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Growth performance and nutrient utilization indices of African Catfish (*Clarias gariepinus* BURCHELL, 1822) Fingerlings fed graded levels of Calabash Seed Cake

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Abstract

The study evaluated the potential of calabash seed cake as a replacement for groundnut cake in the diet of Clarias gariepinus fingerlings. Five isonitrogenous (40% crude protein) and isocaloric (3,260.20 kcal kg⁻¹ metabolizable energy) diets were formulated in which boiled seed cake replaced groundnut cake at 0, 25, 50, 75, and 100%. A total of 300 Clarias gariepinus fingerlings, average weight 2.64 g and average length 4.76 cm were allocated to fifteen experimental tanks, with 20 fish per tank and three replicates per treatment, reared for a period of ten weeks during which growth and nutrient utilization were evaluated. The survival rate (98.33 ± 1.67%) was significantly higher (p < 0.05) in fish fed diet D (75% Calabash seed cake) than in the other four treatments. Survival did not differ significantly (p > 0.05) among diets B (25%), C (50%), and E (100%), but these were significantly higher (p < 0.05) than the control diet (0% Calabash seed cake), which had the lowest survival (83.33±4.41%). The highest mean weight gain (42.13 ± 1.01 g), percentage weight gain (1,601.47 ± 10.42%), and percentage length increase (258.00 ± 7.17%) were recorded in fish fed the diet containing 25% calabash seed cake. However, specific growth rate and condition factor did not differ significantly among treatments (P > 0.05). There were no significant differences in feed conversion ratio (FCR) and protein efficiency ratio (PER) across the graded levels of calabash seed cake (CSC) diets; however, the 25% CSC diet provided the best gross feed conversion efficiency (GFCE) and apparent net protein utilization (AppNPU). It is recommended that boiled seed cake be included in the feed of Clarias gariepinus at 25% for optimum growth performance and feed digestibility. Field experiments, particularly in semi-intensive earthen pond systems, are suggested. Further studies involving boiled seed cake with other cultured species are highly recommended.

Key words: Fish growth, Nutrient utilization, survival, calabash seed cake

Introduction

In Nigeria, the fishery sector plays a significant role in the economy, providing affordable food fish, income, employment, and foreign exchange, especially for riverine communities (NBS, 2017). In 2014, about 1.48 million people were engaged in fisheries (FAO, 2021; World Fish, 2022). Aquaculture is vital for maintaining fish supply, particularly as capture fisheries decline due to unsustainable practices (FAO, 2006). The growth of aquaculture helps bridge the gap between supply and demand, keeping food-fish prices affordable for both rural and urban poor and supporting food security (Huntington and Hassan, 2009). However, aquaculture relies on adequate nutrition, which depends on high-quality feed. Advances in species-specific diet formulations have improved fish nutrition to meet rising demand for affordable, high-quality seafood (Tacon and Metian, 2008; Craig and Helfrich, 2002).

In aquaculture, feed costs drive the majority of operating expenses, making fish nutrition the most costly component (Muthukumar and Kandeepan, 2015). Feed accounts for roughly 70% of aquaculture operations (Anderson *et al*., 1997; Eyo *et al*., 2004; Adekunle *et al*., 2012; Orire and Sadiku, 2014). Feed ingredient quality is a key requirement for producing high-quality feeds, alongside availability, which can be influenced by cost and seasonality (Muthukumar and Kandeepan, 2015). Protein is a non-negotiable nutrient in feed formulation, and it typically constitutes about 50% of feed costs in intensive culture. Thus, selecting appropriate amounts and quality of dietary protein is essential for successful aquaculture practices (Sogbesan and Ugwumba, 2008).

In pursuing economically and environmentally sustainable fish production, researchers are turning to unconventional plant-based protein sources, including seed cake, seed meal, leaves, and other agricultural by-products, valued for their high protein content (Richter *et al*., 2003; Abo-State *et al*., 2014). This move toward non-conventional protein sources in fish feed has gained broad acceptance in commercial aquaculture (Naylor *et al*., 2000). Within this framework, there is a push to formulate fish feeds from inexpensive, locally available, highly nutritious terrestrial resources, such as calabash (*Lagenaria siceraria*) seeds. The crude protein levels of calabash seeds and kernels are comparable to those of other high-protein legumes like soybeans and cowpeas, and the lipid content of calabash seed cake is similar to that of sunflower, soybeans, and cotton seeds (Srbinoska *et al*., 2012). However, effective processing methods are needed to reduce anti-nutritional factors and improve palatability before these seeds can be used in fish diets. This study aims to lay the groundwork for future research on calabash seeds in catfish feed, including feeding trials to evaluate their nutritional value and potential benefits for growth and health.

Materials and Methods

Experimental Site

The experiment was conducted at the Feed mill Laboratory, Teaching and Research Fish Farm of the Department of Fisheries and Aquaculture at the main campus of Usmanu Danfodiyo University, Sokoto. The study site is located on latitude 13^0 07' 78'' N and longitude of 05' 12' 25'' E at 275m above sea level (Google, 2011). The study site is located within the Sudan Savanna vegetation zone of Nigeria. The climate in the area is characterized by a long dry season, cool dry season or harmattan from November to February and hot dry season from March to May. Annual rainfall in the area ranged from 500 to 724 mm which fall during May/June – September/October (Mamman, 2000). The mean relative humidity is 14.9% and 40% between March and June and 41^0 C maximum (Mamman, 2000).

Sourcing and Identification of *Lagenaria siceraria* Seeds

5kg of Calabash (*L. siceraria*) seeds were purchased from Sokoto central market, Sokoto State Plate 3.1. The seeds were identified at the Herbarium of Botany Department, Usmanu Danfodiyo University, Sokoto.

Preparation of *L. siceraria* Seeds

The 5kg of *L. siceraria* seeds were clean and sorted to remove bad seeds from the good ones. The sorted seeds were peeled to remove the outer seed coat and the peeled seeds were taken for boiling.

Processing of *L. siceraria* Seeds

5kg of *L. siceraria* seed sample was boiled in metal cooking pot at a ratio of 1kg: 5litres of water at 100^0C, for duration of 30 minutes. The seeds were shed drying for 24hrs before the oil extraction.

Oil extraction and Preparation of *L. siceraria* seed cakes

Boiled *L. siceraria* seeds weighed 5kg was subjected to oil extraction using a mini oil screw press extraction machine Prasa DO; model DL- 2YJ02, with a capacity of 10kg per hour. Before commencing the extraction process, the machine in built heating element was allowed to heat up to 30 minutes to enable it generate enough heat that eventually facilitate the separation of oil from the seed. The seeds were then gradually introduced into the collection tray which then moves into the extraction chamber, where crushing and oil extraction was achieved. The extruded oil was collected underneath while the flake cake was expelled into a container.

Percentage oil yield was calculated as follow;

Percentage oil yield =

$$\frac{\text{Seed wt prior extraction} - \text{cake wt after extraction}}{\text{seed wt prior extraction}} \text{x100}$$

Sourcing of Ingredients

Fish feed dietary ingredients, such as fish meal, blood meal, maize and ground nut cake were purchased from Sokoto central market. All the feed ingredients were evaluated for proximate composition using standard procedures of AOAC (2006). The proximate composition of the feed ingredients was carried out at the Agric Chemical Lab of Faculty of Agriculture. Usmanu Danfodiyo University, Sokoto.

Diet formulation and production

Feed was formulated using Pearson's square method; five isonitrogenous (40% crude protein) and isocaloric (3,260.20 kcal kg^{-1} metabolizable energy) containing 0%, 25%, 50%, 75% and 100% calabash seed cake represented as A, B, C, D and E, respectively. The proportions of the ingredients were weighed out using sensitive electronic balance (JT2101N model). They were mixed using electric Kenwood Ches mini mixer. Each diet was mixed with warm water to make dough. An electric powered feed pelletizer (50kg capacity/hour) was used to produce pellets of 2mm diameter. The pellets were sundried and kept in air-tight containers until required for feeding. Formulation and proximate composition of the diets were presented in Table 1

Table 1: Formulation and proximate composition of the diets

Ingredients	Diets (substitution levels) %				
	A (0)	B (25)	C (50)	D (75)	E (100)
Fish meal	25	25	25	25	25
Ground nut cake	39.40	31.58	23.80	15.99	0.00
Cottonseed cake	0.00	9.85	19.70	29.55	39.40
Maize	28.30	26.27	23.76	21.77	28.30
Blood meal	2	2	2	2	2
Bone meal	1	1	1	1	1
Palm oil	2.50	2.50	2.50	2.50	2.50
Premix	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100
Determined Analysis (%)					
Moisture	7.00±0.02	8.00±0.06	5.00±0.06	5.80±0.29	8.00±0.23
Ash	12.50±0.28	13.00±0.17	5.00±0.12	12.50±0.23	13.50±0.12
Crude protein	40.03±0.12	39.55±0.03	40.01±0.06	40.10±0.64	40.00±0.01
Lipid	5.55±0.01	6.50±0.12	7.00±0.23	6.50±0.06	7.00±0.06
Fibre	8.0 0±0.06	7.50±0.12	5.00±0.17	10.50±0.12	11.50±0.12
Carbohydrate	26.97±0.09	25.45±0.43	37.99±0.23	24.60±0.27	20.00±0.29
ME (kcal/g)	3.02±4.0.02	3.03±0.01	3.39±.0.43	3.11±0.30	3.07±0.08

Note: CSC= calabash seed cake, ME= Metabolizable energy and CP = crude protein

Sourcing of experimental fish

A total of 300 *Clarias gariepinus* fingerlings, average weight 2.64 g and average length 4.76 cm were purchased from the Hatchery Unit of the National Institute for Fresh water Fisheries Research (NIFFR), New Bussa. Upon arrival, the fish were first conditioned to the water temperature at Teaching and Research Fish Farm of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto. The fish were acclimatized for two weeks and were fed twice daily with a 40% crude protein diet (control) prior to the commencement of the feeding trial.

Experimental set up

Fingerlings were randomly distributed into fifteen circular concrete tanks (1x1m) of $0.79m^3$ volume with 20 fingerlings in each tank. Five experimental diets were randomly allocated to the experimental tanks, in a completely randomized design (CRD) with three replicates per treatment. The water for the experiment was sourced from the borehole in the farm and water in the experimental tanks was half filled and maintained the same levels throughout the experiment.

Experimental Management

Fish were fed at 5% body weight daily and were administered twice daily in two equal rations. The quantities of feeds were adjusted weekly based on the new weight of fish on the day of weight measurement and the experiment was terminated at 10 weeks. The uneaten feeds together with fish faecal residues were siphoned every morning before feeding. The experimental tanks were completely washed every week and fresh water was added to ensure proper water quality monitoring and to maintain at least two third water levels in the experimental units

Fish Growth Measurements

The length and weight of fish in each tank was measured at the commencement and end of the experiment. 20 fish were taken from each tank once a week and weighed with a digital beam balance to determine the weight increase.

Fish Survival and growth indices

Survival rate: This was determined by Cheikyula and Ofojekwu (2003) and Odedeyi (2007):

Survival rate (%) = $\frac{Initial\ number\ of\ fish\ stocked - mortality}{Initial\ number\ of\ fish\ stocked} \times 100$

Weight gain (WG): This was calculated according to Castel and Tiaus (1980).

WG = final body weight (g) – initial body weight (g)

Where; W_f = final fish weight at the end of the experiment, W_i = initial fish weight at the beginning of the experiment

Percentage weight gain (PWG): This was calculated according to Webster and Chhorn (2001)

PWG (%) = $\frac{Wf - Wi}{Wi} \times 100$ Webster and Chhorn (2001)

Where; W_f = final weight (g) and W_i = initial weight (g)

Specific Growth Rate (SGR): Specific growth rate (% body weight / day) was determined as described by Stickney (1979);

SGR (%/day) $= \frac{In_{w2} - In_{w1}}{T_2 - T_1} \times 100$

Where In = Natural logarithm

$W_2 - W_1$ = final and initial weight of fish (g) and $T_2 - T_1$ = period in days

Condition Factor (K): This was determining by using Fulton's formula as described by Bagenal and Tesch (1978);

K = $\frac{100W}{L^3}$

Where; W = final mean body weight (g) and L = mean standard length (cm)

Nutrient utilization indices

Feed conversion ratio (FCR): This was determined as described by Fagbenro *et al.* (1992) and Tutas *et al.* (2013);

FCR = $\frac{F}{Wf - Wo}$

Where; F = weight of food supplied to fish during the study period, Wf = live weight of live weight of fish at the beginning of the study

Gross Feed Conversion Efficiency (GFCE)

$GFCE = \frac{1}{\text{FCR}} \times 100$ Chow *et al*. (1985)

Protein Efficiency Ratio (PER): This index is a measure of nutritive value of dietary protein and was determined as described by Hepher (1988)

$\text{PER} = \frac{weight\ gain\ (g)}{crude\ protein\ consumed}$

Apparent Net Protein Utilization (AppNPU): This was calculated according to Chow *et al*. (1985)

$\text{APPNPU} = \frac{N_b - N_a}{N_i} \times 100$

Where; N_b = Body protein at end of the experiment; N_a =Body protein at the beginning;

N_i =Amount of nitrogen (protein ingested)

Statistical Analysis

The determinations were made in triplicate, parameters obtained were subjected to one way analysis of variance (ANOVA) and, the treatment means was separated for significant differences following the procedure of Duncan Multiple Range Test (Steel and Torrie, 1980). All the analysis was carried out using the computer software Statistical Package for the Social Sciences (SPSS) version 20.0 windows (SPSS, 2013).

Results and Discussion

Growth Response of Experimental Fish

Survival and growth performance of *Clarias gariepinus* fingerlings fed graded levels of Calabash seed cake diets are shown in Table 1. The survival rate was significantly higher ($p < 0.05$) in fish fed diet D (75% Calabash seed cake) than in the other four treatments. Survival did not differ significantly ($p > 0.05$) among diets B (25%), C (50%), and E (100%), but these were significantly higher ($p < 0.05$) than the control diet (0% Calabash seed cake), which had the lowest survival. Figure 1 illustrates the survival trend over the period. Fish fed diet D (75%) survived comparably to those fed diets B (25–100%) up to the sixth week, after which mortality began

Regarding growth parameters, there was an increase in fish fed the diet containing 25% substitution with Calabash seed cake; this finding is similar to Mamman *et al*. (2021), who reported that higher substitution of cottonseed meal with GNC improved growth. The result also showed an impact on the growth performance of Hybrid fingerlings (*Clarias gariepinus* × *Heterobranchus bidorsalis*). This finding was higher than that reported by Mamman *et al*. (2013) when *Clarias gariepinus* was fed Calabash seed meal and by Jibrin *et al*. (2018) when Hybrid fingerlings (*Clarias gariepinus* × *Heterobranchus longifilis*) were fed Calabash seed meal. This suggests greater protein utilization in fish fed diets containing 25% Calabash seed cake.

The observed increase in growth performance in fish fed diets containing 25% Calabash seed cake could be attributed to boiling, the processing method employed for the Calabash seed. However, the reduction in growth performance in fish fed other dietary treatments might not be due to palatability issues, since the diets were accepted by the fish; rather, it may relate to the presence of various anti-nutritional factors (tannin, glycosides, cardiac glycosides, alkaloids, steroids, etc.) in the seed cake. Dienye and Olumuji (2014), Bake *et al*. (2019), and Balogun (2013) reported similar decreases in growth when boiled Moringa seed, Moringa leaf meal, toasted *S. obtusifolia* seed meal, and soaked B. monandra seed meal, respectively, were fed to C. gariepinus. The weight gain trajectory over time is shown in Fig. 2, with the 25% diet (B) outperforming other treatments from week 1 through week 6.

Mortality across all treatments might be attributed to extraneous factors; this observation agrees with Alegbeleye (2005), who reported that mortality may result not only from anti-nutritional factors in the diets but also from extraneous factors such as stress from handling. Significant increases were observed in the condition factor of fish fed experimental diets containing 50–100%

Calabash seed cake; the values increased with higher inclusion levels of Calabash seed cake. This finding is higher than that of Anyanwu (2008), who reported a condition factor of 0.65 for *C. gariepinus* reared in recirculatory system.

Table 1: Survival and growth indices of *Clarias gariepinus* fed experimental diets

Parameters	Graded levels of Calabash seed cakes (%)				
	A (0)	B (25)	C (50)	D (75)	E (100)
Initial fish number	60	60	60	60	60
Final fish number	50	53	53	59	52
Survival rate (%)	83.33±4.41[b]	88.33±1.67[ab]	88.33±4.41[ab]	98.33±1.67[a]	86.66±6.01[ab]
Initial body weight (g)	2.63±0.00	2.63±0.01	2.63±0.00	2.64±0.00	2.64±0.00
Final body weight (g)	43.01±2.68[b]	44.77±1.01[a]	44.47±2.20[a]	39.19±0.76[d]	41.06±2.01[c]
Mean weight gain (g)	40.37±2.68[c]	42.13±1.01[a]	41.84±2.20[b]	36.55±0.75[e]	38.42±2.01[d]
Weight gain (%)	1531.89±10.73[c]	1601.47±10.42[a]	1588.25±13.87[b]	1387.02±19.44[e]	1455.81±14.90[d]
Specific growth rate (%/day)	5.28±0.09	5.34±0.03	5.32±0.07	5.14±0.03	5.21±0.07
Initial body length (cm)	4.71±0.01	4.74±0.02	4.82±0.02	4.77±0.03	4.77±0.04
Final body length (cm)	16.07±0.43[a]	16.96±0.28[a]	14.933±0.24[b]	14.30±0.70[b]	13.63±0.08[c]
Length increase (cm)	11.35±0.42[b]	12.23±0.29[a]	10.11±0.22[c]	9.53±0.72[d]	8.86±0.11[e]
Length increase (%)	241.10±8.63[b]	258.00±7.17[a]	209.80±4.24[c]	199.97±16.35[c]	185.86±3.49[d]
Condition factor (K)	0.99±0.35	1.03±0.0.02	1.33±0.01	1.37±0.18	1.62±0.11

Means in rows having same letters are not significantly different (P>0.05)

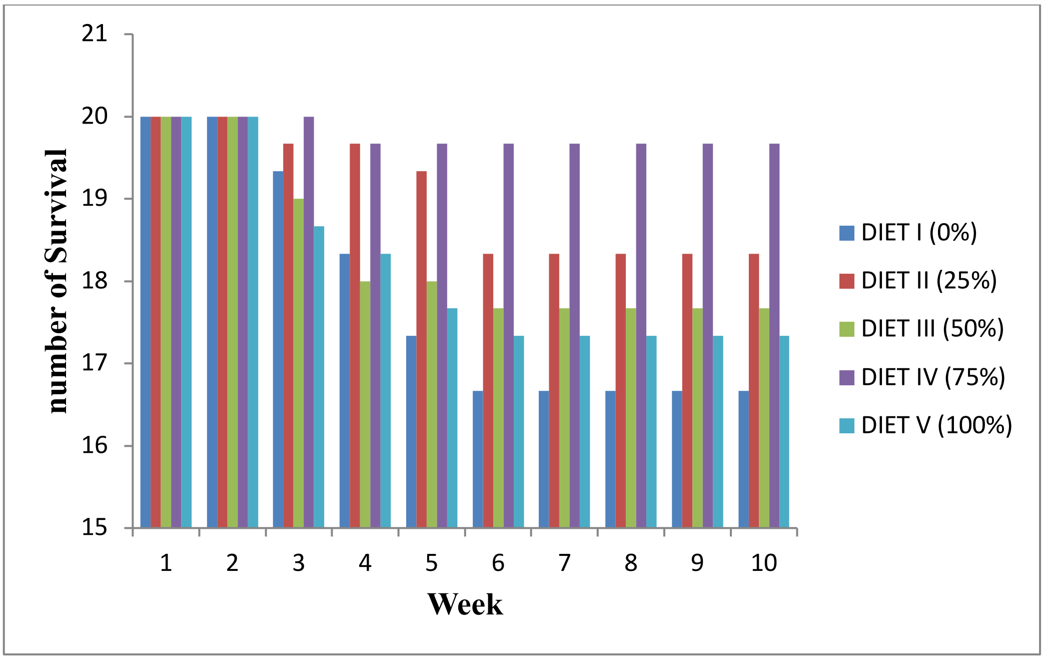

Figure 1: Survival of *Clarias gariepinus* fed graded levels of Calabash seed cake

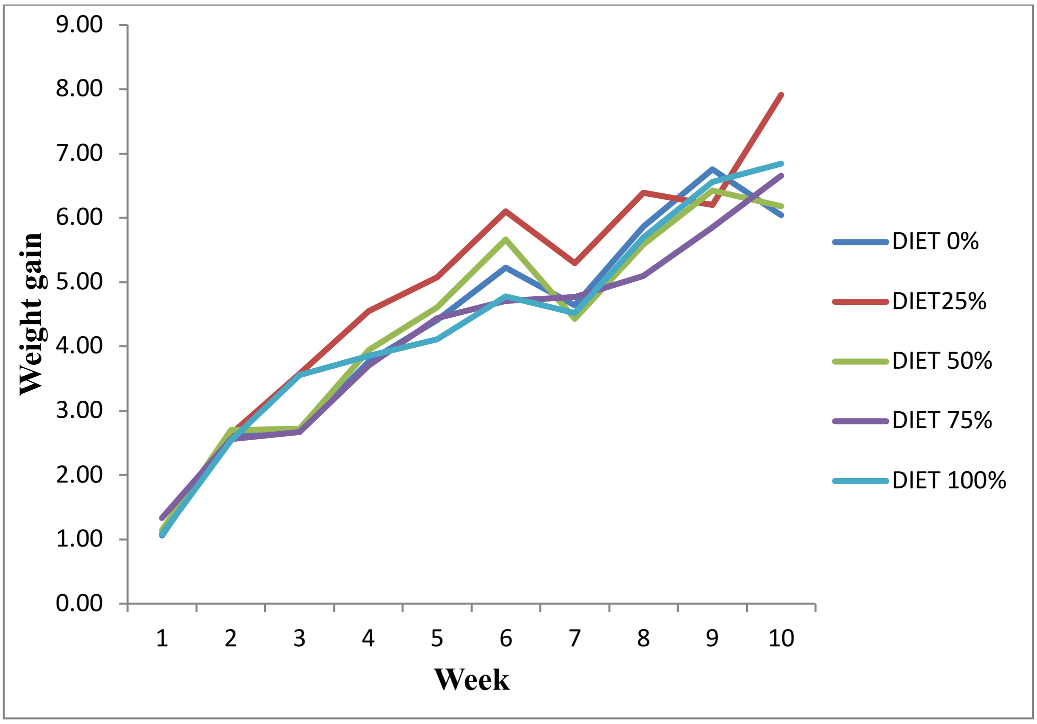

Figure 2: Weekly weight increase

Nutrient utilization indices

Nutrient utilization indices of *Clarias gariepinus* fingerlings fed graded levels of Calabash seed cake diets are presented in Table 2. The low values of feed conversion ratio (FCR) obtained in the present study suggest efficient utilization of Calabash seed cake. The ability of an organism to convert nutrients, especially protein, positively influences growth performance; this is supported by the higher protein efficiency ratio (PER) in fish fed diets with 25–75% substitution of Calabash seed cake for GNC. Stickney (1979) reported that FCR decreases as the efficiency of nutrient utilization increases. However, the findings in this study were better than Bekibele (2005), who reported a poor FCR (2.66) when *Clarias gariepinus* was fed mucuna bean as a plant protein supplement.

The results for feed conversion efficiency (FCE) were higher when compared with Jibrin *et al.* (2018), who observed low FCE in hybrid fingerlings (*Clarias x Heterobranchus longifilis*) fed Calabash seed meal; Mamman *et al.* (2021) reported low FCE in hybrids (*Clarias gariepinus x Heterobranchus bidorsalis*) fed cotton seed meal, and Bekibele (2005) reported low gross feed conversion efficiency when feeding mucuna bean to *C. gariepinus*. These differences could be attributed to the effects of boiling and defatting Calabash seed, which may enhance nutrient availability in the feeds. The protein efficiency ratio (PER) obtained in this study was higher than that reported by Haruna and Zannah (2023), who observed lower PER values when feeding *C. gariepinus* fingerlings with *Moringa oleifera* seed meal, and Mamman *et al.* (2013), who reported lower PER with Calabash seed meal. Similarly, Jibrin *et al.* (2018) reported a lower PER for Hybrid fingerlings (*Clarias gariepinus* × *Heterobranchus longifilis*) fed Calabash seed meal. The higher PER in this study could result from the processing of Calabash seed, which improved nutrient availability in the diets.

These PER results appear to be related to feed intake, which increased with higher levels of seed cake in the diets. PER is influenced by the non-protein energy input of the diet and is a good measure of the protein-sparing effect of lipid and/or carbohydrate (Lie *et al*., 1988; Tibbets *et al*., 2005). De Silva and Anderson (1995) reported that PER reflects how well the dietary protein sources meet the essential amino acid requirements of the fish being fed. They stated that PER is an index associated with fat deposition in the muscle, and that a higher PER can indicate diets that promote fatty fish. However, the PER obtained in this study did not indicate increased fat deposition in the experimental fish.

Table 2: Nutrient utilization indices of *Clarias gariepinus* fed experimental diets

Parameters	Graded levels of Calabash seed cakes (%)				
	A (0)	B (25)	C (50)	D (75)	E (100)
Feed conversion ratio	1.21±0.01	1.29±0.03	1.23±0.01	1.28±0.01	1.23±0.04
Gross Feed Conversion efficiency	77.68±1.17[c]	82.27±0.38[a]	81.10±0.39[b]	78.24±0.51[c]	81.02±2.32[b]
Protein efficiency ratio	2.05±0.01	1.96±0.43	2.02±0.01	1.95±0.03	2.02±0.05
AppNPU	28.34±2.57[c]	34.1±0.92[a]	31.11±2.43[b]	30.31±0.62[b]	23.16±4.63[d]

Mean values in row with same letter are not significantly different ($p > 0.05$)
Note; Apparent net protein utilization = AppNPU

Conclusion and Recommendation

The study on the survival and growth performance of *Clarias gariepinus* fingerlings fed varying levels of Calabash seed cake diets revealed several key insights. The highest survival rate was observed in fish fed a 75% inclusion level of Calabash seed cake, indicating that this dietary formulation may enhance fish resilience. However, in terms of growth parameters, the diets with 25% and 50% Calabash seed cake resulted in the highest final body weights and weight gains, significantly outperforming other treatments. Notably, fish on the 25% diet exhibited the best overall growth performance throughout the trial period, highlighting its potential as an optimal diet for promoting growth. Furthermore, while the specific growth rates and condition factors showed no significant differences across treatments, the trends suggest that a moderate inclusion of Calabash seed cake (25%) is advantageous for maximizing growth in *Clarias gariepinus* fingerlings.

Overall, no significant differences in feed conversion ratio (FCR) or protein efficiency ratio (PER) across the graded Calabash seed cake (CSC) diets; however, 25% CSC gave the best gross feed conversion efficiency and AppNPU. Best practical takeaway: 25% CSC inclusion optimizes nutrient utilization among CSC containing diets, whereas 100% CSC markedly reduces AppNPU and should be avoided for protein retention.

It is recommended that calabash seed cake be included in the feed of *C. gariepinus* at 25% for optimum growth performance. Since this study was carried out under concrete pond experimental conditions, it is recommended that field experiment using the semi-intensive system commonly practiced in earthen ponds may be more desirable and can be explored.

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