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Effect of substituting calabash seed (*Lagenaria vulgaris*) meal for groundnut cake in the diet of catfish (*Clarias* X *Heterobranchus longifilis*) hybrid fingerlings

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Abstract
The effect of substituting calabash seed (*Lagenaria vulgaris*) meal (CSM) for groundnut cake (GNC) on growth performance and survival of *Clarias gariepinus* x *Heterobranchus longifilins* (Hybrid) fingerlings were investigated. Five isonitrigenous (45% crude protein level) diet at 0, 25, 50, 75, and 100% substitution levels of CSM for GNC, numbered I, II, III, IV and V were fed to 15 groups of hybrid fingerlings for 56 days. The CSM contains 5% moisture content, 6% ash content, 40% lipid content, 34.9% crude protein content, 2.5% crude fiber and 16.6% Nitrogen free extract. Fish fed diet I (control) had the highest survival rate of 96.6% followed by 93.3% survival rate of those fed diet II of 25% containing (13.47%) CSM and the lowest survival rate (86.7%) were recorded in fish fed diet III and V containing 27.55% and 57.76% CSM with no significant (P>0.05) difference. Fish fed diet I containing 0% CSM recorded highest weight gain (2.00±0.05g); the lowest weight gain of (0.51±0.12g) was recorded in fish fed diet V containing 57.6% CSM with a significant (P<0.05) difference. Specific growth rate, feed conversion ratio and protein efficiency ratio follow the same trend. The apparent Net protein Utilization (AppNPU) of the dietary treatment ranged from 8.33±0.22g in fish fed diet V containing 57.76% CSM to 3.78±048g in those fed diet III containing 27.55% CSM, with a significant (P<0.05) differences between the dietary treatments. Conclusively, the calabash seed meal (CSM) can be incorporated in the diet hybrid fingerlings at 13.47 to 57.76 CSM with no significant (P<0.05) difference in weight gain, feed conversion ratio and protein efficiency ratio. Therefore, it is expected that proper method of oil extraction should be employed to reduce the oil level of the calabash seed meal (CSM).

Keywords: Calabash seed meal, *Clarias gariepinus* x *Heterobranchus* hybrid fingerlings, growth performance, survival rate

Introduction

Aquaculture is the farming of wide variety of aquatic organisms such as, fishes, crustaceans, mollusks, algae and aquatic plants in fresh, brackish and salt waters [1]. Unlike captured fisheries, aquaculture requires deliberate human intervention in the organisms' productivity, and results in yields that exceed those from natural environment. Some common examples of human intervention include stocking water with seeds (fry/juvenile's organisms), fertilizing the water, feeding of the organisms and maintaining good water quality [2].

The demand of fish is increasing throughout the world due to the recognition of its nutritional value [3]. In addition, the rise of food price and rapid human world population growth increase the demand of fish consumption [4]. For these reason, there are varieties of farming fish species which get a special consideration to increase the world fish population. Fish farming also known as aquaculture plays a major role in Agriculture in Africa and especially in Nigeria [5].

However, with the advent of non-conventional feed resources found abundantly in the country, this problem can be addressed. Non-conventional feed resources (NCFRs) are feeds that are not usually common in the market and are not the traditional ingredients for commercial fish feed production [6, 7]. NCFRs are credited for being non-competitive in terms of human consumption and cheaper to purchase [6]. They are by-products or waste products from agriculture, farm made feeds and processing industries that are able to serve as a waste management in enhancing good sanitation [8]. They may include all types of feed stuffs from animal (silk worm, maggot, termites, earth worm, snails, tadpoles, etc.), plant waste (jack bean, cotton seed meal, soybean meal, cajanus, chaya, duckweed, maize bran, rice bran, palm kernel cake, groundnut cake, brewers waste, etc.) and waste from animals such as animal dung, offal visceral feather, fish silage, bone and blood [6, 9]. All these can be recycled to enhance on fish feed production if is economically justified and technologically possible. The aim of this study was to assess the growth and survival of *Clarias gariepinus* x *Heterobranchus longifilis,* hybrid fingerlings fed diets containing varying levels of calabash seed meal.

Materials and Methods

Experimental Site

The experiment was conducted at the Fish Hatchery of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto. The site is located in Sudan Savanna agro-ecological vegetation zone of Nigeria on latitude 13^0 07' 78'' N and longitude of 5^0 12' 25'' E and on 275m above sea level [10]. The study area is characterized by a long dry season which start from October to May; with cool dry air during the harmattan; (November- February) and hot dry air during March-May. Raining season start in June and ends in September. Annual rainfall in the area ranged from 500 to 724 mm [11]. The mean relative humidity range between 14.9% and 40% during March and June respectively. Ambient temperature can reach up to 41 ^{0}C during April and May and may fall below 20 ^{0}C during December and January.

Calabash Seeds

Calabash (*Lagenaria vulgaris*) seeds were bought from Central Market in Gummi Local Government Area of Zamfara State. The seeds were processed manually at Gummi town; the procedure was the same as applied to groundnut cake oil extraction. The cake was fried, grounded to powdery form and compounded together with other ingredients to form the experimental ration.

Seed Cleaning, Preparation and Conditioning

Calabash seed cleaning was done, in seed cleaning; the materials that are removed include the husks and separating of the seeds from the chaff. This was done manually with the use of a flattened circular aluminium stainless tray. For successful oil extraction to be achieved, cleaning, drying and warming of the seeds were observed. The seeds have been cleaned to ensure that fine dust particles in the seeds are absent. Chaff left in the seed was removed, so as to avoid it from absorbing some of the oil during extraction.

Experimental Diets

Before then, the proximate composition of the feed ingredients were carried out at the Chemical and Physical Laboratory of the Faculty of Agriculture, Usmanu Danfodiyo University, and Sokoto. Percentage protein, fat, moisture, ash, fibre and nitrogen free extract (NFE) were determined for each ingredient following the method of AOAC (2000). The other feed ingredients that formed the ration include: groundnut cake, fish meal, maize, blood meal, bone meal, vitamin and mineral premix, palm oil and table salt.

Five diets containing varying levels of calabash seed meal (CSM) at 0% (Diet 1), 25% (Diet 2), 50% (Diet 3), 75% (Diet 4) and 100% (Diet 5) were formulated following Pearson Square method. The diets contain 45% crude protein.

The appropriate quantities of the ingredients in each diet were weighed and mix thoroughly using electric feed mixer (Kenwood). Each diet was further mix with warm water to make dough. The mix dough was subjected to an electric power feed pelletizer (50/kg capacity/hour) of 2mm in diameter size. The palliated feeds were sundried and broken into smaller sizes appropriately acceptable to fish at the beginning of the experiment and stored before the commencement of the feeding trial.

Experimental Fish

A hybrid fingerlings of an average weight of 2.5g were harvested from Departmental outdoor concrete tanks of Fisheries Hatcheries, Usmanu Danfodiyo University Sokoto (UDUS). The fish were acclimatized for two weeks, after which they were fed with 45% crude protein diets (control). They were then redistributed evenly and randomly to each plastic bowl.

Experimental Setup

One hundred and fifty (150) fingerlings were distributed into fifteen (15) (Forty liters maximum capacity) round bottom plastic bowls at stocking rate of ten fingerlings per bowl. The fish in each bowl was weighed to ensure uniformity in size in each bowl, and the five experimental diets were randomly put to the experimental bowls, in a completely randomized design (CRD) with three replicates. The water source for the experiment was University borehole. Dissolved oxygen supply into the experimental bowls was maintained with the aid of electric aerator connected with series of nozzle horses. The bowls were covered with synthetic net to prevent fish from jumping out.

Experimental Management

Experimental fish in each plastic bowl were fed at 5% body weight for 8 weeks of the feeding trial period. The daily ration was split into two (2) times and fed twice daily at 9: am and 5: pm. The ration was adjusted weekly base on the new weight gain in each plastic bowl. The plastic bowls were clean and uneaten feeds together with faecal residues were siphoned out before feeding. Water level was maintained in each plastic bowl and was washed completely every week, and new water was impounded to each plastic bowl.

Water Quality Analysis

Temperature and pH were monitored in the course of the experiment. Temperature was measured with simple centigrade mercury-thermometer graduated in 0.01 ^{0}C. The temperature readings were taken for both room and water at every feeding time. Hydrogen ion concentration was monitored with pH meter at every feeding period.

Proximate Analysis

The proximate analysis of the feed ingredients, experimental diets, experimental fish and after the experiment were carried out using the standard methods [12]. The analysis includes fat content, protein, ash, moisture, ether extract and crude fiber determinations.

Statistical Analysis

The data on growth and nutrient utilization, economic indices obtained was subjected to analysis of variance (ANOVA) and, the treatment means was separated for significant differences following the procedure of Duncan Multiple Range Test [13]. All the analyses were carried out using the computer software statistical package for the social science (SPSS) version 20. window [14].

Table 1: Gross composition (%) of experimental diet

S/N	Ingredients	Diets				
		I (0%)	II (25%)	III (50%)	IV (75%)	V (100%)
1.	Maize	9.99	8.08	6.09	4.01	1.83
2.	Groundnut Cake	52.66	40.39	27.55	14.10	-
3.	Fish meal	21.06	21.54	22.04	22.56	23.11
4.	Calabash Seed Cake	-	13.47	27.55	42.30	57.76
5.	Blood Meal	10.53	10.77	11.02	11.28	11.55
6.	Bone Meal	2.00	2.00	2.00	2.00	2.00
7.	Palm Oil	2.00	2.00	2.00	2.00	2.00
8.	Salt	0.25	0.25	0.25	0.25	0.25
9.	Vitamin Premix	0.5	0.5	0.5	0.5	0.5
10.	Lysine	0.5	0.5	0.5	0.5	0.5
11.	Methionine	0.5	0.5	0.5	0.5	0.5
	Total	100	100	100	100	100
	Crude Protein Level	45	45	45	45	45
	Calculated Metabolizable Energy (kcalkg^{-1})	3565.2	3782.0	4008.2	4245.1	4493.6

Results

The survival indices of fish fed on the various dietary treatment are presented in Table 2. Fish fed diet 1 (control) had the highest survival rate of 96.6%. The lowest survival rate recorded in the fish fed diet IV of 100% containing (57.76%) CSM of the same ratio with no significant (P>0.05) difference between the treatments.

The highest mean weight gain of 2.00±0.05g was recorded in fish fed diet I (control) at 0% CSM containing (0% GNC substitution). This was followed by 0.80±0.14g on those fish fed diet II containing 13.47% CSM (25% substitution of GNC) with significant (P<0.05) difference between the fish fed diet I (control) and other dietary treatments. The weight gain decreased with decrease in substitution of CSM for GNC in the diets (Table 4). It was lowest (0.51±0.12g) in fish fed diet V containing (57.76%) CSM with a significant (P<0.05) difference in the weight gain of the dietary treatments.

Table 2 shows the feed conversion and nutrient utilization indices of fish fed the experimental diets. Fish feed diet I containing 0% CSM recorded the lowest feed conversion ratio of 4.23±0.05 with a significant (P<0.05) difference with other dietary treatments, while the poorest feed conversion ratio was obtained in fish fed diet V containing (57.76%). The protein efficiency ratio (PER) follows the same trend it as table 2

The App NPU of the dietary treatment ranged from 8.33±0.22 in fish fed diet V containing 57.76% CSM to 3.78±0.48 in those fed diet III containing 27.55% CSM. However, there was significant (P<0.05) differences between the App NPU of the dietary treatment.

The body composition of the experiment fish before and at the end of the feeding experiment is shown in table 3. There was no clear trend in the moisture content of the experimental fish placed on the dietary treatments. The crude protein was highest (30.5) in fish fed diet V containing 57.76% (100%) CSM and this was not significantly (P<0.05) higher than those placed on all the other dietary treatments including the control. However, there was no significant (P>0.05) differences between the proteins levels in fish placed on the dietary treatment it as table 3.

Discussion

The results of the proximate composition of calabash seed cake (Table 2) revealed that the cake meal was high in ether extract and crude protein content with values close to those of [15] who reported 49.31 and 34.47% ether extract and crude protein respectively. The calabash seed meal (CSM) was found to be slightly lower in ash content of (6.0%) and in fiber content (2.5%) [16]. Reported very close values of 8.75% and 3.25% Ash and crude fiber of raw (unprocessed) calabash seeds. The fiber content of calabash seed meal was lower than 5% base line for crude fiber ingredients for monogastric animals [17]. The reason for high lipid content of CSM could have been attributed to the poor quality and inefficiency in oil extraction process.

The highest mean weight gains of 2.00±0.05g (Table 2) was recorded in fish fed diet I containing 0% CSM (0% GNC substitution) and this was higher than the weight gain of the other dietary treatments with calabash seed meal. In the present investigation, the values of weight gain reported were lower than those reported by [18, 19] when fed *C. gariepinus* fingerlings with calabash seed meal and *Amaranthus spinosus* leaf meal. The reduced growth performance in fish fed diet containing high level of CSM (57.76%) diet V (100%) might not be a palatability problem, because the diet was accepted by the fish but might be related to the presence of various antinutritional factors. Calabash seeds have been reported to contain phytic acid, glucosnolates, curcubitacin and cyclopropanoic acid [20, 21, 18].

The carcass composition of the experimental fish after the experiment revealed increase in crude protein, ash and moisture but decrease in ether extract in all the dietary treatment over the initial carcass composition. An inverse relationship between the body lipid and moisture was apparent, which was in line with what is been obtained in previous experiment [22, 18]. This could also be the attribute of the crude protein content of the experimental diets. Protein is essential in the diet of fishes as sources of amino acids which are building blocks of flesh, enzymes, eggs, milk, antibiotics and some hormones [23].

The lower values of Feed Conversion Ratio of 4.23±0.05 to 15.73±3.44 recorded in all the treatments suggested partial efficient utilization of the diets. [24] Reported that that feed conversion ratio becomes lower as efficiency of utilization increases. However, the values recorded in the present study were not too far from [25, 26, 27] who reported the lowest values of FCR of 3.75 and 2.66 when fed *Clarias gariepinus* with water hyacinths and mucuna bean as plant protein supplement.

The protein efficiency ratio recorded in this research study steadily decreased with increased levels of calabash seed meal. However, the significant performance was exhibited by fish fed the control and diet II (13.47%) CSM compared with those fed diet 27.55, 42.30 and 57.76% calabash seed meal.

This may be suggesting that *Clarias gariepinus* x *Heterobranchus longifilis*, a hybrid fingerlings, could not tolerate beyond (25% CSM) level of calabash seed meal in the diet. The crude protein contents of the diets (45%) and the species of fish used in the study were perhaps responsible for the lower PER recorded in the study.

The Apparent Net Protein Utilization was shown in Table 2. The App NPU of the dietary treatment ranged from 8.33±0.22 in fish fed diet V containing 57.76% CSM to 3.78±0.48 in those fed diet III containing 27.55% CSM. However, there was significant (P<0.05) difference between the App NPU of the dietary treatment treatments. Higher values of App NPU were reported in the present study than those reported by [27, 26, 18]. This suggested the fish efficiently utilized the feed fed in body tissue synthesis.

Table 2: Growth and Nutrient Utilization of hybrid fingerlings Fed Calabash Seed Meal

Parameters	Diets				
	1(0%)	**II (25%)**	**III (50%)**	**IV (75%)**	**V (100%)**
No. of fishes	30	30	30	30	30
Mean Initial Body Weight (g)	2.44±0.05^{a}	2.52±0.04^{a}	2.44±0.11^{a}	2.51±0.03^{a}	2.51±0.02^{a}
Mean Final Body Weight (g)	4.44±0.10^{a}	3.32±0.11^{b}	3.19±0.13^{bc}	3.08±0.01^{c}	3.02±0.10^{c}
Mean Weight Gain (g)	2.00±0.05^{a}	0.80±0.14^{b}	0.75±0.23^{bc}	0.58±0.03^{bc}	0.51±0.12^{c}
Percentage Weight Gain (g)	81.89±1.07^{a}	31.77±6.06^{b}	31.05±11.12^{b}	22.94±1.53^{b}	20.40±4.79^{b}
Specific Growth Rate (%)	1.07±0.01^{a}	0.49±0.08^{b}	0.48±0.15^{bc}	0.37±0.02^{bc}	0.33±0.07^{c}
Mean Weekly Feed Intake	8.45±0.18^{a}	7.77±0.30^{b}	7.50±0.05^{b}	7.56±0.09^{b}	7.79±0.16^{b}
Feed Conversion Ratio	4.23±0.05^{c}	9.93±1.66^{b}	10.57±2.83^{b}	13.17±0.76^{ab}	15.73±3.44^{a}
Feed Efficiency Ratio	0.24±0.01^{a}	0.10±0.02^{b}	0.10±0.04^{bc}	0.07±0.01^{bc}	0.07±0.02^{c}
Gross Feed Conversion Ratio	23.65±0.28^{a}	10.26±1.74^{b}	10.02±3.14^{b}	7.61±0.46^{bc}	6.55±1.36^{c}
Protein Efficiency Ratio	4.44±0.10^{a}	1.77±0.32^{b}	1.67±0.52^{bc}	1.28±0.07^{bc}	1.14±0.26^{c}
Mean App. Net Protein Utilization	7.40±0.08^{ab}	6.78±0.29^{ab}	3.78±0.48^{c}	5.00±0.04^{b}	8.33±0.22^{a}

Mean in rows with the same letter are not significantly (P>0.05) different

Table 3: Proximate composition (%) of experimental fish fed calabash seed meal

Parameter	Initial fish	Fish (%) Composition				
		I (0%)	**II (25%)**	**III (50%)**	**IV (75%)**	**V (100%)**
Moisture	53.97	59.26	55.10	58.91	57.01	59.14
Ash	1.5	2.7^{b}	3.0^{b}	3.10^{b}	2.5^{b}	3.2^{a}
Ether extract	3.0	1.5^{b}	1.6^{b}	1.7^{b}	1.6^{b}	1.8^{a}
Crude protein	26.75	30.08	29.80	28.45	29.00	30.5
Nitrogen Free Extract	14.78	5.74^{bc}	10.50^{ab}	7.84^{b}	9.89^{ab}	5.36^{c}

Mean in rows with the same letters are not significantly different (P>0.05)

Conclusion

From the present study, it can be concluded that the calabash seed meal (CSM) with crude protein content of 34.9% and that of crude lipid of 40% could serve as both protein and energy supplement.

The highest weight gain was recorded in fish fed diet containing 0% CSM at 0% level of groundnut cake substitution, followed by 13.47% at 25% level of substitution groundnut cake substitution, while other levels of substitution (50, 75, and 100%) resulted in decreased weight gain. Thus, the CSM can be incorporated in the diet of hybrid fingerlings at 13.47 to 57.76% CSM with no significant (P<0.05) difference in growth and other nutrients utilization parameters.

Recommendations

The finding in the present study recommends further investigation on the utilization of calabash seed as a non-conventional feed ingredient in fish feed for substituting conventional feed materials for fish formulation with respect to more appropriate processing method for oil extraction, its digestibility and effect of Cucurbitacin which is known as anti-nutrient factor of the seed. It is also recommended that the processing method should add more value to the products by increasing oil yields which are alternative use in other household purpose such as cooking. Further studies should be carried out in respect of other generic and species to ascertain the potential of CSM in growth, nutrient utilization and other factors affecting growth and survival.

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