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An Evaluation of Diets Containing Various Levels of Fermented Cottonseed Hull on Digestibility and Growth of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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Abstract

Cotton seed hull, a by-product of cotton seed oil industry, was fermented and evaluated as an ingredient in practical diets for Nile tilapia (*Oreochromis niloticus*) fingerlings reared in hapas suspended in an outdoor concrete cistern. Fermentation resulted in the reduction of condensed tannin and crude fibre levels and an elevation of the crude protein content. Five isonitrogenous (30% crude protein) and isocaloric (19.50 $KJ \cdot g^{-1}$) diets were formulated in which maize meal was progressively substituted at four inclusion levels (15, 30, 45 and 60%) with fermented cottonseed hull. The fish were fed for eight weeks. Results showed a progressive decline in growth performance with an increase in the inclusion level of cottonseed hull. The data showed significant difference ($p<0.05$) in weight gain, specific growth rate and feed conversion ratio among fingerlings fed the different dietary treatments. Result of carcass analysis showed that as the inclusion level of fermented cottonseed hull increased, there was a progressive decrease in body lipid while other carcass parameters did not show any set pattern. It was concluded that cottonseed hull was suitable as a dietary energy supplement for tilapia (*O. niloticus*) fingerlings when incorporated only up to 15% replacement for maize meal. It is suggested, however, that further studies be carried out to reduce the anti-nutritional content of this resource

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Introduction

Cotton (*Gossypium hirsutum*) is a widely cultivated cash crop in the northern and south-western part of Nigeria where it is considered a major oilseed third in rank after soybean and ground nut (FMA 1997). According to Yu et al. (1995), cottonseed hull contains around 900 $g\cdot kg^{-1}$ fibre, 52 $g\cdot kg^{-1}$ condensed tannins and a limited amount of proteins. It has been used extensively in feed for pigs, ruminants and in experimental rats (Yu et al. 1996) but there is a dearth of information on its utilization in fish feeds. A major factor limiting its wider use is the minimal nutritive value, high crude fibre content (Mujahid et al. 2000) and the presence of condensed tannins - a flavonoid polyphenolic compound (Terrill et al. 1992; Yu et al. 1993). Dietary condensed tannins have been shown to reduce body weight gain, impair feed conversion efficiency, and reduce the apparent digestibility of protein and amino acids in monogastric animals (Longstaff and McNab 1991; Jansman 1993; Yu et al. 1996). These collectively have conferred on cottonseed hull's minimal nutritive value in the diets of monogastrics like fish.

Fermentation has been identified as an economic processing method that could be used to improve the nutritional qualities of plant foods (Obizoba and Egbuna 1992; Fransen et al. 1998) and for production of easily digestible product with increased shelf life (Obizoba 1998). It has been used locally in the detoxification and deactivation of anti-nutritional factors in cottonseed and castor oil bean (Odunfa 1985; Sanni and Ogbonna 1991; Obizoba 1998). It is envisaged that fermenting cottonseed hull could increase its crude protein content, and reduce the tannin and crude fibre contents thus improving its nutritive value to fish and other monogastrics. The present study was therefore intended to determine the optimum level of supplementation of maize by cottonseed hull in practical diets for Nile tilapia, *Oreochromis niloticus*

Materials and Methods

Cottonseed hulls were collected as a waste from processed cottonseed meal used in a previous experiment. The cottonseed hulls were sun dried and milled with a locally fabricated hammer mill; the resultant flour was sieved using a 595 µm sieve to remove the chaff and to ensure a homogenous size profile. All other feed ingredients used in the experiment were purchased from a local livestock feed centre.

Six litre (6 L) plastic containers with tight fitting covers were used. Five kilograms of the flour were packed into the plastic container until it was 75% full. The plastic container was then filled with distilled water until the entire flour was covered; then hermetically sealed (air-tight) and allowed to stand for 48 h. Periodic monitoring of the temperature and pH were carried out with a mercury thermometer and Jenway pH meter, respectively. The water was decanted after 48 h and the soaked/fermented flour dried in a drying cabinet (Leec 100) at 38°C for 48 h.

Mixed-sex Nile tilapia (*O. niloticus*) fingerlings (mean weight 5.05 ± .61g) used in the feeding trials were obtained from the production pond of the Department of Aquaculture and Fisheries Management, acclimated for a period of five days inside the hapas and were maintained on a 25% crude protein diet before the commencement of the experiment.

Five diets were formulated in which maize meal was replaced with fermented cotton seed hull at 0, 15, 30, 45 and 60% levels (Table 1). All diets were formulated to be isonitrogenous (30% crude protein) and isocaloric (19.50 $KJ \cdot g^{-1}$). They were pelletized into 2 mm diameter pellets in a Molineux pelletizer, sundried for six hours and stored in labelled polythene bags.

Table 1. Effect of fermentation on the biochemical composition ($g \cdot 100g^{-1}$) of cottonseed hulls.

Parameters	Unfermented	Fermented
Crude protein	$8.62^{b}\pm0.85$	$15.20^{a}\pm0.40$
Crude lipid	$1.23^{a}\pm0.07$	$0.98^{b}\pm0.08$
Crude fibre	$56.67^{a}\pm0.43$	$52.73^{b}\pm0.49$
Ash	$3.45^{a}\pm0.06$	$3.71^{a}\pm0.02$
Condensed tannin	$54.32^{a}\pm0.90$	$23.46^{b}\pm0.70$
Free gossypol	0.64	0.004

Mean of triplicate data in each row with similar superscripts are not significantly different ($p>0.05$)

The feeding trials were conducted in 15 fish hapas (1m x 0.5m x 0.5m) suspended to ¾ of their volume using kuralon twine (No. 15) on carefully arranged bamboo poles in an outdoor concrete cistern (5m x 3m x 1.5m) located at the reverse side of the College of Environmental Resources Management (COLERM) building. The concrete cistern was filled to 5/6 of its volume and was continually supplied with water to sustain optimal environment and preclude primary productivity.

The fingerlings were later randomly distributed at the rate of ten fish per hapa. All fish were fed at 3% body weight daily at 8 am and 4 pm local time for the experimental period of eight weeks. Each diet treatment had three replicates. The fish were batch-weighed weekly with an electronic balance (METLER BD 601) and the amount of feed was adjusted accordingly. At the end of the experiment, five fish from each diet treatment were sacrificed and used for carcass analyses.

Fish growth and nutrient utilization parameters were calculated as follows:

Specific growth rate (SGR) = $(\ln W_2 - \ln W_1)100/\text{Time (days)}$

Weight gain = W_2 - W_1, where W_1 = the initial weight of fish, W_2 = the final weight of fish

Protein efficiency ratio (PER) = Mean Weight Gain /Average Protein Fed

Feed conversion ratio (FCR) = Weight of feed (g)/Weight gain (g)

Net protein utilization (NPU%) = [Protein gain (g wet) / protein intake (g DM)] x 100.

Apparent protein digestibility (%) as $ADC_{protein} = 100 - [100(I_d/I_f^{-1} \times N_f/N_d^{-1})]$, where I_d= chromic oxide in diet, I_f= chromic oxide feaces, N_f = protein in feaces, N_d = protein in diet

All analyses for proximate composition were determined according to the methods of AOAC (1990) as follows. Total Nitrogen (N) was determined by the macro- and micro-kjedahl and procedure and crude protein estimated as N X 6.25. Crude fibre was determined as loss on ignition of dried lipid-free residues after acid-alkaline digestion (Trichloroacetic acid method) Crude lipid was determined after soxhlet extraction of dried samples with petroleum ether. Moisture was determined after oven drying at 110ºC to constant weight. Each data point in the study was the mean value of three samples. Tannin was determined by the Price and Butler Prussian blue method as modified by Graham (1992). Chromic oxide contents of diets and faeces were determined according to the methods of Furukawa and Tsukahara (1966).

The data describing growth performance and feed utilization were analysed statistically by one-way Analysis of Variance (ANOVA) and differences among means were tested for significance (p=0.05) using Duncan's Multiple Range Test.

Results

Soaking of cottonseed hulls resulted in the decline of condensed tannin, crude fibre content, free gossypol and lipid contents and a significant increase in the crude protein content (Table 1). Table 2 shows the proximate composition of feed ingredients, while Table 3 refers to ingredients and proximate composition of experimental diets. All test diets were accepted and actively fed upon by the fish at the first two weeks of the feeding trials. However, a degree of reluctance to feed optimally was observed in the groups fed high (45-60%) inclusion levels of cottonseed hulls as the experiment progressed. No pathological sign as a result of dietary deficiency was observed in all fish used for the experiment. Fish survival remained high ranging between 93 and 100%.

Table 2. Proximate analysis of feed ingredients

Feedstuff	Crude protein (%)	Ether Extract (%)	Crude fibre (%)	Ash (%)
Fish meal	67.67	4.1	1.02	14.8
Soybean meal	45.30	18.0	5.03	4.60
Groundnut cake	34.46	12.80	6.31	13.08
Cotton seed hulls	15.2	9.76	52.73	3.71
Corn	10.81	4.50	3.80	1.40

Monitored water quality parameters were similar in all treatments and were within the range recommended for tilapia and other warm water species (Boyd 1982). The growth response of fish fed the test diets are shown in table 4. The best growth response and nutrient utilization were observed in the group fed the control diet. There was, however, a trend of reduced growth performance and nutrient utilization with increase in fermented cottonseed hull inclusion. Apparent digestibility coefficient for protein was highest in the group fed the control diet and least in the group fed the highest inclusion level of fermented cottonseed hulls. Protein intake was measurably similar; the least value (2.92 g) was recorded in the group fed the highest level of fermented cottonseed hull and was significantly inferior to other treatments. Weight gain of the group fed the control diet was significantly different ($p<0.05$) from those fed experimental diets, those that received diet 1 having 15% replacement level showed superior growth to those of other groups. The least growth was recorded in the group fed the highest inclusion level of fermented cottonseed hull.

The result obtained in the present study demonstrated that maize-based diet (control) performed best in terms of growth, FCR, and PER than

Table 3. Ingredients ($g \cdot 100g^{-1}$) and proximate composition ($g \cdot 100g^{-1}$ dry matter) of diets containing varying levels of fermented cottonseed hull used in the feeding of *Oreochromis niloticus* fingerlings for 56 days. CD – Control Diet, TD1 - Test Diet 1, TD2 – Test Diet 2, TD3 – Test Diet 3, TD4 – Test Diet 4

Ingredient	CD	TD_1	TD_2	TD_3	TD_4
Fish meal	6	6	6	6	6
GNC	8	8	8	8	8
SBM	42.61	41.90	41.18	40.42	39.64
Corn	35.89	31.10	26.12	20.94	15.54
FCSH	-	5.49	11.12	17.14	23.32
Palm oil	5	5	5	5	5
Vit/M premix	1.5	1.5	1.5	1.5	1.5
Methionine	1	1	1	1	1
Lysine	1	1	1	1	1
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Moisture (%)	9.4	9.4	10.0	9.8	10.0
Crude protein (%)	30.24	31.16	30.67	31.46	31.92
Lipids (%)	10.64	10.24	9.82	9.46	9.04
Crude fibre (%)	3.04	4.05	5.06	6.17	7.27
Gross energy($KJ \cdot g^{-1}$)	19.50	19.50	19.50	19.50	19.50

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

Table 4: Growth, feed utilization, nutrient utilization and mortality rate of *Oreochromis niloticus* fingerlings fed varying levels of fermented cottonseed hull for 56 days

Parameters	CD	TD_1	TD_2	TD_3	TD_4
Initial body weight (g)	$6.46^{a}\pm0.16$	$6.02^{a}\pm0.41$	$5.69^{a}\pm0.32$	$5.53^{a}\pm0.09$	$5.53^{a}\pm0.16$
Final body weight (g)	$11.25^{a}\pm0.95$	$9.91^{b}\pm0.31$	$9.25^{bc}\pm0.26$	$8.34^{bc}\pm0.16$	$7.71^{c}\pm0.53$
Weight gain (g)	$4.79^{a}\pm0.19$	$3.89^{b}\pm0.13$	$3.56^{bc}\pm0.16$	$2.81^{cd}\pm0.39$	$2.18^{d}\pm0.04$
SGR ($\% \cdot day^{-1}$)	$0.99^{a}\pm0.05$	$0.89^{a}\pm0.73$	$0.87^{ab}\pm0.12$	$0.73^{ab}\pm0.09$	$0.59^{b}\pm0.05$
Feed intake (g)	$12.10^{a}\pm1.22$	$11.10^{a}\pm0.91$	$10.45^{a}\pm0.77$	$9.72^{a}\pm0.40$	$9.63^{a}\pm0.09$
Protein intake (g)	$3.63^{a}\pm0.41$	$3.33^{a}\pm0.15$	$3.14^{a}\pm0.19$	$2.90^{a}\pm0.23$	$2.92^{b}\pm0.03$
FCR	$2.52^{a}\pm0.33$	$2.85^{a}\pm0.33$	$2.94^{a}\pm0.09$	$3.43^{a}\pm0.49$	$4.46^{b}\pm1.00$
PER	$1.32^{a}\pm0.09$	$1.17^{ab}\pm0.14$	$1.14^{ab}\pm0.13$	$0.97^{ab}\pm0.15$	$0.75^{b}\pm0.04$
$ADC_{protein}$	$82.34^{a}\pm2.92$	$78.46^{ab}\pm0.76$	$78.21^{b}\pm0.93$	$76.00^{b}\pm1.11$	$75.65^{b}\pm1.63$
Survival (%)	93 ± 0.00^{a}	100 ± 0.00^{a}	97 ± 0.00^{a}	100 ± 0.00^{a}	93 ± 0.00^{a}

Mean of triplicate data in each row with similar superscripts are not significantly different ($p>0.05$)

diets containing varying levels of cottonseed hulls in spite of its superior crude protein content. Carcass biochemical composition showed that the group fed different experimental diets had statistically superior crude protein and lipid contents to the initial. There were no significant differences (p>0.05) in carcass protein and moisture contents among dietary treatments. However, there were significant (p<0.05) reductions in carcass lipid contents with increase in soaked cottonseed hull inclusion (Table 5).

Table 5: Proximate carcass composition (g $100g^{-1}$)of *Oreochromis niloticus* fingerlings fed varying levels of fermented cottonseed hull.

	Initial	CD	TD1	TD2	TD3	TD4
Moisture (%)	78.93^{a} ±1.14	77.72^{a} ±4.26	77.86^{a} ±1.38	78.69^{a} ±1.72	78.88^{a} ±1.00	78.30^{a} ±0.23
Crude protein (%)	49.84^{b} ±0.18	59.96^{a} ±1.04	56.62^{a} ±1.22	58.94^{a} ±0.16	58.17^{a} ±0.96	57.46^{a} ±1.18
Crude lipid (%)	8.54^{c} ±0.88	11.25^{a} ±0.11	11.14^{a} ±0.20	10.24^{ab} ±0.39	9.56^{ab} ±0.82	7.55^{bc} ±0.35
Ash (%)	4.90^{a} ±0.55	3.70^{a} ±0.23	2.90^{c} ±0.43	3.10^{b} ±0.57	3.60^{a} ±0.12	3.50^{ab} ±0.24

Mean of triplicate data in each row with similar superscripts are not significantly different (p>0.05)

Discussion

Growth was generally poor in the group of *O. niloticus* fingerlings fed the control and various levels of soaked/fermented cottonseed hull. There was a progressive reduction in feed intake, growth and nutrient utilization with increase in inclusion rate of soaked/fermented cottonseed hull (Table 4). Digestibility also showed a similar trend. Generally, nutrient utilization, a factor of growth is related to digestibility, which in this study was affected by the levels of crude fibre arising from increasing inclusion level of cottonseed hull (Table 3). High fibre content according to Longstaff and McNab (1991) could induce delay in gut emptying time, reduce digestibility and limit nutrient utilization (McCurdy and March 1992) and poor feed utilization (Muller-Harvey and McAllan 1992; Bui et al. 2000). Mwachireya et al. (1999) suggested that high fibre content of plant can be considered an anti-nutritional principle.

According to Harris (1980), the average crude fibre content for optimum growth in energy feed for fish is about 6%, this was corroborated by Leary and Lovell (1975). They suggested that increasing fibre content beyond the basal value could result in reduced growth**.** Lloyd et al. (1978) had earlier reported that the reduced energy availability of high fibre diet, and not the fibre was responsible for depressed growth rates. Crude fibre contents of TD3 and TD4 with high cottonseed hull content were higher than the value recommended by Harris (1980).

Presence of tannin, a known anti-nutritional factor could singly or in combination with crude fibre result in suboptimal growth. Condensed tannins are known to form complexes with protein in feeds reducing its availability and utilization in monogastrics through poor digestibility (Muller-Harvey and McAllan 1992). Tannins are known to interfere with the absorption of iron and other nutrients (Butler 1989) and may also inhibit the activity of digestive enzymes due to the formation of tannin-enzyme complexes, which are biologically inactive (Tamir and Alumot 1969; Longstaff and McNab 1991). Reduction in feed intake was observed among groups fed high inclusion levels of fermented cottonseed hulls; this is often associated with high levels of condensed tannin intake in monogastrics e.g chicken fed on high fibre diets (Nelson et al. 1975). Similar observations were made when ingredients with high fibre levels were fed to *O. niloticus* (Falaye 1987) and *Clarias isheriensis* (Fagbenro 1996). Tannins, according to Mangan (1988) and Salunkhe et al. (1990), could cause increase feacal nutrient especially amino acids, thereby reducing their digestibility. The level of condensed tannin in soaked cottonseed hull was low (Table 1) but considered adequate to influence digestibility and nutrient utilization. Since all test diets were formulated to contain the same crude protein content and were actively fed upon by the fish in all the treatments, the differences in weight gain can only be attributed to the utilization of cottonseed hulls. It is therefore possible that poor growth and reduced feed utilization could be a result of reduced nutrient intake.

Diets containing cotton seed hull are acceptable to Nile tilapia (*O. niloticus*) fingerlings and could be used to supplement maize meal only up to 15% inclusion level for optimum feed conversion and protein utilization. It is suggested that further studies be carried out to reduce the anti-nutritional content of this resource.

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