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Growth Response and Feed Utilization of *Clarias gariepinus* Fingerlings Fed with Bambara Groundnut as protein source.

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Abstract
A 56-day feeding trial was conducted to evaluate the use of Bambara groundnut as plant protein to replace Fish meal (FM) dietary animal protein in *Clarias gariepinus*. A diet with 100% FM (control diet A) as sole protein source was compared to diets with 25%, 50%, 75% and 100% of Bambara Groundnut Meal (BGM) replacement for diets B, C, D and E respectively. The experimental feeds were fed to replicate groups of fingerlings at 5% body weight twice daily. The effect of varied inclusion of BGM was evaluated on the growth performance and feed utilization by the experimental fish species. At the end of the study, the highest percentage weight gain (188.30±25.56), specific growth rate (0.82±0.69) and utilization of feed conversion rate (9.75±1.26) were recorded with fish fed the diet D. The result showed that growth performance and feed utilization were better in 75% BGM inclusion than 100% FM, although there was no significant difference ($P<0.05$) between fish fed diets A, B, C and D. The findings showed that bambara groundnut meal was accepted and digested by the experimental fish as the conventional fish meal. Conclusively, substitution of FM with 75%, 25% and 50% BGM did not compromise growth performance and feed utilization by the fish. However, maximum recommended levels of fish meal replacement by Bambara groundnut meal in diets for *Clarias gariepinus* can be established at 25%- 75% total protein.
Keywords: Growth response**,** Feed utilization, Bambara groundnut, *Clarias gariepinus*

INTRODUCTION

As wild fishing stocks collapse through over-fishing resulting from ever increasing human population, fish farming is growing rapidly. Also, capture fisheries have reached a maximum exploitation level, and increasing supply from aquaculture sector is seen as the only way to maintain the human fish consumption (Chebbaki *et al*., 2010). Ahmad and Diab (2008) reported that feeding cost represents over 50% of the operation cost of fish farming. Fishmeal, because of its high quality protein with balanced amino acid profile is considered the most desirable source of protein ingredient in aqua feeds production. However, fish meal reserve in the world market is changeable and its value is ever increasing. It is likely that fishmeal prices will remain higher than the normal range for the foreseeable future. This may result in high operation cost in fish farming and reduce farmer's interest in investing in aquaculture. According to Sitjà-Bobadilla et al., (2005), the increasing demand, price and world supply fluctuation of fishmeal has emphasized the need for alternative protein sources in aqua feeds. To ensure a sustainable development in aquaculture, there is an urgent need to reduce the dependence on fishmeal as protein source through the introduction of alternative raw materials that will constitute the major protein and lipid sources in fish diets. The main sources of raw materials are likely to be of plant origin because of their abundance and relatively low cost.

Research has shown that fish nutritionists have not relented in their efforts to replace fishmeal with other raw materials that will not compromise the standard of nutritional quality, food safety, profitability, animal health and be sustainably produced to provide good nutritional value for consumers. Some works have been done on the nutritional value of some plants in replacement to supplement dietary animal protein based diets. Among plant protein sources, some studies reported the partial replacement of dietary fish meal with cotton seed meal inclusion at level not exceeding 50% in tilapia diets (Ofojekwu and Ejike, 1984; Mbahinzireki *et al*., 2001 and Agbo *et al*., 2011); corn gluten meal (12 to 26% of the diet) in diets for the *Oncorhynchus mykiss* (Gropp et al., 1979; Alexis et al., 1985; Moyano et al., 1992 and Robaina *et al*., 1995). Alliot *et al*., (1979) found that replacement of fish meal by corn gluten meal at levels up to 20% did not affect growth or feed efficiency ratios in sea bass (*Dicentrurchus labrux*) juveniles. Hasan *et al*., (1997) showed that sesame oil cake could be used at 25-50% in the diet of *Cyprinus carpio*. Sesame oil cake and corn gluten meal was used at 16-48% in different diets of Juvenile Beluga (*Huso huso*) (Abdolreza *et al*., 2012). Bambara groundnut meal (BGM) is a product from Bambara groundnut (*Vigna subterranea L. Verdc*), a novel legume of African origin grown mainly by subsistence female farmers intercropped with major commodities such as maize, millet, sorghum, cassava, yam, peanut and cowpea. Bambara groundnut is the third most important legume after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) in Africa (Sellschop, 1962 and Kay, 1979). Bamishaiye *et al*., (2011) reported that the nuts are known as jugo beans (South Africa), ntoyo ciBemba (Republic of Zambia), Gurjiya or Kwaruru (Hausa, Nigeria), Okpa (Ibo, Nigeria), Epa Roro (Yoruba, Nigeria) and Nyimo beans (Zimbabwe). Brough and

Azam- Ali (1992) reported that Bambara groundnut seed makes a balance food as it contains sufficient quantities of carbohydrate (63%), protein (16.25%) and fats (6.3%) with relatively high proportions of lysine and methionine as percentage of the protein (6.6 and 1.3% respectively). Moreover, Amarteifio *et al*., (2006) stated in his work that Bambara groundnut is a good source of minerals and can be useful in formulating a balanced diet.

The essential amino acid content of Bambara groundnut such as lysine 6.82g/16gN, methionine 1.85g/16gN and cysteine 1.24g/16gN is comparable to that of soybean (6.24g/16gN lysine, 1.14g/16gN methionine and 1.80g/16gN cysteine) (Fetuga *et. al*., 1975). The objective of the present study is to compare the growth and feed utilization of *Clarias gariepinus* fingerlings using Bambara nut as protein source in the diet with fish meal. The aim is to determine a cheaper alternative source of protein in fish feed with a view to reducing the cost of feeding in aquaculture in Nigeria.

MATERIALS AND METHODS

Feed Stuffs- The feed stuffs evaluated were maize, groundnut, Bambara groundnut, soya beans, fish meal, bone meal, salt, palm oil, premix and starch.

Sample Preparation- The feed stuffs were purchased from Metro Vet Consultant Limited situated in Ekiti State and prepared by the production unit. Feed ingredients were finely ground and mixed together in appropriate proportions after which the mixture was pelleted into small sizes and then sun dried.

Experimental Site- The study was carried out at the postgraduate research laboratory of Zoology Department, Ekiti State University, Ado- Ekiti. Sixteen plastic bowls of 50 litres volume were used as tanks. Water was maintained at 30 litres level throughout the experiment and a mosquito mesh was used to cover the bowls to preclude predators from entering into the bowls and fish jumping out as well.

Experimental fish- *Clarias gariepinus* fingerlings were used for the experiment. The fingerlings were bought from a reputable hatchery and acclimatized for two days in a plastic bowl during which they were not fed in order to empty their guts and prepare their appetite for the new feed. The fish were stocked at the rate of ten (10) fingerlings per bowl. Mean weight of fish in each bowl was taken and recorded. Fish were fed at 5% of their body weight twice on a daily basis, that is, morning (08:00 - 09:00 am) and evening (17:00 - 18:00 pm).
Subsequent weight and standard length measurements were taken every week and the rations fed were adjusted according to fish weight gain. Fish mortality was also monitored daily and water in the experimental bowls was renewed totally daily to remove unconsumed food particles and faecal materials to prevent poor water quality.

Experimental Design and Diets- The experiment consisted of five treatments with three (3) replicates representing each treatment including control. Each treatment diet contained Bambara nut seed as fish meal substitute except the control. The four diets were formulated to contain varying percentage of the Bambara nut (*Vigna subterranea*) fortified with different ingredients as shown in Table 1. Each diet was analyzed to determine the proximate composition. The various diets were designated as follows at varying levels of Bambara nut inclusion: 0%, 25%, 50%, 75% and 100% inclusion of Bambara nut for Diet A (control), B, C, D and E respectively.

Sampling- Sampling was carried out weekly to determine the new growth rate and survival using manual weighing balance.

Physico-chemical Parameters of Water- The following parameters were monitored during the experiment: pH, dissolved oxygen, and temperature.

Feed Evaluation Parameters- The initial and weekly mean weights were recorded per treatment.

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

$$\text{Percentage weight gain (\%WG)} = \frac{\text{Final weight} - \text{Initial weight} \times 100}{\text{Initial weight}}$$

$$\text{Percentage specific growth rate (\%SRG)} = \frac{\text{LogeW2} - \text{Loge W1} \times 100}{\text{T2} - \text{T1}}$$

Where:

W2 = Weight of fish at time T2 (final)
W1 = Weight of fish at time T1 (initial)
T = Period of experiment in days

$$\text{Feed Conversion Rate (FCR)} = \frac{\text{Feed consumed}}{\text{Weight gained}}$$

Statistical Analysis- Evaluation of the growth performance and feed utilization parameters were carried out by one way analysis of variance (ANOVA), using the SSP 15.0. The significant differences among mean were determined by the subsequent use of Duncan's Multiple Range Test (DMRT).

RESULTS

Data on mean growth rate and feed conversion ratio of *Clarias gariepinus* fingerlings fed the five different diets (A, B, C, D and E) for 56 days are shown in Table 2. The result shows the weight gained, %weight gain, the specific growth rate (SGR) and feed conversion rate (FCR). Fish fed the diet D had the highest weight gain (33.86±6.15) followed by diet A (25.00±5.37) while diet E gave the least weight gained (15.71±1.34). The highest increase in percentage body weight gain of 188.30 ± 25.56 was achieved with fish fed the diet D followed by diet A with 150.41 ± 38.25. The lowest increase (70.91 ± 9.61) was recorded with diet E. A similar trend was observed for the specific growth rate in which the highest mean of S.G.R (0.82 ± 0.69) was recorded with diet D followed by diet A (0.71 ± 0.12). Fish fed with diet E gave the least S.G.R (0.42±0.05). The evaluation done on the weight gain, %weight gain and S.G.R using one way ANOVA showed no significant difference ($P<0.05$) in all the diets except diet E. Feed conversion ratio was also best in fish fed with diet D (9.75±1.26) while fish fed with diet A had the least FCR (12.18±1.89). However, there was no significant difference ($P<0.05$) between the FCR of fish fed with diet A and D. The proximate composition of *Clarias gariepinus* flesh at the end of the study is shown in Table 3. The result showed no significant difference ($P<0.05$) in moisture (10.28±0.09 and 10.21±0.09) and fat (20.61±0.21 and 20.67±0.39) content between the flesh of fish fed with diet A and D respectively. Also there was no significant difference in the ash content of fish flesh in all the diets except diet C with 14.57±0.64, 14.37±0.83, 14.32±0.86, 14.16±0.85 and 11.08±0.64 for E, B, A, D and C respectively. Diet B (68.96±1.20) had the highest protein content followed by diet A (65.21±1.20) while diet D had the least protein content.

Table 4 shows the proximate analysis of the experimental diets. The moisture content was very high in diet C with (8.04 ± 0.42) and low in diet D with (6.63 ± 0.06) while the Ash value was high in diet E (15.00 ± 0.78) and least in diet A (12.13 ± 0.42). Diet C and E gave the highest and least percentage of fibre content with 3.03 ± 0.42 and 1.97 ± 0.02 respectively. The highest and least fat content was recorded in Diet B (15.29 ± 0.25) and Diet A (13.73 ± 0.23). Diet A gave the highest crude protein (42.67 ± 0.62) while diet B gave the lowest (25.18 ± 0.59). The result of the study showed increase in weight gain of experimental fish with time. Also, very low mortality was observed in all the treatment throughout the period of the experiment.

DISCUSSION AND CONCLUSION

In the present study, fish fed compounded diets actively grew efficiently without external sign of nutritional deficiency because growth performance of fish fed BGM diets at various level up to 75% replacement were similar to those of fish fed the control diet. This shows that BGM contained all the necessary growth factors required by *Clarias gariepinus*. The fish showed good appetite to all the treatment diets as attested to by the increase in body weight and standard length. The observation shows that the fingerlings were able to utilize Bambara nut diet efficiently like conventional fish meal, as high performance of *C. gariepinus* was very exclusive as a result of better acceptance and probably better digestibility of the diet during the experimental period.

The study showed no significant difference ($P<0.05$) between the growth performance (weight gain, % weight gain and specific growth rate) of the fingerlings fed the compounded Bambara nut diets (D, B and C) up to 75% supplement and those fed the conventional fish meal (diet A). This can be attributed to proper utilization of the compounded BGM. Hence, it was able to effect specific growth rate comparable to fish meal. Brough and Azam-Ali (1992) reported that Bambara nut seed makes a balance food as it contains sufficient quantities of

carbohydrate, protein and fats with relatively high proportion of lysine and methionine as percentage of the protein. Amarteifio et al., (2006) reported that Bambara groundnut is a good source of minerals and can be helpful in formulating a balanced diet. The replacement of fish meal based diets with pigeon pea (*Cajanus cajan*) and Bambara nut (*Vigna subterranean* L.) at 50% was found to be suiting with good results especially for Tilapia (Jackson *et al*., 1982).
The result of this study showed the highest replacement of fish meal diet with plant protein source so far, as up to 75% of the compounded meal could replace the conventional fish meal in the absence of growth promoter without affecting the overall growth performance and feed utilization of fish. This work contradicts the report made by Chowdhary et al., (2012) that growth performance and feed utilization efficiency of Asian Catfish (*Clarias batrachus*) fed feeds with animal protein are better than those of plant (Soybeans meal). Agbo et al., (2011) reported that growth performance and feed utilization decreased as Cotton seed meal increased from 25% to 75% in the diet of *Oreochromis niloticus L* ., he then pronounced a maximum of 50% replacement. According to Hassan et al., (1997), Sesame oil cake could be used at 25% to 50% in the diet of *Cyprinus carpio*. Abdolreza (2012) reported the maximum level of 16% to 48% replacement of total protein by Corn gluten meal and Sesame oil cake in the diets for Beluga (*Huso huso*).

The least growth performance was realized from fish fed the diet E (100% BGM). This indicates that BGM cannot be used as a sole protein source for *Clarias gariepinus*. This result supports the work done by Agbo et al., 2011 in which growth of fish fed 75% Cotton seed meal was significantly ($P<0.05$) lower than those of control and the other Cotton seed meal based diets. He reported that up to 50% Cotton seed meal protein could be used to replace fish meal protein in the diet of tilapia without affecting overall growth and feed utilization of fish. However, any replacement beyond that level, will drastically depressed growth. Mbahinzireki et al., (2001) reported depressed growth and even mortality of tilapia when they were fed up to 100% Cotton seed meal of the dietary and recommend an inclusion level of up to 50%. Similarly, Fagbenro and Davies (2000) reported growth retardation and poor feed utilization in tilapia when Cotton seed meal protein replaced 67% of fish meal protein. More so, Ofojekwu and Ejike (1984) reported that Cotton seed meal could not be used as a sole protein source for *Oreochromis niloticus* because the fish exhibited poor growth, food conversion and specific growth rate when fed diets with Cotton seed meal as sole protein source.

In conclusion, it can be projected from the present study that 75%, 25%, and 50% of BGM respectively were satisfactorily acceptable to fish just like the fish meal and could replace fish meal protein in the diet of *Clarias gariepinus* without adversely affecting growth and feed utilization. Also, the BGM diets were more cost effective than fish meal diets. Moreover, due to the fact that the suitability of fish meal replacement with plant protein source varies among fish species and experimental situation in terms of growth performance and feed conversion rate, this work leaves room for specific experiment to be performed for other species of fish using Bambara Groundnut Meal (BGM). This will help to determine the maximum replacement of fish meal with BGM for different species of fish.

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Table 1: Composition of Experimental Diets (Kg)

INGREDIENTS	DIET A Control 0%	DIET B 25% B-nut	DIET C 50% B-nut	DIET D 75%-B-nut	DIET E 100%B-nut
Fish meal	24.72	18.54	12.36	6.18	-
Maize	21.85	21.85	21.85	21.85	21.85
Bambara nut	-	6.18	12.36	18.54	24.72
Ground nut	24.72	24.72	24.72	24.72	24.72
Soya bean	24.72	24.72	24.72	24.72	24.72
Palm oil	1.00	1.00	1.00	1.00	1.00
Bone meal	1.00	1.00	1.00	1.00	1.00
Premix	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Starch	0.50	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**	**100**

Table 2: Mean Growth Rate and Feed Conversion Ratio of *Clarias gariepinus*

Control	0% - A	25% - B	50% - C	75% - D	100% - E
Initial mean Weight	16.66±0.57b	16.84±0.76b	19.97±4.05ab	17.93±0.60ab	22.55±4.89a
Final mean Weight	41.66±5.94ab	41.76±5.72ab	44.85±0.04ab	51.79±6.21a	38.26±6.23b
Weight gain	25.00±5.37a	24.92±4.96a	24.87±5.99a	33.86±6.15a	15.71±1.34b
%Weight gain	150.41±38.25a	147.28±23.40a	124.29±42.56a	188.30±25.56a	70.91±9.61b
S.G.R	0.71±0.12a	0.70±0.78a	0.64±0.16a	0.82±0.69a	0.42±0.05a
F.C.R	12.18±1.89ab	12.14±1.80ab	11.15±0.01ab	9.75±1.26b	11.70±1.81a

Means with the same alphabets across columns are not significantly different at P< 0.05

Table 3: Proximate Analysis of the Flesh of *Clarias gariepinus*

Control	0% - A	25% - B	50% - C	75% - D	100% - E
Moisture	10.28±0.09a	9.89±0.02bc	9.81±0.64c	10.21±0.09a	10.07±0.10ab
Ash	14.32±0.86a	14.37±0.83a	11.08±0.64b	14.16±0.85a	14.57±0.64a
Fat	20.61±0.21b	19.83±0.81c	19.94±0.16c	20.67±0.39b	21.53±0.16a
Protein	65.21±1.20b	68.96±1.20a	60.82±82c	47.30±0.00d	47.82±0.00d

Means with the same alphabets across columns are not significantly different at $P< 0.05$

Table 4: Proximate Analysis of the Experimental Diet

Control	0% - A	25% - B	50% - C	75% - D	100% - E
Moisture	7.33±0.11b	6.79±0.00c	8.04±0.42a	6.63±0.06d	6.80±0.00c
Ash	12.13±0.42b	13.28±0.75ab	12.69±0.61b	13.87±0.77ab	15.00±0.78a
Fibre	2.73±0.15b	2.21±0.09d	3.03±0.42a	2.48±0.09c	1.97±0.02e
Fat	13.73±0.23c	15.29±0.25a	14.24±0.13bc	14.67±0.42ab	14.49±0.40abc
Protein	42.67±0.62a	25.81±0.59d	36.68±0.59b	35.88±0.00b	34.17±0.00d

Means with the same alphabets across columns are not significantly different at $P< 0.05$