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Effect of *Terminalia catappa* on Growth and Heamatology of *Clarias gariepinus* Juveniles

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Abstract: The growth and haematological responses of the African catfish (*Clarias gariepinus* Burchell1822) to graded levels of *Terminalia catappa* (African almond) seed meal (TSM) was investigated. Five isonitrogenous and isoenergetic diets (40% crude protein) containing 0, 25, 50, 75 and 100% TSM were formulated. *Clarias gariepinus* juveniles (30.7±0.75) stocked at 10 fish/50 L plastic troughs were fedtwice daily for 112 days. Temperature, pH and dissolved oxygen were the determined water quality variables. Data obtained were analyzed using Analysis of Variance (ANOVA), correlation and LSD and was separated at 5% probability level. The highest Mean Weight Gain (MWG) was at 50 and 0% and did not follow a specific order of increase. Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), Protein Intake (PI) were significantly higher ($p<0.05$) at 0 and 50% TSM inclusion. Percentage Survival Rate (% SR) was unrelated to the treatment diets. There were slight differences in the values of haematological parameters of the *C. gariepinus* at the different inclusions of TSM. Physicochemical variables in the experiment were within levels recommended for the culture of fresh water fish.

Key words: Terminalia seed meal, haematology, *Clarias gariepinus*, PER, PI

INTRODUCTION

Protein requirements are fundamental in fish diet and as such the requirement need to be met in the right quality and quantity in order to obtain the desired growth in cultured fish species. High quality protein according to Fagbenro and Adeparusi (2003) can be used to maintain an active metabolism in fish. Fish protein is among the most quality sources of animal protein but are often the major contributor to the high fish feed cost and as such there is the need to search for alternative protein sources to replace fishmeal in fish feed. Several studies have been carried out using protein of plant origin in fish diet (Alegbeleye *et al*., 2001; Keembiychetty and de Silva, 1993; Osuigwe *et al*., 2005; Sotolu, 2008; Sotolu and Faturoti 2008). According to Regost *et al*. (1999) and Fournier *et al*. (2004), a mixture of plant protein sources is however more appropriate than the incorporation of a single plant source because of improved amino acids profile. Interest in the utilization of *Terminalia catappa* as source of raw materials for feed ration formulation is growing among nutritionists especially because of its very high protein content (19-22%) and oil (50-52%). *T. catappa* is a large, deciduous tree with smooth grey bark and whorled branches that form a canopy and is found in tropical and subtropical regions. The timber is moderately easy to saw and work, polishes well has wide applications and is classed as a good constructional timber (Inbaraj and Sulochana, 2006). The fruit is large (2-3 inches), edible, fleshy, green (unripe) and yellow or red (when ripe). The fruit has a husk (34.08%), a porous and fibrous pericarp (8.97%) and an exocarp which is relatively thin and smooth while the hard endocarp (46.63%) enclosed an edible kernel (10.32%) (Untwal and Kondawar, 2006; Inbaraj and Sulochana, 2006; Muhammad and Oloyede, 2004). The leaves of *T. catappa* have been reported to have medicinal values (Lin *et al*., 1999; Chen *et al*., 2000). The fruit is edible, fleshy, green (unripe) and yellow or red (when ripe). The exocarp is relatively thin and smooth and the endocarp is hard. When cracked, a kernel is obtained and this can be consumed as well. The kernel of *T. catappa* according to Ratnasooriya and Dharmasiri (2000) has aphrodisiac activity and may be useful in the treatment of certain form of sexual inadequacies especially premature ejaculation.

This present study investigates the growth and haematological responses of the African catfish (*C. gariepinus* Burchell 1882) juveniles to *Terminalia catappa* Seed Meal (TSM) used as a partial

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substitute for fish meal in compounded fish diet. The effect of the formulated diet on physico-chemical parameters of the culture environment was also investigated.

MATERIALS AND METHODS

Collection and processing of *Terminalia catappa*: Large collection of specimen of *T. catappa* (African almond) was obtained towards the end of the rainy season within Njala University Campus. Samples were first sun-dried for a period of 1 month and thereafter the shell (endocarp) was crushed using hammer and stone to remove the fleshy seeds. The dried seeds were roasted in a hot pan filled with clean river sand. The roasted seeds of *T. catappa* were further dried before finally grinding preparatory to apportioning and blending.

Collection and preparation of feedstuff: The ingredients used along with *T. catappa* in this study include fishmeal (FM 62% CP), yellow maize, soya beans, vitamin premix (methionine) and starch as binder. The feedstuffs were bought at a nearby market. The soya bean was pre-processed by roasting before utilization to rid it of growth inhibitors. All ingredients were finely grinded, blended with vitamin premix and starch and thereafter extruded using a meat mincer. *Clarias gariepinus* juveniles used for this study were obtained from River Tia (Njala axis of Moyamba district). The fish samples were acclimated in a plastic trough for 3 days. After acclimation ten juveniles of *C. gariepinus* mean weight 30.7±0.75 were randomly selected and distributed into the five treatments. The fish weight was determined with the aid of Soehnle Electronic Kitchen Balance (Model Art-Nr. 65055). Each treatment consists of three replicates of 50 L capacity plastic trough and the set-up was a completely flow-through set-up. A constant inflow and out flow of 2.5 L water volume/minute was maintained throughout the study. Each of the treatment was assigned to one of the experimental diets containing different levels of *T. catappa* seed meal 0% (control), 25, 50, 75 and 100%, respectively. Fish were fed twice daily (morning and evening) for 124 days between 9.00-09.30 h and 19.00-19.30 h at 5% body weight with the daily ration divided into two. Clearing of faecal materials, cleaning of troughs and changing of water were done every 3 days while fish sampling was done bi-weekly. Mean weight changes were determined with Soehenle Kitchen Electronic Balance (Model Art-Nr 65055). Fish mortality was recorded daily and is accounted for in adjustment of feeding and mean weight measure. Water quality variables determined for this study were temperature (T°C), Dissolved Oxygen (DO) and pH and were determined bi-weekly using Pometer pH-009 (III) Pen type meter, Hanna portable instrument model H198204 No. 227075. Dissolved oxygen was determined after Boyd (1979) titrimetrically and was proved with Jenwey 970 DO_2 m (No. 970-201).

Feed utilization: Data were collected weekly on fish growth performance and nutrient utilization by determining Mean Weight Gain (MWG), Feed Intake (FI), Specific Growth Rate (SGR), Percentage Survival Rate (% SR), Protein Efficiency Ratio (PER), Feed Conversion Ratio (FCR) and Protein Intake (PI) using recommended procedures.

Haematological study: After 124 days haematological studies was carried out on the fish samples. Fish haematology was carried out using standard procedures (Svobodova *et al*., 1991; Wagner *et al*., 1997). Initial blood samples were collected prior to feeding trial that is 0th day while the final blood samples were collected from the fish in triplicates on the last day of the experiment (124th day). The fishes were taken out individually using a small hand net and placed belly upward on a table. Blood samples of about 4 mm were collected from the caudal peduncle with the aid of a 2 cm^3 plastic hypodermic syringe. The blood sample was dispensed into Ethylene Di-Amine Tetra Acetic Acid (EDTA) an anti-coagulant placed in a plastic sample bottles. The use of plastic syringe is a necessary precaution with fish blood because contact with glass result in decreased coagulation time. The haematological indices of Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) were calculated using the total Red Blood Cell count (RBC), Haemoglobin concentration (Hb) and Haematocrit (HCT).

Statistical analysis: Data collected were subjected to Analysis of Variance (ANOVA) and correlation analysis using the SPSS package Version 10 and significant mean differences were separated at 5% probability level using Duncan multiple range test.

RESULTS AND DISCUSSION

Diet ingredients, proximate composition and mineral contents of formulated feed used for the experiment are presented in Table 1. Chemical components determined for the diets include crude protein, crude fat, crude fiber, ash and dry matter while the mineral contents of the diets are Na, K, Ca, P, Mg (determined as %), Fe, Zn and Cu (determined as mg/kg). The proximate analysis of

Table 1: Diet formulation, proximate composition and mineral contents of *Terminalia catappa* seed meal and diet

Diet composition	Diet 1 (0%)	Diet 2 (25%)	Diet 3 (50%)	Diet 4 (75%)	Diet 5 (100%)
Fish meal	14.70	11.10	7.35	3.65	0.00
T. catappa meal	0.00	3.65	7.35	11.10	14.70
Yellow maize	30.00	30.00	30.00	30.00	30.00
Soybean meal	54.80	54.80	54.80	54.80	54.80
Vitamin	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Chemical composition (%) on a dry matter basis					
Crude protein	40.68	38.55	39.01	39.01	39.79
Crude fat	5.26	4.97	4.86	4.79	4.83
Crude fibre	4.37	4.89	4.97	5.12	5.39
Ash	15.47	14.69	14.78	14.88	14.97
Dry matter	91.76	90.72	90.78	90.86	91.23
Mineral content (% and mg/kg)					
Na (%)	0.57	0.64	0.73	0.79	0.88
K (%)	1.37	1.41	1.51	1.68	1.79
Ca (%)	1.18	1.23	1.28	1.35	1.41
P	1.87	1.97	2.04	2.16	2.27
Mg (%)	0.42	0.61	0.74	0.83	0.96
Fe (mg kg^{-1})	314.20	296.20	318.70	326.50	343.70
Zn (mg kg^{-1})	41.20	36.80	44.30	46.80	49.20
Cu (mg kg^{-1})	7.80	1.30	15.40	16.70	17.30

Table 2: Proximate composition of processed unmixed TSM

Parameters determined (%)	Percentage composition (%)
Crude protein	24.50
Crude fat	28.00
Ether extract	36.00
Ash	6.00
Dry matter	2.34

Table 3: Growth and nutrient utilization of experimental fish fed varying inclusion of TSM diets and mean water quality parameters

	Percentage inclusion level of TSM seed meal				
Parameters	0%	25%	50%	75%	100%
Culture period (days)	112.00	112.00	112.00	112.00	112.00
Initial mean weight (g)	30.00	30.50	31.20	31.70	30.00
Final mean weight (g)	58.30	42.10	61.70	51.90	36.20
MWG (g)	28.30	11.60	30.50	20.20	6.20
FI (g)/Fish	36.40	30.50	36.40	30.50	27.50
SGR	0.26	0.13	0.26	0.19	0.07
PER	1.94	0.95	2.09	1.66	0.56
FCR	1.27	2.63	1.19	1.51	4.44
PI	14.56	12.20	14.56	12.20	11.00
Survival rate (%)	70.00	67.00	60.00	87.00	73.00
pH	7.50	7.60	7.70	7.70	7.30
Temperature (°C)	29.00	27.00	29.00	28.00	27.00
Oxygen (mg L^{-1})	5.00	5.00	5.10	5.00	5.10

Terminalia catappa Seed Meal (TSM) is presented in Table 2. Growth and nutrient parameters of the fish fed with graded *Terminalia catappa* seed meal diets and mean water quality parameters of the study are shown in Table 3. The results of the feeding trials showed that the experimental fish responded to all the diets irrespective of their composition. The fish effectively utilized the *T. catappa* seed meal diet for growth.

Growth and nutrients utilization was higher at 50 and 75% inclusion of TSM, respectively and this compared favourably with the control diet (0% TSM). According to Muhammad and Oloyede (2004), the seed of *T. catappa* is very rich in protein (19-22%) and oil (50-52%). *Terminalia catappa* has been found to have multi use properties. The leaf, bark and fruits are used in treating dysentery, rheumatism, cough and asthma. The fruit is also helpful in the treatment of leprosy and headaches and the leaves are specifically used in getting rid of intestinal parasites, treatment of eye problems, wounds and liver problems (Kritikar and Basu, 1991; Corner, 1997; Anonymous, 1976). According to Nagappa *et al.* (2003) and Moody *et al.* (2003), the fruit and the leaves have been scientifically proven to have anti-diabeticand anti sickling properties. The chloroform and methanol extracts of the bark and root displayed strong antimicrobial activities (Pawar and Pal, 2002). The amino acid and mineral profile of this seed has equally been documented (Osagie, 1998). Feed intake which is a determinant of fish performance did not decrease consistently with the *T. catappa* seed meal inclusion as recorded by several researchers in experiment using other plant protein materials. The order of feed intake was 0% and 50>25% and 75>100%.

The importance of feed intake by fish as a determinant of fish performance has been strongly emphasized (Preston and Leng, 1987; Faturoti, 1989; Pillay, 1990). Anderson *et al.* (1984) and Keembiychetty and de Silva (1993) pointed out the possibility of protein sparing effects by other nutrients in a feed that is as more energy was supplied for metabolism through other nutrients, more protein is available for fish growth and tissue development. All diets used in the trial produced higher values of fish carcass protein and lipid than initial values however with marginal differences among the treatments indicating different utilization of the diets. The relatively high values of crude protein could be viewed alongside the research by Alegbeleye *et al.* (2001) who reported that effective utilization of Bambara groundnut at varying inclusion was responsible for variation in Heteroclarias carcass protein and lipid. Crude fiber was not found in the fish carcass composition and this has been associated with effective utilization of experimental diets (Sotolu, 2008). The physico-chemical variables determined for the study were within the values recommended for the culture of fresh water species (Omitoyin, 2007).

Carcass and haematological composition of experimental fish fed TSM are shown in Table 4. Haematological parameters determined include PVC, Hb, RBC, WBC, MCV, MCHC, Lym and Neut. The result of the study showed a slight decrease in the values of haematological parameters of the experimental fish at the different level of TSM inclusion. Haemoglobin and Parked Cell Volume (PCV) have been suggested as tests that is

Table 4: Carcass and haematological composition of experimental fish fed TSM diet for 112 days

Parameters (%)	Initial values of fish	Final values at different TSM inclusion rates				
		0%	25%	50%	75%	100%
Crude protein	51.68	52.7	52.6	53.8	52.2	51
Crude fat	4.67	1	1	1	2	1
Ether extract	1.06	23	22.3	25.7	23	27.7
Ash	13.76	17.3	18	14	18	14.7
Dry matter	92.81	2.22	2.28	2.22	2.22	2.22
Haematocrit						
PVC (%)	8	6.3±2.52	9.3±2.09	7.3±4.04	5.3±2.52	6.3±2.09
Hb (g/100 mL)	2.7	2.0±0.73	3.0±0.82	2.4±1.42	1.7±0.91	2.0±0.75
RBC ($\times10^6$/mL)	2.02	0.75±0.48	0.94±0.78	0.94±0.80	1.17±0.78	1.43±0.86
WBC ($\times10^3$/mL)	0.8	3.6±2.36	3.6±2.62	2.4±1.78	3.3±2.32	2.4±1.61
MCV (fl)	39	33±4.36	81.3±35.23	100.0±40.58	65.3±46.31	71.0±70.66
MCH (pg)	13	44.7±44.41	26.0±12.77	31.3±11.01	21.7±15.96	21.3±15.82
MCHC (%)	3	3.0±0.00	3.0±0.00	3.0±0.00	3.0±0.00	3.0±0.00
Lym (%)	40	26.0±5.29	25.3±4.51	26.0±6.00	27.0±6.24	27.0±7.00
Neut (%)	59	73.0±4.58	73.7±5.13	72.3±5.03	71.7±5.86	72.3±6.50

can be carried out on routine basis in fish hatchery as a check on fish health status (Klinger *et al*., 1996). Haematological characteristics have been widely used in clinical diagnosis of diseases and pathologies of human and domestic animals. The applications of haematological techniques have proved valuable for fishery biologist in assessing the health of the fish (Fagbenro and Adeparusi, 2003) and monitoring stress response (Soivio and Oikari, 1976). Some of the haematologicalparameters measured in this study were slightly low and this could be associated the condition under which the fish were kept. In a stress situation, erythrocyte count has been known to be the first parameter to be affected. Increase in WBC (leucopomia) as observed in the fish fed *T. catappa* seed meal diet is attributed to increase in the production of leucocytes in the haematopoietic tissue of the kidney and perhaps the spleen. According to Wedmemayer and Wood (1974), the primary consequences of observed changes in leucocyte count in stressed fish are the expression of the immune system and increased susceptibility to disease.

CONCLUSION

In conclusion, *C. gariepinus* performed better at 50% TSM inclusion and as such it is recommended as the best level of inclusion.

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