EVALUATION OF *Leucaena* AND *Gliricidia* LEAF PROTEIN CONCENTRATE AS SUPPLEMENTS TO BAMBARA GROUNDNUT (*Vignas subterranean*(L. Verdc) IN THE DIET OF *Oreochromis niloticus*

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Abstract

A 56-day growth trial was conducted to assess the response of *Oreochromis niloticus* fed either cooked or toasted bambara groundnut (Bam-nut) supplemented with either *Leucaena leucocephala* or *Gliricidia sepium* leaf protein (LP) as either total or partial replacement for groundnut cake in the control diet. Either of the LP supplied equi-protein in the diets and all diets were formulated at 30% crude protein. Fish (average weight 8.4g) were randomly assigned in triplicates of 30 fish per dietary treatment and fed at 5% body weight daily for 8 weeks.

Fish fed Bam-nut supplemented with Gliricidia was superior in growth, nutrient utilization, crude protein digestibility, carcass crude protein, least feed conversion ratio and best feed efficiency ratio. Fish fed cooked Bam-nut supplemented with Gliricidia had higher weight gain, protein efficiency ratio, specific growth rate and net protein utilization. The least values in fish growth, nutrient utilization, ash digestibility, carcass lipid and ash occurred in fish fed combination of bambara groundnut and groundnut cake. Toasted Bam-nut supplemented with leaf protein gave the best crude protein digestibility followed by the fish fed a mixture Bam-nut and groundnut cake.

Introduction

In order to reduce the cost of a balanced fish diet, locally available ingredients such as agricultural by-products and plant proteins should be included in the diet or substituted for expensive animals plant protein sources. Bambara groundnut (Bam-nut) is a leguminous plant similar to the common groundnut/peanut (*Vigna unguilata*). Like the groundnut, it is grown for its underground seeds (Stephens, 1994). The seeds may be eaten raw when immature but the hard mature seeds need to be roasted or boiled to produce sweet and pleasant tasting meal. The seed contains 14-24% protein, about 60% carbohydrate, and it is higher in essential amino acids, methionine than most other grain legumes. (Brough *et al.*, 1993). It contains 6-12 % oil, which is lesser than the amount in peanuts. Bambara groundnut, though underutilized African legume, has global availability especially in hostile tropical environments (Heller *et al*., 1997). Bam-nut is one of the indigenous grains of sub-Saharan Africa, favoured in terms of nutritional value and tolerance to adverse environmental conditions. It is the third most important legume after groundnut and cowpea. Despite these, its full economic significance has not been determined (FAO, 2002).

Research interest has been focused on different leaf meals as protein sources in animal feeds (Agbede and Aletor, 2003; Wouters, 1994). Constraints to enhanced utilization of seeds and leaves of fodder trees includes high fibre content, presence of anti-nutrients (ANF) and deficiencies of some essential amino acids (D'Mello, 1987). Processing of leaf into leaf protein concentrate reduces fibre content and some ANFs. Concentrates are feedstuffs high in digestible nutrients and low in fibre (AERLS, 1987). Better use of plants to deal with problems of poverty, food and nutritional security, income generation and environmental health would revive such plants that are currently neglected as increased demand would encourage farmers to increase production. *Gliricidia sepium* and *Leuceana leucocephala* are widely used as forages. Recently, trials have been tried in broilers (Agbede and Aletor, 2003a) and in human weaning food (Agbede and Aletor, 2003b).

Materials and methods

Sources and processing of ingredients

Bam-nut was purchased locally from the market while *Leucaena* and *Gliricidia* leaves were freshly plucked from the wild in the Federal University of Technology forest. All other ingredients were purchased from a reputable feed mill in Akure. The fish meal used was imported Danish fishmeal (Type FF) from Denmark. The groundnut cake was from Taraku Mills, Makuru, Benue State. All ingredients were ground into powdery form using an industrial milling machine.

Processing of bam-nut

Six out of the seven diets to be formulated included Bam-nut.

Toasting

Bam-nut was put in a heavy metallic frying pan lined with sand to a depth of 40cm and allowed to warm up for 20 minutes. At 55^{o}C, the beans were added and toasted. The nuts were turned to prevent burning. After toasting it was milled with a grinding machine. The source of fire was gas and the toasting took about 10-15 minutes.

Cooking

The Bam-nut were washed, put in cooking pot and boiled for forty minutes on a gas cooker at ATP (100^{o}C, 760 mm/Hg). Table 1 shows the proximate composition of the major ingredients.

Table 1. Proximate analysis of feed component (% dry weight).

Ingredients	Crude protein	Lipid	Crude fibre	Ash	Moisture content	NFE
Cooked Bam-nut	20.47	10.09	3.51	11.63	11.42	42.88
Gliricidia leaf	50.05	11.85	4.37	4.78	10.78	18.23
Brewery dry grain	32.40	7.97	8.84	21.33	10.72	18.74
Leucaena leaf	34.38	12.50	10.60	6.59	11.06	24.87
Toasted Bam-nut	18.69	11.16	6.47	3.21	11.08	49.39
Groundnut cake	45.40	7.42	7.58	5.18	11.28	23.14
Fish meal	72.91	8.07	15.03	4.63	2.61	6.75
Rice bran	11.51	10.96	34.83	11.56	10.11	21.03
Yellow maize	10.43	2.43	35.78	1.76	10.85	38.75

Processing of Gliricidia and Leucaena leaf protein concentrate

Flow chart for leaf protein fractionation used in this study followed that of Fellows (1987).

Feed formulation and preparation

The control diet was formulated for 30% crude protein using Pearson square method. Six other experimental diets with crude protein levels of 30% were formulated using the trial and error method based on the composition of ingredients inTable1. In the other six diets the groundnut cake in the control was either totally or partially replaced. The following diets were prepared:

Diet 1 (CTR) - Control
Diet 2 (CBL) - cooked bam-nut and *Leuceana* protein concentrate
Diet 3 (TBL) - toasted bam-nut and *Leuceana* protein concentrate
Diet 4 (CBG) - cooked bam-nut and *Gliricidia* protein concentrate
Diet 5 (TBG) - toasted bam-nut and *Gliricidia* protein concentrate
Diet 6 (CBGC) - cooked bam-nut and groundnut cake (ratio 4:1)
Diet 7 (TBGC) - toasted bam-nut and groundnut cake (ratio 4:1)

Fixed quantities of fishmeal (20.83%) were added to each diet. Groundnut cake in the control diet was totally or partially replaced at equi-protein level where necessary maize quantity was adjusted to accommodate differences (Table 2). *Leucaena* LP had higher fibre than *Gliricidia* LP hence lesser quantity of rice bran was added to *Leucaena* LP based diets. Bam-nut had more lipid than the groundnut this was balanced up in the formulation to prepare an isocaloric diet. This did not affect the energy content as the other ingredients had approximately equivalent nitrogen free extract.

Table 2. Gross composition of experimental diets for O*reochromis niloticus.*

Ingredients	CTR	CBL	TBL	CBG	TBG	CBGC	TBGC
Fishmeal 65% CP	20.83	20.83	20.83	20.83	20.83	20.83	20.83
Yellow maize	25.00	0.36	0.36	0.36	0.36	0.36	0.36
Groundnut cake	39.17	-	-	-	-	11.11	11.11
Bam-nut	-	42.13	42.13	42.13	42.13	56.00	56.00
Brewery dry grain	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Gliricidia	-	-	-	15.31	15.31	-	-
Leucaena	-	21.68	21.68	-	-	-	-
Oyster shell	2.00	2.00	2.00	2.00	2.00	1.00	1.00
Vegetable oil	2.50	2.50	2.50	2.50	2.50	3.00	3.00
Rice bran	1.00	1.00	1.00	7.37	7.37	-	-
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bonemeal	3.00	3.00	3.00	3.00	3.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00	2.00	0.50	0.50

Culture system

The experiment was carried out in glass tanks of 70 x 45 x 45 cm (L x B x H) with water volume maintained at 50 cm level. A total of 21 glass tanks were used.

Experimental fish

Tilapia (*Oreochromis niloticus*) fingerlings with an average weight of 8.4 g were obtained from the Ondo State Agricultural Development Project farm, (ADP) in Akure. A total of 630 fingerlings were purchased and distributed at 30 per tank into the 21 experimental glass tanks. These fishes were allowed to acclimate and fed with the control diet for 7 days before the experiment started.

Feeding

The fingerlings were distributed at 25 per tank in triplicates and fed twice between 8.00-9.00am and between 15.00-16.00pm daily at 5% body weight for eight weeks (56 days). Left-over feed was removed 30 minutes after feeding. Faeces in each tank was siphoned out four hours after feeding (Adeparusi and Jimoh, 2001) with a white muslin cloth, sun-dried and stored in the refrigerator, until adequate quantity was obtained for proximate analysis.

Chemical analysis

Proximate analyses of diets and fish (initial and final) were determined using AOAC (1995). Acid insoluble ash contents of test diets and faeces were determined using the methods described in (Halver, 1997). Nitrogen free extract (NFE) was determined by subtraction method as:

% NFE = (100 - % Crude protein + % Ether extract + % Ash + % Moisture)

Gross energy of the different diets and their respective faecal samples were determined using their calorific values: protein, lipid and carbohydrate.

Biological evaluation

`The initial and weekly mean weights were recorded per treatment.

(i) Weight gain = Final weight of fish - Initial weight of fish

(ii) Percentage weight gain = $\frac{\text{Mean weight gain X 100}}{\text{Mean weight initial}}$

(iii) Specific growth rate (SGR) was calculated as:

$$\text{SGR (\% per day)} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \text{ X } \frac{100}{1}$$

where:

W_2 = Weight of fish at time T_2 (final)

W_1 = Weight of fish at time T_1 (initial)

(iv) Protein efficiency ratio (P.E.R.) = $\frac{\text{Weight gain by fish}}{\text{Protein intake by fish}}$

(v) Net Protein Utilization = $\frac{\text{Protein gain by fish}}{\text{Protein intake}}$

(vi) Protein intake per fish = Total feed consumed multiplied by the % crude protein in feed

(vii) Feed conversion ratio (FCR). This was calculated from the relationship of feed intake and wet weight gain.

$$\text{FCR} = \frac{\text{Total feed consumed by fish}}{\text{Weight gain by fish}}$$

(vii) Feed Efficiency Ratio (FER) = $\frac{\text{Weight gain per fish}}{\text{Food fed}}$

Determination of digestibility coefficient

Digestibility was calculated based on the percentage of AIA in feed and in faeces and the percentage of nutrients in the diet and faeces

$$\text{Digestibility} = 100 - 100 \frac{\%\text{ A I A in feed}}{\%\text{ A I A in faeces}} \times \frac{\%\text{ nutrient in faeces}}{\%\text{ nutrient in feed}}$$

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using the SPSS version 11 (Statistical Package for Social Sciences Version 11) and SAS System. Where significant difference ($P<0.05$) occurred, treatment means were comparedusing Duncan Multiple Range Test.

Results

Table 3 shows the growth and nutrient utilization of *O. niloticus* on the test diets. There was a general increase in weight gain of fish on all the diets from the first week to the last week of the experiment (Fig 1.). There were significant differences ($P<0.05$) in the mean weight gain (MWG), specific growth rate (SGR) and net protein utilization (NPU) of fish fed the different diets. There were no significant differences in the feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) of fish on all diets ($P>0.05$). Highest MWG, SGR and NPU were in found in fish fed CBG. The least MWG and SGR, FCR, PER and FER were observed in fish fed TBGC while the least NPU was observed in fish fed CBGC.

Table 4 presents the apparent digestibility coefficient of fish on each of the diets. There were significant differences ($P<0.05$) in crude protein; lipid and ash digestibility in fish fed the different diets. The highest crude protein digestibility was found in fish fed TBL followed by those fed TBG and lowest in the CTR diet. The highest lipid digestibility was in fish fed TBG. Fish fed on diets CRT, CBL and TBL has similar lipid digestibility as well as fish fed CBG, TBG, CBGN and TBGN. Highest ash digestibility was found in fish fed CTR diet and lowest in fish fed TBGC diet. There were significant differences ($P<0.05$) between the carcass composition of fish at the start and at the end of feeding trial (Table 5). Highest carcass protein was found in fish fed TBG followed by fish fed CBG and lowest in fish fed CBGC. There were no significant differences ($P>0.05$) in carcass protein gain of fish on the CTR and CBL. Carcass lipid gain was highest in fish fed the CTR diet and lowest in those fed TBGC. However there were no significant differences ($P>0.05$) in the lipid gain of fish on TBL, TBG and those fed CBGC. Ash gain was highest in fish fed CBGC and lowest on those fed the CTR.

Table 3. Growth and nutrient utilization of O*reochromis niloticus* fed different diets of bam-nut.

Parameters	CTR	CBL	TBL	CBG	TBG	CBGC	TBGC
Mean weight gain	3.34[b] ± 0.85	3.77[ab] ± 0.21	3.7[ab] ± 0.707	4.45[a] ± 0.00	4.36[a] ± 0.07	3.74[ab] ± 1.131	3.06[b] ± 9.192
FCR	1.086[a] ± 1.037	1.098[a] ±1.101	1.295[a] ±1.26	1.131[a] ±1.021	1.051[a] ±1.310	1.268[a] ±1.403	1.37[a] ±1.734
PER	0.426 ±0.042	0.421 ±0.049	0.370± 0.127	0.45 ±0.042	0.424 ±0.052	0.38 ±0.106	0.313 ±0.091
FER	92.15 ±0.014	91.34 ±0.012	75.12 ± 0.047	88.96 ±0.014	95.19 ±0.016	81.49 ±0.038	70.94 ±0.028
SGR	1.09[cd] ±0.042	1.640[ab] ±0.007	1.257[c] ±0.07	1.763[a] ±0.086	1.706[ab] ±0.084	1.402[bc] ±0.336	0.860[d] ±0.014
NPU	72.18[b] ±0.92	73.44[b] ±0.047	71.80[b] ±0.016	95.94[a] ±0.014	94.4[a] ±0.038	66.04[c] ±0.028	67.31[c] ±0.014

Mean values in the same row with similar superscript letters are not significantly different (P>0.05).

Table 4. Apparent digestibility coefficient in each diet (%).

Parameters	CRL	CBL	TBL	CBG	TBG	CBGN	TBGN
Crude protein	80.65[g] ±0.22	81.56[f] ±0.23	88.27[a] ±0.23	84.26[e] ±0.24	87.03[b] ±0.25	86.93[c] ±0.24	85.21[d] ±0.24
Lipid	98.49[b] ±0.37	99.48[b] ±0.37	99.42[b] ±0.38	99.65[a] ±0.38	99.78[a] ±0.40	99.72[a] ±0.40	99.69[a] ±0.41
Ash	95.15[a] ±0.40	74.55[e] ±0.41	87.51[b] ±0.42	67.53[f] ±0.42	86.25[c] ±0.43	75.91[d] ±	65.35[g] ±0.43

Mean value in the same row with similar superscript letters are not significantly different (P>0.05).

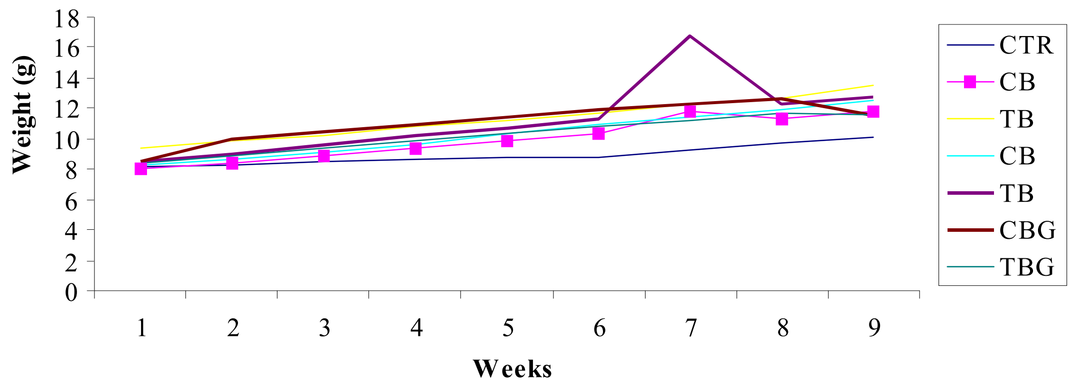

Figure 1. Weekly weight (g) of *O. niloticus* fed bambara groundnut supplemented with leaf protein.

Table 5. Proximate composition of fish carcass before and after experiment.

Nutrients	Initial	CTL	CBL	TBL	CBG	TBG	CBNC	TBNC
Crude protei n	60.32[g] ±029	63.00[d] ±027	63.00[d] ±030	63.87[c] ±030	71.75[b] ±029	73.50[a] ±031	60.38[f] ±031	61.13[e] ±029
Lipid	6.71[f] ±0.11	12.90[a] ±0.10	12.60[b] ±0.10	11.10[d] ±0.11	11.40[c] ±0.10	11.00[d] ±0.10	11.00[d] ±0.11	9.10[e] ±0.11
Ash	12.45[f] ±0.48	14.90[e] ±0.49	15.67[c] ±0.50	16.29[b] ±0.51	11.29[g] ±0.49	11.31[h] ±0.48	17.34[a] ±0.50	15.44[d] ±0.49
NFE	20.52[a] ±0.18	9.20[d] ±0.18	8.73[e] ±0.19	8.74[e] ± 0.20	5.56[f] ±0.19	4.19[g] ±0.18	11.28[c] ±0.20	14.33[b] ±0.21
Dry matter	79.13	89.96	90.32	90.07	90.78	89.76	91.56	91.28

Mean values in the same row with similar superscript letters are not significantly different (P>0.05).

Discussion

Based on the response of fish in this study, cooking of bam-nut increased the bioavailability of nutrients for growth and nutrient utilization of *O. niloticus* than in crude protein digestibility and carcass crude protein accumulation. Igbedon (1994) reported that boiling had the greatest effect on reduction of phytic acid in bam-nut. This could be due to the leaching of anti-nutrient when cooking or soaking in water. Higher lipid deposit in the carcass of fish fed with cooked bam-nut showed that better growth in terms of weight indices were due to lipid rather than crude protein gain. Either of the processing methods could be employed for bam-nut depending on the production goal of the farmer whether for high weight gain or better protein gain.

Digestibility of crude protein and ash of fish fed toasted Bambara and groundnut cake were higher than those of cooked Bambara and groundnut cake thus, confirming the fact that cooking is a better processing method. This trend differs from the response reported in pigeon pea, lima bean and African yam bean when fed to *Clarias gariepinus* and *Oreochromis niloticus* (Adeparusi, 1994; Adeparusi and Olute, Adeparusi and Jimoh, 2001), where cooking gave the best fish performance. Relatively lower performance of *O. niloticus* on *Leucaena* LPC could be due to residual presence of mimosine which might not have been totally removed with LPC processing. In evaluating *Leucaena leucocephala* leaf meal as a protein source in Indian major carp, *Labeo rohita*, Hassan *et al.* (1994) observed a trend of reduced performance with the highest growth, in terms of weight in fish fed diets with 25% soaked *Leucaena* diets. But the response on *Leucaena* LPC was still better than that of fish on the groundnut control and the mixture of groundnut and Bam-nut. This shows that inclusion of either of the LPCs used in this study gave a better response in *O. niloticus*. Vogt *et al.* (1986) found out that growth and survival of the tiger prawn was better when fed diets in which 20% of the soybean was replaced with *Leucaena leucocephala* leaves. Ghatnekar *et al.* (1983) reported that diets with 30- 65% *Leucaena leucocephala* had no adverse effects on growth or reproductive behaviour of Mossambique tilapia (*S. mossambicus*) and Indian major carps (*Labeo rohita, Cirrhinus mrigala,* and *Catla catla*) although the diets did not improve their performance over that of a standard diet. When Rahman *et al*. (1988) fed Nile tilapia (*T. nilotica*) a diet containing 25% *Leucaena leucocephala* leaves; the fish grew more slowly than those on a standard rat diet. The leaves also caused alterations in the female gonads. The response of *O. niloticus* in this study is encouraging on *Leucaena leucocephala*.

Better performance in growth, nutrient utilization and carcass crude protein deposition in *O. niloticus* fed diets with LPC showed that leaf proteins made from *Gliricidia* and *Leucaena* could be viable means of improving fish feed especially for herbivorous fish like *O. niloticus*. Tilapias are omnivorous and will readily accept feed containing animal or plant materials (Arrignon, 1998). In the wild, they feed on chironomid larvae, worms, algae, zooplankton and bacteria. From the size of 5 cm, they are almost entirely herbivorous (Trewavas, 1983). Combination of bam-nut (an underutilized legume) and LPC could encourage both the production of *O. niloticus* and these shrubs as integral parts of a fish farm. This would maximize the benefits derived from a given piece of land as these plants could be planted on the dyke. Such trials have been practiced in Southeast Asia where farmers either toss in whole leaves or use fish meal (Wouter, 1994). Agbede and Aletor

(2003) reported a decline in the weight gain, average feed consumption as well as feed efficiency as the level of *Gliricidia* LPC inclusion increased in the diet of broiler chicks. Bolongan and Coloso (1994) reported a non-significant difference in the growth and survival of milkfish, *Chanos chanos*, fed diets with *Leucaena* or other leaf meals. Results from this study shows that LPC from *Gliricidia* or *Leucaena* could therefore be regarded as a potential dietary supplement in the diet of *O. niloticus*.

Conclusion

Fish fed the LPs gave a better growth performance, nutrient utilization and digestibility than either groundnut or a mixture (1:4) of groundnut with bam-nut. *Gliricidia* LP gave a higher growth performance in O*reochromis niloticus* than *Leucaena* LP while fish fed *Leucaena* LP had a better protein gain. These leaf proteins would therefore be a good supplement in Bam-nut diets for O*reochromis niloticus*.

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