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RESEARCH PAPER

Potentials of Bambara Groundnut (*Vigna subterranean*) Protein Concentrate as a Viable Option to Fishmeal in the Diets of African Catfish (*Clarias gariepinus*) Juveniles

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Abstract

Given the sustainability challenges associated with the usage of fishmeal (FM) in aquafeed, bambara groundnut (BG) protein concentrate (BGPC) was investigated as FM substitute in the diets of African catfish, *Clarias gariepinus*. Protein from BG was precipitated at its iso-electric point and the product was used to replace FM at 0 (Control, $BGPC_0$), 33 ($BGPC_{33}$), 66 ($BGPC_{66}$) and 100% ($BGPC_{100}$). The diets, which contained similar crude protein (40%) and energy (19.5kJ/g), were fed to three groups of juveniles *C. gariepinus* (mean weight, 10.07±0.02g) for 45 days. The growth performance of the group fed $BGPC_{66}$ was similar ($P>0.05$) to other groups. Feed utilization parameters were identical ($P>0.05$) across groups. Lymphocytes increased significantly ($P<0.05$) in the BGPC-fed groups compared to the control. The $BGPC_{100}$-fed group had higher ($P<0.05$) total protein and globulin than the control group. Hepatic and serum aspartic (AST) and alanine (ALT) transaminases, serum glucose and respiratory burst activity were not significantly ($P>0.05$) affected by dietary BGPC. Intestinal amylase activity was significantly ($P<0.05$) lowered by supplementing 66 and 100% BGPC in *C. gariepinus* diet. This study revealed that up to 66% of FM can be substituted by BGPC in feed for *C. gariepinus* without eliciting significant growth, nutrient metabolism and health compromise.

Introduction

Aquaculture plays a significant role in achieving the Sustainable Development Goals; especially those relating to poverty alleviation, hunger eradication, and creation of gainful employment (FAO, 2017; Garlock et al., 2022). The importance of aquaculture has become more evident given the increasing population, particularly in developing nations, and the stagnation of production from capture fisheries over the last two decades (FAO, 2022). Feed is a major input in fish farming, representing as much as 75% of operating costs (El-Sayed et al., 2015). Since the protein requirements of most aquaculture species at different stages of development are high, the ingredients used to compound most aquafeed are those equally high in crude protein with balanced amino acids to satisfy the requirements of different fish species. In this regard, fishmeal (FM) represents the gold standard due to its highly digestible crude protein and palatability advantage (Turchini et al., 2019). However, the focus of research in aquaculture nutrition over the years has been on strategies to reduce or eliminate the use of FM in aquafeed because of various associated challenges. Firstly, many stakeholders in the aquaculture industry have questioned the rationale of harvesting fish to feed fish, for which reason some have labeled aquaculture as a net consumer rather than a fish producer (Huntington

and Hasan, 2009). Besides, the use of high quantities of FM in aquaculture diets is no longer sustainable. This is because the fisheries from which FM is derived have been over-exploited, leading to a decline in its supply (FAO, 2022). Such supply crisis has been aggravated by El Nino Southern Oscillation, a global climate phenomenon that prevents the upwelling of nutrients to support the fisheries from which FM are derived (Bertrand et al., 2020). The FM supply crisis is even direr in developing countries where most utilized FM are imported and consequently subjected to the vagaries of foreign exchange. Accordingly, the inadequate supply and scarcity of FM, which is a major ingredient in aquafeed have triggered a chain of reactions such as increased production cost, increased cost of aquaculture products, and ultimately crippling the achievement of the sustainable development goals. Further, FM supplies more nutrients such as nitrogen and phosphorus than is required in fish diets; the excess is usually released into the aquatic ecosystem where it poses a risk of eutrophication (Hernández et al., 2016). Meanwhile, many investigators alluded to the immense opportunities for aquaculture growth, if feed could be provided at a reduced price (Subasinghe et al., 2009; Garlock et al., 2020). Given the foregoing, there is ongoing advocacy for a shift from heavy reliance on FM towards more sustainable alternatives, especially those of plant origin that are abundant, renewable, and cost-effective. Bambara groundnut (BG), a drought-tolerant, inexpensive, and underutilized legume could be a viable option to FM in aquafeed. It can grow in marginal soil and other harsh environmental conditions (Mateva et al., 2023). Among food legumes, it comes only behind cowpea and groundnut in order of importance (Hillocks et al., 2012). It has little relevance in the human food system because of its associated hard-to-cook factor which results in increased energy expenditure and lower nutritional quality relative to other legumes; for which reason it has been classified as underutilized and is generally regarded as a lost crop (Mubaiwa et al., 2017; Mateva et al., 2023). Although Nigeria is the leading producer of BG, it is also generally cultivated in most tropical and sub-tropical countries and some parts of Latin America and Asia (Mubaiwa et al., 2017). BG is rich in carbohydrates and contains widely varied lipids (1.4-9.7%) and crude protein (14-24%); depending on the cultivar, genetic makeup and growing condition (Tan et al., 2020; Maphosa et al., 2022). It is rich in lysine, leucine, phenylalanine and glutamic acid among many other significant amino acids; but it is limiting in methionine and tryptophan (Adebowale et al., 2011; Bamishaye et al., 2011). In addition to some key amino acid limitations, the crude protein content reported for BG (14-24%) is incomparable to that of FM (which could sometimes contain as high as 72% crude protein) and as such cannot effectively replace it. Attempt to incorporate BG alongside sesame seed meal in *C. gariepinus* diets by Enyidi et al. (2014) necessitated a high and impractical dietary level (53%) of FM. Adewunmi and Odeyemi (2018) could not achieve similar crude protein composition in their experimental diets when they attempted to replace FM with BG in the diets of *C. gariepinus*. Katya et al. (2017), however, reported that more than 50% of wheat flour could be substituted with BG in the diets of Nile tilapia, *Oreochromis niloticus*; thus, suggesting that BG can be utilized as a carbohydrate source in fish diets. Another limitation of BG is its associated antinutritional factors such as condensed tannins, phytic acid, amylase and trypsin inhibitors that could interfere with nutrient digestibility and utilization (Mune et al., 2011). Thus, to make BG competitive as an option to FM, there is a need for one or a combination of processing interventions that can address these limitations. The processing option explored under the current investigation was protein extraction. Such processing has been reported to improve the nutritional value of BG, especially the crude protein and amino acid profile, while reducing its antinutrients (Eltayeb et al., 2011; Adewunmi et al., 2022). To our knowledge, there is no report on the use of BGPC in fish diets. Hence, this study examined the implications of dietary substitution of FM with BGPC on growth, haemato-biochemical response, and activities of digestive and metabolic enzymes of African catfish, *C. gariepinus* juveniles.

Materials and Methods

Preparation of Bambara Groundnut Protein Concentrate

Bambara groundnut was purchased at Bodija Market, Ibadan, Oyo State, Nigeria and milled using a locally fabricated hammer mill. The iso-electric precipitation method described by Shamna et al. (2015) was used in preparing bambara groundnut protein concentrate. Briefly, cleaned BG was ground into flour and dispersed in distilled water (1:10 w/v) after which the pH of the medium was adjusted to 10 using 1 N sodium hydroxide to facilitate protein solubility. The solution was stirred for 1 hour at room temperature and thereafter centrifuged (Heraeus Megafuge 8R, Thermo Fisher Scientific, USA) at 3500 g, 4°C for 20 minutes. The supernatant was collected and its pH was adjusted to 4.0 with 1 N hydrochloric acid with continuous stirring at room temperature, in order to precipitate the protein. The precipitated protein was recovered by centrifuging at 3500 g, 4°C for 20 minutes and oven-dried at 40°C. The dry matter recovery (DMR) and protein recovery (PR) of the concentrate were calculated as previously reported in Olude et al. (2023).

$$\text{Protein recovery (\%)} = \frac{\text{Crude protein of the BGPC mass (g)}}{\text{Crude protein of the BG mass (g)}} \times 100$$

$$\text{Dry matter recovery (\%)} = \frac{\text{Weight of BGPC obtained (g)}}{\text{Initial weight of BG extracted (g)}} \times 100$$

Feed Ingredients and Preparation of Feeds

Adequate quantities of the feed ingredients required for the experiment were obtained from Funsaab Nigeria Limited, Oko-oba, Agege, Lagos, Nigeria. The major feed ingredients procured were fish meal, groundnut cake, soybean meal and maize. Minor ingredients such as butylated hydroxytoluene, choline chloride and carboxymethyl cellulose (CMC) were sourced from the Fish Nutrition Laboratory of the Department of Marine Sciences. Four isonitrogenous (40% crude protein), isolipidic (10.8% crude lipid) and iso-energetic (19.5 kJ/g gross energy) diets were formulated (Table 1) using BGPC to replace fishmeal at different levels. The control diet had no BGPC ($BGPC_0$) whereas diets $BGPC_{33}$, $BGPC_{66}$ and $BGPC_{100}$ had the FM portion of the control diet replaced with BGPC at 33, 66 and 100% levels. The major ingredients were carefully measured alongside CMC per each treatment and thoroughly mixed, after which hot water was added to make dough. The dough was wrapped with foil paper and autoclaved (Prestige Clinical Autoclave, Series 2100) at 121˚C and 15 psi (1.05 kg cm^{-2}) for 30 minutes to ensure sterility and enhance nutrient availability. After autoclaving, the vitamins/minerals premix with the remaining micro-ingredients were added with the vegetable oil to the dough and mixed thoroughly. The diets were pelletized with a manual pelletizing machine through a 2mm die. The pelletized diets were air-dried for a week and subsequently packed in properly labeled plastic bottles until use.

Experimental Site, Design and Feeding

The feeding experiment was conducted in the Department of Marine Sciences' experimental unit located at the Biological Garden of the University of Lagos in Akoka using 12 transparent plastic bowls (0.55 x 0.33 x 0.21) m^3 filled with 26 litres of fallowed tap water from the University's Water Unit. African catfish, *C. gariepinus*, juveniles were bought from Iceberg Agricultural Consult in Ikotun, Lagos, Nigeria, and maintained on a commercial diet (Skretting, 2mm) for 2 weeks before the commencement of the feeding trial to acclimatize them to the condition of the experimental unit. Following that, six juveniles of similar sizes (average weight of 10.07±0.02 g) were selected into each of the twelve rearing containers. The prepared diets were randomly assigned to three groups of fish and fed twice daily (9.00 am and 5.00 pm) at 4% biomass for 45 days. The fish were weighed (Camry EK 5055) every 15^{th} day till the completion of the experiment and new fish weights were used in adjusting feed gift. To maintain the ideal environment for rearing, the water in the container was changed every other day. Monitored pH (6.7 to 8.1), water temperature (27.3-28.0˚C) and dissolved oxygen (7.6-9.0 mg/l) were within acceptable limits for *C. gariepinus* juveniles. Three fish were sacrificed per replicate for the study of body proximate composition after the feeding trial; five numbers were earlier sacrificed and stored at -20˚C for the same purpose before the experiment commenced.

Proximate and Amino Acid Analysis

The proximate composition of feed ingredients, feed and fish carcass were determined according to AOAC (2005). Moisture was determined by drying the samples to a constant weight in a hot-air oven set at 105°C. Determination of crude protein followed the micro-Kjeldahl method in which samples were digested with concentrated H_2SO_4 using copper sulphate as a catalyst. The digest was distilled over 40% NaOH and the distillate was titrated with standard 0.1 N HCl acid. Auto-fat extraction system (HTC, Tecator, Sweden) using petroleum ether as solvent was used to determine the ether extract in the samples. The fibre content was determined by passing the sample kept in dried fritted crucibles through a Fibertec hot/hydrolysis unit and Fibertec cold extraction unit after which the samples were ashed for 3 hours at 500˚C. Total ash was calculated from the loss in ignition after burning samples

Table 1. Composition (g/kg) of Experimental Diets

Ingredients	$BGPC_0$	$BGPC_{33}$	$BGPC_{66}$	$BGPC_{100}$
Fish Meal	330	220	110	0
BGPC	0	110	220	330
GNC	180	162	128	100
SBM	180	180	180	180
Maize	199.8	217.8	251.8	279.8
Vegetable oil	60	60	60	60
CMC	10	10	10	10
Vit. / Min. Premix*	20	20	20	20
BHT	0.2	0.2	0.2	0.2
Methionine	10	10	10	10
Lysine	10	10	10	10

*Radar vitamin/mineral premix supply/100g diet – vitamin A palmitate, 1000 IU; cholecalciferol (D), 1000 IU; α-tocopherol acetate (E), 1.1mg: menadione (K), 0.02 mg; thiamine B1, 0.63mg; riboflavin (B2), 0.5mg; pantothenic acid, 1.0mg; pyridoxine (B6), 0.15mg; cyanocobalamine (B12), 0.001mg; nicotinic acid, 3.0mg; folic acid, 0.1mg; choline, 31.3mg; ascorbic acid (C), 0.1mg; ferrous sulphate, 0.05mg; copper sulphate, 0.25mg; manganese sulphate, 6.00mg; cobalt chloride, 0.5mg; zinc sulphate, 5.0mg; sodium selenite, 0.02mg. BGPC, Bambara groundnut protein concentrate; GNC, Groundnut cake; SBM, Soybean meal; CMC, Carboxymethyl cellulose.

in a muffle furnace set at 500°C for 6 hours. Nitrogen-free extract was determined by deducting the sum of other proximate components from 100. The method of Hušek (1991) was used for the analysis of amino acids. For total amino acid extraction, 100 mg of the dry sample was hydrolyzed with 5 ml of 6 M HCl at 110°C for 24 hours. The hydrolyzed extract was filtered, pH-adjusted, mixed with an internal standard, and diluted to 10 ml with Milli-Q water. The solution was filtered (0.45 µm) and dried. The dried sample (1 mg) was dissolved in water and ethanol/pyridine solution, mixed with ethyl chloroformate, and shaken with dichloromethane and NaCl for extraction. A 1 µL aliquot was injected into a GC2010 Plus system (Shimadzu, Tokyo, Japan) with a flame ionization detector. Separation was performed on a CP-Sil 19 CB column, with helium as the carrier gas, and temperature programmed from 140°C to 280°C. Amino acids were identified by retention times and quantified using L-p-chlorophenylalanine as an internal standard.

Haematology and Serum Biochemistry

After the trial, blood samples were drawn from the caudal vein of three fish per replicate and emptied into heparin-coated bottles for haematological analysis and plain bottles for biochemical analysis of the serum. The haematological study was done by following the methods described in Blaxhall and Daisely (1973). Total erythrocytes and leucocytes were counted in the Neubauer counter after diluting appropriately in citrate fluid. Haemoglobin concentration was assayed using the cyanmethaemoglobin method while haematocrit was estimated by drawing well-mixed blood into microhaematocrit tube after which it was centrifuged at 10,500 rpm for 5 mins. The leucocyte differentials (heterophils, lymphocytes, eosinophils, monocyte, basophils) were counted separately and estimated in percentages after a thin smear of the sample was made on a grease-free slide, dried, stained with Giemsa stain and observed using oil immersion objective lens. The erythrocyte indices of mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were computed using standard relationships (Meyer et al., 1992). The blood samples for serum biochemical analyses were allowed to clot after keeping them at room temperature for an hour. The serum was collected from the upper layer after centrifuging the clotted blood at 2500 g, 4°C for 15 minutes. The biuret method (Tietz, 1995) was followed to determine the total protein in the serum while bromocresol green method (Doumas et al., 1972) was used for serum albumin. Globulin was estimated as the difference between total protein and albumin. The method of Schmidt and Frankel (1963) which measured the absorbance of oxaloacetate hydrazone and pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine was used to determine aspartate aminotransferase (AST) and alanine aminotransferase (ALT), respectively. The colourimetric glucose oxidase/peroxidase (GOD/POD) method was used to determine serum glucose (Thomas, 1998).

Respiratory Burst Activity

Respiratory burst activity of the leucocyte was assayed using the nitroblue tetrazolium (NBT) test as described by Stasiak and Baumann (1996). Freshly collected 50 µl blood of the experimental fish was incubated in an ELISA plate for 1 hour to encourage adhesion of red cells after which the supernatant was removed and the plate washed with phosphate buffer solution. The plate was read (Labtech LT-4500-6F microplate reader) at 620 nm using 0.2% NBT as coloring agent after dissolving the blue formazon precipitate formed with 2N KOH and dimethyl sulfoxide.

Tissue Enzyme Analysis

The muscle, liver and intestine were removed carefully from three fish per replicate, weighed and homogenized in chilled sucrose solution (0.25 M). The homogenate was centrifuged at 5000 rpm for 10 min at 4°C and the supernatant was stored at −20°C before enzyme analysis. Intestinal protease activity was determined by the casein digestion method described by Drapeau (1974). The dinitro salicylic acid (DNS) method of Rick (1974) was used to determine intestinal amylase activity using 2% (w/v) starch solution as substrate. Muscle and liver aspartate aminotransferase (AST) and alanine aminotransferase were measured by the method of Wooten (1964). The activities of LDH and MDH in the muscle and liver were measured using the techniques used by Wroblewski and Ladue (1955) and Ochoa (1955), respectively. The total protein of each tissue homogenate for enzyme tests was calculated was assayed by the lowry method (Lowry et al., 1951).

Growth Performance and Diet Utilization

The following growth and nutrient utilization parameters were calculated as measures of the effectiveness of utilization of BGPC in the diet of *C. gariepinus* as previously captured in Olude et al. (2023).

$$\text{Mean weight gain (MWG,\%)} = \text{Final weight} - \text{Initial weight}$$

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

$$\text{Specific growth rate (SGR,\%/day)} = \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Experimental period (days)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry feed fed (g)}}{\text{Total wet weight gained (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Protein fed (g)}}$$

$$\text{Protein productive value (PPV,\%)} = \frac{\text{Final fish body protein} - \text{Initial fish body protein}}{\text{Protein fed (g)}} \times 100$$

$$\text{Hepatosomatic Index (HSI)} = \frac{\text{Liver weight (g)}}{\text{Body weight(g)}} \times 100$$

$$\text{Intestinal Somatic Index (ISI)} = \frac{\text{Weight of intestine (g)}}{\text{Body weight(g)}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{No.of fish remaining at the end of experiment}}{\text{No.of fish stocked}} \times 100$$

Data Analysis

One-way analysis of variance (ANOVA) was used to analyze all of the data collected throughout the experiment. Tukey's HSD test was used to compare treatment averages at a 95% significant level to ascertain the extent of variation declared by the ANOVA. The analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Advanced Statistics 20.0), and the data were reported as mean±standard error.

Results

On a dry matter basis, 23% of the extracted BG was recovered in BGPC, with 86.34% of the initial protein retained in the extracted biomass; The crude protein content of the BGPC was 770.50 g/kg. A total of 286.37 g/kg of indispensable amino acids and 484.14 g/kg of dispensable amino acids; respectively representing 37 and 63% of the total amino acid were documented in BGPC (Table 2). BGPC was rich in cysteine (114.60 g/kg) and ornithine (103.00 g/kg) which were the highest amino acids (by quantity) observed in BGPC. Conversely, the least amino acids observed in BGPC were asparagines and serine (14.13 g/kg), and threonine and methionine (15.69 g/kg). The nutritional and gross energy compositions of experimental diets presented in Table 3 showed similarity. The growth progression (Figure 1) of *C. gariepinus* juveniles fed the experimental diets was similar in the first 15 days after which there was a noticeable distinction in growth among the

Table 2. Amino Acid Compositions (g/kg ingredient) of Bambara Groundnut Protein Concentrate

Amino acid	BGPC
Valine	28.49
Leucine	79.31
Isoleucine	23.23
Threonine	15.69
Methionine	15.69
Phenylalanine	24.83
Lysine	57.26
Histidine	23.48
Tryptophan	18.39
Arginine	nd
Cysteine	114.60
Tyrosine	29.17
Glycine	76.59
Alanine	23.48
Proline	21.16
Hydroxyproline	15.96
Serine	14.13
Glutamic acid	22.21
Glutamine	22.70
Asparagine	14.13
Aspartic Acid	27.01
Ornithine	103.00
TIAA	286.37
TDAA	484.14
TSAA	130.29

TIAA- Total indispensable amino acid; TDAA – Total dispensable amino acid; TSAA – Total sulphur amino acid; nd – not determined

Table 3. Proximate Composition (g/kg) and Gross Energy (kJ/g) of Ingredients and Experimental Diets

	$BGPC_0$	$BGPC_{33}$	$BGPC_{66}$	$BGPC_{100}$	Fish meal	BGPC	BG
Moisture	92.5	102.1	93.6	109.0	52.0	28.0	49.0
Crude protein	410.0	390.5	410.1	397.4	695.0	770.5	205.3
Ether extract	104.0	105.2	108.7	113.1	124.0	30.7	70.5
Total ash	72.0	77.8	73.6	72.9	119.0	54.0	48.7
Crude fibre	81.0	85.9	86.6	86.8	-	0.2	23.5
NFE	240.5	238.5	227.4	220.8	10.0	116.6	603.0
Gross energy	19.4	19.0	19.8	19.8	28.5	22.7	11.3

NFE, Nitrogen Free Extract; BGPC, Bambara groundnut protein concentrate; BG, Bambara groundnut

dietary groups. The groups that received $BGPC_0$ and $BGPC_{33}$ progressed at a similar rate and were distinctively higher than those of $BGPC_{66}$ and $BGPC_{100}$ for the rest of the experimental period. This trend is reflected in the parameters of growth depicted by significant (P<0.05) poorer performance in the group that received $BGPC_{100}$ in terms of PWG (203.31±1.18%) and SGR (2.46±0.09 %/day) relative to those of $BGPC_0$ and $BGPC_{33}$. However, the nutrient utilization parameters were similar (P>0.05) across dietary groups with FCR, PER and PPV ranging from 1.02±0.03 to 1.19±0.07, 2.37±0.06 to 2.13±0.13 and 36.85±0.70 to 34.09±0.70 respectively (Table 4). Although $BGPC_{100}$ significantly (P<0.05) increased the hepato-somatic index compared to diet $BGPC_0$, there was no particular trend observed among the dietary groups. The control group's intestinal somatic index was comparable (P>0.05) to those of other dietary groups. Dietary treatment neither resulted in any fish mortality throughout the experimental period nor significantly (P>0.05) affected carcass proximate composition. There was a beneficial increase in the carcass crude protein and lipid contents of the different experimental groups over the levels therein at the start of the experiment (Table 5). Generally, a trend in which the experimental fish responded similarly (P>0.05) to dietary treatment was also observed in the haematological study (Table 6). Haematocrit, haemoglobin, erythrocyte and leucocyte counts, and associated blood indexes were similar in the control and treated groups. However, the lymphocytes of the groups that received diets containing different levels of BGPC increased significantly (P<0.05) relative to that of the control group. Total protein and globulin of the serum followed a similar trend to that of the lymphocytes; relative to the control group, the group that received $BGPC_{100}$ had significantly (P<0.05) higher levels of total protein and globulin (Table 7). The albumin fraction of the total protein, the serum transaminases (AST and ALT), serum glucose and respiratory burst activity of the leucocyte were not significantly (P>0.05) influenced by BGPC inclusion in the diet of *C. gariepinus*. The tissue metabolic enzyme activity presented in Table 8 showed that hepatic ALT, AST, LDH and MDH were not affected (P>0.05) by dietary substitution of FM with BGPC. The muscle AST and MDH were not influenced (P>0.05) by dietary treatment. However, there was a significant increase in muscle ALT in the group that received $BGPC_{33}$ and $BGPC_{66}$ compared to the control group. The muscle LDH also increased with increasing dietary BGPC; the increase was

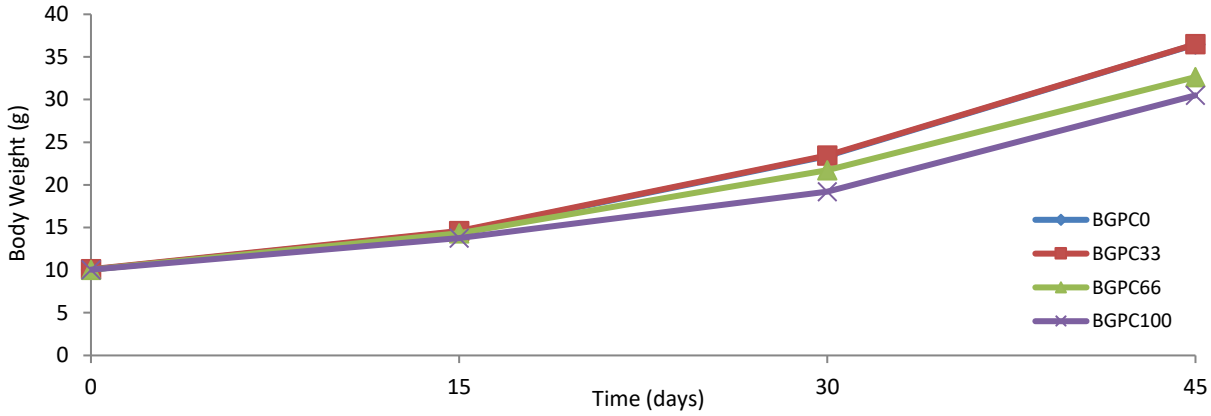

Figure 1. Growth Progression of *Clarias gariepinus* Juveniles Fed Increasing Dietary Inclusion of Bambara Groundnut Protein Concentrate (BGPC) for 45 days

Table 4. Growth, Nutrient Utilization and Survival of *Clarias gariepinus* Fed Increasing Dietary Inclusion of Bambara Groundnut Protein Concentrate (BGPC) for 45 days

Parameters	$BGPC_0$	$BGPC_{33}$	$BGPC_{66}$	$BGPC_{100}$
Initial weight(g)	10.06±0.06	10.11±0.06	10.06±0.06	10.06±0.06
Final weight(g)	$36.47±1.25^b$	$36.50±1.13^b$	$32.64±0.40^{ab}$	$30.50±1.20^a$
Mean weight gained(g)	$26.41±1.22^b$	$26.39±1.09^b$	$22.58±0.46^{ab}$	$20.44±0.89^a$
Percentage weight gained (%)	$262.63±1.16^b$	$260.93±9.75^b$	$224.67±5.73^{ab}$	$203.31±1.18^a$
Specific growth rate (%/day)	$2.86±0.07^b$	$2.85±0.06^b$	$2.62±0.04^{ab}$	$2.46±0.09^a$
Feed intake (g/fish/day)	$26.85±0.76^b$	$26.97±0.36^b$	$25.84±0.25^{ab}$	$24.11±0.38^a$
Feed conversion ratio	1.02±0.03	1.02±0.03	1.14±0.02	1.19±0.07
Protein intake (g/fish/day)	$11.01±0.31^b$	$10.52±0.14^b$	$10.59±0.10^b$	$9.58±0.15^a$
Protein efficiency ratio	2.37±0.06	2.50±0.08	2.13±0.04	2.13±0.13
Protein productive value (%)	36.85±0.70	36.97±0.75	33.62±0.04	34.09±0.70
Hepatosomatic index	$1.24±0.07^a$	$1.47±0.07^{ab}$	$1.34±0.03^{ab}$	$1.48±0.06^b$
Intestinal somatic index	3.05±0.17	3.11±0.10	3.00±0.15	3.08±0.23
Survival	100±0.00	100±0.00	100±0.00	100±0.00

Values across the rows with different superscripts are significantly different (P<0.05)

Table 5. Proximate Carcass Composition (g/kg Wet Weight) of *Clarias gariepinus* Juveniles Fed Increasing Dietary Inclusion of Bambara Groundnut Protein Concentrate (BGPC) for 45 days

	Initial	$BGPC_0$	$BGPC_{33}$	$BGPC_{66}$	$BGPC_{100}$
Moisture	758.2	732.5±7.5	728.4±8.6	723.0±7.0	734.7±5.3
Crude protein	116.1	141.2±0.8	142.1±2.1	143.3±1.8	142.0±2.0
Crude lipid	61.5	83.5±5.5	86.0±4.0	89.0±3.0	79.6±1.6
Ash	42.1	42.5±2.5	37.4±5.0	39.5±6.0	43.2±3.7

Values across rows with same superscript did not differ significantly (P>0.05).

Table 6. Haematological Response of Juvenile *Clarias gariepinus* to Increasing Dietary Inclusion of Bambara Groundnut Protein Concentrate (BGPC)

	$BGPC_0$	$BGPC_{33}$	$BGPC_{66}$	$BGPC_{100}$
Haematocrit (%)	26.50±2.50	34.00±1.00	31.50±0.50	26.50±0.50
Haemoglobin (g/dl)	9.77±1.87	11.43±0.24	10.47±0.16	9.85±0.75
Erythrocyte count (x 10^6/ µl)	1.12±0.14	1.44±0.12	1.39±0.02	1.12±0.03
Leucocyte count (x 10^3/µl)	1.50±0.10	1.60±0.30	1.60±0.20	1.70±0.40
Heterophils (%)	39.50±1.50	30.50±1.50	35.00±3.00	34.50±2.50
Lymphocyte (%)	57.50±1.50^a	66.50±1.50^b	62.50±1.50^{ab}	67.50±1.50^b
Eosinophils (%)	2.50±0.50	2.50±0.50	3.00±0.00	2.50±0.50
Monocyte (%)	0.50±0.50	0.50±0.50	1.00±0.00	0.50±0.50
Basophils (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MCHC (g/dl)	28.90±4.31	33.64±0.30	33.24±0.02	37.24±3.53
MCH (pg)	68.32±8.11	79.51±4.70	75.32±0.07	87.75±8.62
MCV (fl)	237.52±7.37	236.23±11.88	226.61±0.34	235.57±0.79

Values across the rows with different superscripts are significantly different (P<0.05). MCHC, Mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

Table 7. Serum Biochemical Response and Respiratory Burst Activity of Juvenile *Clarias gariepinus* to Increasing Dietary Inclusion of Bambara Groundnut Protein Concentrate (BGPC)

Parameters	$BGPC_0$	$BGPC_{33}$	$BGPC_{66}$	$BGPC_{100}$
Total protein (g/L)	4.13±0.02^a	4.96±0.05^{ab}	4.44±0.33^{ab}	5.58±0.30^b
Globulin (g/L)	2.37±0.06^a	2.47±0.17^a	2.68±0.30^{ab}	3.34±0.23^b
Albumin (g/L)	1.77±0.04	2.50±0.22	1.76±0.30	2.24±0.52
ALT (u/L)	76.50±3.50	83.50±0.50	80.50±2.50	86.00±4.00
AST (u/L)	77.50±2.50	77.50±3.50	80.50±1.50	83.50±2.50
GLU (mg/dL)	79.70±6.30	89.85±5.55	84.10±4.60	87.45±2.95
RBA	0.22±0.01	0.24±0.03	0.24±0.04	0.21±0.01

Values across the rows with different superscripts are significantly different (P<0.05).
ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GLU, Glucose; RBA, Respiratory burst activity.

significant in the groups fed $BGPC_{66}$ and $BGPC_{100}$ relative to those of $BGPC_0$ and $BGPC_{33}$. Intestinal protease activity was not influenced by varied inclusion of BGPC in the diets of *C. gariepinus*, whereas intestinal amylase activity was significantly lowered by supplementing 66 and 100% BGPC in *C. gariepinus* diet (Figure 2).

Discussion

Many plant-derived proteins have limitations, including low crude protein, imbalanced amino acids, poor digestibility due to high fiber, and anti-nutritional factors. These factors have necessitated the need for processing interventions that could delimit their use as novel and alternative ingredients in aquafeed. The protein concentration intervention in this study successfully increased BG's crude protein content to levels comparable to FM, suggesting BGPC as a potential FM substitute. Protein concentration is a well-established procedure previously used to increase the crude protein content of novel ingredients, among many other nutritional advantages it confers on the final product. For instance, Prabu et al. (2021) prepared cottonseed protein concentrate from cottonseed meal and found a remarkable enhancement (64.97%) in the crude protein of the raw material, from 42.11% to 69.47%. Similar observations were documented by various authors who prepared protein concentrate from different legumes (Wright and Bumstead 1984; Onyago 2022; Neji et al., 2022). The result obtained in the present study showed about 26% higher crude protein relative to the outcome (61.1% crude protein) of Adewunmi et al. (2022) when they prepared protein concentrate from BG. The result of the present study that documents an increase of 275.30% in the crude protein of the raw material (from 20.53 to 77.05%) is

thus in line with previous reports and relatively more remarkable. This result is cheering since most fish, including *Clarias gariepinus* used in the present study, require high dietary crude protein. It has been argued that fish do not have protein requirements *per se* but rather a properly balanced amino acid necessary for growth and other metabolic activities (Miles and Chapman, 2007). In this regard, it is always important to assess the composition of amino acids of novel ingredients when evaluating them in a fish diet. The dispensable and non-dispensable amino acids reported under the current investigation were similar to those reported by Prabu et al. (2021) and Adewunmi et al. (2022). The result obtained in the present study also corroborates those of other investigators that established that methionine is limiting in BG (Adebowale et al., 2011; Bamishaye et al., 2011) and transforming it into concentrate did not improve the situation (Adewunmi et al., 2022). The observed value (15.69 g/kg) of methionine under current investigation was similar to 17.7 g/kg reported by Adewunmi et al. (2022) and higher than 12.7 g/kg reported by Kudre et al. (2013). Methionine deficiency in fish diets has been reported to impair growth and feed efficiency (Elesho et

Table 8. Metabolic Enzyme Activity of *Clarias gariepinus* Juveniles Fed Increasing Dietary Inclusion of Bambara Groundnut Protein Concentrate (BGPC) for 45 days

	ALT (L)	ALT (M)	AST (L)	AST (M)	LDH (L)	LDH (M)	MDH (L)	MDH (M)
$BGPC_0$	6.58±0.92	14.84±1.10^a	34.61±5.36	34.84±0.41	7.81±1.53	6.25±0.34^a	1.46±0.28	1.71±0.64
$BGPC_{33}$	6.98±1.0	22.09±1.52^b	33.31±1.22	34.12±0.11	6.61±1.77	9.75±0.32^a	1.21±0.06	1.07±0.04
$BGPC_{66}$	6.70±0.81	21.26±3.30^b	33.64±1.24	35.31±1.85	8.19±0.03	13.9±2.73^b	1.31±0.52	1.40±0.33
$BGPC_{100}$	6.33±1.15	16.43±2.63^{ab}	32.59±0.81	33.53±1.65	7.20±1.50	16.23±1.40^b	1.21±0.25	1.84±0.74

Values across the columns with different superscripts are significantly different (P<0.05).
ALT, Alanine aminotransferase (nanomoles of sodium pyruvate released/min/mg protein at 37°C); AST, Aspartate aminotransferase (nanomoles of oxaloacetate released/min/mg protein at 37 °C); LDH, Lactate dehydrogenase (unit min^{-1} mg $protein^{-1}$ at 37°C); MDH, Malate dehydrogenase (unit min^{-1} mg $protein^{-1}$ at 37 °C); L, Liver; M, Muscle

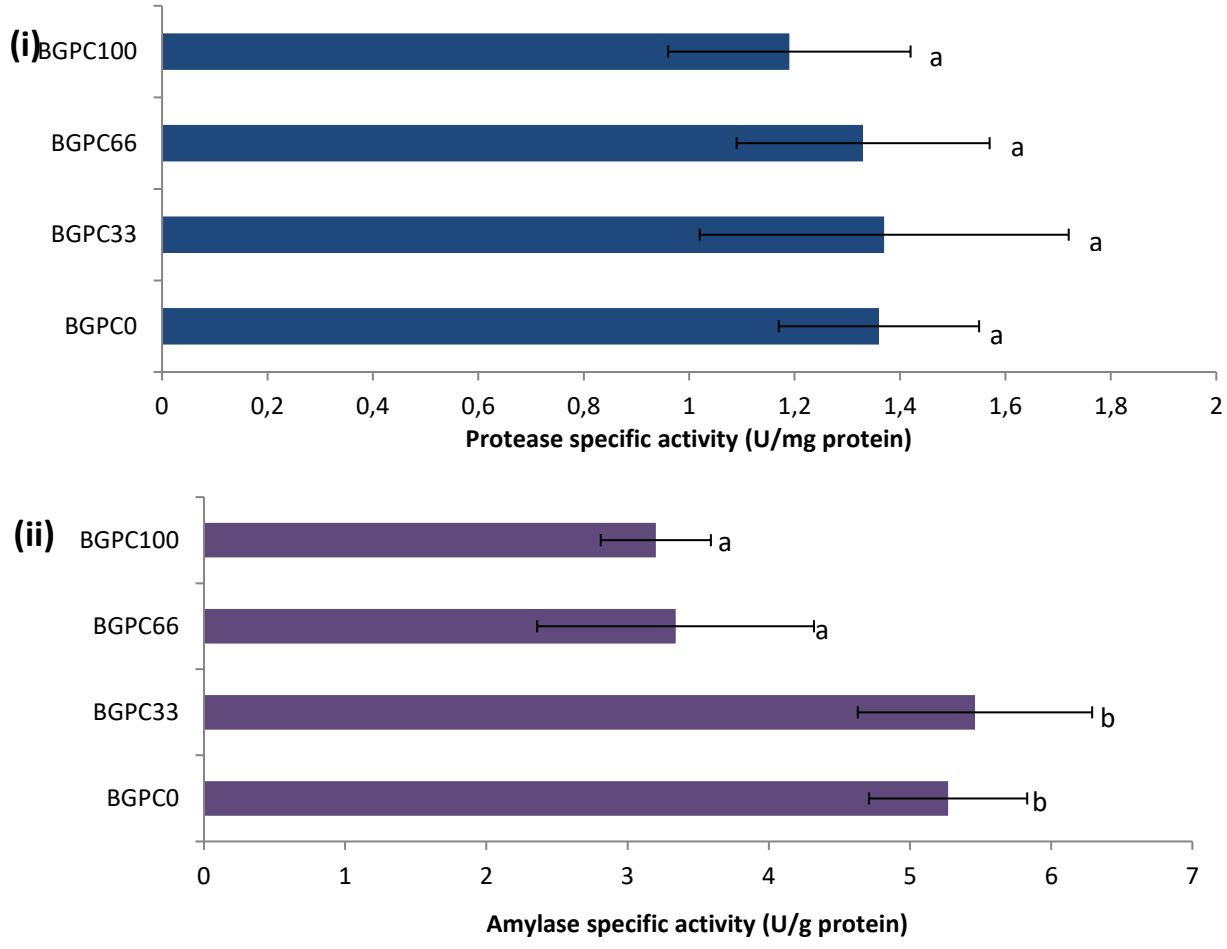

Figure 2. Intestinal (i) Protease (ii) Amylase Activity of *Clarias gariepinus* Juveniles Fed Diets in which Fishmeal was Substituted with Increasing Dietary Inclusion of Bambara Groundnut Protein Concentrate (BGPC). Means with different letter differ significantly (P<0.05)

al., 2021). The considerably high cysteine (114.6 g/kg) in the present study might represent a major nutritional boost. This is because supplementation of cysteine in the diet can spare 40–60% of the methionine needed to meet the total sulphur amino acid (TSAA) requirements and high methionine requirements in some fish species could be attributed to low dietary levels of cysteine (Jobling, 2011).

Replacement of FM in aquafeed has been the focus of many investigations in the aquaculture industry to ensure sustainability. Under current investigation, it is gratifying to note that no mortality was recorded in all the experimental groups throughout the feeding trial; suggesting that the tested diets did not contain any toxic components at levels that could elicit morbidity and mortality. The present investigation revealed that BGPC cannot completely replace FM in the diet of *C. gariepinus* without compromising growth. When this was done in the present study, it resulted in significant growth depression. The result of the present study agrees with the outcome of a similar investigation utilizing plant-derived ingredients as a substitute for FM in aquafeed. For example, Slawski et al. (2011) fed graded levels of rapeseed protein concentrate as a substitute to FM and documented growth compromise of common carp at 66 and 100% replacement levels. Zhang et al. (2019) also recently reported that levels beyond 34% soy protein concentrate, supplementing 60% of FM resulted in inferior growth and diet utilization in rice field eel, *Monopterus albus*. Zhao et al. (2021) similarly substituted FM with graded levels of concentrated dephenolized cottonseed protein and observed improved growth of *Oncorhynchus mykiss* only up to 50% replacement level. A similar result was obtained with juvenile hybrid grouper when low-gossypol cottonseed protein concentrate replaced FM (Ye et al., 2020). Factors such as poor palatability and repulsive smell which limit feed intake; antinutrients; and deficiency of important amino acids are some of the factors previously adduced to poor utilization of plant proteins. In the present study, feed intake was significantly lower in the group that received $BGPC_{100}$ and this may be responsible for the observed result and the result suggests that *C. gariepinus* may be averse to taking diets with no FM. Although the antinutritional profile of BGPC tested in the present study was not evaluated, Mune et al. (2011) observed appreciable levels of polyphenols, phytates, trypsin inhibitors and alpha-amylase inhibitors in BGPC. They particularly noted that alpha-amylase inhibitors in BG remained unchanged after processing it into concentrate. Going by the result of intestinal digestive enzyme activity in the present study, it is unlikely that trypsin inhibitors that may be residual in BGPC played a significant role in the observed result since protease activity in the different experimental groups was statistically similar. Shamna et al. (2015) had earlier attributed significantly reduced protease activity in rohu to the effects of trypsin inhibitor arising from dietary jatropha protein concentrate. On the other hand, the alpha-amylase inhibitor could have been responsible for significantly lowered amylase activities in the $BGPC_{66}$ and $BGPC_{100}$ experimental groups which could have resulted in their measurable and significant inferior growth, respectively. In an *in vitro* study, Natarajan et al. (1992) demonstrated the vulnerability of carp and tilapia's gut amylase to amylase inhibitors from wheat.

Deficiency of important amino acids is another factor previously advanced for limited utilization of plant proteins in fish diet. In this context, the consideration would be methionine which is known to be generally limiting in legumes and observed to be one of the least amino acids documented in BGPC under current investigation. However, it is doubtful that deficiency of methionine played a role in the observed growth depression when BGPC completely replaced FM. This is because the diets were supplemented with crystalline methionine such that its dietary content would be over 21.4 g/kg recently suggested by Elesho et al. (2021) as the methionine requirement for *C. gariepinus*. Further, the BGPC contained a high quantity of cysteine which Jobling (2011) documented could spare as high as 60% of methionine. Additionally, the results of protein utilization (PER and PPV) and carcass crude protein composition do not suggest deficiency of amino acids, since they were similar across all the experimental groups.

A fundamental consideration when evaluating novel ingredients such as BGPC assessed in the present study is the health implication of their inclusion in the fish diet. Such health implications are often assessed using different blood parameters (Seibel et al., 2021). Many reports revealed varying health compromises consequent of FM replacement with plant proteins. Jahanbakhshi et al. (2012) reported a significant reduction in haemoglobin when they fed sturgeons with increasing levels of plant protein in place of FM. In the present study, there was no significant influence of dietary BGPC on the haematology of *C. gariepinus* juveniles; indicating that the health of the fish was not compromised, even when BGPC completely replaced FM. One of the leucocyte differentials, lymphocytes, in the BGPC-fed groups increased over the control. A similar trend was also observed in serum total protein and globulin. Lymphocytes, total protein and globulin are recognized for their crucial immunological roles (Magnadottir, 2010; Magadan et al., 2015). Their enhancement in the group that received BGPC under the current investigation may not be unconnected with rich bioactive components, mainly polyphenolic compounds, previously reported in BG (Okafor et al., 2022). Such compounds are known to have significant health-promoting effects.

The serum aminotransferases are commonly used indicators of liver function and health. These enzymes are found in high concentrations in liver cells and are released into the bloodstream when liver cells are damaged or stressed (Xie et al., 2021). In the present

study, serum AST and ALT were not substantially affected by dietary replacement of FM with BGPC, unlike similar studies where plant-derived ingredients were reported to induce hepatic damage. For instance, Zhang et al. (2022) observed significantly elevated transaminases, which they attributed to gossypol, when they replaced 45% FM with low-gossypol cottonseed meal in the diet of juvenile turbot.

Serum glucose can be influenced by diet and metabolism (Seibel et al., 2021). Its elevation has sometimes been associated with energy stress triggered by the release of stress hormones, such as catecholamines, to cope with stress (Shamna et al., 2015). In the present study, the inclusion of BGPC instead of FM in *C. gariepinus*' diet did not elevate serum glucose. This suggests that the different experimental groups were not under any form of energy stress. Contrastingly, the muscle LDH in the group that received $BGPC_{66}$ and $BGPC_{100}$ was significantly raised and trended similarly to those of intestinal amylase activity. Elevated tissue LDH is usually attributed to increased anaerobic metabolism occasioned by stress imposed on normal energy-release mechanisms (Murray et al., 2000). Physical stress arising from handling or other forms of physical exertion, energy deficit caused by impaired carbohydrate digestion, hypoxic conditions or nutritional imbalance have been previously associated with the activation of anaerobic glycolysis for energy, leading to higher LDH activity (Shamna et al., 2015; Gopan et al., 2020; Olude et al., 2023). Although the source of the stress is not established in this study, it may also have resulted from the earlier postulation on the possible impairment of carbohydrate digestion by alpha-amylase inhibitor; leading to limited energy availability. However, other stress biomarkers of carbohydrate metabolism assessed in the present study (muscle and liver MDH and liver LDH) showed similarity across dietary groups and corroborated the result of serum glucose. The activity of hepatic and muscle enzymes (AST and ALT) involved in protein utilization in the groups that received BGPC was either similar to or higher than that of the control-fed group. This result is in line with those of protein utilization parameters and further supports our earlier opinion that there was no amino acid deficiency arising from feeding BGPC to *C. gariepinus* juveniles. Reduced activity of tissue AST and ALT has previously been associated with impaired protein utilization as a consequence of dietary plant-derived ingredients (Luo et al., 2012).

Conclusions

This study demonstrated the possibility of substituting 66% (220 g/kg) FM with BGPC, in the diet of *C. gariepinus* juveniles, without eliciting substantial growth, diet utilization and health compromise. Beyond this level, there was an obvious limitation in the utilization of BGPC by *C. gariepinus* juveniles. It was suggested that an alpha-amylase inhibitor might be responsible for the result but further investigation is required to ascertain the factor(s) that might have caused the limitations observed in the use of BGPC as a complete replacer of FM and proffer adequate strategies toward resolution of such factor(s).

Ethical Statement

This study complied strictly with the guidelines provided by the University of Lagos Animal Care and Use Research Ethics Committee (UNILAGACUREC) for the use and care of laboratory animals.

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Author Contribution

OO: Conceptualization, Supervision, Formal Analysis, Writing-review and editing; AA: Project Administration, Investigation, Writing-original draft; AF: Investigation, Visualization, Methodology, Writing-original draft; PO: Investigation, Data Curation, Methodology, Writing-original draft.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional or personal interest that could have influenced the work reported in this manuscript.

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References

Adebowale, Y. A., Schwarzenbolz, U., & Henle, T. (2011). Protein Isolates from Bambara Groundnut (*Voandzeia subterranea* L.): Chemical Characterization and Functional Properties. *International Journal of Food Properties*, 14(4), 758–775. https://doi.org/10.1080/10942910903420743

Adewumi, O. O., Felix-Minnaar, J. V., & Jideani, V. A. (2022). Physiochemical and Nutritional Characteristics of Ready-to-Use Therapeutic Food Prepared Using Bambara Groundnut-*Moringa oleifera* Leaf Protein Complex. *Foods*, 11(12), 1680. https://doi.org/10.3390/foods11121680

Adewumi, A., & Odeyemi, O. (2018). Evaluation of Bambara Nut (*Vigna subterranea*) as Feed Ingredient in the Diet of the Catfish, *Clarias gariepinus* (Burchell 1822).

International Journal of Sciences, 4(09), 14–20. https://doi.org/10.18483/ijsci.1784

Association of Official Analytical Chemists (2005). Official Methods of Analysis of Association Official Analytical Chemists, 18th ed. Gaithersburg, MD.

Bamishaye, O.M., Adegbola, J.A., & Bamishaye, E.I. (2011). Bambara groundnut, an under-utilized nut in Africa. *Advanced Agricultural Biotechnology*, 1, 66-72.

Bertrand, A., Lengaigne, M., Takahashi, K., Avadi, A., Poulain, F., & Harrod, C. (2020). *El Niño Southern Oscillation (ENSO) effects on fisheries and aquaculture*. Vol. 660. Food & Agriculture Org.

Blaxhall, P. C., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5(6), 771–781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Doumas, B.T., Arends, R.L., Pinto, & P.C. (1972). Determination of serum albumin. In *Standard Methods of Clinical Chemistry*, (Vol. 7, pp. 175-188). Elsevier.

Drapeau, G. R. (1976). Protease from *Staphyloccus aureus*. Part B: *Proteolytic Enzymes*, 469–475. https://doi.org/10.1016/s0076-6879(76)45041-3

Elesho, F. E., Sutter, D. A. H., Swinkels, M. A. C., Verreth, J. A. J., Kröckel, S., & Schrama, J. W. (2021). Quantifying methionine requirement of juvenile African catfish (*Clarias gariepinus*). *Aquaculture*, 532, 736020. https://doi.org/10.1016/j.aquaculture.2020.736020

El-Sayed, A.-F. M., Dickson, M. W., & El-Naggar, G. O. (2015). Value chain analysis of the aquaculture feed sector in Egypt. *Aquaculture*, 437, 92–101. https://doi.org/10.1016/j.aquaculture.2014.11.033

Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A.A., & Abu-Salem, F.M. (2011). Chemical composition and functional properties of flour and protein isolate extracted from bambara groundnut (*Vigna subterranean)*. *African Journal of Food Science*, 5(2), 82-90.

Enyidi, U.D., Pirhonen, J., & Vielma, J. (2014). Effects of sesame seed meal and Bambara nut meal on growth, feed utilization and body composition of juvenile African catfish *Clarias gariepinus*. *Iranian Journal of Fisheries Science*, 13(4), 998-1013. https://dorl.net/20.1001.1.15622916.2014.13.4.17.3

Food and Agriculture Organization (2017). FAO and the SDGs. Indicators: Measuring up to the 2030 Agenda for Sustainable Development. Rome: FAO, 39 pp. http://www.fao.org/3/a-i6919e.pdf

FAO (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., Ropicki, A., Smith, M. D., & Tveterås, R. (2019). A Global Blue Revolution: Aquaculture Growth Across Regions, Species, and Countries. *Reviews in Fisheries Science & Aquaculture*, 28(1), 107–116. https://doi.org/10.1080/23308249.2019.1678111

Garlock, T., Asche, F., Anderson, J., Ceballos-Concha, A., Love, D.C., Osmundsen, T.C., & Pincinato, R.B.M., (2022). Aquaculture: The missing contributor in the food security agenda. *Global Food Security,* 32, 100620.

Gopan, A., Sahu, N. P., Varghese, T., Sardar, P., & Maiti, M. K. (2020). Karanj protein isolate prepared from karanj seed cake: effect on growth, body composition and physiometabolic responses in *Labeo rohita* fingerlings. *Aquaculture Nutrition*, 26(3), 737-751.

Hernández, A. J., & Roman, D. (2016). Phosphorus and nitrogen utilization efficiency in rainbow trout (*Oncorhynchus mykiss*) fed diets with lupin (*Lupinus albus*) or soybean (*Glycine max*) meals as partial replacements to fish meal. *Czech Journal of Animal Science*, 61(No. 2), 67–74. https://doi.org/10.17221/8729-cjas

Hillocks, R.J., Bennett, C., & Mponda, O.M. (2012). Bambara nut: A review of utilisation, market potential and crop improvement. *African Crop Science Journal*, 20(1).

Huntington, T.C., & Hasan, M.R. (2009). Fish as feed inputs for aquaculture–practices, sustainability and implications: a global synthesis. *FAO Fisheries and Aquaculture Technical Paper*, 518, 1-61.

Hušek, P. (1991). Rapid derivatization and gas chromatographic determination of amino acids. *Journal of Chromatography A*, 552, 289–299. https://doi.org/10.1016/s0021-9673(01)95945-x

Jahanbakhshi, A., Imanpoor, M. R., Taghizadeh, V., & Shabani, A. (2012). Hematological and serum biochemical indices changes induced by replacing fish meal with plant protein (sesame oil cake and corn gluten) in the Great sturgeon (*Huso huso*). *Comparative Clinical Pathology*, 22(6), 1087–1092. https://doi.org/10.1007/s00580-012-1532-4

Katya, K., Borsra, M. Z. S., Kuppusamy, G., Herriman, M., & Azam Ali, S.N. (2017). Preliminary study to evaluate the efficacy of bambara groundnut (*Vigna subterranea*(L.) Verdc.) meal as the dietary carbohydrate source in Nile tilapia, *Oreochromis niloticus*. *Madridge Journal of Aquaculture Research & Development*, 1(1), 13–17. https://doi.org/10.18689/mjard-1000103

Kudre, T. G., Benjakul, S., & Kishimura, H. (2013). Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *Journal of the Science of Food and Agriculture*, 93(10), 2429–2436. https://doi.org/10.1002/jsfa.6052

Lovrien, R., & Matulis, D. (1995). Assays for Total Protein. *Current Protocols in Protein Science*, 1(1), 3.4.1–3.4.24. https://doi.org/10.1002/0471140864.ps0304s01

Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biology Chemistry*, 193(1), 265 - 275.

Luo, Y., Ai, Q., Mai, K., Zhang, W., Xu, W., & Zhang, Y. (2012). Effects of dietary rapeseed meal on growth performance, digestion and protein metabolism in relation to gene expression of juvenile cobia (*Rachycentron canadum*). *Aquaculture*, 368-369, 109–116. https://doi.org/10.1016/j.aquaculture.2012.09.013

Magadan, S., Sunyer, O. J., & Boudinot, P. (2015). Unique Features of Fish Immune Repertoires: Particularities of Adaptive Immunity Within the Largest Group of Vertebrates. *Results and Problems in Cell Differentiation*, 57, 235–264. https://doi.org/10.1007/978-3-319-20819-0_10

Magnadottir, B. (2010). Immunological Control of Fish Diseases. *Marine Biotechnology*, 12(4), 361–379. https://doi.org/10.1007/s10126-010-9279-x

Maphosa, Y., Jideani, V. A., & Maphosa, L. (2022). Bambara groundnut production, grain composition and nutritional value: Opportunities for improvements. *The Journal of Agricultural Science,* 1–44. https://doi.org/10.1017/s0021859622000521

Mateva, K.I., Tan, X.L., Halimi, R.A., Chai, H.H., Makonya, G.M., Gao, X., Shayanowako, A.I.T., Ho, W.K., Tanzi, A.S., Farrant, J., Mabhaudhi, T., King, G.J., Mayes, S., Massawe, F. (2023). Bambara groundnut (*Vigna subterranea* (L.) Verdc.). In *Neglected and Underutilized Crops* (pp. 557-615). Academic Press. https://doi.org/10.1016/B978-0-323-90537-4.00021-1

Miles, R.D., & Chapman, F.A. (2007). The concept of ideal protein in formulation of aquaculture feeds: FA144/FA144, 3/2007. *EDIS*, 2007(11).

Mubaiwa, J., Fogliano, V., Chidewe, C., & Linnemann, A. R. (2016). Hard-to-cook phenomenon in bambara groundnut (Vigna subterranea (L.) Verdc.) processing: Options to improve its role in providing food security. *Food Reviews International*, 33(2), 167–194. https://doi.org/10.1080/87559129.2016.1149864

Mune, M.M.A., Minka, S.R., Mbome, I.L., & Etoa, F.X. (2011). Nutritional potential of Bambara bean protein concentrate. *Pakistan Journal of Nutrition*, 10(2),112-119.

Murray, R. K., Granner, D. K., Mayes, P. A., & Rodwell, V. W. (2000). Glycogen metabolism. In D. A. Bames, J. Ransom, & J. Roche (Eds.), *Harper's biochemistry* (25th ed., pp. 199–207). New York, NY: McGrawHill Education

Natarajan, M., Ross, B., & Ross, L. G. (1992). Susceptibility of carp and tilapia α-amylase to purified wheat amylase inhibitor. *Aquaculture*, 102(3), 265–274. https://doi.org/10.1016/0044-8486(92)90153-c

Neji, C., Semwal, J., Kamani, M. H., Máthé, E., & Sipos, P. (2022). Legume Protein Extracts: The Relevance of Physical Processing in the Context of Structural, Techno-Functional and Nutritional Aspects of Food Development. *Processes*, 10(12), 2586. https://doi.org/10.3390/pr10122586

Jobling, M. (2011). National Research Council (NRC): Nutrient requirements of fish and shrimp. *Aquaculture International*, 20(3), 601–602. https://doi.org/10.1007/s10499-011-9480-6

Jimenez-Lopez, J. C., & Clemente, A. (2022). Legumes Research Volume 2. In IntechOpen eBooks. *IntechOpen*. https://doi.org/10.5772/intechopen.104821

Ochoa, S. (1955). *Methods of Enzymology*, (Colowick, S.P. & Kapalan, N.O. ed), Vol. 1, 735 pp. Academic press Inc. New York, USA.

Okafor, J. N. C., Jideani, V. A., Meyer, M., & Le Roes-Hill, M. (2022). Bioactive components in Bambara groundnut (*Vigna subterraenea* (L.) Verdc) as a potential source of nutraceutical ingredients. *Heliyon*, 8(3), e09024. https://doi.org/10.1016/j.heliyon.2022.e09024

Olude, O.O., Sahu, N.P., Sardar, P. & Nuzaiba, P.M. (2023). Utilization of valorized cassava leaf meal as an alternative feedstuff to defatted soybean meal in feed for rohu, *Labeo rohita* fingerlings. *Bioresource Technology Reports*, 22: 101400. https://doi.org/10.1016/j.biteb.2023.101400

Prabu, D.L., Vijayagopal, P., Ebeneezar, S., & Muniswaran, B.R. (2021). Cottonseed protein concentrate as an alternate protein source for fishmeal replacement in aquafeeds: Production optimisation and its nutritive profile. *Indian Journal of Fisheries*, 68 (4), 51-57.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clininical Pathology*, 28:56-63.

Rick, W., & Stegbauer, H. P. (1974). α-Amylase measurement of reducing groups. In *Methods of enzymatic analysis* (pp. 885-890). Academic Press.

Sarma, P.R. (1990). Red cell indices. In: Walker HK, Hall WD, Hurst JW, editors. *Clinical Methods*: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths.

Seibel, H., Baßmann, B., & Rebl, A. (2021). Blood Will Tell: What Hematological Analyses Can Reveal About Fish Welfare. *Frontiers in Veterinary Science*, 8. https://doi.org/10.3389/fvets.2021.616955

Shamna, N., Sardar, P., Sahu, N.P., Pal, A., Jain, K., & Phulia, V. (2015). Nutritional evaluation of fermented Jatropha protein concentrate in *Labeo rohita* fingerlings. *Aquaculture Nutrition*, 21(1), 33–42. https://doi.org/10.1111/anu.12138

Slawski, H., Adem, H., Tressel, R.P., Wysujack, K., Koops, U., Wuertz, S., & Schulz, C. (2011). Replacement of fish meal with rapeseed protein concentrate in diets fed to wels catfish (*Silurus glanis* L.). *Aquaculture Nutrition*, 17(6), 605–612. https://doi.org/10.1111/j.1365-2095.2011.00857.x

Stasiak, A.S., & Baumann, C.P. (1996). Neutrophil activity as a potential bioindicator for contaminant analysis. *Fish & Shellfish Immnunology*, 6, 37-39.

Subasinghe, R., Soto, D., & Jia, J. (2009). Global aquaculture and its role in sustainable development. *Reviews in Aquaculture*, 1(1), 2–9. https://doi.org/10.1111/j.1753-5131.2008.01002.x

Tan, X. L., Azam-Ali, S., Goh, E. V., Mustafa, M., Chai, H. H., Ho, W. K., Mayes, S., Mabhaudhi, T., Azam-Ali, S., & Massawe, F. (2020). Bambara Groundnut: An Underutilized Leguminous Crop for Global Food Security and Nutrition. *Frontiers in Nutrition*, 7. https://doi.org/10.3389/fnut.2020.601496

Thomas, L. (Ed.). (1998). *Clinical laboratory diagnostics: use and assessment of clinical laboratory results*. TH-books Verlagsgesellschaft.

Turchini, G. M., Trushenski, J. T., & Glencross, B. D. (2018). Thoughts for the Future of Aquaculture Nutrition: Realigning Perspectives to Reflect Contemporary Issues Related to Judicious Use of Marine Resources in Aquafeeds. *North American Journal of Aquaculture*, 81(1), 13–39. https://doi.org/10.1002/naaq.10067

Wooten, I. D. P. (1964). Microanalysis. *Medical biochemistry*, *98*, 101-107.

Wright, D., & M. Rachel Bumstead. (1984). Legume proteins in food technology. *Philosophical Transactions of the Royal Society of London*, 304(1120), 381–393. https://doi.org/10.1098/rstb.1984.0033

Wroblewski, F., & Ladue, J. S. (1955). Lactic Dehydrogenase Activity in Blood. *Experimental Biology and Medicine*, 90(1), 210–213. https://doi.org/10.3181/00379727-90-21985

Xie, R., Amenyogbe, E., Chen, G., & Huang, J. (2021). Effects of feed fat level on growth performance, body composition and serum biochemical indices of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus polyphekadion*). *Aquaculture*, 530, 735813–735813. https://doi.org/10.1016/j.aquaculture.2020.735813

Ye, G., Dong, X., Yang, Q., Chi, S., Liu, H., Zhang, H., Tan, B., & Zhang, S. (2020). Low-gossypol cottonseed protein concentrate used as a replacement of fish meal for juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂): Effects on growth performance, immune responses and intestinal

microbiota. *Aquaculture*, 524, 735309–735309. https://doi.org/10.1016/j.aquaculture.2020.735309

Zhang, J., Zhong, L., Peng, M., Chu, W., Liu, Z., Dai, Z., & Hu, Y. (2019). Replacement of fish meal with soy protein concentrate in diet of juvenile rice field eel *Monopterus albus*. *Aquaculture Reports*, 15, 100235–100235. https://doi.org/10.1016/j.aqrep.2019.100235

Zhang, X., Zhou, H., Liu, C., Mai, K., He, G., & Wang, X. (2022). Fishmeal substitution with low-gossypol cottonseed meal in the diet for juvenile turbot (*Scophthalmus maximus* L.): Effects on growth, nutrients utilization and haematological responses. *Aquaculture Reports*, 24, 101149. https://doi.org/10.1016/j.aqrep.2022.101149

Zhao, W., Liu, Z.-L., & Niu, J. (2021). Growth performance, intestinal histomorphology, body composition, hematological and antioxidant parameters of *Oncorhynchus mykiss* were not detrimentally affected by replacement of fish meal with concentrated dephenolization cottonseed protein. *Aquaculture Reports*, 19, 100557–100557. https://doi.org/10.1016/j.aqrep.2020.100557