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Evaluation of nutritional and toxicological effects of *Treculia africana* (Decne.) seed flour-supplemented diets on *Clarias gariepinus* (African catfish) fingerlings

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Abstract
A feeding trial was conducted to investigate the nutritional and toxicological effects of full fat *Treculia africana* seed flour- supplemented diets on growth performance, nutrient utilization, survival, histopathology and blood parameters of *Clarias gariepinus* juveniles. One hundred and fifty fingerlings of *C. gariepinus* of average weight of 2.55.96 ± 0.13 g were stocked and fed at 3 % body weight per day for 42 days. Five experimental diets containing 35 % crude protein in which groundnut cake (GNC) meal was replaced by full fat *T. africana* seed flour meal at 0 % (TAF0), 15 % (TAF15), 30 % (TAF30), 45 % (TAF45) and 60 % (TAF60) were formulated and compounded. Each treatment had three replicates using 10 catfish per 40-litre capacity plastic bowl. There were no significant differences (P>0.05) in protein efficiency rate and feed intake among the control and experimental fish. Packed cell volume was highest in fish on TAF45 (45 %) and was higher than fish fed TAF60 (60 %). Histopathology result showed no visible lesions in some of the tissues of fish fed with the experimental diets. Full fat *T. africana* seed flour might not be toxic to catfish; rather it seemed to be a nutritional source.
Keywords: *T. africana, C. gariepinus*, blood parameters, nutrient utilization, toxicology

1. Introduction

Treculia africana is found in many areas including parts of West and Central Africa and belongs to the family Moraceae (Osabor *et al.*, 2009; Osujo and Owei, 2010). The seed of *T. africana* has been described as an underutilized plant resource with a potential for use in novel foods and industry (Shittu and Raji, 2011) and pastries (Onyekwelu and Fayose, 2007). *T, africana* is a multipurpose tree crop and primarily useful for its nutritious, starchy fruit. It is the main staple crop in many areas of the Pacific and supplements other staple foods for home consumption elsewhere. It generally has little commercial use but is becoming an export crop in the Caribbean. It originated in the western Pacific, with New Guinea and associated islands such as the Bismarck Archipelago being the centre of diversity for wild seeded forms of *Artocarpus altilis*. Seedless *T. africana* is widely distributed throughout the tropical world (Osujo and Owei, 2010). It is also widely cultivated in South-West states of Nigeria with present level of production in the South-Western Nigeria estimated at about 10 million tons dry weight per year with potentials to exceed 100 million tons every year (Adewusi *et al*., 1995). The chemical composition and mineral element content of *T. africana* seeds and seed oil has already been reported by Ajayi (2008).

Aquaculture refers to the breeding, rearing and harvesting of plants and animals in all types of water environments. Early catfish producers depended primarily on natural pond organisms to provide nutrients that are essential for fish growth. Fish production was often enhanced by the addition of fertilizers to pond water to stimulate the growth of natural food organisms. Prepared feeds, mixtures of feedstuffs processed into various forms, were used to supplement natural productivity. Supplemental feeds were largely steam-pelleted feeds that provided protein and energy, but were generally deficient in micronutrients such as vitamins, minerals, and essential fatty acids. Requirements for some micronutrients were met from those present in feed ingredients and/or natural foods (Li, 1996). There is an ongoing effort to produce fish feeds that meet dietary requirements at reasonable cost (El Dakar *et al.*, 2008; Malik, 2009). The utilization of nonconventional feedstuffs of plant origin has been limited owing to the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponegin, momosine, cyanoglycosides, linamarin to mention a few despite their nutrient values and low cost implications (Sogbesan *et al*., 2006). These antinutritional factors negate growth and other physiological activities at higher inclusion levels (Oresegun and Alegbeleye, 2001).

Though *T. africana* is useful as food for man especially as “ukpa” in Ibo speaking areas of Nigeria, little information is available concerning its use in fish nutrition. This study was therefore carried out to evaluate the toxicological effects, if any, and possible usefulness of *T. africana* seed flour in the diets of *C. gariepinus* as partial replacement for groundnut cake. This is in continuation of previous work on seed flour/cake and their nutritional/industrial applications in fish/animal feeding (Ajayi *et al*., 2012; Olaifa *et al*., 2012).

2. Materials and methods

2.1 Collection and preparation of *Treculia africana* fruits and seeds

Mature seeded *T. africana* fruits were collected from the Botanical Garden of the University of Ibadan, Oyo State, Nigeria.The fruits were stacked in a heap and allowed to ferment for some days. The fermented fruits were macerated and washed in running water to remove the slimy and jelly-like flesh. Cleaned seeds were air-dried, dehulled and ground to fine flour with pestle and mortar before use in formulating the experimental rations.

2.2 Experimental conditions for the fish

One hundred and fifty fingerlings of *Clarias gariepinus* (mean body weight: 2.45g ± 0.03) were obtained from the Teaching and Research Farm of Department of Aquaculture and Fisheries Management, University of Ibadan, Oyo State, Nigeria. They were kept in fifteen circular 40-litre plastic bowls for seven days while feeding with an imported commercial feed in order to acclimatize them to the new environment. After acclimatization, the fish were divided into groups of 10 fish per bowl to the 15 circular bowls (three bowls of replicates per treatment). Each bowl was filled with 35 liters of de-chlorinated water and covered using synthetic nets to prevent the fish from jumping out of the bowl and protect them from foreign materials and predators. The quality of the water used during the study was monitored weekly for temperature, dissolved oxygen and pH.

2.3 Fish feed formulation and preparation

The feed ingredients purchased for this study included fishmeal, soybean meal, maize, wheat offal, vitamin/mineral premix, millet, starch, dicalcium phosphate, salt, vegetable oil and groundnut cake (Table 1). These ingredients were mixed together to produce a 35 % crude protein diet containing *T. africana* as: 0 (control), 15, 30, 45 and 60 % and serving as partial replacement of groundnut cake in the diets representing dietary treatments 1 – 5. Each diet mixture was treated separately, extruded through a 1/4mm die mincer of Hobart A-200T pelleting machine (Hobart GmbH, Rben-Bosch, Offenburg, Germany). The diets were sun-dried, broken mechanically into suitable sizes for the fish, packaged in labelled polythene bags and stored before use.

2.4 Feeding of fish

Fish were fed by hand twice daily at 3 % body weight with the required portion for each day divided into 2 equal parts and presented twice daily. The mean weights and lengths of fish in each treatment was recorded weekly using a digital scale (model EHA 251) and a 12- cm ruler respectively. The experiment lasted for 42 days.

2.5 Determination of fish growth, performance and proximate analysis of fish and experimental diets

Fish performance and nutrient utilization were determined according to the methods of Olaifa *et al*. (2012). Mean weight gain (MWG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR), protein intake (PI) and fish survival rates (SR %) were also recorded.

2.6 Proximate analysis of experimental diets and fish after experiment

T. africana seed flour, fish carcass and experimental diets were analyzed for crude protein, lipid, moisture, fiber and ash content using the methods of AOAC (2000) in all treatments at the end of the feeding trial.

2.7 Fish growth and survival

For this experiment, growth was expressed as weight gain, relative growth rate, specific growth rate, condition factor, survival rate, protein efficiency ratio and feed conversion ratio and nitrogen metabolism.

Mean Weight Gain (MWG) = $W_1 - W_0$

Where:

W_0 =initial mean weight
W_1 =final mean weight

Specific Growth Rate (SGR) = $\frac{(\text{Ln } W_1 - W_0)}{T} \times 100$

Where:

Ln = Natural log
W_0 = initial mean weight
W_1 = final mean weight
T = time interval

.

Relative Growth Rate(RGR) = $\frac{\text{Weight gain by fish(g)}}{\text{Initial body weght(g)}} \times 100$

Condition Factor (K) = $\frac{W \times 100}{L^3}$

Where:
W = final weight
L = Final standard length

Survival Rate (S) % = $\frac{N_1}{N_0} \times 100$

Where:
N_1 = final number of fish at the end of experiment
N_0 = initial number of fish at the beginning of experiment.

Feed Conversion Ratio (FCR) = $\frac{\text{Dry weight of feed}}{\text{Fish weight gain}}$

Nitrogen Metabolism (NM) = $\frac{(0.549)(a+b)h}{2}$

Where:
a = initial mean weight of fish (g)
b= final mean weight of fish (g)
h= experimental period in days

Protein Efficiency Ratio (PER) = $\frac{\text{Wet body weight gain (g)}}{\text{Crude protein fed (g)}}$

2.8 Haematological Study

Blood samples were collected into heparinised bottles through the cardiac puncture of fish from each treatment. The capillary tubes were micro - centrifuged and relative packed cell volume (PVC) was measured to determine the percentage heamatocrite value. Other heamatological parameters assessed included white blood cell, heamoglobin, platelets, monocytes using the method described by Jain (1986).

2.9 Tissue pathology

Histopathology of the gill, kidney and liver were carried out. Gills, kidneys and liver samples were collected and fixed in formalin and then passed through a series of dehydration in graded concentrations of xylene. Sections were taken out and assessed using the methods of Jain (1986).

2.10 Statistical analysis*:* The biological and chemical data obtained were subjected to the statistical analysis of Variance (ANOVA) and the difference in mean was determined by the use of Duncan Multiple Range Test (Duncan, 1955).

3. Results and Discussion

3.1 Water quality

Water temperature in the experimental systems ranged from 28.0 – 29.0 ^{o}C, dissolved oxygen ranged from 2.0-2.2 mg/l while pH ranged from 7.8 and 8.2 (Table 2). The water quality parameters in all the treatments were within the tolerable ranges for catfish culture (Chuapoehuk, 1999). Moreover, fish responded favourably to the experimental diets in all treatments from the beginning to the end of the trial.

3.2 Proximate composition of diets

The proximate composition of diets fed to *C. gariepinus* fingerlings are presented in Table 3. The proximate composition of the experimental feeds differed significantly ($p<0.05$). Moisture content increased at TAF15 and TAF45 with values of 10.25 % and 9.96 % respectively. Crude protein was highest in diet TAF0 (control) with value of 34.9 % and lowest in diet TAF60 with a value of 30.20 %. This is lower than crude protein obtained in

commercial fish feeds in Nigeria; it is however higher than the one reported for *Gnetum africanum* 17.50% (Ekop, 2007). Fat content was lowest in diet TAF30 and increased as TAF increased in diet.

3.3 Proximate composition of fish after experiment

The proximate composition of fish after the feeding trials is shown recorded in Table 4 and reveals a higher protein content in TAF0 with 57 % than all experimental diets. It was followed by 54 % in TAF30. The crude protein value is lower than 22.0 % reported by Souza *et al.* (2007).

3.4 Mineral element of the fish after experiment

The mineral composition of the fish after experiment is shown in Table 5. The result shows that TAF 0 had the highest value of potassium (299 ppm), followed by TAF 45 (181.50 ppm) while the least value is contained by TAF 45 (149.50 ppm). The highest calcium content was observed in TAF 15 (268 ppm) followed by the control/TAF 0 (256.50 ppm) and TAF 45 had the lowest value (152 ppm). All fish showed traces of lead in their flesh including the control. Onyia *et al*. (2010) has a similar report in literature that potassium is the dominant mineral in their studies.

3.5 Growth response and protein utilization efficiency

Growth response, and protein utilization efficiency by catfish fed on *T. africana* inclusion are summarized on Table 6; it reveals that the best overall weight gain was obtained in fish fed with TAF0 (control) diet and the least weight gain was recorded in fish fed with TAF60 %. The responses of fish to the different diets showed that growth and nutrient utilization differed significantly ($p \leq 0.05$) among the treatments. There were no differences in survival of fish among treatments. Edward *et al*. (2010) stated that a condition factor above 1 indicates better utilization of feeds by the fish for growth and development and for sound health.

3.6 Heamatology of fish

The heamatological analyses were also represented in Table 7. The result shows that there was no significant differences in all the blood parameters of the fish fed control diet (TAF0) and experimental diet. However there were changes in the blood either above or below the values obtained before the experiment. Packed cell volume, haemoglobin, red blood cell count, platelets and neutrophils decreased while the mean corpuscular volume, mean corpuscular haemoglobin, lymphocytes and erythrocyte sedimentation rate increased at the end of the experiment above initial values. The changes in the haematological parameters of the fish during the experiment could be due to the presence of anti nutritional factors in the feeds. Fasasi *et al.* (2003) reports that antinutritional factors like tannins, phytates and oxalate might not have been completely removed during processing. Blood parameters indicate the physiological states of the fish (Babatunde *et al.*, 1992). Blood biochemistry can also be used as health indicators in fish (De Pedro *et al.*, 2005; Satheeshkumar *et al.*, 2011).

3.7 Histopathology result of the fish after the experiment

Table 8 shows the summary of histopathology of the fish after the experiment. There were no lesions in the gill tissues except in TAF 45 which showed mild mucosal congestion. No lesions were observed in the kidney of the fish. However, the most visible changes were exhibited by the liver. This is because the liver is responsible for dealing with all chemicals within the body. The liver is the organ involved in the metabolism, detoxification and excretion of chemicals and xenobiotics in the body (Pathan *et al.*, 2010). Vacuolation has been observed to be a common response to the presence of chemicals in fish (Clearwater *et al*., 2002; Shaw and Handy, 2006).

4. Conclusion

This study showed that *Treculia africana* seed flour without any further processing could be included in the diets of fingerings of *C. gariepinus* at 30 % level without compromising growth and nutrient utilization. Beyond the 30 % inclusion level, there was reduction in growth and nutrient utilization.

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Table 1. Gross composition of experimental diets (%)

Ingredients	**TAF 0**	**TAF 15**	**TAF 30**	**TAF 45**	**TAF 60**
	Control 0 %	15 % TAF	30 % TAF	45 %TAF	60 % TAF
Fishmeal	10.13	10.13	10.13	10.13	10.13
Soy bean	20.26	20.26	20.26	20.26	20.26
Groundnut cake	40.52	34.44	28.36	22.29	16.21
Millet	7.03	7.03	7.03	7.03	7.03
Wheat offal	7.03	7.03	7.03	7.03	7.03
Maize	7.03	7.03	7.03	7.03	7.03
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Vegetable oil	2.00	2.00	2.00	2.00	2.00
Starch	1.00	1.00	1.00	1.00	1.00
T. africana seed flour	0.00	6.08	12.16	18.23	24.31
Total	100	100	100	100	100

Table 2. Weekly water quality parameter of the experimental plastic bowl

Parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Mean
Temperature	29	28.5	29	28	29	29	28.75±0.38
pH	7.8	8.2	8.0	7.9	8.0	8.0	7.98±0.12
D.O	2.0	2.2	2.1	2.0	2.0	2.1	2.06±0.07

D O= Dissolved Oxygen

Table 3. Proximate composition of formulated diets (%)

Composition %	**Control**	**TAF 15**	**TAF 30**	**TAF 45**	**TAF 60**
Crude protein	34.95 ± 0.12^{a}	33.78 ± 0.12^{b}	33.41 ± 0.25^{b}	32.32 ± 0.12^{c}	30.28 ± 0.12^{d}
Crude fibre	6.34 ± 0.04^{a}	4.74 ± 0.06^{c}	5.00 ± 0.02^{b}	$5.31\pm0.0.03^{b}$	4.37 ± 0.03^{c}
Crude fat	16.49 ± 0.01^{c}	18.19 ± 0.11^{a}	14.82 ± 0.03^{d}	18.05 ± 0.08^{a}	17.65 ± 0.04^{b}
Moisture content	8.51 ± 0.37^{b}	10.25 ± 0.24^{a}	7.95 ± 0.12^{c}	9.96 ± 0.08^{a}	8.43 ± 0.03^{b}
Ash content	9.72 ± 0.09^{b}	9.61 ± 0.06^{b}	10.14 ± 0.23^{c}	9.16 ± 0.05^{b}	7.68 ± 0.17^{a}
NFE	30.49 ± 0.21^{b}	28.18 ± 0.17^{a}	33.61 ± 0.22^{c}	30.49 ± 0.16^{b}	35.94 ± 0.16^{c}

Superscript with different letters in the same row are significantly different ($p<0.05$), NFE = Nitrogen free extract

Table 4. Proximate composition of fish on dietary inclusion of *T. africana* seed flour after experiment

Composition (%)	**Control**	**TAF 15**	**TAF 30**	**TAF 45**	**TAF 60**
Crude protein	57	52	54	52	51
Moisture content	11.9	8.1	10.5	9.4	9.6
Ash content	4.0	3.0	3.7	3.3	3.1
Crude fat	10.2	8.2	9.5	9.2	9.0
Crude fibre	0.9	1.2	1.1	0.7	1.0
NFE	16	27.5	21.2	25.4	26.3

Superscript with different letters in the same row are significantly different ($p<0.05$), NFE = Nitrogen free extract

Table 5. Mineral composition of fish after treatment (ppm)

Minerals	**Initial**	**Control**	**TAF 15**	**TAF 30**	**TAF 45**	**TAF 60**
Calcium	170.00	256.50	268.0	152.00	158.50	171.00
potassium	130.50	299.00	152.50	149.50	181.50	152.00
Sodium	38.00	85.00	56.00	57.00	70.00	46.00
Magnesium	13.10	47.00	24.25	17.60	21.95	20.50
iron	19.70	90.05	19.70	25.6	29.45	21.90
zinc	0.47	1.12	0.83	0.57	0.75	0.89
lead	0.30	0.33	0.23	1.19	0.25	2.50
Manganese	1.00	7.65	3.60	2.80	3.30	2.50

Table 6. Growth, feed utilization and % survival rate of cultured fish

Paramerters	Control	TAF 15	TAF 30	TAF 45	TAF 60
Initial mean weight (g)	2.54±0.08^{a}	2.45±0.10^{a}	2.52±0.15^{a}	2.59±0.09^{a}	2.49±0.05^{a}
Final mean weight (g)	10.15±0.19^{a}	8.01±0.08^{b}	9.25±0.51^{c}	7.14±0.15^{d}	7.01±0.03^{d}
Mean weight gain(g)	7.61±0.12^{a}	5.58±0.06^{c}	6.71±0.41^{b}	4.54±o.15^{d}	4.53±0.07^{d}
Mean length gain (cm)	3.60±0.08^{a}	3.61±0.02^{a}	3.75±0.12^{a}	3.60±0.14^{a}	3.76±0.05^{a}
Percentage weight gain (%)	25.03±0.41^{c}	30.33±1.15^{c}	27.29±1.19^{b}	36.31±1.33^{a}	35.37±0.79^{a}
Feed conversion ratio	2.06±0.25^{c}	2.09.64±0.45^{b}	2.09.45±0.41^{b}	2.09±0.41^{a}	2.39±0.66^{a}
Specific growth rate	3.29±0.04^{a}	2.84±0.08^{bc}	3.09±0.10^{ab}	2.41±0.08^{d}	2.68±0.20^{cd}
Protein efficiency ratio	0.22±0.00^{a}	0.16±0.00^{c}	0.20±0.02^{b}	0.13±0.00^{d}	0.15±0.00^{d}
Condition factor (K)	16.34±1.01^{a}	11.82±0.75^{bc}	12.82±1.43^{b}	9.78±0.86^{c}	9.72±1.26^{c}
Nitrogen metabolism(x10^{2})	1.45±0.03^{a}	1.20±0.01^{c}	1.35±0.07^{b}	1.12±0.02^{c}	1.16±0.05^{c}
Survival %	98	95.5	97	96	97

Superscripts with different letters in the same row are significantly different ($p<0.05$)

Table 7. Haematology of the fish on *T. africana* seed flour-based diets at the end of the experiment

Parameters	Initial	control	TAF 15	TAF 30	TAF 45	TAF 60
PCV (%)	28	8.67±3.0^{a}	9.33±4.0^{a}	14.00±7.0^{a}	13.67±2.1^{a}	12.33±5.5^{a}
HB (g/dl)	9.2	3.17±1.1^{a}	3.03±1.4^{a}	4.60±2.2^{a}	4.4±0.6^{a}	4.00±1.8^{a}
RBC(x10^{12}/ L)	8.24	1.71±0.3^{a}	1.92±0.2^{a}	2.07±0.7^{a}	2.18±0.2^{a}	1.95±0.2^{a}
WBC(x10^{9}/L)	8.7	3.53±0.7^{a}	2.73±0.8^{a}	3.8±1.1^{a}	2.66±0.4^{a}	3.13±0.9^{a}
PLA (X10^{9}/L)	8.0	2.67±0.01^{a}	3.33±0.1^{a}	4.00±0.2^{a}	3.67±0.1^{a}	3.33±0.1^{a}
MCV (Fl)	33	49.33±8.7^{a}	47.33±14.9^{a}	65±9.5^{a}	62±3.6^{a}	61±21.9^{a}
MCH (Pg)	11	16.00±3.0^{a}	14.3±6.1^{a}	18.0±14.5^{a}	19.67±1.1^{a}	10.67±3.5^{a}
LYMPH (%)	60	79.67±1.5^{a}	79.67±1.5^{a}	81.0±1.0^{a}	79.67±1.5^{a}	78.67±2.1^{a}
NEUT (%)	39	19.67±1.5^{a}	17.67±1.5^{a}	18.33±0.5^{a}	20.33±2.0^{a}	18.33±1.5^{a}
MONO	01	1.33±0.6^{a}	1.0±0.0^{a}	1.33±0.6^{a}	1.00±0.0^{a}	1.33±0.6^{a}
ESR (mm/hr)	02	2.33±1.5^{a}	4.33±2.1^{a}	4.33±2.5^{a}	5.00±1.0^{a}	6.33±0.5^{a}

Superscript with different letters in the same row are significantly different ($p<0.05$)
PVC=Packed cell volume, HB= Heamoglobin, RBC= Red blood cell, WBC= White blood cell, MCV= Mean corpuscular volume, MCH= Mean corpuscular heamoglobin, MONO=Monocyte, ESR=Erythrocyte sedimentation rate, LYMPH=Lymphocyte, PLA=Platelets

Table 8. Summary of histopathology of the tissues of the control and experimental fish

Tissue	Control	TAF 15	TAF 30	TAF 45	TAF 60
Gill	No visible lesion seen	No visible lesion seen	No visible lesion seen	There is mild sub mucosal congestion	No lesion seen
Kidney	No visible lesion seen	No visible lesion seen	No visible lesion seen	No visible lesion seen	No visible lesion seen
Liver	No visible lesion seen	There is moderate portal and central congestion . There is diffuse vacuolar degeneration	There is moderate diffuse vacuolar degeneration of hepatocytes	There is severe diffuse vacuolar degeneration of hepatocytes	There is moderate portal and central venous congestion.. There is severe diffuse vacuolar degeneration