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REPLACEMENT VALUE OF ELEPHANT EAR TREE [*Enterolobium cyclocarpum* (Jacq.) Griseb.] SEED MEAL FOR SOYBEAN MEAL IN THE DIETS OF AFRICAN CATFISH (*Clarias gariepinus* Burchell: 1822) FINGERLINGS

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ABSTRACT

An eight-week feeding trial was conducted in a partial flow through system to assess the nutritive value of raw *Enterolobium cyclocarpum* (RECSM) and autoclaved *Enterolobium cyclocarpum* (AECSM) seed meals as dietary protein replacement for Soya bean meal in practical diets for *Clarias gariepinus* fingerlings. Seven isonitrogenous (35% crude protein) diets with mean metabolizable energy (ME) of 363.72Kcal/100g were formulated in which mature RECSM and AECSM were used to replace soya bean meal each at three inclusion levels (15, 30 and 45%), namely, TD_1, TD_2, TD_3, TD_4, TD_5 and TD_6, respectively. The control diet was without the seed meals. Survival was generally high between 83 and 95%. Water quality was within the optimal range for cultured warm water fish species. Autoclaving resulted in increased crude protein (from 21.60 to 26.20%) and lipid (from 5.82 to 7.64%) contents and decreased crude fibre (from 11.53 to 8.20%). The best growth performance was recorded in the group fed test diet TD_1 (15% AECSM), while the least performance was recorded in the group fed TD_6; every other growth and nutrient utilization parameter followed this trend. Nutrient digestibility displayed progressive reduction with increase in inclusion levels of RECSM and AECSM. In view of its potentials in fish feed, further studies are necessary on techniques like dehulling for reducing the levels of crude fibre of the meal.

Keywords: *Clarias; Enterolobium* seed meal; Growth and nutrient utilization

INTRODUCTION

The major challenge faced by the Nigerian fish farming industry, especially the small scale sector is the provision of good quality fish feed at affordable cost. This has become more complicated with the competitive demand on soybean as a dietary protein source for human and livestock nutrition and the decline in national production of this staple food (USDA, 2008). Some studies have been carried out on cheap and available alternative dietary protein sources for *C. gariepinus*. These include unconventional animal resources like poultry waste (Omitoyin, 1996), cultured invertebrates (Omoyimi, 2003), and

grasshopper meal (Alegbeleye *et al*., 2011). Other researchers have also assessed the use of locally available dietary sources of plant origin (especially legumes) in the diets of cultured fishes such as *Mucuna atterima* (Obasa *et al.*, 2004), bambara groundnut (Alegbeleye *et al*., 2001), cotton seed (Arowosoge, 1987) and oil seeds (Fagbenro, 1998) to supplement or partially replace soya bean. The results of these studies show growth to be similar to those obtained with the various soybean-based diets.

The major limitations in the extensive use of plant protein sources, according to Tacon (1993), is their low crude protein content, poor amino acid profile and high crude fibre levels, and all these are further complicated by various levels of antinutritional factors like phytates, anti-trypsin and haemagluttinins. These toxic substances can be detoxified through various processing methods such as autoclaving, toasting, cooking, soaking, extrusion, dehulling and fermentation (Ziena *et al*., 1991; Gomes *et al*., 1992; Danielson *et al*., 1992; Proulx *et al*., 1993; Erbas *et al.*, 2005).

Enterolobium cyclocarpum (Jacq.) Griseb is one of the largest trees in the dry forest formation of Mexico and Central America, reaching up to 3m in diameter and 40 m in height with a huge spreading crown. It is a fast growing timber yielding species of artisanal importance, and so popular that it is adopted as the national tree of Costa Rica. (Janzen, 1981). The tree is particularly popular for its thick, contorted, indehiscent pods which bear the extremely hard seeds.

Very little is known on the use of the plant in animal husbandry in spite of its nutritive value, except its use in the control of protozoans (Ivan *et al.*, 2004; Navas *et al*., 1992). According to Babayemi (2006), the foliage is yet to be accepted by ruminants in Nigeria. Research studies are at the level of experimental trials **(**Proll *et al*., 1998; Iyayi *et al*., 2004; Grant *et al*., 1993; Babayemi, 2006; Siddhuraju and Becker, 2005). However, its nutritional composition makes its trial as a dietary protein source attractive. This preliminary study therefore aimed to evaluate the effect of replacing soybean meal with variously-processed *Enterolobium cyclocarpum* seed on the growth, nutrient utilization and body composition of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

This experiment was conducted in a static rearing system consisting of twenty-one cylindrical plastic containers (30 litres each) with perforated cover knitted with mosquito net and placed under erected bamboo shade located behind the College of Environment Resources Management (COLERM) building in the University of Agriculture, Abeokuta. The plastic bowls were filled to 20 litres of the volume and were continually supplied with bore-hole water to sustain optimal environment. The water was introduced in a splash form for better aeration.

African catfish (*Clarias gariepinus*) fingerlings (4.5g ±.46) used for the experiment were obtained from Stephen Fish Farm, Obantoko, Abeokuta. They were acclimatized for a period of 14 days inside a glass fiber trough in the Department of Aquaculture and Fisheries Management of the University.

Diet Formulation and Preparation

All feed ingredients, except *E. cyclocarpum* seeds, were purchased from University of Agriculture, Abeokuta (UNAAB) Feed Mill, Kotopo, Abeokuta. The seeds were collected from the Gene Bank Arboretum, International institute of Tropical Agriculture

(IITA), Ibadan. The dry seeds were cleaned of debris, cracked into smaller pieces in a wooden mortar, milled in a locally-fabricated hammer mill and sieved with a fine mesh. The meal was divided into two batches; a batch was stored raw as raw *Enterolobium cyclocarpum* seed meal (RECSM) while the other was packed in a polythene bag and autoclaved in a medical autoclave (Prestige Clinical Autoclave, Series 2100) at 126^{o}C and 21psi for 15 minutes and sun-dried. This batch was stored as autoclaved *Enterolobium cyclocarpum* seed meal (AECSM)

Seven isonitrogenous (35%) diets with mean metabolizable energy (ME) of 363.72Kcal 100g^{-1}) were formulated to contain the autoclaved and raw seed meal each at three inclusion levels (15, 30 and 45%). Autoclaved seed meal (AECSM) were tagged TD_1,TD_2 and TD_3 and same levels of raw seed meal (RECSM) tagged diets TD_4, TD_5 and TD_6. The feed was palletized into 2mm diameter size using a HV6 Moulinex pelletizing machine, sun-dried and stored in tagged polythene bags.

Experimental Set-up and Management

A total of 150 fingerlings were weighed individually at the start of the experiment, and were randomly distributed at the rate of 10 fingerlings per bowl. Each of the seven experimental diets was randomly assigned to triplicate groups of fish. All fish were fed at 3% body weight twice daily at 8-9am and 4-5pm local time for the period of the experiment (8 weeks). Uneaten feed particles and wastes were siphoned out and water loss was appropriately replaced. The fish were batch-weighed weekly with an electronic balance and the amount of feed was adjusted based on weight increment.

Chemical Analysis

All analyses for proximate composition of ingredients and carcasses were determined according to the methods of AOAC (1990). Total Nitrogen (N) was determined by the Micro-Kjeldal procedure and crude protein estimated as N x 6.25. Crude lipid was determined after Soxhlet extraction of dried samples with petroleum ether. Crude fiber was determined as the loss of ignition of dried lipid-free residues after acid-alkaline digestion (Trichloro acetic acid TCA) method. Moisture was determined after oven–drying at 110^{o}C to constant weight. Carbohydrate (as NFE) was calculated as follows: NFE = 100 - (%protein + %lipids + %ash + % fibre). Faeces were collected daily in the last three weeks of the feeding trial; collected faeces were pooled according to treatment, then dried and stored in tagged cellophane bags for analysis. Chromium (III) oxide contents of the diets and faecal materials were determined by the method described by Schurch *et al*. (1950).

Data Analysis

The growth performance and feed utilization were analysed according to Olivera *et al.,* (1990) as follows:

Specific Growth Rate = $Ln\frac{\mathrm{W2 - LnW1}*100}{Time\ (day)}$

W_1 = Initial weight (g)
W_2 = Final weight (g)
Ln = Natural logarithm

$$\text{Protein Efficiency Ratio} = \frac{\text{Mean Weight Gain (g)}}{\text{Average Protein Fed}}$$

$$\text{Average Protein Fed} = \frac{\text{Feed intake} * \text{\% crude protein of feed}}{\text{Weight gain (g)} = W2 - W1}$$

$$\text{Feed Conversion Ratio} = \frac{\text{Weight of feed (g)}}{\text{Weight gained (g)}}$$

The apparent digestibility coefficients (ADCs) were calculated with the formula of Castel and Tiews (1980):

$$ADC_{\text{Dry matter}}\ (\%) = 100 * \left(1 - \frac{\%Cr2O3 \text{ in diet}}{\%Cr2O3 \text{ in faeces}}\right)$$

$$ADC_{Nutrient}(\%) = 100\ (1 - ((Cr2O3 \text{ in diet}) * (\%\text{nutrient of energy in faeces}) + (\%Cr2O3 \text{ in faeces}) * (\%\text{nutrient or energy in diet})$$

$$\text{Mortality Rate (\%)} = \frac{\text{No. of dead fish at the end of the experiment}}{\text{No. of fish at the beginning of the experiment}} * 100$$

$$\text{Survival Rate (\%)} = \frac{\text{No. of fish remaining alive at the of experiment}}{\text{No. of fish at the beginning of the experiment}} * 100$$

$$\text{Protein productive value (PPV) (\%)} = \frac{\text{Protein gained (g)}}{\text{protein fed (g)}} * 100$$

The growth performance and feed utilization were analyzed statistically by one way Analysis of Variance (ANOVA) according to Steel and Torrie (1980). The differences among means were tested for significance (P = 0.05) using Duncan's Multiple Range Test (Gomez and Gomez, 1985).

Water Parameters

Essential physico-chemical parameters of the culture system were monitored twice weekly during the course of the feeding trial. Water temperature (26.5-29.0^{o}C) was determined with mercury- in- glass thermometer; dissolved oxygen (DO) (4.8 – 5.70mgl^{-1}) and pH (6.24-7.87) were monitored with Electric Jenway pH meter. Ammonia (NH_3) (0.14-0.45mgl^{-1}) and alkalinity (157-175 mgl^{-1}) were by the titrimetric determination of total alkalinity (Thomas and Lynch, 1960)

RESULTS

Autoclaving affected the proximate composition of the seed meal (Table 1). There were measurable increases in crude protein content (21.60- 26.20%) and lipid content (5.82-7.64%), and a decrease in the crude fibre content (11.53 to 8.20%). This affected the proximate composition of the diets, especially the relative values of crude fibre (Table 2).

All test diets were accepted and actively fed upon by the fish at the first fortnight of the experiment; consequently the rate of feed intake in the group fed the raw meals

decreased. However, no pathological sign due to dietary deficiency was observed in all fingerlings fed the different experimental diets. Generally, the group fed diets containing the autoclaved seed meal (TD_1, TD2 & TD_3) performed better than the groups fed raw seed meal and control diets. The best growth performance (Table 3) was observed in the group fed 15% inclusion level of autoclaved meal (TD_1), while the least growth performance was observed among those that received 45% raw meal (TD6). This pattern was observed in all other growth and nutrient utilization parameters like average weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV). Percentage survival was generally high (85-95%) and was non-differential, that is, was not affected by dietary levels of the seed meals.

Apparent digestibility coefficients (dry matter, lipid and protein decreased with increase in inclusion levels of the seed meals.

The body composition of the fish at the beginning (initial) and end of the experiment are presented in Table 4. Carcass composition was not clearly affected by diet composition as there were no significant differences ($P>0.05$), except in the moisture content. Moisture content increased while carcass lipid decreased with the level of the seed meal in the diets. There was an inverse relationship between body moisture and lipid levels, and ash content did not present any defined trend among the different groups.

Table 1: Proximate composition of feed ingredients

Feedstuff	Composition (%)				
	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Ash
Fish meal	68.00	10.24	-	6.96	14.80
Soya bean	42.00	18.00	5.00	30.4	4.60
Raw *Enterolobium* seed meal	21.6	1.57	11.53	64.08	1.22
Autoclaved *Enterolobium* seed meal	26.2	1.69	8.20	62.72	1.19
Groundnut cake	45.00	8.80	4.31	30.21	13.08
Maize	10.00	5.50	1.40	81.7	1.40

DISCUSSION

The effect of processing on the proximate composition of *E. cyclocarpum* seed was considered positive, because it resulted in the enhancement of the nutritive value (Table 1) as observed in previous studies by Igbedioh *et al*. (1994), Giami and Ikpimi (1992), Siddhuraju *et al.* (1996) and Umoren *et al.* (2005). Observations from their studies were similar to the observed increase (Table 1) in the protein and lipid contents and decrease in crude fibre content.

The nutritive values of the raw and autoclaved seed meals (Table 2) suggest 15% AECSM inclusion level without adverse effect on growth and nutrient utilization. However, the growth and feed utilization of the fish fed AECSM were better than those fed RECSM. Apparent digestibility values for dry matter, protein and lipid for the control and TD_1 were numerically superior to all those of RECSM and higher levels of AECSM. This could be as a result of various levels of anti-nutritional elements in *E. cyclocarpum* seed such as trypsin inhibitors, phytic acids and alkaloids (Proll *et al*., 1998; Iyayi *et al*., 2004) haemaggluttin,

(Grant *et al*., 1993) and saponin (Babayemi, 2006), in fact saponin could be toxic to fish at higher inclusion levels (Siddhuraju and Becker, 2005)

Table 2: Composition of experimental diets (g $100g^{-1}$)

Ingredient	----------Autoclave----------				----------Raw----------		
	CD	TD_1	TD_2	TD_3	TD_4	TD_5	TD_6
Gross Composition Fish meal	150	150	150	150	150	150	150
Groundnut cake	200	200	200	200	200	200	200
Soybean meal	317.1	291.1	260.5	224.2	298.0	274.5	244.6
Raw *Enterolobium* seed meal	-	-	-	-	52.6	117.6	200.1
Autoclaved *Enterolobium* seed meal	-	51.4	111.7	183.5	-	-	-
Maize	247.9	222.5	192.8	157.3	214.4	172.9	120.3
Palm oil	25	25	25	25	25	25	25
*Vit. / Mineral Premix	15	15	15	15	15	15	15
Methionine	10	10	10	10	10	10	10
Lysine	10	10	10	10	10	10	10
Starch	10	10	10	10	10	10	10
Common Salt	10	10	10	10	10	10	10
Chromic oxide	5	5	5	5	5	5	5
Dry Matter Proximate composition	90.47	90.65	90.18	90.72	90.96	90.29	91.02
Crude protein	34.86	35.09	35.84	36.22	35.04	36.21	35.92
Ether extract	3.43	3.03	3.25	3.30	3.17	3.41	3.27
Crude fibre	5.42	6.48	8.28	9.48	7.22	9.48	10.34
Ash	6.21	5.82	6.23	6.00	6.22	6.32	6.14
NFE	50.08	55.40	46.40	45.00	48.35	44.58	44.33
+M Energy kcal $100g^{-1}$	370.63	389.23	358.21	354.58	362.09	358.85	350.43

*(B12): 0.5mg; Panthothenic Acid: 1.0mg; Phyridoxine (B6): 0.15mg; Cyanocobalamine (B 12): 0.001mg; Nicotinic Acid: 3.0mg; Folic Acid 0.1mg; Choline: 31.3mg; Ascobic Acid (C): 0.1mg; Iron Radar Vitamin. Premix. Supply /100g Diet. Palmat A: 1000Iu; Cholecalcifero (D): 1000Iu; G-Tocopherolacetate (E): 1.1mg; Menacilione (K): 0.02mg; Thiamine B1: 0.63mg; Riboflvin (Fe): 0.05mg; Cu: 0.25mg; Mn: 6.00mg; Co: 0.5mg; Zn: 5.0mg; Sn 0.02mg ; + Gross energy was

calculated by using equivalent factors of 4.0, 9.0 and 4.0 kcal/g for CP, Lipid/EE and NFE respectively (Lee and Putnam, 1973)

Table 3: Growth performance and feed utilization of *C. gariepinus* fingerlings fedxperimental diets with graded levels of *E. cyclocarpum* seed meals

Paramerter	Diet			
	----------Autoclaved----------			
	CD	TD_1	TD_2	TD_3
Initial body wt. (g)	4.65±0.05[a]	4.05±0.05[a]	4.55±0.05[a]	4.50±0.20[a]
Final body wt. (g)	16.85±0.65[a]	16.20±0.30[a]	15.65±0.95[a]	16.10±0.40[a]
Weight gain (g)	12.20±0.70[a]	12.25±0.35[a]	11.10±0.90[a]	11.60±0.20[a]
%Weight gain	262.56±17.87[ab]	302.62±12.38[a]	243.77±17.1[ab]	258.09±7.03[ab]
SGR %	2.30±0.09[ab]	2.49±0.06[a]	2.20±0.09[ab]	2.28±0.04[ab]
FCR	1.10±0.04[a]	1.06±0.07[a]	1.13±0.06[a]	1.12±0.04[a]
PER	2.61±0.08[a]	2.72±0.16[a]	2.54±0.12[a]	2.57±0.08[a]
PPV	45.17±1.81[a]	45.20±2.76[a]	41.89±2.13[a]	43.16±0.04[a]
ADC_{dm}	79.23±3.08[a]	78.67±0.84[a]	79.09±2.85[a]	77.00±1.12[a]
ADC_{lipid}	82.33±2.17[a]	82.87±0.54[a]	80.90±3.00[a]	80.00±1.50[a]
$ADC_{protein}$	84.57±1.43[a]	83.22±0.95[a]	79.65±4.25[a]	78.26±0.56[a]
Survival %	90	85	95	95
		Diet		
		----------Raw----------		
	CD	TD_4	TD_5	TD_6
Initial body wt. (g)	4.65±0.05[a]	4.40±0.20[a]	4.05±0.05[a]	4.70±0.20[a]
Final body wt. (g)	16.85±0.65[a]	13.95±0.25[a]	14.45±0.05[a]	15.40±0.60[a]
Weight gain (g)	12.20±0.70[a]	9.55±0.05[a]	10.40±0.00[a]	10.70±0.40[a]
%Weight gain	262.56±17.87[ab]	217.45±8.75[b]	256.83±3.17[ab]	227.71±1.18[b]
SGR (%)	2.30±0.09[ab]	2.06±0.05[b]	2.28±0.02[ab]	2.12±0.11[b]
FCR	1.10±0.04[a]	1.24±0.06[a]	1.15±0.02[a]	1.15±0.01[a]
PER	2.61±0.08[a]	2.32±0.10[a]	2.50±0.04[a]	2.49±0.03[a]
PPV	45.17±1.81[a]	38.32±2.65[a]	38.70±0.89[a]	39.26±2.01[a]
ADC_{dm}	79.23±3.08[a]	76.24±1.86[a]	77.23±1.81[a]	72.10±0.90[a]
ADC_{lipid}	82.33±2.17[a]	81.98±4.22[a]	76.77±1.37[a]	72.13±1.20[a]
$ADC_{protein}$	84.57±1.43[a]	79.56±0.65[a]	76.24±1.28[a]	76.78±1.97[a]
Survival (%)	90	95	90	95

Means on the same row with the same letter are not significantly different (P>0.05). SGR (%)- specific growth rate; FCR- food conversion ratio; PER-protein efficiency ratio; PPV- protein productive value; ADC_{DM}- apparent digestibility coefficient dry matter; ADC_{lipid}- apparent digestibility coefficient lipid; $ADC_{protein}$- apparent digestibility coefficient protein

Table 4: Proximate carcass composition (% wet weight) at the start and end of experiment.

Parameter (%)	Diet				
	----------Autoclaved----------				
	Initial	CD	TD_1	TD_2	TD_3
Moisture	75.89	71.67±0.40^{c}	71.95±0.50^{c}	72.04±0.18^{c}	72. 40±0.07^{bc}
Crude Protein	14.31	16.49±0.15^{a}	16.03±0.05^{a}	15.84±0.08^{a}	16.13±0.34^{a}
Crude Lipid	5.16	6.93±0.06^{a}	6.55±1.00^{a}	6.56±0.11^{a}	5.73±0.17^{b}
Ash	6.06	4.72±0.06^{a}	4.54±0.11^{a}	4.45±0.21^{a}	4.64±0.19^{a}
	Diet				
	----------Raw----------				
	Initial	TD_4	TD_5	TD_6	
Moisture	75.89	73.89±0.25^{ab}	73.04±0.06^{abc}	74.39±0.39^{a}	
Crude Protein	14.31	15.82±0.31^{a}	15.16±0.07^{a}	15.33±0.68^{a}	
Crude Lipid	5.16	5.29±0.06^{b}	5.33±0.09^{b}	5.21±0.23^{b}	
Ash	6.06	4.89±0.11^{a}	5.06±0.16^{a}	5.13±0.07^{a}	

Values are means of three replicates (n=3) diets, means on the same row followed by the same superscript are not significantly different (P>0.05).

The best response of the different groups (Table 4) was observed in the group fed the various levels of autoclaved meals, while the least growth was recorded in those fed the highest (45%) inclusion level of raw seed meal. Autoclaving is known to enhance the nutritive value of ingredients through improved digestibility and nutrient release in plant-based ingredients (Proulx *et al*., 1993; Mubarak, 2005; Siddhuraju and Becker, 2005). Possible reasons for the poor performance of diet TD_4 could be due to the presence of residual anti-nutritive factors that include enterolobin (Castro-FariaNeto *et al*., 1991), protease inhibitors lectins and ribosomes inactivating proteins (Gatehouse *et al*., 1999; Gatehouse *et al*., 1984; Gatehouse and Boulter, 1983). All these are known to reduce growth in monogastrics, especially fish (Fagbenro, 2000). They are also known to be heat labile (Ravindran *et al*., 1996). There was no significant difference ($P > 0.05$) in the weight gain, specific growth rate (SGR) and food conversion ratio (FCR) among the dietary treatments. However, the superior performance of the group fed autoclaved meals (15, 30 & 45%) over the control is noteworthy, since soybean is reputed to have superior nutritive value (Robinson and Li, 1993); however, soybean could also be limited by imbalance in amino acid profile, presence of residual trypsin inhibitors (Storebakken *et al*., 2000), and it is also known to contain about 20-30% non-starch polysaccharide (NSP) (Bach-Knusden, 1997). According to Forde-Skjaevrvik *et al*. (2006), the soluble part of this fraction could

increase digesta viscosity, thus reducing nutrient absorption by reducing proper mixing of enzymes with digesta as earlier observed in poultry (Choct *et al*., 1996).

The growth performance and the high survival rate (90-95%) among the group that received the various levels of RECSM appears unusual, considering the presence of anti-nutritional factors and high crude fibre content. These could suggest that the species is tolerant to low levels of antinutritional factors. This had been observed in previous studies (Balogun and Ologhobo, 1989) The growth performance of this group agreed with previous studies on feeds with high crude fibre content (Usmani *et al.*, 2003; Mukhopadhyay and Ray, 1999).

The extent of the effect of heat treatment on the anti-nutritional factors was not determined in this study, but the proximate composition of the autoclaved seed showed that it enhanced the nutritive value of seed meals, as evidenced by the lower PPV and PER values recorded for the groups that received RECSM.

The results from the experiment showed that both raw and autoclaved *E. cyclocarpum* seed meal could be used to supplement soya bean meal in practical diets for *C. gariepinus* fingerlings. However, heat treatment improved the bioavailability of nutrients. A major constraint to the utilization of *Enterolobium* seed is the hard coat and high crude fibre content which make it difficult to process to a meal, and this could cause poor digestibility. Subsequent studies could consider some adaptable methods that could enhance the relevance of this seed in fish nutrition.

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