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PRELIMINARY EVALUATION OF FERMENTED CASTOR (*Ricinus communis*) SEED MEAL IN THE DIETS OF *Clarias gariepinus x Heterobranchus bidorsalis* HYBRID FINGERLINGS.

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ABSTRACT

The potential of using fermented castor seed (*Ricinus communis*) meal (FCSM) as a dietary protein source in the diets of hybrid *Clarias gariepinus x Heterobranchus bidorsalis* fingerlings was studied. FCSM level was increased at the expense of soybean meal (SBM) in isonitrogenous (30%) and isocaloric (metabolizable energy (ME) of 409.22Kcal/100g) diets. SBM was replaced at 0, 25, 50, 75 and100% levels by FCSM. Each diet was fed for 56 days to triplicate groups of fingerlings in a completely randomized design using floating hapas in an outdoor concrete cistern. Growth was best in the group of fingerlings fed the control diet, but this was not significantly ($P<0.05$) better than the group fed TD_1 6.98% FCSM diet level; while it was least in the group fed 32% FCSM level. Every other growth and feed nutrient utilization parameter followed this trend. Survival was 60 to 86% and non-differential between the dietary treatments. Carcass protein significantly decreased with increased FCSM level, and carcass lipid showed inverse relationship with protein and moisture levels. Results showed that FCSM can be incorporated up to about 7% in the diet of the hybrid fingerlings. However, further studies are required on techniques of deoiling prior to fermentation and use of antioxidants.

Keywords: *Clarias gariepinus* x *Heterobranchus bidorsalis*; Castor seed meal; Growth and nutrient utilization.

INTRODUCTION

Research into cheap and available dietary protein sources to supplement or replace expensive dietary ingredients in competitive demand has been the focus of various researches in recent time. These include the evaluation of terrestrial animal by-products like poultry by-products **(**Abdel-Warith *et al.,* 2001; Omitoyin, 1996), fishery by-products (Nwana, 2003), plant protein sources like aquatic plants (Fasakin *et al*., 2001), legumes and oilseeds (Obasa *et al*., 2006; Fagbenro, 2004), bambara groundnut (Alegbeleye *et al*., 2001) and *Parkia biglobosa* seed (Oso *et al*., 2011). The most viable alternatives however, are dietary protein sources of plant origin.

Plant dietary protein products are nevertheless constrained by a list of factors which include low protein level, imbalance in amino acid profile, high crude fibre content and presence of antinutritional factors. Soybean had been variously processed as dietary protein source in the feed of cultured fish (Forster, 2003; Bhart *et al.*, 2009). However, its extensive use in feeds has been limited by inadequate local production and competing demand in human nutrition (IITA, 1990) and livestock feed industry, and therefore competitive pricing/cost (USDA, 2008).

Since some plants with potentials as ingredients in fish diets have some relevance in human nutrition, it is sensible to embark on the evaluation of underutilized plants with limited relevance in human nutrition. Variously-processed oilseeds and their products such as vegetable oil and spread are described as nutritious, although energy dense, and are good sources of vitamins D and E (McKevith, 2005).

Castor seed (*Ricinus communis*) (Family: Euphorbiaceae) is a typical example of an underutilized oilseed (Enujiugha and Ayodele-Oni, 2008). It is one of the most visible underutilized plants in South-western Nigeria, thriving in wild plantations, in disturbed land like abandoned construction sites and dung hill. It is considered nutritious (Weiss, 1983), and contains about 18-46% crude protein (depending on the method of processing) and 50% oil (Negi, 1996). However, its use in livestock feed is limited by the presence of at least three toxic factors: the protein ricin allergen; a protein polysaccharide; and ricinine, a mildly toxic alkaloid (Kodras *et al*., 1949). Attempts at detoxifying castor seed included steam treatment at $5kg/cm^2$ for 15-30 minutes, but this was found to be unsatisfactory as the product could only be optimally included to 10% level in sheep feed (Punj, 1988). Variously-processed castor oil seeds have been studied in the diets of ruminants, rabbits, pigs and chicks. Fermentation seems to be the only effective means of detoxification (Obizoba, 1998). When fermented, castor oil seed is a veritable soup condiment favoured by the Igbos of the South-east of Nigeria (Odunfa, 1985).

Fermentation is a method that could enhance the nutritive value of castor oilseed. Oso *et al*. (2011) assessed the nutritive value of fermented castor oil seed in cockerel chicks and concluded that it could be included in starter diet only to $50gkg^{-1.}$

In view of the potential of castor oilseed as a dietary protein ingredient, the present study was undertaken to determine the nutritive value of fermented castor oilseed to *Clarias gariepinus x Heterobranchus bidorsalis* hybrid.

MATERIALS AND METHODS

Experimental System and Fish

The experiment was carried out in fish hapa nets (1m x 0.5m x 0.5m) suspended by bamboo poles in an outdoor tank (5m x 3m x 1.5m) located at the College of Environmental Resources Management (COLERM) building, University of Agriculture, Abeokuta, Nigeria.

Fifteen (15) hapas (1m x 0.5m x 0.5m) were immersed to ¾ of their volume, using Kuralon twine (No.15), tied to carefully-arranged bamboo poles into a concrete cistern (3 x 5 x 1.5m). The concrete tank was filled to 75% of its volume and continually supplied with water from a tube well to sustain optimal environment and to preclude primary productivity. The water was introduced in a splash for better aeration.

Hybrid fingerlings of *C. gariepinus* x *H. bidorsalis* (0.87g± 0.24) used in the feeding trial were obtained from Iki Fish Farm, Obantoko, Abeokuta. The fingerlings were

acclimatized a fortnight prior to the feeding trial in 5 glass aquaria (1 x 1 x 1.5m) in the wet laboratory, and were maintained on a formulated diet containing 25% crude protein before the commencement of the feeding trial.

Feed Preparation

All feed ingredients (Table 1) were purchased from University of Agriculture,. (UNAAB Agro Allied Industry Ltd.) Kotopo, Abeokuta, except the castor seeds which were harvested from wild plantations around the city of Abeokuta. The cooked castor seed was fermented according to the method described by Weiss (1983). The cooked castor seeds were crushed and subjected to a mechanical screw press to expel the oil. It was then poured into a pot container, covered with pre-heated banana leaves and subjected to continuous natural fermentation for between 24 and 96 hours, after which it was sun-dried.

Five diets (Table 2) were formulated to be isonitrogenous (30% crude protein) with metabolizable energy of approximately 409.22kcal/100g. Soybean was progressively replaced with fermented castor seed meal (FCSM) meal at 25, 50, 75 and 100% levels. The feeds were pelletized into 2mm diameter size using a HV 6 Moulinex pelletizing machine, sun-dried for some days and stored in labeled polythene bags.

Experimental Procedure

The acclimated experimental fish were starved for 24 hours prior to the commencement of feeding and weighed individually. The fingerlings were randomly distributed at the rate of 10 fish per hapa.

The five experimental diets were randomly assigned to triplicate groups of fish. All fish were fed at 5% body weight twice daily for 10 weeks at 8-9am and 4-5 pm local time. The fish were batch-weighed weekly with an electronic balance (METTLER BD 601), and the amount of feed was adjusted based on weight measurement. At the end of the experiment, five fish from each dietary treatment were sacrificed for carcass analysis.

Essential physico-chemical parameters were monitored at the beginning and twice weekly in the course of the experiment. Water temperature (28.4-30^{o}C) was determined with mercury- in-glass thermometer; dissolved oxygen (4.8 – 5.80 mgL^{-1}) and pH (6.83-7.50) were monitored with Electric Jenway pH meter. Ammonia (NH_3) (0.12-0.29mgL^{-1}) and alkalinity (155-173 mgL^{-1}) were determined by the titrimetric determination of total alkalinity (Thomas and Lynch, 1960)**.** These values were within the range recommended for Clariids (Swann, 2006)

Chemical Analyses

Feed ingredients, experimental diets and fish carcasses (initial and final) were analysed for their proximate composition by (AOAC, 1990). Crude protein (N x 6.25) was analysed by Kjeldhal methods, crude lipid was determined by extraction with petroleum ether in a soxhlet apparatus, ash content was determined at 450^{o}C in a Gallenkamp Muffle Furnace while crude fibre was determined as loss on ignition of dried lipid-free residues after acid-alkaline digestion (Trichloroacetic) acid method. Moisture content was determined after oven-drying in a Gallenkamp oven (BS 250 size 2) at 105^{o}C to constant weight. Peroxide value was determined by measuring the amount of iodine formed by the reaction of the peroxide (product of oil rancidity) with iodide ion. Iodine liberated was titrated with sodium thiosulphate. Indicator was starch solution (Salunkhe *et al.*, 1992). Faeces were collected daily in the last three weeks of the feeding trial, and collected faeces

were pooled according to treatment, 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

Nutrient utilization parameters were calculated as described by Olivera *et al*, (1990):

$$\text{Weight gain (\%)} = 100 * (\text{(final body weight – initial body wt) / (initial body wt)})$$

$$\text{SGR (\% day)} = 100 * \frac{(\log \text{finial body wt} - \log \text{initial body wt})}{\text{time(day)}}$$

$$\text{Feed conversion ratio} = \frac{feed\ intake\ (dry\ weight)g}{\text{fish wt (fresh weight) g}}$$

$$\text{Protein efficiency ratio} = \frac{\text{fish (fresh weight)g}}{\text{protein intake (g)}}$$

The growth performance and feed utilization were analyzed statistically by one-way Analysis of variance (ANOVA), and the differences among means were tested for significance (P = 0.05) following Duncan's Multiple Range Test (Gomez and Gomez, 1985).

RESULTS

Effect of Fermentation

Fermentation resulted in the elevation of the crude protein content of deoiled castor seed from 31.41% (raw) to 35.58% (fermented), and crude fibre content from 6.46% to 7.90%, declined lipid content from 38.20% to 12.45% and a significant increase in rancidity as depicted by high peroxide value ranging from 20.12 in control diet to 119.15 meq peroxide kg^{-1}-in TD_4 (Table 1).

Experimental fish fed actively all through the feeding trial. Mortality appeared non-differential as it was highest (40%) in the group fed the control diet, therefore handling stress was suspected.

Growth and Nutrient Utilization

The mean growth response and feed utilization indices are shown in Table 3. All fish in the different groups fed actively in the first fortnight of the feeding trial, feeding however decreased in the groups receiving diets with high (50, 75 and 100%) seed meal inclusion levels. The group fed the control diet showed superior growth in terms of weight gain (7-13g) (Table 3) and did not differ significantly over the groups that received diets TD_1 (6.98%), but differed significantly ($P<0.05$) from the groups that received diets TD_2(14.6%), TD_3 (22.96%) and TD_4 (32.17%)**.** However, weight gain generally decreased as inclusion of FCSM increased. The specific growth rate of fish (SGR) showed the same

trend as weight gain, so also was protein efficiency ratio (PER). Feed conversion ratio (FCR) of TD_2 (2.44) was slightly higher than TD_3 (2.31).

Table1: Proximate composition (%) of ingredients

Ingredient	Composition %				
	Crude Protein	Ether extract	Crude fibre	NFE	Ash
Fish meal	67.76	8.00	1.00	4.00	14.89
Soya bean meal	45.30	18.00	5.00	26	4.60
Raw castor seed meal	31.41	38.20	5.46	21.46	15.42
Fermented castor Seed meal	35.58	12.45	7.90	13.65	11.60
Corn	10.81	5.50	1.40	79.60	1.40
Ground nut cake	36.46	8.80	4.31	30.21	13.08

Table 2: Gross (%) and proximate compositions of the experimental diets

Ingredient	DIET				
	CD (0%)	$TD_{1\ (25\%)}$	$TD_{2(50\%)}$	$TD_{3(75\%)}$	$TD_{4(100\%)}$
Fishmeal	6	6	6	6	6
Soybean meal	44.56	39.53	34.08	28.06	21.45
Groundnut cake	6	6	6	6	6
Corn	34.94	32.99	30.82	28.06	25.88
Fermented castor seed meal	-	6.98	14.6	22.96	32.17
Palm oil	3	3	3	3	3
Vitamin/Mineral premix	1.5	1.5	1.5	1.5	1.5
Methionine	1	1	1	1	1
Lysine	1	1	1	1	1
Starch	1	1	1	1	1
Moisture	5.72	6.16	6.45	6.76	6.82
Crude Protein	31.20	29.78	30.06	31.66	31.81
Crude lipid	6.43	9.34	12.65	13.77	15.21
Ash	6.25	5.65	5.20	5.11	5.67
NFE	50.40	49.07	45.64	42.70	40.49
PV meq peroxide GE	20.12	55.76	76.55	109.24	119.15
Gross energy (kcal/100)[1]	448.72	462.61	481.10	488.35	479.91
Metabolizable energy (kcal/100g)	384.27	399.46	416.65	421.37	424.37

1. Metabolizable energy was calculated by using the physiological equivalent factors where 1g of CP, Lipid/EE and NFE (Carbohydrate) yields 4.0, 9.0 and 4.0 kcal/g respectively (Lee and Putnam, 1973)

Table 3: Growth performance and feed utilization efficiency of the fingerlings fed castor seed meal

Parameter	Diet $\overline{x}$ ±				
	CD	$TD_{1(25\%)}$	$TD_{2(50\%)}$	$TD_{3(75\%)}$	$TD_{4(100\%)}$
Initial weight	.87±0.02^{a}	0.89±0.04^{a}	0.85±0.03^{ab}	0.89±0.03^{a}	0.85±0.02^{a}
Final weight	8.00±0.44^{a}	7.84±1.07^{a}	4.10±0.26^{b}	3.12±0.14^{b}	2.58±0.05^{b}
Wt gain	7.13±0.42^{a}	6.95±1.03^{a}	3.25±0.29^{b}	2.23±0.17^{b}	1.73±0.07^{b}
PER	2.29±0.02^{a}	2.14±0.14^{a}	1.37±0.01^{b}	1.44±0.00^{b}	1.30±0.04^{b}
SGR	3.98±0.09^{a}	3.87±0.17^{a}	2.81±0.17^{b}	2.40±0.15^{bc}	2.13±0.08^{c}
FCR	1.46±0.02^{e}	1.67±0.02^{d}	2.44±0.02^{b}	2.31±0.01^{c}	2.58±0.07^{a}
Protein fed	3.11±0.15^{ab}	3.47±0.49^{a}	2.37±0.20^{bc}	1.55±0.12^{cd}	1.34±0.02^{d}
Food fed	10.37±0.51^{ab}	11.55±1.61^{a}	7.90±0.65^{bc}	5.15±0.39^{cd}	4.45±0.05^{d}
Survival	60±0.78^{bc}	86.0 ±1.94^{a}	72±1.68^{c}	69±2.75^{c}	79±1.35^{b}

Value in the same row with same letters are not significantly different (P<0.05)

Body Composition

Table 4 shows the carcass proximate composition of experimental fish at the start and end of the feeding trial. Moisture content of initial fish was measurably highest (77.32%), and least (72.75%) in the group fed the control diet. Crude protein content was least (11.07%) in the initial fish and highest (15.56%) in the control. Crude lipid content was generally high in those groups that received high (22.96% and 32.17%) inclusion levels of the FCSM.

Table 4: Carcass composition of the fingerlings fed various levels of fermented castor seed meal.

Param-eter	Diet					
	Initial	CD	$TD_{1\ (25\%)}$	$TD_{2\ (50\%)}$	$TD_{3\ (75\%)}$	$TD_{4\ (100\%)}$
Moisture	77.32±1.08^{a}	76.75±0.36^{ab}	73.29±0.34^{b}	77.67±1.25^{a}	73.84±0.58^{ab}	72.25±0.21^{ab}
Crude protein	11.07±0.75^{c}	15.56±1.32^{a}	15.35±0.67^{a}	14.62±0.33^{ab}	14.87±0.45^{ab}	13.52±0.21^{bc}
Crude lipid	4.92±0.34^{b}	6.14±0.32^{b}	6.30±0.12^{ab}	7.08±0.05^{a}	7.67±1.33^{a}	8.03±0.07^{a}
Ash	5.76±0.32^{a}	4.12±0.10^{e}	4.21±0.10^{cd}	5.76±0.35^{ab}	5.64±0.18^{ab}	5.19±0.10^{ab}

Values in the same row with same letters are not significantly different (P<0.05)

DISCUSSION

The results of this preliminary study demonstrate the potential of fermented castor seed meal (FCSM) as a dietary protein source that can partially replace soybean meal in the practical diets of the hybrid fingerlings. The partial water flow through of the holding concrete cistern ensured that water quality in the rearing medium remained within the optimal range prescribed for the culture of Clariids. Results showed that growth and nutrient utilization was optimal in the groups that received the control diet and TD_1 (6.98% FCSM). Growth and nutrient utilization were however significantly ($P<0.05$) negatively affected by increased inclusion levels of FCSM. This was in spite of the positive effects of fermentation on castor seed meal (Table 2). Previous studies have demonstrated (Aslani *et al*., 2007). poor growth of fish and livestock fed severally-processed castor seed meal (Okorie *et al*., 1987; Anandan *et al*., 2004; Audi *et al*., 2005; Oso *et al*., 2011) due to residual toxic principles like ricine, ricinine, allergen and other antinutritional factors.

The high lipid content, storage at ambient and high peroxide and pH values of the resultant diets (Table 3) could have contributed to the poor growth recorded in this study. Oxidative rancidity results in the conversion of oil to a variety of toxic aldehydes, ketones, peroxides and acids which could be responsible for the off-flavours and odours consistent with the destruction of fat soluble vitamins (Halliwell and Chirico, 1993). This could also affect the bio-availability of some essential nutrients like lysine, tryptophan and vitamins and reduce nutrient utilization in monogastrics (Wedemeyer, 1996; Chae *et al*., 2002). Peroxide value of 5-10 meq peroxide kg^{-1} is considered safe for monogastrics, thus, the values (20-119 meq peroxide kg^{-1}) recorded in this study are considered high.

In this experiment, groups of fish that received high levels of FCSM also exhibited poor feed intake, as observed in Eurasian perch (*Perca fluiatilis*) by Kestermont *et al*. (2001). The severely-depressed growth recorded in this study could also be because juvenile fish were used as observed by Ketola *et al*., (1989) that young fish were vulnerable or prone to oxidative rancidity. Many researchers have also demonstrated the adverse effects of feeding rancid oil and oxidative rancidity in young culture fish species like hybrid tilapia (*Oreochromis niloticus* x *O. aureus*), *Clarias gariepinus* and Atlantic halibut (*Hypoglossus hipoglossus*) and even in black tiger shrimp (*Penaeus monodon*) (Baker and Davies, 1997; Huang and Huang, 2004; Martins *et al.,* 2007; Laohabanjong *et al.,* 2009)

There was a trend of increased carcass lipid with increase in inclusion levels of FCSM; protein was highest in the group that received the control diet and least in those that received the highest inclusion level of FCSM (Table 4). Excess carcass lipid could be associated with poor or reduced growth rate related to dietary energy, as earlier observed in cultured *C. gariepinus* (Machiels and Henken, 1985), Indian Major Carps (Hassan *et al*., 1995; Hassan and Jafri, 1996). Based on the foregoing, it could be suggested that rancidity could have resulted in the poor growth performance and diet utilization of the group of the fingerlings fed high inclusion levels of the FCSM.

CONCLUSION

This study demonstrated the potential of castor seed meal as dietary protein source in the diets of *Clarias gariepinus* x *Heterobranchus bidorsalis* hybrid and the effects of high degree of rancidity / lipid oxidation of fermented castor seed meal on feed intake, growth, feed utilization and survival of the fingerlings. Oxidative rancidity resulted in poor growth performance. Further studies are suggested to consider the probable effect of deoiling prior to fermentation and the use of synthetic antioxidants.

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