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Research Article

Growth and Haematological Responses of the African Catfish (*Clarias gariepinus* burchell 1822) Juveniles to Graded Levels of *Terminalia catappa* (African Almond) Seed Meal Based-Diets

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Received: July 20, 2015; **Published:** August 20, 2015

Abstract

Fish nutritionists worldwide are concerned with the formulation of least-cost diets that supplies the nutrients needed by fish for growth and production. Consequently the search for alternative protein source as a substitute for fish meal in fish diet is intensified since fishmeal constitute a significant proportion of the costs incurred in fish feed production. The present study was therefore carried out to determine the effect of graded levels of *Terminalia catappa* (African almond) seed meal (TSM) on growth and haematological responses of the African catfish (*Clarias gariepinus* Burchell 1822). 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 litre 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) was 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. In conclusion, *C. gariepinus* performed better at 50% TSM inclusion and as such it is recommended as the best level of inclusion.

Keywords: *Terminalia Seed Meal; haematology; Clarias gariepinus*

Introduction

Clarias gariepinus is considered to be one of the most important tropical fresh water species for aquaculture whose potential as been hardy and tolerant to a wide range of environmental conditions have been documented [1,2]. They are also known to be omnivorous in their food habits and are disposed to accepting unconventional dietary feeds. Conventional fish feeds are usually very expensive because of the high cost of fishmeal which constitutes more than 50% of total cost of feed production. Unconventional feed stuff sourcing especially of plant origin is meant to explore and utilize other resources which are cheaper and less competitive [3,4]. 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 [5] 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 [6-11]. According to Regost., *et al*. [12]; Fournier., *et al*. [13] 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 [14]. 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%) [14-16]. The leaves of *T. catappa* have been reported to have medicinal values [17,18]. 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 Ratnassooriya., *et al*. [19] 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 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 one 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 three days. After acclimation ten (10) 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 50L capacity plastic trough and the set-up was a completely flow-through set-up. A constant inflow and out flow of 2.5L 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 9.00h - 9.30h and 19.00 hr - 19.30 hr 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 three days while fish sampling was done bi-weekly. Mean weight changes were determined with Soehnle 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 meter (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 [20,21]. Initial blood samples were collected prior to feeding trial that is 0^{th} day while the final blood samples were collected from the fish in triplicates on the last day of the experiment (124^{th} day). The fishes were taken out individually using a small hand net, and placed belly upward on a table. Blood samples of about 4 millimeters 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

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 *Terminalia catappa* seed meal (TSM) is presented in Table 2. The mineral contents of the experimental diets increase with increasing addition of *T. catappa* seed meal.

Growth and nutrient parameters of the fish fed with graded *T. catappa* seed meal diets and mean water quality parameters of the study are shown in Table 3. Oxygen, Temperature and pH determined for the study were within the recommended range for the culture of African Catfish.

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). Feed intake which is a determinant of fish performance did not decrease consistently with the *T. catappa* seed meal inclusion as recorded by several authors in experiment using other plant protein materials. The order of feed intake was 0% and 50% > 25% and 75% > 100%.

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.

Diet composition	Diet 1 (0%)	Diet 2 (25%)	Diet 3 (50%)	Diet 4 (75%)	Diet 5 (100%)
Fish meal	14.7	11.1	7.35	3.65	0
T. catappa meal	0	3.65	7.35	11.1	14.7
Yellow maize	30	30	30	30	30
Soybean meal	54.8	54.8	54.8	54.8	54.8
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)	314.2	296.2	318.7	326.5	343.7
Zn (mg/kg)	41.2	36.8	44.3	46.8	49.2
Cu (mg/kg)	7.8	1.3	15.4	16.7	17.3

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

Parameters determined (%)	Percentage composition (%)
Crude protein	24.5
Crude fat	28.0
Ether extract	36.0
Ash	6.0
Dry matter	2.34

Table 2: *Proximate composition of processed unmixed TSM.*

Parameters	Percentage inclusion level of TSM seed meal (%)				
	0%	25%	50%	75%	100%
Culture period (Days)	112	112	112	112	112
Initial mean weight (g)	30	30.5	31.2	31.7	30
Final mean weight (g)	58.3	42.1	61.7	51.9	36.2
MWG (g)	28.3	11.6	30.5	20.2	6.20
FI (g)/fish	36.4	30.5	36.4	30.5	27.5
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	12.2	14.56	12.2	11.0
Survival rate (%)	70	67	60	87	73
pH	7.5	7.6	7.7	7.7	7.3
Temperature (°C)	29	27	29	28	27
Oxygen (mg/L)	5.0	5.0	5.1	5.0	5.1

Table 3: *Growth and nutrient utilization of experimental fish fed varying inclusion of TSM diets and mean water quality parameters.*
MWG: Mean Weight Gain, FI: Feed Intake, SGR: Specific Growth Rate, PER: Protein Efficiency Ratio, FCR: Food Conversion Ratio, PI: Protein Intake

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	51.0
Crude fat	4.67	1.0	1.0	1.0	2.0	1.0
Ether extract	1.06	23.0	22.3	25.7	23.0	27.7
Ash	13.76	17.3	18.0	14.0	18.0	14.7
Dry matter	92.81	2.22	2.28	2.22	2.22	2.22
Haematocrit						
PVC (%)	0.8	6.3 ± 2.52	9.3 ± 2.09	7.3 ± 4.04	5.3 ± 2.52	6.3 ± 2.09
Hb (gm/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 (x 10^6/ml)	2.02	0.75 ± 0.48	0.94 ± 0.78	0.94 ± 0.80	1.17 ± 0.78	1.43 ± 0.86
Wbc (x 10^3/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 (%)	03	3.0 ± 0.00	3.0 ± 0.00	3.0 ± 0.00	3.0 ± 0.00	3.0 ± 0.00

Table 4: *Carcass and haematological composition of experimental fish fed TSM diet for 112 days.*
PVC: Parked cell volume; Hb: Haemoglobin; Rbc: Red blood cell; Wbc: Red blood cell (Leucopomia); MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration

Discussion

Clarias gariepinus used for the trial responded favourably to all the experimental diets, and are found also to effectively utilize them 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). 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 [22-24]. Anderson., *et al*. [25]; Keembiyehetty and De-Silva [8] 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 work of Alegbeleye., *et al*. [7] 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 [10].

According to Muhammad and Oloyede [16] 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 [26-28]. According to Nagappa [29] and Moody [30] the fruit and the leaves have been scientifically proven to have anti-diabetic and anti - sickling properties. The chloroform and methanol extracts of the bark and root displayed strong antimicrobial activities [31]. The amino acid and mineral profile of this seed has equally been documented [32].

The results obtained showed that mineral contents of the experimental diets increase with increasing addition of *T. catappa* seed meal. Potassium according to Malik [33] is an essential nutrient and has an important role is the synthesis of amino acids and proteins while magnesium is essential for enzymatic reactions and also regulate the acid - alkaline balance in the body [34]. Calcium besides being important in bone formation together with magnesium aids in photosynthesis and carbohydrate metabolism.

There was a slight decrease in the values of haematological parameters of the *Clarias gariepinus* fed with *T. catappa* seed meal compared to the control diet. This corroborates the report of Joshi., *et al*. [35] on the effect of toxicant on blood parameters in freshwater tallest fish. The increase observed in the haematological parameters of fish fed with control diet was also in consonance with the findings of Joshi., *et al*. [36] that survival of fish can be correlated with increase in antibody production which helps in their survival and recovery. The decrease in blood parameters of fish fed *T. catappa* seed meal is indicative of blood loss and are lower than those reported in the literature for the African catfish [37,38].

Haemoglobin and parked cell volume (PCV) have been suggested as tests that can be carried out on routine basis in fish hatchery as a check on fish health status [39]. 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 [5] and monitoring stress response [40]. Some of the haematological parameters measured in this study were slightly low and this could be associated with the condition under which the fish were kept.

Decrease in RBC and haemoglobin values in *Clarias gariepinus* fed *T. catappa* seed meal in this study is similar to the observations of Joshi., *et al*. [41]; Gill and Pant [42] in *Clarias batrachus* exposed to different toxicants. A decrease in haematological parameters has been recorded for *Oreochromis mossambrius* exposed to copper and zinc [43]. Inability of fish to meet its protein requirement might inhibit erythrocyte production or increase its rate of destruction and with low haemoglobin level come a decrease in the ability of the fish to meet occasional dietary demands.

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 Wademeyer and Wood [44] the primary consequences of observed changes in leucocyte count in stressed fish are the expression of the immune system and increased susceptibility to disease.

In conclusion the study clearly revealed that inclusion of TSM at 50% gives better growth performance and as such substituting it with fish meal in fish diet at that rate showed great promise with potential to attenuate the cost of fish feed. *Terminalia catappa* seed meal contains very important nutrients and minerals that enhance biochemical activities of vertebrate organs. The experimental diet did not cause water quality issues and also with minimal effect on the haematology of the trial fish. It is however recommended that the seed be defatted before using it in fish feed formulation. Defatting the seeds of *T. catappa* would probably spare more protein and reduce the excess fat that has the tendency of polluting the culture media [45].

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