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Groundnut shell meal as a partial replacement for maize in diets of *Clarias gariepinus* juveniles

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Abstract The effect of varying inclusion levels of groundnut (*Arachis hypogaea*) shell meal on the performance of *Clarias gariepinus* juvenile and feed floatation was investigated. A total of 2000 juveniles of *C. gariepinus* with uniform measurements (mean weight and length 8.24 g and 6.7 cm) respectively, were acclimatized by immersion into a 2-ppm potassium permanganate solution for 20 mins and randomly sorted in quadruplicate (100 fish/tank measuring 1.5 m X 1 m X 0.3 m) and fed the experimental feed based on percentage body weight. At the end of 56 days of study, changes in the growth rate and haematology were ascertained, while the duration of floatation was taken regularly. Significant differences (P<0.05) were observed among the growth parameters. Although the best was observed in groundnut shell meal (GMS) at 4%, body weight gained feed conversion ratio and survival rate (37.14g, 1.61, and 96% respectively). Blood profile revealed optimum performance at GSM at 8% (PCV; 39.07, HB; 14.06 and WBC; 6511.3), while RBC was highest in GSM at 6% (7.411). Floatation duration also favored GSM 6% (160 seconds). Conclusively, a groundnut shell meal inclusion level of 4% is recommended to improve the performance of *C. gariepinus* and floatation of locally formulated fish feed.

Keywords: *Clarias gariepinus,* Floatation, Groundnut shell, Haematology, Performance

Introduction

Fish farming involves raising fish in tanks or enclosures (József *et al.*, 2019). However, the greatest challenges in aquaculture are the high cost of feed ingredients which makes the feed industries and farmers compromise quality for availability and affordability (FAO, 2008; Ekelemu and Irabor, 2013; Nwachi and Irabor, 2015; Jamabo and Dasimeokuma, 2020; Irabor *et al.*, 2021a). Fish feed manufacturing is an essential consideration in both subsistence and large-scale fish farming since it affects both growth efficacy

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and wastage (Tsevis, and Azzaydi, 2000; Little *et al.*, 2016; Irabor *et al.*, 2022). Also, substances in feed forms consumed by fish have been reported to significantly influence the blood profile, thereby the well-being of fish (Adeparusi and Ajayi, 2004; George *et al*., 2007; Yue and Zhou, 2008; Akintayo *et al*., 2008; Irabor *et al.*, 2016; Irabor *et al.*, 2021b).

Extruded fish diets have grown in popularity in the last decade (Giovanni *et al.,* 2019). Extruded feed is more stable in water and has greater floating qualities. digesting ease, growth, low water absorption potential, and zero waste of basic materials (Amalraaj, 2010; Avramescu *et al.*, 2020) and greater energy content compared to locally formulated non-extruded feeds (Johnson and Wandsvick, 1991; Weththasinghe *et al.*, 2020). Whereas non-extruded feeds pollute the environment and cause poor growth efficiency due to feeding waste which corresponds to a high feed conversion ratio (Johnson and Wandsvick, 1991; Juan Baztan *et al.*, 2018, Irabor *et al.*, 2022).

Animal nutritionists are faced with the production of locally formulated aqua feeds that are readily available, and nutritionally adequate at a reduced cost (Mahmoud and Shunsuke, 2019; Geetanjali *et al.*, 2020; Irabor *et al.*, 2021b). The search for an alternative cheaper aqua feed had led to the use of groundnut shells as possible floating material.

Groundnut (*Arachis hypogaea*) as a major agro-product is predominantly grown in all parts of Nigeria (Bappah *et al.,* 2019). The estimated production for the year 2019 hits about 3 million metric tonnes and this upgrades Nigeria to the major producer in entire West Africa and third in the world ranking (Kebede, 2020). The presence of groundnut nearly all over Nigeria makes its waste (shell) readily available in massive quantities, especially in the Northern and Southern parts of the country (Sadaa *et al.*, 2013; Shittu *et al.,* 2018).

Several studies have been done on the nutritive compositions of groundnut shells which had better potential compared to some other natural fibres (Pham *et al*., 2019). These fibre qualities of groundnut shells are categorized into significant levels of roughage, hemicellulose and some carbohydrates (37%, 18.7%, and about 2.5%), respectively and the carbohydrate content aids its fermentation efficacy and improves produce (Jaishankar *et al.,* 2014; Irabor *et al.,* 2021a).

The use of locally formulated feed has been harnessed by most farmers due to the expensive nature of commercially pellet extruded feed. Nevertheless, the issue of feeds sinking within seconds of introduction into the culture medium remains challenging causing severe pollution, stunted growth, diseases, and capital loss. Therefore, to achieve a sustainable aquaculture sector where production processes are not compromised to the detriment of the fishes, the need to harness agricultural waste to boost the floatation quality of locally formulated feed is paramount. The study determined the effect of groundnut shell meal (GSM) on the performance of *C. gariepinus* and the floatation potential of locally formulated feeds.

Materials and methods

Description of the study area

This research was carried out in Fishery Laboratory, Delta State University, Asaba Campus located in Oshimili South Local Government Area of Delta State, Nigeria. It lies between longitudes 6^0E and 8^0E and between latitudes 4^0N and 10^0N.

Procurement of feed ingredients and formulation of diets

Feed ingredients used for the feed formulation were procured as follows: - fish meal, soyabean and groundnut cake (protein sources), maize (energy source), vitamin premix, lysine, methionine, and salt (minerals), and starch (binder). All ingredients were procured from the popular Abraka market in Asaba Delta State and were milled into a fine mixture. Groundnut shells were identified and collected from groundnut sever within the area, then milled into powder form and preserved in an airtight container. The groundnut shell granule was added to the trial diet at levels 0, 2, 4, 6 and 8%, milled with other ingredients, mixed and pelleted to size (2mm) with a multiple phase model pelletizer. Fish samples were weighed biweekly using a sensitive scale to regulate feed mass in every tank for body weight changes.

The feed constituents utilized in diet formulation at a crude protein level of 40% (Table 1). The feed trial was carried out for fifty-six (56) days (June - August 2021).

Experimental fish and set up

The research was carried out using two thousand juveniles of *C. gariepinus* which were purchased from best fish farm, a notable farm in Asaba. The morphometric measurements were taken (weight with triple bean balance and lengths with calibrated meter rule) in the laboratory The fish had an average initial weight and length of 8.24 g and 6.7 cm, respectively. Before acclimatization, fish were immersed in a 2-ppm potassium permanganate solution for 20 mins to prevent infection due to stress during transportation. Fish were stocked in a 6 m X 4 m X 2.4 m tank with well-oxygenated water and subjected twice a day (7:30 am and 5:30 pm) feeding schedule using Coppens feed (2 mm) for two weeks before commencement of the experiment.

Table 1. Composition of the different feed ingredients

Ingredient	0% GSM	20% GSM	40% GSM	60% GSM	80% GSM
Fish meal	35.0	35.0	35.0	35.0	35.0
Soya beans	23.0	23.0	23.0	23.0	23.0
Groundnut cake	9.00	9.00	9.00	9.00	9.00
Maize	29.0	23.2	17.4	11.6	5.80
Groundnut Shell	0.00	5.80	11.6	17.4	23.2
Vitamin premix	1.50	1.50	1.50	1.50	1.50
Bone meal	1.50	1.50	1.50	1.50	1.50
Lysin	0.20	0.20	0.20	0.20	0.20
Methionine	0.10	0.10	0.10	0.10	0.10
Salt	0.20	0.20	0.20	0.20	0.20
Binder	0.50	0.50	0.50	0.50	0.50

Experimental design

The acclimated fish were separated into quadruplicates of twenty-five juveniles per tank measuring 1.5 m X 1 m X 0.3 m with an adequate water holding capacity. Each labelled 0% GSM_{1-4} (control), 2% GSM_{1-4}, 4% GSM_{1-4}, 6% GSM_{1-4}, and 8% GSM_{1-4} in accordance with the diets to be administered. Tanks 2% GSM_{1-4}, 4% GSM_{1-4}, 6% GSM_{1-4}, and 8% GSM_{1-4} were fed to test diets, while GSM $0\%_{1-4}$ was fed diet with no inclusion of the test ingredient.

Feeding routine

The locally formulated feed constituting the different proportions of groundnut shell concentration was administered to fish samples. The feeding was performed adequately (5% body weight) twice daily between 7:30 am and 5:30 pm. To avoid contamination, adequate water replenishing (one-third) on weekly basis with fresh water was ensured.

Measurement of growth parameters

The body weight of the samples was measured at the end of the trial (56days) to evaluate the growth parameters. The sampling was done by draining entirely the tanks through the outlets, after which the fish were gently harvested using a scoop net. The weight and length of the fish were measured appropriately with the aid of a sensitive gram scale and calibrated meter rule respectively. Fish were promptly returned to their respective tanks after each sampling.

Determination of growth indices and food utilization were all determined using methods as prescribed by Nwachi and Irabor (2022).

Weight gain = final weight of sampled fish – the initial weight of sampled fish.

Mortality was calculated as $M = (N_0 – N_1)$, Where; No = number of fish at the start of the experiment and N_1 = number at the end of the experiment.

Specific growth rate (SGR) in percentage body weight per day was calculated as

$$SGR = 100 \frac{(LnW_2 – LnW_1)}{t}$$

Where; W_2 = Final weight, W_1 = Initial weight, t = Period of experiment in days, and Ln = Base of the natural logarithm.

Protein efficiency ratio (PER) was calculated as PER = Weight gain (g)/Feed fed (g). Feed conversion ratio (FCR) was calculated as Food eaten (g)/Weight gain (g).

Haematological analysis

The effect of the additive (groundnut Shell Meal) on the blood parameters of the sampled fish species was determined at the end of the trial. The following parameters; packed cell volume (PCV), haemoglobin (HB), Red Blood Cell (RBC), and White Blood Cell (WBC) were determined using Kone *et al.* (2019) methods. PVC **is** used as a microhaematocrit centrifuge by the wintrobe's and Westergreen's method with commercially available heparinized capillary tubes of 75mm. A blood sample was drawn by capillary action into microhaematocrit tubes then synthetic sealant was used to seal one end. Sealed tubes were centrifuged in a microhaematocrit centrifuge at 1200g for 10 minutes and placed in a chart where final reading for PCV using a Microhaematocrit reader was obtained.

Hb = T x C x DF/A x 100 (Kone *et al.,* 2019).

Where: T = Test Absorbance, A = Standard Absorbance, C = Concentration of cynamethaemoglobin, DF = Dilution Factor.

RBC **=** N x DF x 10^2/A x A (Kone *et al.,* 2019).

Where: N = Number of Cells Counted, DF = Dilution Factor, A = Area Chamber counted, D = Depth of Chamber

WBC = N x DF x 10^2/A x A (Kone *et al.,* 2019).

Where: N = Number of Cells Counted, DF = Dilution Factor, A = Area Chamber counted, D = Depth of Chamber.

Floatation test

Five transparent experimental glass tanks measuring 2 m x 2 m x 1.6m were labeled as follows 0%, 2% 4% 6%, and 8% GSM), and used to evaluate the feed floatation capability of the formulated diets. A total of 50 pellets from each diet were introduced into the tanks based on the labels and a stopped watch was used to record the time taken for all 50 pellets to sink. Percentage pellets afloat were calculated as % Pellet afloat = final no. of pellets afloat/initial no. of pellets afloat x 100 (Solomon *et al*., 2011).

Statistical analysis

All data were statistically analyzed using the ANOVA statistical method from SPSS version 23, with Duncan's multiple range tests to separate the mean. The difference between the mean was compared for the significance ($P \leq 0.05$).

Results

Proximate composition of groundnut shell

The result of proximate analysis carried out on groundnut shells revealed that moisture, carbohydrate, and crude protein were 5.69, 25.69, and 5.29, while ether extract, crude fibre, crude ash, and nitrogen-free extracts also recorded 0.53, 34.77, 4.6 and 23.43 respectively (Table 2).

Antinutritional components of groundnut shell

The antinutritional constituents of GSM recorded that phytate and oxalate are significantly high 361.8 and 219 while trypsin inhibitor and cyanogenic glycoside were 26 and 1.62 respectively (Table 3).

Proximate composition of the test diets

The proximate analysis of the dietary treatments revealed that crude fibre was the only parameter to show a progressive decrease as inclusion levels appreciated across the treatments. The crude fibre values ranged from 5.87 – 8.92 where the least and highest are 0% GSM and 8% GSM. The crude protein values ranged from 38.95 – 40.38 where the least and highest were 8% GSM and 0% GSM. A similar trend as recorded in CP was also observed for crude lipids, moisture, and total ash respectively (Table 4).

Table 2. Proximate composition of groundnut shell

Parameters	Percentage (%)
Moisture	5.69
Carbohydrate	25.69
Crude protein (CP)	5.29
Ether Extract (EE)	0.53
Crude Fibre (CF)	34.77
Crude Ash	4.6
Nitrogen Free Extract (NFE)	23.43

Table 3. Antinutritional properties of groundnut shell

Parameters	**mg/100 g**
Oxalate	219
Phytate	361.8
Cyanogenic glycoside	1.62
Trypsin inhibitor (TUI/mg)	26

Table 4. Proximate composition of feeds with varying inclusion levels of GSM

Parameters	**0% GSM**	**2% GSM**	**4% GSM**	**6% GSM**	**8% GSM**
Crude protein (%)	40.38	39.52	39.36	39.08	38.95
Crude lipid (%)	5.09	5.02	4.86	4.64	4.48
Crude fibre (%)	3.87	5.96	6.99	7.83	9.92
Moisture content (%)	9.87	8.96	8.63	7.98	7.58
Total ash (%)	4.93	4.56	4.29	3.92	3.57
NFE	35.41	34.95	34.35	33.84	33.59

Performance and nutrient exploitation of C. gariepinus fed with various levels of groundnut shell meal

The performance and nutrient using *C. gariepinus* subjected to feed with various levels of groundnut shell meal during the period of study is shown in Table 5. The mean value for feed intake was highest in 0% GSM, while the least value was observed in 8% GSM. 4% GSM had the highest final weight (49.37g), body weight gain (37.14g), protein efficiency ratio (0.62), specific growth rate (6.87%), and survival rate (96%), while 8% GSM had the least values (27.18g, 14.94g, 0.42, 5.73% and 63%) respectively. GSM 4% had the least mean value for feed conversion ratio (1.61), while 8% GSM had the highest mean value (2.36).

Haematological profile of C. gariepinus juveniles fed experimental feed

The blood characteristics of experimental fish were analyzed and mean separation is expressed in differences in Table 6. It revealed that a significant difference (P<0.05) was shown across treatments. Nevertheless, PCV, HB, and WBC were highest in 8% GSM with mean values (39.07, 14.06, and 6511.3, respectively), while 0% GSM was least with mean values (32.09, 11.02, and 4706, respectively). The observed RBC means value (7.411) was optimum in 6% GSM, while 8% GSM was least (4.692).

Table 5. Performance and nutrient exploitation of *C. gariepinus* fed with various levels of GSM on 56days

Parameters	0% GSM	2% GSM	4% GSM	6% GSM	8% GSM
IW (g)	12.24±0.4	12.24±0.83	12.23±0.2	12.25±0.1	12.24±0.9
FW (g)	46.42±0.4^{b}	46.31±0.6^{ab}	49.37±0.21^{a}	33.17±1.0^{a}	27.18±0.3^{ab}
BWG (g)	34.18±0.4^{b}	34.07±0.6^{ab}	37.14±0.70^{a}	20.92±1.1^{a}	14.94±0.1^{ab}
FI/56Day/Fish (g)	63.28±0.4	61.60±0.6	59.92±0.2	45.36±0.21	35.28±0.21
FI/Day/Fish (g)	1.13±0.11^{a}	1.10±1.39^{b}	1.07±1.43^{b}	0.81±1.12^{c}	0.63±1.06^{d}
FCR	1.85±0.16	1.81±0.25	1.61±0.58	2.17±0.9	2.36±0.23
PER	0.54±0.91	0.55±0.62	0.62±0.3	0.46±0.2	0.42±0.16
SGR (%)	6.75±0.2	6.75±0.1	6.87±0.35	6.11±0.30	5.73±0.65
SR (%)	94	92	96	79	63

GSM: groundnut shell meal; IW: initial weight; FW: final weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio; SGR: specific growth rate; SR: survival rate.

Table 6. Haematological profile of *C. gariepinus* juveniles fed experimental feed

Parameters	0% GSM	2% GSM	4% GSM	6% GSM	8% GSM	SEM	Sig.
PCV	32.09^{c}	37.50^{b}	38.53^{ab}	38.45^{ab}	39.07^{a}	1.064	0.001
HB	11.02^{c}	13.14^{b}	14.52^{ab}	13.89^{ab}	14.06^{a}	0.367	0.001
WBC	4706^{e}	6313.07^{b}	6241^{c}	5708.14^{d}	6511.3^{a}	272.03	0.000
RBC	4.957^{c}	4.769^{c}	6.102^{b}	5.411^{a}	4.692^{c}	1.023	0.051

a-d; mean within the row with different superscripts differs significantly (p<0.05).
Legends: PCV: Packed Cell Volume, HB: Haemoglobin, WBC**:** White Blood Cell, RBC**:** Red Blood Cell

Duration of floating

The floatation characteristics of all diets (0% GSM, 2% GSM, 4% GSM, 6% GSM, and 8% GSM), when subjected to floating trials, are shown in Table 7. Amongst the experimental diets, there was a progressive increase in the percentage of pellets afloat after 60 seconds as the inclusion level of GSM increased though a sudden reduction was observed in 8% GSM. At the 6% GSM inclusion level, the highest value (54%) of pellet float was recorded, while the least value (8%) was observed at the 2% GSM inclusion level.

Water parameter

The recorded parameters within the study period (Table 8). GSM 4% had the lowest values for pH and temperature (6.12 and 28.5 °C respectively), while the highest was observed at GSM 0% (6.8 and 32.1 °C respectively). Dissolved oxygen was higher in GSM at 4% (6.35) than the others, while GSM at 0% had the smallest (5.93).

Table 7. Evaluation of floatation characteristics of formulated diets

Parameters	0% GSM	2% GSM	4% GSM	6% GSM	8% GSM
Initial no. of pellet	50	50	50	50	50
Final no. of pellet at 60 seconds	50	4	18	27	12
% pellet afloat	100	8	36	54	24

Table 8. Summary of pH, Dissolved oxygen, and temperature across the tank

Parameter	0%GSM	2%GSM	4%GSM	6% GSM	8%GSM
pH	6.8	6.39	6.12	6.57	6.61
Dissolve Oxygen (mg/L)	5.93	6.11	6.35	6.18	6.02
Temperature	32.1	30.4	28.5	29.6	29.9

Discussion

Results obtained from the study showed that the inclusion of groundnut shell meal from 2% in fish feed can aid buoyancy and improve growth. This result supported similar works carried out in melon shell (Obi *et al*., 2013), baobab leaf meal (Eze and Eyo, 2018), cassava flour, and brewer yeast (Onada and Ogunola, 2019) where growth rate improved significantly with their inclusions in the feed of fish. The observed weight gain across sample groups fed varying diets of groundnut shell meal is also buttressed by the findings of Oduntan *et al.* (2019), where increased weight gain was reported for *C. gariepinus* fed different levels of plantain peel additives. A similar trait was observed by Agbabiaka *et al*. (2013) when maize was substituted at various levels for plantain peel. Oladipupo and Salami (2020) reported high growth performance when watermelon peel was fed at various inclusion levels to *C. gariepinus.* The same growth pattern was observed in *C. gariepinus* fed varying concentrations of walnut shell (Ayoola and Omoile, 2019).

Feed utilization (feed intake, feed conversion ratio, protein efficiency ratio, and specific growth rate) demonstrated a significant difference ($P<0.05$) between diets, particularly when groundnut shell meal inclusion levels were raised. Eze *et al.* (2019) reported increased values for feed utilization as the percentage of moringa leaves and methionine increased in the feed fed to *C. gariepinus* fingerlings. However, there was a slight reduction from inclusion level 6% which possibly resulted from reduced palatability of feed due to increased fiber content. Nevertheless, the sinking nature of locally formulated feed gives rise to loss in water-soluble nutrients (vitamins and amino acids) and water pollution from the uneaten food. This is in harmony with Oduntan *et al.* (2019) and Oladipupo and Salami (2020) who respectively reported reduced feed utilization in *C. gariepinus* fed increased inclusion levels of plantain peel and watermelon peel. The study's finding contradicted that of Adegbesan *et al.* (2019), who reported enhanced feed consumption in C. gariepinus fed increased ginger root powder meal.

The survival rate was reported to be significantly higher at a 4% inclusion level and showed the feed was readily digestible and absorbable by fish samples. A similar finding was recorded by Oladipupo and Salami (2020) in their study, where diets containing watermelon peels at varying inclusion levels were fed to *C. gariepinus* fingerlings. In another study, Adaniyi *et al.* (2018) reported optimum survival rate in *C. gariepinus* fed diets with Tamarind pulp and leaf meal as additives.

The health status of fish species is significantly expressed via blood profile (Muttappa *et al.,* 2015; Irabor *et al.,* 2021b). The variations in the blood profile of the sampled fish revealed the immune-stimulatory and antibiotic potential of groundnut shells, especially with the proportionate increase in feed additive (GSM) with WBC count. In line with this, Omeje *et al.* (2019) also reported increasing WBC count of *Oreochromis mossambicus* fed inclusion levels of pawpaw seed meal. Similarly, Asian sea bass and rainbow trout had high WBC when fed increased levels of maize cob (Borkovic *et al.,* 2004; Rashidian *et al.*, 2020).

Generally, the triggered HB within fish-fed treated diets suggested a high influx of oxygen which is healthy for fish. Although it disagreed with the report by Omeje *et al.* (2019) who reported that low HB in *O. mossambicus* fed pawpaw seed meal. The trend observed for PCV in the study was in contrast with Kakwi and Olusegun (2020) who stated a significant decline of PCV in *Cyprinus carpio* as the inclusion level of *Mucuna pruriens* seed increased. Although at GSM 6% there was a significantly decreased which could be increased physiological stress on fish (Kumar *et al.,* 2019). The differences observed in RBC across treatments agree with the observations of Millet *et al.,* (2005), Khan *et al.* (2006), and Rezaei *et al.* (2009) who stated that on *Oreochromis niloticus* fed varied inclusion levels of maize cob.

The floatation potential of the feeds revealed significant differences ($P<0.05$) across experimental diets. The best floatation quality was expressed in

GSM 6%. It revealed a progressive relationship between inclusion level and floatation performance. The same trend was recorded by Onada and Ogunola (2019) when the inclusion of cassava flour and brewer yeast increased, and floatability increased. Also, Eze and Eyo (2018) reported an increased baobab leaf meal concentrate in the fish diet led to higher floating time. Although at GSM 8%, a drop-in floating time was observed. Generally, the result did not correspond with the report of Momoh *et al.* (2016) who had decreased floatation period, as the inclusion level of yeast was reduced in test diets. The optimum level of inclusion of groundnut shell meal (GSM) in the diet of fish feed was 4%, beyond which the immune system of fish is affected negatively. Considering growth performance and feed floatation quality, the best was achieved at a 4% inclusion level.

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