficial ones, thereby leading to reduction in nutrient utilization in such animal and concomitantly reduced growth. This was however not observed in the present study with a high inclusion level of 200gKg^{-1} instead growth was better as the level of inclusions increases, these differences is probably due to plant origin of the white kola as compared the synthetic nature of OTC. This study also recorded higher value of body protein in all the experimental treatments compared with the body protein of fish before feeding trial but was not statistically significant among the fish after the experiment (P<0.05), this is an indication that the experimental diets were well utilized.

Osuigwe *et al.,* (2005) reported that the hematological parameters of fish are affected by a range of factor which includes size, age, physiological status, environmental conditions and dietary regime (e.g. quality and quantity of ingredients, protein sources, vitamins and probiotics). Although the hematological parameter varied significantly in this study they were still within the normal range for the species. The white blood cell values increased significantly as inclusion levels of white kolanut meal increased and the values were found to differ significant when compared with the control diet (P<0.05). An increase in White Blood Cells (WBC) and lymphocyte count (lymph) according to Oyawoye and Ogunkunle (1998) is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system. However in this study increased WBC

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Table 4: Hematological Parameters of *Clarias gariepinus* Fed Kola *nitida* Meal.

Blood Parameter	Initial	DT1	DT2	DT3	DT4	DT5
WBC ($x10^9$/L	11.10±0.201^d	123.85±0.25^c	136.55 ±0.35^b	134.60±0.80^b	144.10±0.70^a	146.00±0.20a
Hb(g/dl)	1.05±0.15^d	6.852±0.25^c	7.10±0.30^b	6.65±0.35^c	7.20±0.30^b	7.40±0.20a
RBC($x10^{12}$L)	0.23±0.02^e	1.28±0.03^d	1.40±0.06^c	1.42±0.03^c	1.65±0.15^a	1.49±0.03^b
PCV(%)	2.85±0.02^e	18.05±0.25^d	19.00±0.40^c	20.20±0.40^b	21.45±0.25^a	21.20±0.03^a
MCV( )	128.30±1.10^e	146.10±0.70^b	139.70±0.40^d	149.35±0.85^a	142.30±0.80^c	146.80±1.00^b
MCH(pg)	57.70±0.60^a	53.10±0.30^b	51.40±0.70^c	49.60±0.70^d	50.65±0.65^{cd}	50.25±0.95^{cd}
MCHC (g/dl)	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^c
RDW- (%)	13.05±0.35^a	12.35±0.25^b	11.00±0.20^c	11.75±0.25^c	9.15±0.35^d	10.10±0.40^{cd}
RDW-SD(FL)	76.85±0.45^a	64.60±0.40^c	57.30±0.40^d	71.90±0.30^b	57.60±0.80^d	66.85±1.05^c
PLT(x10 g/L)	11.00±1.00^f	30.50±0.50^a	18.00±1.00^d	17.50±0.50^e	22.50±1.80^c	26.00±1.00^b
MPV (F1)	0.00±0.0^f	7.00±0.20^d	7.90±0.30^a	7.15±0.50^c	6.60±0.30^e	7.25±0.35^b
PDW	0.00±0.0^e	17.20±0.10^c	18.00±0.10^a	16.95±0.25^d	17.15±0.25^c	17.55±0.5^b
PCT	01.00±0.0^a	0.02.75±0.00^b	0.02±0.00^b	0.02±0.00^b	0.02±0.00^b	0.02±0.00^b

Means in the same row with the different superscripts differ significant ($P<0.05$).

WBC = White blood cells
MCV = Mean cell volume
HGB = Haemaglobin
MCH = Mean cell Haemoglobin
RBC = Red Blood Cell Count
MCHC = Mean cell Haemoglobin concentration
PCV = Packed cell volume
RDW-CV = Red blood width cell volume
RDW-SD = Red blood cell with sedimentation
PLT = platelet count
PDW = platelet Distribution width
MPV = Mean mullet volume
PCT = platelet Grit.

with increase white kola inclusion is suggestive that white kolanut meal provides antibiotic properties that probably can make the fish less vulnerable to disease. The increase in Haemoglobin and Red Blood Cells value can enhance the oxygen carrying capacity in fish blood. This result is similar to the reported work of Dada and Ikuenenawo (2009).

Conclusion

Optimal growth and weight gain in this present study was obtained at an inclusion level of 200g kg^{-1} of white kolanut meal. It is concluded that white kolanut meal can be used a growth promoting agent in the diet of *Clarias gariepinus* with better haemoglobin variables up to 200gkg-1, however futher study should be conducted to investigate if at higher inclusion levels, white kolanut meal could impair growth or lower immune responses in *Clarias gariepinus*.

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