Effects of Replacing Soybean Meal with Fermented Bambara Groundnut Meal (*Vigna subterranea)* Meal in the Diet of *Clarias gariepinus* Fingerlings

Original Research Article

ABSTRACT

Background: Efforts to reduce fish feed cost have been geared towards fishmeal replacement with cheap ingredients. However, the price of soybean which is a supplementary protein source in fish diet is also soaring due to impact of covid-19 pandemic couple with competition by man and livestock; this therefore calls for a search for its alternatives. Bambara groundnut (BG), although highly nutritious and climate resistant crop, is described as a neglected and under-utilized crop in most countries of Africa.

Aims: To ascertain the effect of replacing soybean meal with Fermented Bambara Groundnut Meal (FBGM) on the growth performance and feed utilization of *C. gariepinus*.

Study Design: Completely Randomized Design (CRD) was used with five treatments and three replicates. Diet FBGM00 with no FBGM was used as control. Various replacement levels were designation as FBGM25, FBGM50, FBGM75 and FBMG100 for 25, 50, 75 and 100% respectively.

Place and Duration of Study: Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike; Central Labouratory, University of Uyo, Nigeria, between May 2021 and August 2021.

Methodology: We fermented *Vigna subterranea* for 72 hours, air-dried, weighed and oven dried at 60˚C for 24 hours, followed by cooling. Dried flour of FBGM and other ingredients were analyzed for proximate composition used in the formulation of experimental diets. These were milled at 2 mm pellets, sundried and stored at 10 per cent moisture content, in black polythene bags. Diets were fed to one hundred and fifty (150) fingerlings of catfish (*C. gariepinus*) with mean weight 50±2.35 for 84 days. Growth performance, feed utilization and survival rate parameters were calculated and statistically compared.

Results: Physico-chemical parameters fluctuated slightly but were within the optimum range. The highest daily weight gain (85.29±14.46 g) was recorded in FBGM75 while the lowest (68.29±8.49 and 69.86±8.73 g) were recorded in the control diet (FBGM00) and FBGM25 respectively. The specific growth rate, daily weight gain, feed conversion ratio, protein efficiency ratio and percentage survival rate also followed the same pattern. However, no significant differences (P>.05) were detected in all these parameters.

Conclusion: Fermented Bambara Groundnut Meal can totally replace soybean meal in the diet of *C. gariepinus*, without adversely affecting growth performance, feed utilization and survival rate. However, the optimum replacement level lies in FBGN75. More researches are needed on the adults or grow out stages to unveil its effects on the grow out stage and brooder and different processing methods employed and compared to the best processing method that will be mild enough to increase the nutrient content while removing the phytochemicals.

Keywords: Legume; ingredient; processing; survival; feed; least-cost.

1. INTRODUCTION

In Nigeria, fish is widely accepted by the populace, thereby making the demand for it to be on the increase. In recent time, a good number of fish consumed by Nigerians is from aquaculture because the conventional fish catch from ocean and rivers are continually declining due to over fishing and environmental hazards [1]. Jamiu and Ayinla [2] reported that feed accounts for minimum of 60% of the total cost of fish production in Africa and a major factor that determines the viability and profitability of fish farming enterprise. As aquaculture production becomes more and more intensive in Nigeria, fish feed will be a significant factor towards increasing the productivity and profitability of aquaculture [3]. The need to intensify fish culture, so as to meet the ever-increasing demand for fish has made it essential to develop suitable diets either in supplementary forms for earthen ponds or as complete feed in tanks and other artificial enclosures [4].

High fish feed cost is primarily due to high protein requirement of fish (35-42%) which is derived majorly from fishmeal. Foreign fishmeal (menhaden -72% crude protein) now cost about three thousand naira (₦3000.00) per kg in Nigeria. Efforts to reduce fish feed cost have therefore been geared towards fishmeal replacement with cheap ingredients. However, the price of soybean which is a supplementary protein source in fish diet is also soaring due to impact of covid-19 pandemic [5] couple with competition by man and livestock; this therefore calls for a search for its alternatives also hence the study on Bambara groundnut. Bambara groundnut (BG), although highly nutritious and climate resistant crop, is described as a neglected and under-utilized crop in most countries of Africa.

Bambara groundnut (*Vigna subterranean* (L.) *Verdc*) is a legume grown mainly in the Middle Belt region and Enugu State of Nigeria [6]. Bambara groundnut seed has been reported to contain 14-24% crude protein [7,8], about 60% carbohydrate, and it is higher in essential amino acids like isoleucine, leucine, lysine, phenylalanine, threonine, valine and methionine than most other grain legumes including groundnut [9,10]. It contains 6-12% of oil that is lesser in peanuts. Bambara groundnut has not been effectively cultivated or underutilized especially in hostile tropical environments [11]. The utilization of Bambara nut is limited by the presence of growth inhibitors such as trypsin and chymotrypsin inhibitors, phytates, nitrates and cyanogen [12]. Traditional processing techniques such as soaking, pouting or cooking have limited effects on the improvement of protein quality of tree legumes [13,14]. According to Adamu et al., [15], fermentation is one of the oldest methods of food preservation known to man. In Nigeria and most African countries, condiments such as fermented locust bean (Iru), fermented melon seed (Ogiri), fermented Bambara seed (Daddawa), fermented cotton seed (Ogiri) and fermented pigeon pea are widely used to season food. Fermentation improves the nutrient content of feedstuff. For instance, the proximate composition revealed variations between unfermented and fermented seeds of Bambara groundnut with lipid increasing from 1.67 to 13.33%; protein value from 6.53 to 11.44%; then carbohydrate decreased from 77.8 and 49.46%. The completion of fermentation is indicated by the formation of mucilage and overtones of ammonia produced as a result of the breakdown of amino acids during the fermentation [16].

The aim of this research is to study the effect of substituting soybean meal with varying dietary levels of fermented Bambara Groundnut Meal (FBGM) on the growth performance, feed utilization and survival rate of *C. gariepinus*.

2. MATERIALS AND METHODS

2.1 The Study Area

This research work was conducted in fish farm of the Department of Fisheries and Aquatic Resources Management of Michael Okpara University of Agriculture, Umudike. Umudike lies between latitude 5029'N and 7033'E. The average temperature of the area is 26^{o}C, maximum being 32^{o}C and the minimum 22^{o}C. Umudike is 122 m (499 ft) above sea level. It has an average rainfall (21698.8 mm) which is obtained within 148-155 days. The relative humidity is 50-95%. Umudike is within the humid rainforest zone characterized by long duration (7-12 months) of rainfall and short period of dry season.

2.2 Collection and Processing of Ingredients

Vigna subterranea seed, Soya bean seeds, yellow maize seeds were procured from open market at Eke Aku Market in Igbo Etiti Local Government Area, Enugu State and fishmeal was Denmark and taken to the laboratory of the Department of Fisheries and Aquatic Resources Management for proper processing.

2.3 Fermentation of *Vigna Subterranea* Seed

Samples were prepared according to the method of Shlini and Siddalinga Murthy [17]. Seeds of *V. subterranea* were threshed mechanically with a mortar and pestle, and soaked for 14 hours, washed thoroughly to remove seed testa and bad ones. Then seeds were fermented for 72 hours by placing them in an airtight container (Plastic container of about 9-liter size, containing about 5 liters of water), air-dried, weighed and oven dried at 60˚C in a paper bag for 24 hours followed by cooling. All the ingredients and the dried seeds of Bambara but were milled at Umuaraga Street in Umudike and sieved using a 0.5 mm mesh sieve [18]. The dried flour of fermented Bambara nut meal and other ingredients were taken for proximate analyses and the result presented in Table 1.

2.4 Feed Formulation

Diets were formulated using feed formulation software for windows (Winfeed 2.8) which formulates feed by linear programming technique [19]. All diets were formulated on dry matter basis using the proximate compositions of the feed ingredients. Diets formulated are shown in Table 2.

Table 1. Proximate composition and digestible energy of the feed ingredients used in this experiment

Feedstuff	Content (%)										(Kcal kg^{-1} DM)
	DM	**CP**	**CF**	**EE**	**Ash**	**P**	**Ca**	**NFE**	**LS**	**MT**	**DE**
FM	91.12	52.89	3.11	5.78	21.90	2.89	5.14	16.32	4.85	2.62	2861.00
SBM	88.50	88.50	6.50	3.50	5.67	0.20	0.20	31.33	2.80	0.60	2230.00
YMM	88.51	7.31	2.00	3.20	0.51	0.09	0.01	76.59	0.30	0.18	3432.00
FBNM	90.76	21.92	3.78	7.16	4.54	1.45	1.34	64.9	1.09	1.22	2574.00

FM=fishmeal; SBM=soybean meal; YMM=yellow maize meal; FBNM=fermented bambara nut meal; DM=dry matter CP=crude protein; CF=crude fibre; EE=ether extract; P=phosphorus; Ca=calcium; NFE=nitrogen free extract; LS=lysine; MT=methionine; DE=digestible energy

Table 2. Ingredients and nutrient compositions of the experimental diets

Ingredient (g/kg)	F1 (FBGM00)	F2 (FBGM25)	F3 (FBGM50)	F4 (FBGM75)	F5 (FBGM100)
Fermented Bambara Groundnut meal	--	119.8	239.7	359.5	479.3
Soyabean meal	479.3	359.5	239.6	119.8	--
Fishmeal	280.7	280.7	208.7	208.7	208.7
Yellow maize meal	240	240	240	240	240
Fish oil	2	2	2	2	2
Sodium chloride	0.5	0.5	0.5	0.5	0.5
Biomix (mg)	2.5	2.5	2.5	2.5	2.5
Proximate composition (%)					
Crude protein	40	40	40	40	40
Ether extract	4.09	4.09	4.09	4.09	4.09
Crude fibre	3.73	3.73	3.73	3.73	3.73
Ash	4.41	4.41	4.41	4.41	4.41
Nitrogen free extract	67.17	67.17	67.17	67.17	67.17
Methionine	1.51	1.51	1.51	1.51	1.51
Phosphorus	0.45	0.45	0.45	0.45	0.45
Calcium	0.63	0.63	0.63	0.63	0.63
Digestible energy	3476.23	3476.23	3476.23	3476.23	346.23

2.5 Feed Milling and Drying

Diets formulated in percentage were then converted to weight basis. The ingredients were measured using Camry kitchen weighing balance into a mixer where they were mixed thoroughly. Five percent of cassava starch was used as a binder. Hot water was then added into the mixture and mixing continued. After 10 minutes, they were then pelleted using manual pelletizer of 2 mm die ring and dried accordingly. Vitamin premix was dissolved in liquid hexane at 100 mg: 0.25 liter of liquid hexane. The use of liquid hexane was to ensure that all the vitamins dissolve since some of the vitamin are fat soluble and cannot dissolve in water. This was sprayed on the pellets according to different treatments and then air-dried for 15 minutes [20].

2.6 Experimental Fish

One hundred and fifty (150) fingerlings of catfish (*C. garienpinus*) for the experiment were obtained in chapel of revelation church in Michael Okpara University of Agriculture Umudike and transported with 9 liters of white bucket to the fish farm. The journey was done in the evening to avoid stress of hot weather. On arrival at the farm, the fish were allowed to rest for an hour and then acclimatized for two weeks (14 days) before stocking. During this period, the fish were held in a concrete tank of 2x1.5x2 m size and were fed ad libitum with commercial feed containing 40 percent crude protein.

2.7 Experimental Procedures

Completely randomized design (CRD) was used in the experiment. There were three replicate aquaria for each treatment. Five treatments were made. Treatment 1 (FBGM00) with no Fermented Bambara Groundnut Meal (FBGM) was used as control. Various replacement levels were designed thus; Treatment 2 (FBGM25), Treatment 3 (FBGM50), Treatment 4 (FBGM75) and Treatment 5 (FBMG100) for 25, 50, 75 and 100% respectively.

2.8 Stocking, Feeding and Water Management

The stocking density of the fingerlings were ten 10 fish aquarium tank^{-1} (84x40x40 cm which equals 134.4 liters). The water level was 120 liters. According to stocking density in aquarium tank, 112 to 120 litres aquarium should contain 8-15 fish depending on size. The mean body weight of *C. gariepinus* stocked was 50±2.35 g. Fish were transferred into the aquaria glasses using scoop net. Aquaria were randomly arranged to receive similar treatment and by homogeneity. Feeding frequency recommended by Marimuthu et al. [21] were adopted. The fishes were fed twice daily (8.00 and 18.00 hours) with their various experimental feeds at five (5) percent body weight. Culture water was changed weekly in order to avoid contamination of the water by the uneaten feed and faeces. Feeding response were monitored and recorded. Physico-chemical parameters such as pH, temperature, ammonia and dissolved oxygen were monitored thrice a week to ensure optimum water quality.

2.9 Data Collection

Data on fish growth were recorded fortnightly. The weight of individual fish was obtained with an electronic top loading weigh balance (Mettle Toledo, model PB 602). The experimental tanks were inspected daily to remove dead fish. Daily weight gain, feed conversion ratio, specific growth rate and survival were calculated thus:

i) Daily Weight Gain (g) (DWG) = (FW – IW)/N, Where: DWG=Daily weight gain, FW=Final weight of fish, IW=Initial weight of fish, N=number of days of the experiment.
ii) Specific Growth Rate (SGR): = (In FBW – In IBW)/N × 100, Where: FBW= Final body weight at each harvest, IBW= Initial body weight, In= Natural logarithm N=Number of days
iii) Feed Conversion Ratio (FCR) = Dry weight of feed fed (g)/Weight gain (g).
iv) Survival Rate (SR) in % = (Total fish number harvested/ Total fish number stocked) 100
v) Protein Efficiency Ratio (PER) = Weight gain/protein intake.

2.10 Physico-Chemical Parameters

Physico-chemical parameters such as pH, temperature, ammonia and dissolved oxygen were checked thrice a week to ensure optimum water quality. Every week water quality was monitored according to APHA, [22], throughout the experimental period.

2.11 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA). The means from the various treatments were compared for significant differences (P<0.05), using Duncan's multiple range test with the aid SPSS V.19 for windows.

3. RESULTS AND DISCUSSION

3.1 Water Quality

Physico-chemical parameters of the cultured water are shown in Table 3 and fluctuated slightly. These were within the optimum range recommended for the culture of freshwater fishes in the tropical region [23]. The range of values measured for the pH, dissolved oxygen (DO), temperature and ammonia concentration of water fell within the range for optimal fish production. Generally, water quality monitoring revealed that the difference in the mean weight of *C. gariepinus* in the five treatments was not as a result of the difference in the physico-chemical parameters.

3.2 Effect of Fermentation on the Proximate Composition of Fermented Bambara Groundnut Meal

The proximate composition of FBGM in this study (Table 1) compares favorably with that of Adamu et al., [24] whose proximate composition revealed variations between unfermented and fermented seeds of Bambara nut with lipid having a value of 1.67 and 13.33%; protein value 6.53 and 11.44%; then carbohydrate was 77.8 and 49.46% respectively. In this study, the lipid level was 7.14%, crude protein level was 21.92% and carbohydrate was 64.9%. This agrees with the fact that fermentation can enhance the nutritional value of a feedstuff. According to Enyidi and Etim [25], Fermentation of the plant ingredients can reduce the ANF and improve feed utilization and growth rate of fish.

3.3 Effect of Substitution of Fermented Bambara Groundnut Meal on Growth Performance, Feed Utilization and Survival of *C. gariepinus* Fingerlings

Table 4 shows the growth response and nutrient utilization of *C. gariepinus* fed diets substituted with varying levels of fermented Bambara Groundnut Meal (FBGM) for 84 days. The highest daily weight gain was recorded in diet FBGM75 while the lowest was seen in the control diet (FBGM00) and FBGM25. The specific growth rate also followed the same pattern. Result of the analysis shows that there was no significant difference (p>.05) among the final mean weights of fish in the different treatments. Values for feed conversion ratio were not significantly different (*P>.05*) in all the treatments though slightly higher in diet FBGM75 and lower the control diet (FBGM00). The highest protein efficiency ratio was achieved in FBGM75 while the lowest was achieved in FBGM00 though they were statistically similar. However, the rates of fish survival in all the experimental treatments were grossly similar. The lack of significant

Table 3. Physico-chemical parameters of tank water during the 84 days culture period

Parameter	FBGM00	FBGM25	FBGM50	FBGM75	FBGM100
Dissolved oxygen (mg l^{-1})	5.93±0.05	5.72±0.49	5.91±0.47	5.72±0.49	5.72±0.49
Morning temperature (^oC)	25.94±0.25	25.70±0.30	25.77±0.34	25.86±0.31	25.84±0.34
pH	7.14±0.11	7.10±0.12	7.09±0.89	7.09±0.81	7.10±0.89
Ammonia (mg l^{-1})	0.04±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01

Table 4. Growth performance and feed utilization of *C. gariepinus* fed different experimental diets

Parameter	Experimental diets (%)				
	FBGM00	**FBGM25**	**FBGM50**	**FBGM75**	**FBGM100**
Growth performance					
Initial mean weight	$49.33±0.01^a$	$50.33±0.33^a$	$50.00±0.12^a$	$49.33.00±0.12^a$	$49.00±0.02^a$
Final mean weight	$68.29±8.49^a$	$69.86±8.73^a$	$70.86±11.13^a$	$85.29±14.46^a$	$75.17±16.20^a$
DWG (g $fish^{-1}$)	$0.23±0.01^a$	$0.23±0.03^a$	$0.25±0.02^a$	$0.43±0.1^a$	$0.31±0.02^a$
Specific Growth Rate	$0.38±0.12^a$	$0.39±0.01^a$	$0.40±0.02^a$	$0.65±0.02^a$	$0.51±0.01^a$
Feed utilization					
Feed	$0.31±0.14^a$	$0.36±0.01^a$	$0.31±0.03^a$	$0.65±0.0311^a$	$0.54±0.03^a$

conversion ratio					
Protein efficiency ratio	1.01±0.0215[a]	1.15±0.261[c]	1.24±0.282[b]	1.45±0.261[c]	1.36±0.261[c]
Survival rate	70.0±1.41[a]	78.6±1.01[a]	71.4±1.35[a]	87.1±0.61[a]	82.9±0.81[a]

[a] Means in the same row with common letter are not different at P>0.05
DWG=Daily weight gain

differences between mean final weight, DWG, FCR, SGR and survival rate of catfish fed experimental diets is indicative of cost saving and benefits of FBGM.

Many studies however, attempted to replace fishmeal with Bambara Groundnut and found out that fish fed the experimental diets showed increase in weight without any sign of nutritional deficiency. Fish fed Bambara groundnut meal (BGM) diets, at various level up to 50% replacement recorded appreciable weight gain, percentage weight gain and specific growth rate, comparable to the control diet [26]. Oso et al. [27] also reported that replacement of fishmeal with BGM up to a level of 75% was satisfactorily acceptable to the fish just like the fish meal and could replace fish meal protein in the diet of *C. gariepinus*, without adversely affecting growth and feed utilization. Enyidi and Mgbenka [28] also reported that Bambara nut waste meal can supplement up to 59% of fishmeal in the diets of the larvae of *C. gariepinus*. This shows that Bambara groundnut (BGN) contains necessary growth factors required by *C. gariepinus*. The fish showed good appetite to all the treatment diets as attested to by the increase in body weight. The observation shows that the juveniles were able to utilize Bambara nut diet efficiently like conventional fish meal. This might also be due to good digestibility of the diet.

Generally, the fish showed good appetite to all the treatment diets, attested to by the increase in body weight (Fig. 1). The growth trend shows that there was similar growth pattern until the 4th sampling date when there was general mortality. However, growth resumed during the 5th sampling.

Replacement of soybean meal/cake with other ingredients have yielded varying results in fish. For instance, Adeniji et al., [29] studied the potentials of oven dried *Amaranthus spinosus* leaf meal as partial replacement for soybean cake in the diet of Nile Tilapia, revealed no significant difference (*P*>.*05*) in feed and protein intake. Fish fed on *Amaranthus spinosus* leaf

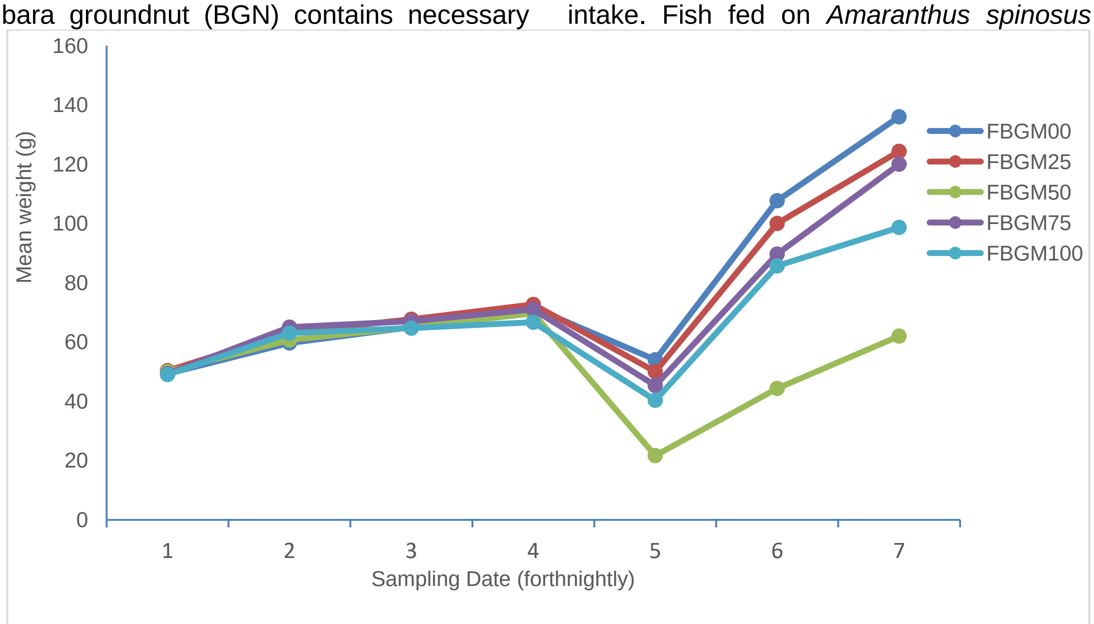

Fig. 1. Growth trend of *Clarias gariepinus* fingerlings during the 84 days culture period.

meal diets had significant *(P<.05)* higher survival percentage, while that on soybean cake meal (control diet) recorded significant (*P*<.*05*) better weight gain, average daily rate of growth, efficient feed and protein utilization as well as average final weight. Abdalbast et al., [30] studied the feasibility of carob seed germ meal (CSGM as a soybean meal (SBM) replacement in the diet of red tilapia hybrid. The survival and growth of red tilapia were unaffected by the dietary CSGM inclusion up to 30%. Growth and feeding efficiencies were significantly reduced at 40% CSGM inclusion. In conclusion, a dietary inclusion of only up to 20% untreated CSGM was recommended for red hybrid tilapia. Solomon et al., [31] conducted the feeding to investigate the effect of replacing about 40% soybean meal (at 58.8% inclusion) with *C*. *ensiformis* in the diet of *C. gariepinus*. The result obtained after 56 days revealed that fingerlings could tolerate up to 20% replacement without significant effect on growth and nutrient utilization. Beyond this, growth was significantly reduced. Survivals of the fish also follow a similar trend as stated above. It was concluded that dietary inclusion of raw *C*. *ensiform* meal should not be beyond 11% (or 20% replacement for soybeans meal included at 58.8% in the diet of *C*. *gariepinus*. Solomon, et al., [32] also tested the performance of *C. gariepinus* fed dried brewer's yeast slurry meal (DBYM) based diets. Fingerlings of *C. gariepinus* were five diets with increasing substitution of soybean meal with 25%, 50%, 75%, and 100% of dried brewer's yeast and a control without dried brewer's yeast (0% substitution) for 8 weeks. Growth and utilization parameters such as weight gain, feed conversion ratio, protein efficiency ratio, and specific growth rate differed significantly. It was concluded that, the optimal range of inclusion and substitution of soybean meal with DBYM in *C. gariepinus* feed is between 1% and 14% of dry matter. These findings and many others show that other ingredients can replace soybean meal in the practical diet of *C. gariepinus* but not 100% replacement as compared to this present finding with fermented Bambara Groundnut meal.

4. CONCLUSION

The price of soybean which is a supplementary protein source in fish diet is soaring, hence the study on FBGM. This study has demonstrated that fermentation can increase the nutrient composition and reduces the anti-nutritional value of BG. Fermented Bambara Groundnut Meal has been demonstrated to to have the potentials of replacing soybean meal to a level of up to 100%, in the diet of *C. gariepinus* fingerlings without adversely affecting growth and feed utilization. However, diet four (4) with 75 percent replacement gave the optimum Daily gain, specific growth rate and feed conversion ratio. This convey the meaning though not significantly different 75 percent replacement level seems to be the most sustainable. Bambara groundnut seed has been shown to make a balanced food as it contains sufficient quantities of nutrients (carbohydrate, protein and fats) necessary for fish growth and therefore recommended for use as a soybean total replacer in fish culture. More researches are needed on the adults or grow out stages to unveil its effects on the grow out stage and brooder and different processing methods employed and compared to the best processing method that will be mild enough to increase the nutrient content while removing the phytochemicals.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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