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Response of African Catfish, *Clarias gariepinus*; Burchell 1822 to diets of African Yam Bean (*Sphenostylis stenocarpa*) Subjected to two processing methods

Ogunji, Johnny[1], Ozigbo Joan[1], Ossai, Angela[1] and Ochang Stephen[1,2]
[1]Department of Fisheries and Aquaculture, Ebonyi State University Abakaliki Nigeria, [2]Department of Fisheries and Aquatic Science, Cross River University of Technology Calabar, Nigeria
Correspondence: Email: ogunjijo@yahoo.com

Abstract
This study assesses response of African Catfish, *Clarias gariepinus* to diets of African yam bean (*Sphenostylis stenocarpa*) (AYB) subjected to two processing methods. Mature AYB was boiled and fermented. Their meals were evaluated as a protein sources for *Clarias gariepinus* fingerlings. Seven diets were formulated to contain 44.07 ±0.48% crude protein (Mean ± SD) and 19.03±0.05 kJg^{-1} gross energy (Mean ± SD) respectively. Fishmeal in the diets was substituted with each of the two processed AYB meals at 40%, 45% and 50% levels. Control diet did not contain AYB. Nine fingerlings (initial average weight 12.28±0.18g) were stocked per experimental tank. Experimental diets were fed to triplicate groups of catfish fingerlings at 10% body weight for 56 days. Results showed that Specific growth rate (SGR) was highest at 45% replacement of fermented AYB (3.32±0.20) compared to control (3.17±0.44). However, all fish fed Boiled AYB diets performed well also. Mean values for haematological parameters (PCV, HB, WBC and RBC) significantly increased ($P<0.005$) above the initial status and control group. Haematological values for fish fed 40% inclusion level of fermented and boiled AYB in diet were the highest. This study shows that AYB processed by fermentation and boiling are effective and can enhance fish growth. However, Fermentation process seems to have improved fish performance better. Based on the histological results processed AYB should not be included beyond 45% inclusion level.

Introduction
Thorarinsdottir et al. (2011) indicated that the aquaculture industry is the fastest growing food production industry in the world and approximately 50% of all fish consumed by humans is from aquaculture. They however observed that the main cost factor in aquaculture is the cost of feed. Corroborating this fact Ogunji et al. (2008a) reported that the high prices of fishmeal in world markets have necessitated the search for substitute protein sources. According to Tacon and Metian (2008) fishmeal has been adjudged the most important and expensive protein ingredient used in aquafeeds. Fishmeal has been replaced with cheap plant proteins (Jackson et al. 1982; Tacon and Jackson 1985; Webster et al., 1992; Ogunji and Wirth 2001). One of the major local feed stuffs that meet the protein needs of most species is soybean meal (Ogunji, 2004). This feed ingredient and most others, have become relatively expensive also due to their use in fish feed as it competes with human and livestock feeds.

African yam bean (AYB) is one of the tropical legume seed that has been scarcely used in fish feed production in spite of its crude protein content. Amino acid analysis indicates that lysine and methionine levels in the protein are equal to or better than those of soybeans (Obatolu et al., 2001). However, like other legume beans, its nutritive value is masked by the occurrence of antinutritional factors (ANFs) such as alkaloids, flavonoids and saponins (Asuzu and Undie, 1986). The objective of this study therefore, was to determine the effect of two processing methods (boiling and fermentation) of African yam bean (*Sphenostylis stenocarpa*) on the growth performance, heamatology and intestinal mocusa of African catfish *Clarias gariepinus*.

Materials and Method
The experiment was carried out at the Department of Fisheries and Aquaculture, Ebonyi State University, Abakaliki Nigeria. Mature African yam bean was purchased from a local farmer. The yam bean was subjected to two processing methods: Boiling and Fermentation respectively to detoxify anti-nutrient present. The method described by Obatolu *et al.,* (2001) was used.

Fish meal and African yam bean meal were used as the major dietary protein sources in the diets. Seven diets were formulated to contain 44.07 ±0.48% crude protein (Mean ± SD) and 19.03±0.05 kJg^{-1} gross energy (Mean ± SD) respectively (Table 1). Fishmeal in the diets was substituted with each of the two processed AYB meals (Fermented – F; Boiled – B) at 40%, 45% and 50% levels. Control diet did not contain AYB (Table 1). The dry diet component

including vitamin C and oil were thoroughly mixed. Water was added and the feed was pressed into pellets of 2mm diameter. The pelleted feed was sun dried to reduce moisture content. This was done to enhance quality and was stored in refrigerator until use (Ogunji *et al.* 2008b).

A total of 189 catfishes (initial average weight 12.28±0.18g)) were acclimatized for seven days. They were weighed and distributed among 21experimental tanks at a rate of nine fish per tank with 10 litres of water. Test diets were randomly assigned using completely randomized design to triplicate lots of ten litre capacity aquaria. The fish were fed a restricted ration of 10% body weight per day in two portions for 56 days in static water. Quantity of feed was adjusted forth nightly after bulk weighing of experimental fish. The aquaria were cleaned and water completely replaced by siphoning every other day to avoid fouling. Water temperature, dissolved oxygen, Nitrate and pH were monitored and remained relatively stable. Temperature was maintained at 28 ± 0.19 ^{o}C, dissolved oxygen between 7.61±0.90 mg/L Nitrate 0.00 mg/L and pH between 7.61±0.65. No critical values were detected in any of the tanks. Experimental data, samples of fish and feedstuff were analyzed at the end of experiment.

Blood samples were collected at the commencement, and at the end of the experiment from the caudal vein into an EDTA litium tube. The blood was analyzed to determine the packed cell value (PCV) with microhaematocrit using heparnized capillary tube (25mm). Red blood cell (RBC) and white blood cell (WBC) counts were determined as described by Blaxhal and Diasley (1973). Hemoglobin (Hb) concentration was determined by the methods described by Wedemeyer and Yasutake (1977).

At commencement and end of the experiment, 2 fish were dissected the intestine removed and preserved in 10% buffer solution to retain the structural integrity of the cells and tissue. The dealcoholized tissues were impregnated in molten paraffin wax for 3 hours. After the impregnation, tissues were embedded using a deposable embedding mold and allowed to cool. Before sectioning the embedded tissues were placed on ice for proper sectioning. The tissues were then sectioned using Lecia rotary micotome and then transferred to the flattening table (hot plate) from the water bath to dry. Finally, the sections were stained using A & E staining procedure prescribed by Baker *et al.* (1989).

The amino acid cum proximate analyses of feed stuff and feed samples were carried out. Amino acid in samples was determined spectorophotometrically using ninhydrin chemical reaction (Schroeder et al. 1990). Protein (N x 6.25) was determined by the Kjeltec System (Tecator) and crude fat by Soxtec System HT (Tecator) using petroleum ether. Ash was determined by burning in a muffle furnace at 550 C for 10 hours. Gross energy was calculated using the following factors: crude protein = 23.9 kJ/g, crude lipids = 39.8 k/Jg and NFE = 17.6 kJ/g (Schulz et al. 2005).

At the end of the experiment, all fish was weighed and data obtained from triplicate tanks were used to calculate weight gains, specific growth rate (SGR), feed conversion ratio (FCR) and Percentage body weight. Weight gain = final weight – initial weight, SGR = (LnW2 - LnW1)/(T2-T1)100 where W1 and W2 = initial and final weight of fish and T1 and T2 = time in days. FCR = $\frac{Feed\ \ fed}{live\ \ weight\ \ gain}$

Percentage body weight (% BW or PBWG (-%) = $\left(\frac{W_2 - W_1}{t_2} - t_1\right) \times 100$; Where: W_2 = final weight of fish, W_1 = initial weight of fish and (T_2-T_1) = time (day).

All growth and heamatological data were subjected to one way analysis of ;variance (ANOVA). The significance of difference between means was determined by Duncan's Multiple Range test ($p<0.05$) using SPSS for windows (version 17). Values were expressed as means ±SE.

Result

The proximate composition of experimental diets is presented on Table 2. Results of the amino acid showed that processing AYB by fermentation increased amino acid values while boiling process decreased the content. Similar observation was made in the proximate analysis in comparison with raw AYB.

The growth response of *C. gariepinus* fed boiled and fermented African yam bean meals at two varying levels of dietary incorporation is shown in (Table 3). With respect to the values of FCR and SGR best growth response was obtained at 45% inclusion levels of fermented yam bean meal. However, the values are not significantly different from fish fed control diet and BD2 (Boiled diet 2). Values of Control and FD2 are in most cases not significantly differently ($P>0.05$).

Table 8 shows the mean value and standard error (SE) of all blood parameter for each feeding group. The blood indices in each treatment varied significantly ($P<0.005$) higher than control and initial. Among the treatment group, PCV, Hb, WBC and RBC content of fish fed fermented diet, at 40% inclusion (FD1) was significantly higher ($P<0.005$).

Figure 1 a – c selectively shows the normal intestinal mucosa of African catfish and that of fish fed experimental diet FD3 and BD1. Fish fed with BD1, had wide lamina propria. Intestinal mucosa of fish fed with FD_3 showed degradation and blunting of epithelial cell.

Table 1: Diet Composition (%)

Ingredients	**FD1**	**FD2**	**FD3**	**BD1**	**BD2**	**BD3**	**Control**
Fishmeal	34	33	32	34	33	32	41
African Yam Bean	40	45	50	40	45	50	0
Maize	9.5	5.5	1.5	9.5	5.5	1.5	42.5
Soy Bean	15	15	15	15	15	15	15
Vitamin C	1	1	1	1	1	1	1
Cordliver Oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100

F = fermented; B = boiled

Table 2. Proximate composition (%) of experimental diets[1]

	Fermented			Boiled			Control
	40%	45%	50%	40%	45%	50%	0%
Dry matter	92.84±0.01	93.05±0.01	92.91±0.01	91.73±0.01	91.67±0.01	91.72±0.01	93.54±0.01
Crude protein	44.34±0.03	44.63±0.03	44.72±0.02	43.62±0.02	43.62±0.02	43.72±0.04	43.82±0.03
Crude fat	4.55±0.01	4.66±0.01	4.61±0.01	4.27±0.01	4.33±0.01	4.24±0.01	4.40±0.01
Ash	13.47±0.01	13.39±0.01	13.57±0.01	12.85±0.01	12.96±0.01	13.06±0.01	13.56±0.01
NFE[2]	37.64±0.01	37.32±0.04	37.10±0.02	39.26±0.02	39.19±0.03	38.98±0.06	38.23±0.04
Fibre	3.37±0.01	3.25±0.01	3.30±0.01	3.16±0.01	3.22±0.01	3.13±.0.01	3.58±0.01
Energy[3]	19.04±0.00	19.09±0.00	19.06±0.00	19.04±0.00	19.02±0.00	18.99±0.00	18.95±0.00

1. Values are mean of triplicate determination ± standard error. 2. NFE = 100 – (%protein + %fat +%ash) 3. Gross energy = crude protein = 23.9 kJ/g, crude lipids = 39.8 k/Jg and NFE = 17.6 kJ/g (Schulz et al. 2005)

Table 3. Growth performance of *Clarias gariepinus* fingerlings fed diets with fermented and boiled African yam bean at varying levels of incorporation*

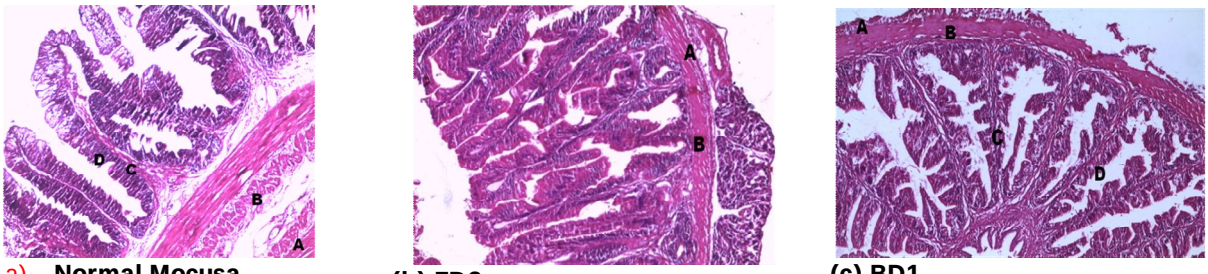
	Fermented			Boiled			
	FD_1	**FD_2**	**FD_3**	**BD_1**	**BD_2**	**BD_3**	**Control**
inclusion	40%	45%	50%	40%	45%	50%	0%
Initial weight (g)	1.38± 0.00^{b}	1.34± 0.00^{a}	1.37± 0.00^{ab}	1.37± 0.01^{ab}	1.36± 0.01^{ab}	1.37± 0.01^{ab}	1.36± 0.0^{ab}
Final weight (g)	6.02± 0.65^{ab}	8.26± 0.89^{b}	4.40± 1.34^{a}	5.06± 1.22^{ab}	4.86± 0.45^{ab}	6.96± 0.62^{ab}	8.48± 1.86^{b}
Weight gain (g)	4.63± 0.65^{ab}	6.91± 0.89^{b}	3.03± 1.34^{a}	3.69± 1.20^{ab}	3.50± 0.46^{ab}	5.59± 0.62^{ab}	7.12± 1.86^{b}
Feed conversion ration (FCR)	3.39± 0.41^{a}	2.38± 0.32^{a}	6.26± 2.77^{a}	5.11± 2.08^{a}	3.79± 0.25^{a}	2.87± 0.23^{a}	2.65± 0.79^{a}
Specific growth Rate (SGR %)	2.61± 0.19^{ab}	3.32± 0.20^{a}	1.91± 0.56^{a}	2.20± 0.49^{b}	2.26± 0.17^{ab}	2.88± 0.16^{ab}	3.17± 0.44^{a}
Percentage body weight gain (%)	335.91± 47.89^{ab}	516.39± 68.26^{a}	220.72± 97.37^{b}	268.29± 85.78^{ab}	258.96± 36.71^{ab}	406.79± 44.96^{ab}	524.12± 138.99^{a}

*Values (mean ±SE) on the same horizontal line with different superscript letters are significantly different ($P<0.05$) from each other. SE is standard error.

Table 4: Hematological paramenter of *Clarias gariepinus* fingerling fed experimental diets*.

Experimental diets	**PCV (gcl/l)**	**HB (%)**	**WBC x (10^3 cells/mm^3)**	**RBC x (10^3 cells/mm^3)**
BD1	26.5±0.29^{e}	8.70±0.17^{e}	18700.0±230.9^{f}	11.47±0.09^{e}
BD2	24.0±0.00^{c}	8.07±0.03^{c}	17700.0±57.7^{c}	11.17±0.03^{c}
BD3	25.0±0.00^{d}	8.27±0.03^{cd}	18150.0±28.9^{de}	11.27±0.03^{cd}
FD1	27.5±0.29^{f}	9.17±0.03^{f}	19150.0±28.9^{g}	11.70±0.06^{f}
FD2	26.0±0.00^{e}	8.40±0.00^{d}	18350.0±28.9^{ef}	11.40±0.06^{de}
FD3	24.5±0.29^{cd}	8.17±0.15^{cd}	17900.0±230.9^{cd}	11.27±0.09^{cd}
Control	21.5±0.29^{b}	7.27±0.03^{d}	15350.0±28.9^{b}	8.47±0.03^{b}
Initial	14.5±0.29^{a}	4.87±0.09^{a}	10450.0±28.9^{a}	5.77±0.03^{a}

*Values represent treatment mean ±SE; Values on the same column with differentsuperscript letters are significantly different ($P<0.005$) from each other. PCV = packed cell volume, Hb = hemoglobin, WBC = white blood cell counts, RBC =Red blood cell count.



a) **Normal Mocusa** **(b) FD3** **(c) BD1**

Figure 1 a – c: Photomicrograph showing: (a) normal mucosa of African Catfish from the experiment (b) fish fed Diet FD3 with observed degradation of mucosa. (c) Fish fed Diet BD1 without degradation Magnification: X 500. **A**= Serous Membrane, **B**= Muscularis, **C**= Lamina Proprria **D** = Mucosal Epithelium

Discussion

African Yam Bean (AYB) used for diet formulation in this study was subjected to two different processing methods to remove anti-nutrient present. Results revealed that food consumption by fish throughout the experiment was the same. A mean striking time of 0.66±0.10 showed no difference in feed acceptability by the different dietary inclusions of the two processed feed. This could be due to the presence of flavonoids which enhanced the palatability irrespective of presence of alkaloids. Johnson (2001) reported that flavonoids are a large and complex group of phenolic compounds that contribute to the flavour and colour of vegetables and fruits, and account for most of the dissolved solids in beverages such as tea, coffee and wine. Asuzu and Undie (1986), reporting on toxic components of yam bean indicates the presence of alkaloids, flavonoids and saponins.

Fermenting process improved crude protein and fat content of AYB while boiling decreased the crude protein and fat content. Bressani *et al.* (1987) reported that heat treatment not only reduced the level of lysine but also destroyed methionine (both essential amino acids) in jack bean, thus reducing the biological value of JBSM protein.

Fish groups fed fermented yam bean at 45% level (FD2) performed better than the control. The values were not significantly different (P>0.05). Specific growth rate (SGR) was (3.32±0.20) compared to control (3.17±0.44) while FCR was 2.38±0.32 and control 2.65± 0.79. At 40% of FAYB (FD1) and the three dietary inclusion of boiled yam bean values were not significantly different but at 50% inclusion of FAYB fish performance was the poorest. The values are higher than the SGR of 2.28 reported by Fagbenro and Jauncey (1995) for *C. gariepinus* juveniles fed fish silage blended with hydrolyzed feather meal. Ogunji *et al.,* (2008b) posit that removal of undesirable components is essential for the enhancement and effective utilization of plant nutrients in animal feed. In confirmation, Mubarak (2005) argued that cooking as processing method improves the protein quality by either destroying or inactivating heat labile anti-nutritional factors.

Mean values for haematological parameters (PCV, HB, WBC and RBC) significantly increased (P<0.005) above the initial status and control group. Haematological values for fish fed 40% inclusion level of fermented and boiled AYB in diet were the highest. Ogunji et al. (2008c) reported that stressful conditions in fish and in mammals are associated with decreased growth, haematocrit (packed cell volume) and haemoglobin values, increased whole blood glucose (hyperglycaemia) and plasma cortisol concentrations. As such the experimental diet may not have introduced any stress factor to the fish.

However, damage to epithelial mucosa of the fish was observed in groups fed 45% inclusion level of boiled and fermented AYB diet respectively. This study shows that fermentation and boiling are effective method of reducing anti-nutrient in AYB and can enhance fish growth. Nevertheless, based on the histological results, processed AYB should not be included beyond 45% inclusion level.

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