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Growth Response and Diet Utilization of *Clarias gariepinus* (Clariidae) Fed Diets Containing Cassava Leaf Protein Concentrate as Plant Protein Source

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Abstract

Cassava leaf protein concentrate (CLPC) was evaluated as a dietary protein source for *Clarias gariepinus* (6.4±0.4g). A control diet contained solvent-extracted soybean meal (SBM), which was stepwise substituted at 20-80% with CLPC in test diets. Catfish fingerlings were fed at 5% body weight/day for 70 days in triplicate diet treatments. No catfish mortality occurred in all treatments. Where CLPC substituted up to 40% of dietary SBM, no adverse effects occurred in growth response and diet utilization. Catfish growth declined and diet was poorly utilized beyond this substitution level, caused by a reduction in protein and energy digestibility. Haematological parameters of catfish in all treatments were similar (P >0.05). Histological sections of the liver in catfish fed diets containing CLPC substitution up to 40% of dietary SBM showed normal architecture, while those fed higher levels showed slight to severe alterations due to residual anti-nutrients.

Keywords: *Manihot esculenta* Utilisation, Plant protein source, *Clarias gariepinus*.

Introduction

The use of soybean products (meal, protein concentrate) in feed formulation is increasingly unjustified in economic terms; as it is increasingly being used for food by the human population and for feed by poultry and livestock (Tacon *et al*., 1998; Fagbenro and Davies, 2004). Hence, there is need to exploit cheaper, locally and commonly available plant protein sources to substitute soybean meal for sustainable aquaculture production. Considerable amounts of cassava leaves waste away on farmlands after harvesting of the tubers (the main commercial product). The leaves have been recognized as a cheap and abundant source of protein in livestock and poultry diets (Ravindran, 1993).

Cassava leaves have high protein content (average 21%, Ravindran, 1993) and contain higher levels of many of the essential amino acids

than levels occurring in soybean meal (Gómez and Valdivieso, 1984); suggesting that cassava leaves represent a potential protein source in aquafeeds (Fagbenro, 2013). Converting cassava leaves into cassava leaf protein concentrate (CLPC) reduces fibre content and increases protein quality (Müller, 1977; Telek and Martin, 1983; Stupak *et al*., 2006; Oresegun *et al.*, 2016).

CLPC contains 45-50 g/kg crude protein and low antinutrients (Müller, 1977; Ravindran, 1993). In previous studies, CLPC produced from cultivar (TME 419) was similar to solvent-extracted soybean meal (SBM) in terms of nutrient composition and digestibility (Oresegun *et al*., 2016; Fagbenro and Olurole, 2016). CLPC was evaluated as dietary protein and SBM replacer in diets for tilapias, *Oreochromis niloticus* and *Tilapia zillii* and found to be suitable as SBM replacer (Fagbenro *et al*., 2017), and is yet to be evaluated for the African catfish, *Clarias gariepinus*, the most widely cultivated fish in Nigerian aquaculture. This study further investigates the effects of CLPC substituting SBM as dietary protein source on mortality, growth response, diet utilization, nutrient digestibility, liver histology, and haematological properties of *C. gariepinus*.

Materials and Methods

Fresh leaves of cassava (*Manihot esculenta* Crantz) (cultivar TME 419) were collected from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The leaves were weighed, washed, and pulped with a leaf pulping machine. This process was followed by crushing in water (1:3 w/v at a pH 8.5) and filtered as described by Modesti *et al*. (2007). The separated leaf juice was heated at 90-100°C for 10 minutes to coagulate leaf protein fraction, which was separated by filtration and subsequently dried at atmospheric temperature.

SBM was obtained from a commercial feedstuff market in Lagos, Nigeria. Proximate composition of SBM and CLPC was determined using AOAC (2000). Hydrogen cyanide, tannic acid and phytic acid contents of CLPC were determined using Francis *et al*. (2001) methods. Gross energy content was determined by direct combustion in an adiabatic bomb calorimeter (A 305 Gallenkamp, UK). Lysine and methionine contents were determined using an LKB 4151 Alpha^{+} amino acid analyzer after treating the hydrolysate with 6 mol. L^{-1} HCl under reflux for 24 h at 110 °C.

Five dry diets (420g/Kg crude protein, 120g/Kg crude lipid, 22 MJ/Kg gross energy) were formulated and prepared. A control diet (CD1) contained SBM providing 60% of total protein. The SBM component was substituted at 20%, 40%, 60% or 80% with CPLC in test diets (CD2, CD3, CD4, CD5) (Table 1). All the ingredients were milled to <250µm using a laboratory mill, mixed thoroughly in a Hobart A120 steam pelleted and extruder (Hobart, Troy, Ohio, USA) to obtain a homogeneous mixture and passed through a 2mm die to obtain strands, which were oven-dried at 60°C for 24 h. Dried strands were broken into 2mm sizes and stored at -20°C. Proximate composition of diets was determined according to AOAC (2000) methods and gross energy content was determined by bomb calorimetry.

Groups of 20 apparently healthy full siblings of African catfish, *C. gariepinus* (6.4±0.4g) were stocked into flow-through 60-litre capacity glass tanks filled with aerated water (flow rate, 1 litre/minute). Each diet was fed to catfishes in triplicate tanks at 5% body weight/day for 70 days. Fish mortality was monitored daily, total fish weight in each tank was determined at two-week intervals. Water temperature and dissolved oxygen concentration were measured daily while pH was monitored weekly. Alkalinity, hardness, phosphate, nitrite, and nitrate concentrations were determined according to Stirling *et al*. (1985). Fish carcass composition was determined using AOAC (2000) methods. Growth response and diet utilisation indices were determined as:

Weight gain = Final weight of fish - the Initial weight of fish.

Percentage weight gain = Mean weight gain/Mean initial weight x 100.

Feed conversion ratio (FCR) = Total feed consumed by fish/Weight gain by fish.

Ten *C. gariepinus* were stocked into 20-litre plastic tanks filled with aerated water. Each diet

Table 1: Ingredient and Proximate Compositions(g/kg) of the Experimental Diets

	Control diet CD1	Test diets			
		CD2	CD3	CD4	CD5
Fish (herring) meal	200	200	200	200	200
Bovine blood meal	100	100	100	100	100
Soybean meal (solvent-extracted)	400	320	240	160	80
Cassava leaf protein concentrate (CLPC)	0	80	160	240	320
Cassava starch (gelatinized)	180	189	198	208	217
Corn oil: Cod liver oil (1:1)	70	61	52	42	33
Vitamin-Mineral premix[1]	30	30	30	30	30
Carboxy-methyl cellulose (binder)	20	20	20	20	20
Crude protein	423.6	423.9	424.2	424.6	424.9
Crude lipid	100.7	100.8	100.9	100.1	100.2
Crude fibre	24.8	27.2	29.6	32.0	34.4
Total ash	48.3	46.5	44.6	42.8	40.9
Gross energy (MJ/kg)	22.22	22.25	22.28	22.30	22.34

[1]Vitamin-Mineral mix (g/kg): Manufactured by DSM Nutritional Products Limited, Basle, Switzerland - Vitamin A, 1600 IU; vitamin D, 2400 IU; vitamin E, 160 mg; vitamin K, 16 mg; thiamine, 36 mg; riboflavin, 48 mg; pyridoxine, 24 mg; niacin 288 mg; panthotenic acid, 96 mg; folic acid, 8 mg; biotin, 1.3 mg; cyanocobalamin, 48 mg; ascorbic acid, 720 mg; choline chloride, 320 mg; calcium 5.2 g; cobalt, 3.2 mg; iodine, 4.8 mg; copper, 8 mg; iron, 32 mg; manganese, 76 mg; zinc, 160 mg; Endox (antioxidant) 200 mg.

CD1 = (0% CLPC); CD2 = (20% CLPC); CD3 = (40% CLPC); CD4 = (60% CLPC); CD5 = (80% CPLC).

was assigned to duplicate tanks and fishes were fed at 5% body weight/day for 14 days. On day 15, faeces were collected from anaesthetized fish (2.5 ml quinaldine/litre) eight hours after feeding using the dissection method. Dry matter, crude protein and gross energy were determined in triplicate samples of diets and faeces. Nutrient digestibility was determined using the acid insoluble ash method (Atkinson *et al*.,1984) as:

$$100 - 100 \frac{(\text{AIA in feed} \times \text{nutrient in faeces})}{(\text{AIA in faeces} \times \text{nutrient in feed})}$$

Fishes were anaesthetized with 200 mg/litre MS222, their caudal peduncles severed and the blood was collected. Thereafter, 2 ml blood was transferred into a tube containing ethylene-diamine-tetra-acetic acid (EDTA) as an anticoagulant. The supernatant plasma was collected and stored at 4°C prior to the determination of erythrocyte count, leucocyte count, haematocrit, haemoglobin concentration and erythrocyte sedimentation rate according to Svobodova *et al*., (1991).Livers often fish specimens/treatment were removed, weighed and later fixed in 1:10 buffered formalin solution, dehydrated in graded levels of ethanol, cleared with xylene, blocked in paraffin, sectioned at 5µ thickness, placed on glass slides, stained with haematoxylin and eosin, examined under a light microscope, and interpreted using Chinabut *et al*. (1990).

Data were subjected to one-way analysis of variance (ANOVA) test ($P < 0.05$). Duncan's multiple-range test was applied to quantify the differences among treatments using Statgraphics 5^+ package (Manugistics Inc. and Statistical Graphics Corp, MD, US., 1994).

Results

Nutrient composition of CLPC (from cultivar TME 419) is similar to that of SBM (Table 2). Cassava leaves contain cyanogenic glycosides (linamarin and lotaustralin) which on hydrolysis give HCN and appreciable levels of other antinutrients notably phytin and tannin. These antinutrients were degraded to non-deleterious

Table 2: Nutrient and Anti-nutrient Compositions of Soybean Meal (SBM) and Cassava Leaf Protein Concentrate (CLPC)

	SBM	CLPC
Dry matter (g/kg)	889	989
Crude protein (g/kg)	485	489
Crude lipid (g/kg)	19	133
Crude fibre (g/kg)	37	67
Total ash (g/kg)	62	39
Gross energy (MJ/kg)	18.92	21.35
Calcium (%)	0.28	0.71
Phosphorus (%)	0.70	0.59
Lysine (g/kg protein)	32	68
Methionine (g/kg protein)	8	25
Cyanide (g/kg)	ND	0.98
Phytates (%)	ND	1.63
Tannin (%)	ND	1.07

ND – not determined

Table 3: Water Quality Parameters during the Study

Parameters	Range
Temperature	25-27.2^{o}C
Dissolved oxygen	5-7.6mg/litre
pH	7.5-9.1
Alkalinity	122-135 mg/litre
Hardness	290-320 mg/litre
Phosphate	0.2-0.5 mg/litre
Nitrite	0.12-0.15 mg/litre
Nitrate	2-4 mg/litre

levels in the CLPC (Table 2). Proximate composition and gross energy content of the experimental diets (Table 1) showed that no differences occurred in crude protein, crude lipid, crude fibre and ash contents of diets. Crude protein and gross energy contents of catfish diets were 423.6-424.9 g/Kg and 22 MJ/Kg, respectively.

Values of water quality parameters monitored during the feeding trials are presented in Table 3. No abnormal fish behaviour or signs of stress were observed in fishes in all treatments during feeding trials. Fish survival in all treatments during the feeding trials was 100%. During the feeding trials, *C. gariepinus* became accustomed to the test diets within five days.

Mean final weight, mean weight gain and % weight gain of *C. gariepinus* fed with test diets containing CLPC variety up to 40% substitution of SBM were similar ($P > 0.05$) to those fed with the control diet. Average Daily Gain (ADG) and FCR values were also similar ($P > 0.05$) (Table 4). This study reveals that the conversion to CLPC apparently degraded antinutrients and fibre contents of diets thereby increasing nutrient availability. *Clarias gariepinus* in all diet treatments had high ACPD values (Table 4).

Except for higher Erythrocyte Sedimentation Rate (ESR) values ($P < 0.05$) in *C. gariepinus* fed diets containing CLPC at 80% substitution of SBM, there were no differences ($P > 0.05$) in the other haematological parameters measured (Table 5).

Table 4: Growth Response, Diet Utilization and Nutrient Digestibility of *Clarias gariepinus* fed with the Experimental Diets

	CD1	CD2	CD3	CD4	CD5
Mean initial weight (g)	6.2±0.3	6.2±0.3	6.2±0.3	6.2±0.3	6.2±0.3
Mean final weight (g)	44.5±0.5^{a}	44.0±0.4^{a}	40.9±0.4^{a}	34.8±0.5^{b}	30.3±0.2^{b}
Mean weight gain (g)	38.3±2.5^{a}	37.8±2.3^{ab}	34.7±2.3^{b}	28.6±2.3^{c}	24.1±2.4^{c}
% weight gain	617.7^{a}	609.7^{ab}	559.7^{b}	461.3^{c}	388.7^{c}
Feed Conversion Ratio	1.42^{bc}	1.45^{b}	1.48^{b}	1.63^{a}	1.67^{a}
Feed intake (g/fish/day)	1.3^{a}	1.3^{a}	1.1^{ab}	1.0^{b}	0.8^{bc}
Apparent nutrient digestibility (%)					
Dry matter	89.3±2.0^{a}	85.1±2.0^{a}	84.8±2.3^{a}	75.0±1.8^{b}	70.9±1.5^{b}
Crude protein	85.8±1.3^{a}	85.2±2.4^{a}	85.0±2.4^{a}	79.1±1.3^{b}	75.6±2.4^{b}
Gross energy	72.5±0.8^{a}	72.1±2.0^{a}	71.5±2.0^{a}	67.2±0.8^{b}	60.6±2.0^{b}

Survival = 100%. Mean values in a row with different superscripts are significantly different ($P<0.05$).
CD1 = (0% CLPC); CD2 = (20% CLPC); CD3 = (40% CLPC); CD4 = (60% CLPC); CD5 = (80% CPLC).

Table 5: Haematological Properties of *Clarias gariepinus* fed with the Experimental Diets

	CD1	CD2	CD3	CD4	CD5
Haematocrit (g)	28.2±1.5	28.7±1.1	28.3±1.5	28.6±1.0	28.5±1.5
Haemoglobin concentration (g/dl)	2.1±0.2	2.4±0.3	2.3±0.3	2.4±0.2	2.3±0.3
Erythrocyte count ($x10^{12}$/litre)	2.2±0.1	2.3±0.1	2.0±0.1	2.1±0.1	2.4±0.1
Leucocyte count ($x10^{9}$/litre)	53.3±6.4	52.5±5.4	52.6±6.2	53.0±7.0	52.9±8.1
Erythrocyte sedimentation rate (%)	$43.1±2.1^{a}$	$44.3±3.0^{a}$	$43.7±2.8^{a}$	$54.9±5.6^{b}$	$56.0±7.0^{b}$

Mean values in a row with different superscripts are significantly different ($P<0.05$).
CD1 = (0% CLPC); CD2 = (20% CLPC); CD3 = (40% CLPC); CD4 = (60% CLPC); CD5 = (80% CPLC).

There were no disruptions to liver tissues in *C. gariepinus* fed with diets CD1, CD2 and CD3; but slight to severe vascular and fatty changes occurred in *C. gariepinus* fed with diet CD4 and CD5 in which CLPC substituted 60% or 80% of SBM, respectively.

Discussion

The antinutrients in CLPC is much lower than values in the corresponding leaf meal (Agbede, 2006, Oresegun *et al*., 2016). Hydrocyanide (HCN) levels<5.3-80 g/Kg is regarded as safe limits for animal feeds (Ravindran, 1993). The inherent antinutrients and toxic substances may limit their use, as they interfere with nutrient digestibility and uptake (Francis *et al*., 2001). If leaves are not processed properly, the cyanide content remains (Ngudi *et al*., 2003) and impact on the health of cultured species. P*rocessing into CLPC reduced the crude fibre content and increased protein quality* (Oresegun *et al.,* 2016). Crude protein and gross energy contents of catfish diets were within the nutrient requirements range for *C. gariepinus* as recommended by Wilson and Moreau (1996).

Values of water quality parameters monitored during the feeding trials were within the acceptable limits/range for the culture of the African catfish (Viveen *et al*., 1985; Britz, 1988). According to Pereira-da-Silva and Pezzato (2000), acceptance of diet by fish as a result of poor palatability is a major problem when plant protein sources are used in aquafeeds. Ogino *et al*., (1978) and Nour *et al*. (1985) showed that leaf protein concentrates produced from ryegrass and water hyacinth partially substituted casein and fish meal in diets for rainbow trout (*Salmo trutta*) and mirror carp (*Cyprinus carpio*), respectively. Soliman (2000) reported that water hyacinth protein concentrate meal could safely substitute 30% of fish meal protein in red tilapia (*Oreochromis niloticus*) diets. Chavez *et al*., (2016) included water hyacinth leaf protein concentrate, substituting up to 75% of SBM for white shrimp, *Litopenarus vannamei*, and recorded beneficial effects at 25% on growth compared to a control diet. The conversion to CLPC apparently degraded antinutrients and reduced fibre contents of diets thus increasing nutrient availability. This study reveals that the conversion to CLPC apparently degraded antinutrients and fibre contents of diets thereby increasing nutrient availability.

The high ACPD values (Table 4) is indicative of the ability of catfishes to digest protein in the diets. ACPD values of CLPC-based diets in *C. gariepinus* are similar to values reported for diets containing soybean meal by *C. gariepinus* (86.9%) (Fagbenro*,* 1998) and the dwarf African catfish, *C. isheriensis* (90%) (Fagbenro*,* 1996). Apparent gross energy digestibility (AGED) values of diets containing CLPC (Table 4) are lower than the values reported for full-fat soybean meal-based diets (77.4%) in *C. gariepinus* (Fagbenro, 1998) and 85% in *C. isheriensis* (Fagbenro, 1996).

Except for ESR values, the other haematological parameters measured were within the normal range for *C. gariepinus* (Svobodova *et al*., 1994). However, Oresegun and Alegbeleye (2001, 2002) reported variations in some haematological parameters/indices in Nile tilapia fed with dry practical diets containing cassava peels and the variations were attributed to the presence of residual antinutrients; which were ameliorated when the diets were supplemented with DL-methionine.

The slight to severe vascular and fatty changes in *C. gariepinus* fed with diet CD4 and CD5 in

which CLPC substituted 60% or 80% of SBM, respectively. are presumably due to presence of residual anti-nutrients in the diets which may have caused disturbance in metabolism and/or mobilization of fat as previously suggested for common carp, and the Indian major carp fed high dietary levels of *Leucaena* leaf meal or mustard seed cake (Hossain and Jauncey, 1988; Hasan *et al*., 1991,1994). Fatty changes also occurred in livers of Coho salmon (Higgs *et al*., 1979), Chinook salmon (Higgs *et al*., 1982), and common carp (Hossain and Jauncey 1989) fed with diets containing rapeseed meal or mustard oil cake.

Conclusion

Cassava leaf protein concentrate (CLPC) produced from TME 419 cultivar supported good growth and diet utilization without histological disorders up to 40% substitution of solvent-extracted soybean meal (SBM) in *Clarias gariepinus* diets; beyond which growth response and diet utilization were compromised, due to reduced protein and energy digestibility. When CLPC substituted >40% of SBM in diets, it elicited histological alterations in liver tissues. Residual antinutrients were mainly responsible for these effects; and will almost certainly limit the use of CLPC beyond this level in *C. gariepinus* diets. The suitability of CLPC as dietary protein in catfish will depend on reduction/removal of inherent anti-nutrients as well as improving digestibility.

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