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Effect of Bambara Seed (*Vigna subterranea*) Meal on Growth Performance, Physiology and Histology of *Clarias gariepinus* Juveniles

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ABSTRACT: This experiment was conducted to evaluate the effects of replacing roasted Bambara bean with soybean in the catfish diet. One hundred and sixty-eight *Clarias gariepinus* juveniles (8.69±0.28 g) were allocated to four treatments in replicates. Experimental diets were formulated at 40% crude protein with three inclusion levels of Bambara meal diet BMD (15%, 30% and 45%), respectively, and a soybean control diet, fed twice daily at 3 % body weight for 8 weeks, in a completely randomized experiment. Growth indices of Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Intake (FI), Feed Conversion Ratio (FCR) and Percentage Weight Gain (PWG) were measured. Haematological parameters, digestive enzymes, intestinal morphometry and organ histology of liver, kidney and intestine were also investigated. Results showed that growth indices of *Clarias gariepinus* decreased with increased levels of BMD. The highest weight gain (83.73) was in the control diet and the least (65.66) in BMD45%, while SGR, FCR and PWG were not significantly different ($p>0.05$) in the experiment. Carcass protein and haematological parameters were highest at BMD45%, and serum biochemical parameters highest in control diet. Digestive enzymes acted best on BMD45%, while intestinal morphometry was highest in the control diet and significantly different from dietary treatment. Organ histology showed no observable lesions in the control treatment, but BMD15%; BMD45% kidney was affected by the diet, while the BMD30% showed liver degeneration and intestinal villi atrophy. Roasted BMD can best be included at 15% and up to 45% when WG, SGR, FCR and PWG are considered.

Keywords: Bambara seed, Digestive enzymes, Intestinal morphometry, Haematology, Histology

Introduction

Underutilized crops especially of African origin are yet to be fully exploited as food and feed despite the abundance in terms of production and nutrient potential. Bambara plant is a food crop of tropical Africa, and an underutilized crop of the tropics (Fasoyiro *et al.*, 2012). The nutritional value is a factor that allows scientists to focus interest on Bambara as feedstuff in fish nutrition. Bbebe (2019) highlighted research works in which the use of Bambara seed in different animal nutrition has paved way for its inclusion in livestock diet despite its low economic value. The by-products of Bambara seed are also utilized as fish feed (Yusuf *et al.*, 2024). According

to Bamishaye *et al.*, (2011), it is a leguminous crop with other important food crop of soybean and groundnut with the botanical name *Vigna subterranean* belongs to Plantea of the family Fabaceae and sub family of Faboidea. It is consumed in roasted and boiled form by humans (Orire *et al.*, 2015).

The use of Bambara in the nutrition of different species of fish, highlighted the importance to the need for its use to replace expensive feedstuffs of fishmeal and soybean which are major nutrient source in feed formulation. Aliu and Egwemi (2022) stated that soybean is at times imported to meet local livestock demand hence need for local available feed resources which are underutilized. Processing methods to reduce the anti-nutrients and phytochemicals in Bambara seed have made claims for its inclusion as feed with impact on fish growth and survival. Tiamiyu and Solomom (2008); Enyidi and Mgbenka (2015); Adewunmi and Odeyemi (2018); Salisu *et al.*, (2024) Olude *et al.*, (2025) documented Bambara seed diet to replace fishmeal in diet of catfish at various life stages. While Enyidi *et al.*, (2017) reported that it can partly substitute fishmeal and completely replace soybean meal. Katya *et al.*, (2017) studied the meal as energy feedstuff with replacement of wheat flour. Feedstuff combination in the form of different blends can serve a complementary effect on studied species, and Ishiwu *et al.*, (2020) reported blend of pigeon pea+ soaked Bambara + Fishmeal fed to catfish. Aliu and Egwemi (2022) included bambara in the diet of *H. bidorsalis* fingerlings. From literature there was a gap on the effect of feeding BMD on blood, digestive enzyme, intestinal morphology and tissue histology of catfish fed with BMD diet. This study hereby documented the growth performance of juvenile catfish fed roasted Bambara seed up to 45% inclusion as soybean replacement, and the supplementation effect hereby reported.

Materials and methods

The experiment was conducted at Fish Nutrition Laboratory, Department of Forestry, Wildlife and Fisheries, Faculty of Agricultural Production and Renewable Resources, College of Agricultural Sciences, Olabisi Onabanjo University, Nigeria. *C. gariepinus* juveniles (Burchell, 1822) with average weight of 8.69±0.28g were stocked in rectangular plastic tank of 54 litres (0.6 m × 0.3 m × 0.3 m) at 14/fish/tank in a complete randomized experimental design. The experimental water was changed every two days and the experiment lasted for 8 weeks. Bambara bean (*Vigna subterranean*) was purchased from market and roasted. The roasted bean was used in formulating three dietary treatments BMD15%, BMD30%, BMD45% along with other feedstuff and a control diet in replacement for soybean (Table 1). Diet was fed at 3% body weight. The feed and fish were analyzed for nutritive value (AOAC, 2005). The fish were weighed weekly and weight recorded used in calculating growth indices.

Table 1: Composition of bambara diets fed to *Clarias gariepinus* juveniles

Ingredients	Control	BMD (15%)	BMD (30%)	BMD (45%)
Fishmeal	38.12	38.12	38.12	38.12
Bambara meal	0	5.72	11.44	17.15
Soybean meal	38.12	32.4	26.68	20.97
Maize	20.92	20.92	20.92	20.92
Premix	0.6	06	0.6	0.6
Oyster Shell	2	2	2	2
Salt	0.25	0.25	0.25	0.25

Growth indices of the experimental fish

Weight Gain = $W_2 - W_1$

Specific Growth Rate (SGR) (%/day) = $\frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100$

Ln = natural logarithm.

W_1 = Initial weight (g) of fish at time T_1 (beginning of the experiment)

W_2 = final weight (g) of fish at time T_2 (end of the experiment)

T = time in days

Food Conversion Ratio = $\frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$

Survival Rate (%) = $\frac{\text{Initial no. of fish stocked} - \text{Mortality}}{\text{Initial no. of fish stocked}} \times 100$

Percentage Weight Gain = $\frac{\text{Mean weight gain}}{\text{Initial weight}} \times 100$

Haematology assay: Samples for haematological examination were obtained by collecting blood from fish (n=3 per treatment) deprived of feed for 24hs using syringe and needle. The needle was introduced on the ventral midline between the anal opening and the beginning of the anal fin to access the cardinal vein beneath the vertebral column. The blood samples collected were preserved in an EDTA bottle before being taken to the laboratory for further examination.

Intestinal morphology: Intestinal morphology was conducted according to Sule et al. (2023) in which three numbers of *C. gariepinus* were randomly selected from each treatment and slaughtered for intestinal histomorphometry. Tissues for samples of morphological measurement were rinsed in physiological saline. Morphological analysis was performed on formalin-fixed intestinal samples. The samples were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The sections were visualized using a light microscope. Villus height and crypts depth were measured and analyzed using Image-ProPlus 7.0 software, and 5 well-oriented villi and crypts from each sample were selected for measuring villus height and crypt depth.

Histological assay: Samples for histological examination were taken from fish (n = 3 per treatment). Fish were dissected and samples of liver, kidney and intestines taken and preserved in Bouin's fluid. For microscopy examination, samples were dehydrated in graded ethanol prior to embedding in paraffin wax. From each wax block, multiple sections (5µm thick) were cut with a microtome and then mounted on glass slides. Multiple sets of sections were stained with haematoxylin and eosin. Tissue structures of the samples were examined under light microscope and images captured with a digital camera. For the intestines, the images were further analyzed to assess intestinal villi height, villi width and muscle thickness.

Statistical analysis: Completely Randomized Design was used while data collected were subjected to one-way ANOVA statistical analysis using SPSS 20.0. Determination of significant difference among individual means was determined at P = 0.05 means compared by Duncan's multiple range tests.

Results

The roasted Bambara bean possess the parameters highlighted in Table 1, with crude protein over 20%, high anti-nutritional factor in alkaloid, tannin, moderate flavonoid, phytate; with low saponin and oxalate.

Table 2: Composition of roasted Bambara meal

Parameters	Bambara meal	Anti-nutritional factors	(mg/100g)
Crude protein	21.95	Saponin	11.37
Fat	6.45	Tannin	391.33
Crude fibre	3.65	Alkaloid	1413.16
Ash	2.78	Phytate	41.94
Moisture content	7.53	Flavonoid	65.96
Carbohydrate	57.64	Oxalate	5.15

Nutritional content of the formulated feed (Table3) revealed that the protein ranged 40.9% - 42.96%, fat 6.40% - 7.40%, crude fibre 3.40% - 3.95%, ash 5.80% - 7.00% and moisture content 10.14% - 11.04%

Table 3: Nutritional composition of feed

Parameters	Control	BMD (15%)	BMD (30%)	BMD (45%)
Crude protein	40.90	42.96	40.67	41.53
Fat	6.40	7.40	7.25	6.45
Crude fibre	3.95	3.65	3.40	3.60
Ash	7.00	5.83	6.25	5.80
Moisture content	11.44	10.22	11.05	10.14

The experimental fish responded to diet with varied performance (Table 4). The final weight gain, weight gain followed the same trend as control diet significantly differed (P<0.05) from BDM45%. There was no significant difference from BDM15% and BDM30% with respect to FWT and WG. In all treatment there was no significant difference (P>0.05) in total feed intake, SGR, FCR. Control fish showed the highest Percent weight gain and least survival rate.

Table 4: Growth performance of *C. gariepinus* fed Bambara meal-based diets

Indices	Control	BMD (15%)	BMD (30%)	BMD (45%)
IWT	8.59±0.23	8.79±0.38	8.79±0.17	8.59±1.82
FWT	92.32±6.84 ^a	87.46±2.26 ^{ab}	79.82±4.16 ^{ab}	74.26±0.99 ^b
WG	83.73±6.62 ^a	78.67±2.04 ^{ab}	71.03±4.32 ^{ab}	65.66±2.76 ^b
TF	57.18±4.73	56.08±2.72	50.64±1.84	47.62±0.21
SGR	4.23±0.89	4.11±0.06	3.94±0.13	3.94±0.43
FCR	0.68±0.01	0.71±0.02	0.72±0.03	0.73±0.03
PWG	1071.31±53.18	997.74±34.39	910.76±64.49	964.98±44.15
SR	78.57±8.25	90.48±2.38	95.24±2.38	92.86±4.12

^{abcd} means on the same row with different superscript are significantly different (p<0.05) IWT; initial weight, FWT; final weight, WG; weight gain, TF; total feed, SGR: specific growth rate, FCR: feed conversion ratio, PWG: percentage weight gain, SR; survival rate.

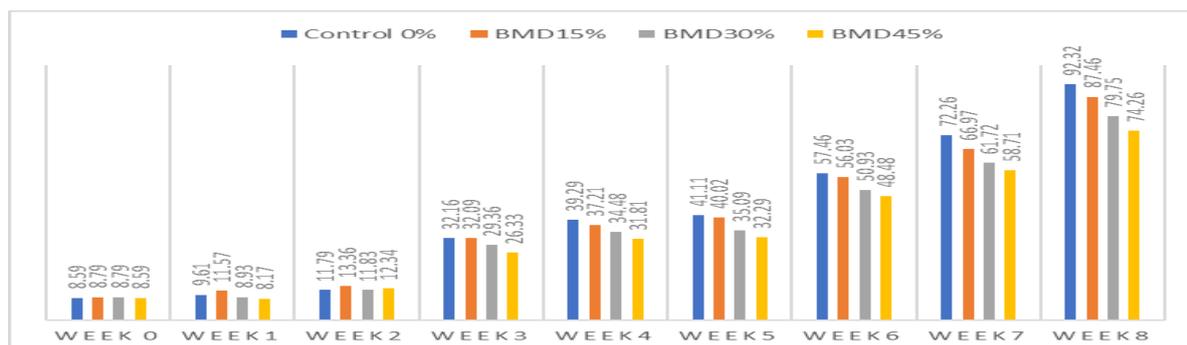


Fig. 1: Weekly growth rate of *C. gariepinus* juveniles fed bambara meal-based diets.

Carcass composition analysis (Table 5) showed varied indices among the treatments and initial fish. Carcass protein was improved over all treatments and control at BMD45%. No significant difference was noticed in fat content in BMD 15% and BMD30%, while ash was least in initial fish with no significant difference in dietary treatments.

Table 5: Carcass composition of *C. gariepinus* juveniles fed bambara meal-based diets

Indices	Initial fish	Control	BMD (15%)	BMD (30%)	BMD (45%)
Protein	39.82±0.06 ^c	40.29±0.04 ^c	50.29±0.03 ^b	49.95±0.07 ^b	72.85±0.21 ^a
Fat	6.21±0.03 ^b	6.55±0.15 ^b	7.05±0.15 ^a	7.00±0.02 ^a	5.55±0.05 ^{bc}
Ash	4.55±0.02 ^d	6.70±0.20 ^a	6.35±0.05 ^b	6.05±0.15 ^{bc}	6.25±0.15 ^{bc}
Moisture	9.27±0.04 ^{bc}	11.00±0.10 ^a	9.92±0.08 ^{bc}	10.24±0.27 ^b	8.67±0.08 ^c

^{abc} means (SD) on the same row with different superscript are significantly different (p<0.05).

Haematological parameters (Table 6) of experimental fish showed that PCV, HB, RBC, WBC, platelet, MCHC and Lym in fish fed BDM45% were significantly different from others. Control fish blood parameters was high in MCV, MCH, HET, and MON with significant difference to other treatments.

Table 6: Haematological parameters of *C. gariepinus* juveniles fed bambara meal-based diets

Indices	Control	BMD (15%)	BMD (30%)	BMD (45%)
PCV %	24.00±4.00 ^b	23.50±1.50 ^c	28.50±0.50 ^a	30.50±0.50 ^a
HB g/dL	7.60±1.10 ^c	7.70±0.07 ^c	8.75±0.15 ^b	10.00±0.30 ^a
RBC x10 ⁶ /μL	2.15±0.61 ^b	2.11±0.45 ^c	2.73±0.03 ^a	2.87±0.04 ^a
WBC μL ⁻¹	12350±1050.00 ^b	11975±1475.00 ^b	13650±400.00 ^a	13525±325.00 ^a
Platelet x10 ⁶ /μL	118000±12000.00 ^b	115500±5500.00 ^c	117000±5000.00 ^c	120000±3000.00 ^a
MCV fl	11.59±1.41 ^a	11.51±1.74 ^a	10.44±0.07 ^b	10.63±0.03 ^b
MCH pg	3.69±0.53 ^a	3.75±0.47 ^a	3.21±0.02 ^c	3.49±0.06 ^b
MCHC %	0.32±0.01 ^b	0.33±0.01 ^a	0.31±0.00 ^c	0.33±0.01 ^a
LYM %	55.00±5.00 ^c	55.00±2.00 ^c	63.50±5.50 ^b	67.00±1.00 ^a
HET %	38.50±4.50 ^a	37.50±2.50 ^{ab}	31.00±6.00 ^c	26.00±2.00 ^d
MON %	3.50±1.50 ^a	2.50±0.50 ^c	3.00±1.00 ^b	3.00±0.00 ^b
EOS %	3.00±1.00 ^b	4.00±0.00 ^a	3.00±1.00 ^b	3.00±0.00 ^b
BAS %	0.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00

^{abc} means (SD) on the same row with different superscript are significantly different (p<0.05). PCV: packed cell volume, HB: haemoglobin, RBC: red blood cells, WBC: white blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular volume, MCHC: mean corpuscular haemoglobin concentration, LYM: lymphocytes, HET: heterophil, MON: monocytes, EOS: eosinophil, BAS: basophil.

The serum biochemical properties of blood sample of experimental fish (Table 7) revealed no difference in albumin, glucose and creatinine. The control fish total protein, globulin, triglyceride and ALT were significantly high. Also, BMD45% possess high cholesterol and AST which differs from other treatments.

Table 7: Biochemical blood parameters of *C. gariepinus* juveniles fed bambara meal-based diets

Indices	Control	BMD (15%)	BMD (30%)	BMD (45%)
Total Protein (g/dL)	5.90±1.30 ^a	5.65±0.05 ^{bc}	5.50±0.30 ^c	5.80±1.00 ^b
Albumin (g/dL)	1.15±0.35	1.15±0.05	1.15±0.05	1.15±0.25
Globulin (g/dL)	4.75±0.95 ^a	4.50±0.00 ^b	4.35±0.25 ^{bc}	4.65±0.75 ^{ab}
A/G ratio	0.24±0.03 ^c	0.26±0.02 ^{ab}	0.27±0.01 ^a	0.25±0.02 ^{bc}
Cholesterol (g/dL)	168.50±1.50 ^b	170.00±0.00 ^a	167.50±0.50 ^{bc}	170.50±2.50 ^a
Triglyceride(g/dL)	62.00±14.00 ^a	60.50±15.00 ^b	56.00±6.00 ^c	61.50±16.50 ^{ab}
Glucose (mg/dL)	300.50±3.50	302.00±0.00	300.00±0.00	302.50±5.50
Creatinine (m.mol/L)	0.55±0.05	0.60±0.02	0.60±0.06	0.60±1.00
BUN (mg/dL)	6.10±0.30 ^c	6.60±0.20 ^a	6.35±0.35 ^b	6.35±0.85 ^b
AST (iu/L)	67.00±12.01 ^b	65.00±3.00 ^c	63.50±7.50 ^{cd}	68.50±8.50 ^a
ALT (iu/L)	17.00±1.00 ^a	16.50±0.50 ^{ab}	16.00±1.00 ^b	17.00±2.00 ^a
ALP (iu/L)	178.00±2.00 ^c	187.00±3.00 ^a	176.00±6.00 ^d	185.50±10.50 ^{ab}

^{abc} means (SD) on the same row with different superscript are significantly different (p<0.05)

BUN: blood urea nitrogen, AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphate

The enzymes that assisted in the digestive process were also studied (Table 8). The diet BMD45% was acted upon by the enzyme amylase, maltase, pepsin and lipase with significant difference from other diets, while BMD30% showed the impact of sucrase and chymotrypsin on the diet and different from other diets.

Table 8: Digestive enzymes of *C. gariepinus* juveniles fed bambara meal-based diets

Parameters	Control	BMD (15%)	BMD (30%)	BMD (45%)
Amylase	2.63±0.24 ^d	5.27±0.17 ^c	7.53±0.65 ^b	8.65±0.06 ^a
Sucrase	1.25±0.18 ^c	0.68±0.68 ^d	10.59±0.44 ^a	4.43±0.59 ^b
Maltase	1.32±0.11 ^c	0.49±0.01 ^d	1.89±0.47 ^b	4.24±0.12 ^a
Chymotrypsin	0.50±0.45 ^c	0.18±0.21 ^d	6.24±2.35 ^a	5.09±1.71 ^b
Pepsin	0.21±0.19 ^d	1.59±2.43 ^b	0.85±0.09 ^c	2.09±0.22 ^a
Lipase	28.81±14.22 ^{ab}	25.36±11.19 ^c	18.01±0.30 ^d	29.03±0.45 ^a

^{abc} means (SD) on the same row with different superscript are significantly different (p<0.05)

The intestinal morphology of catfish fed BMD diet (Table 9) indicated that control fish was significantly different to other treatments with respect to villi width, CD, CW and MT, while villi height was highest in BMD15%.

Table 9: Intestinal morphology of *C. gariepinus* juveniles fed bambara meal-based diets

Indices	Control	BMD (15%)	BMD (30%)	BMD (45%)
Villi height	1664.67±112.09 ^c	1863.25±140.64 ^a	1752.12±24.17 ^b	1720.25±26.79 ^{bc}
Villi width	204.92±3.65 ^a	184.25±6.49 ^c	171.63±27.36 ^d	196.27±29.25 ^b
CD	423.80±35.09 ^a	353.81±73.18 ^b	425.89±62.63 ^a	296.30±10.13 ^c
CW	205.87±3.14 ^a	183.32±5.47 ^b	174.72±35.11 ^c	184.38±20.23 ^b
MT	275.25±9.36 ^a	211.80±33.94 ^b	191.34±2.49 ^c	216.65±2.09 ^b

^{abc} means (SD) on the same row with different superscript are significantly different (p<0.05) CD: cryptal depth, CW: cryptal width, MT: muscle thickness.

The histology of liver, kidney and intestine (Table 10) showed that control fish was not affected by the diet. The kidney of fish fed BMD15% and BMD45% was affected by the roasted Bambara meal, while BMD30% affected both the liver and intestine of juvenile catfish.

Table 10: Organ histology of *C. gariepinus* juveniles fed bambara diets

Treatments	Liver	kidney	Intestine
Control	No observable lesions	No observable lesions	No observable lesion.
BMD 15%	No observable lesions	Patchy tubular epithelial degeneration	No observable lesions
BMD 30%	Multiple foci of hepatocellular degeneration	No observable lesions	Moderate atrophy of villi
BMD 45%	No observable lesions	Patchy tubular epithelial degeneration	No observable lesions

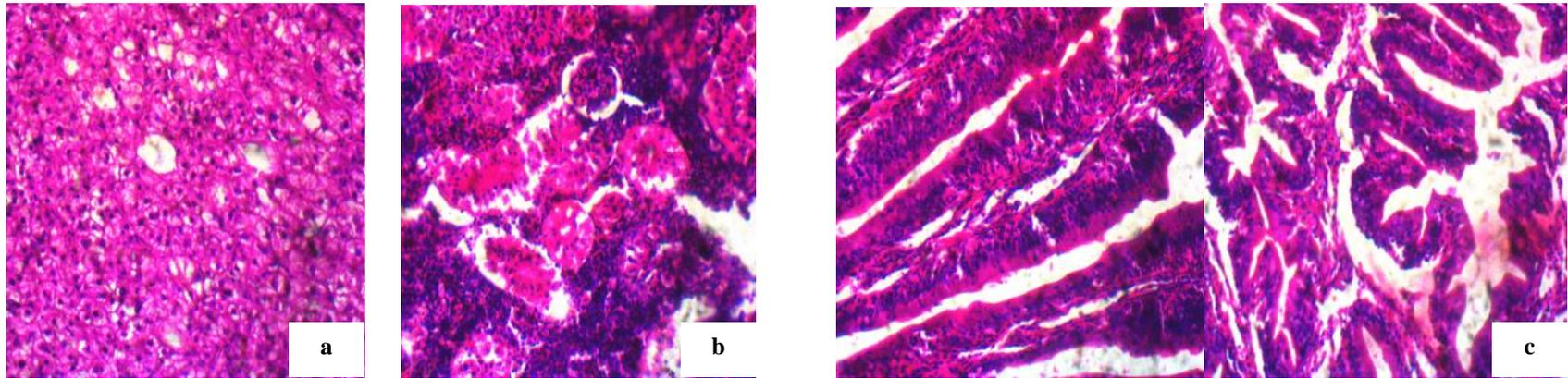


Plate 1: Micrograph organs of Control *C. gariepinus* juveniles fed bambara diets (a) Liver- No observable lesion. (b) Kidney- No observable lesion. (c) Intestine- No observable lesion. HE x400

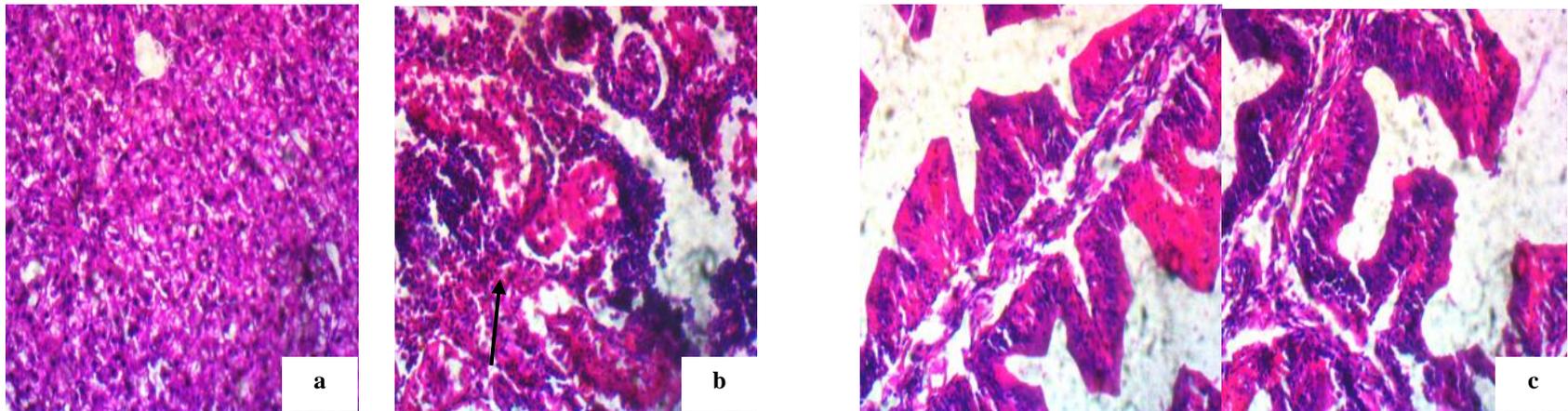


Plate 2: Micrograph organs of BMD 15% *C. gariepinus* juveniles fed bambara diets (a) Liver- No observable lesion. (b) Kidney- Patchy tubular epithelial degeneration (c) Intestine- No observable lesion. HE x400

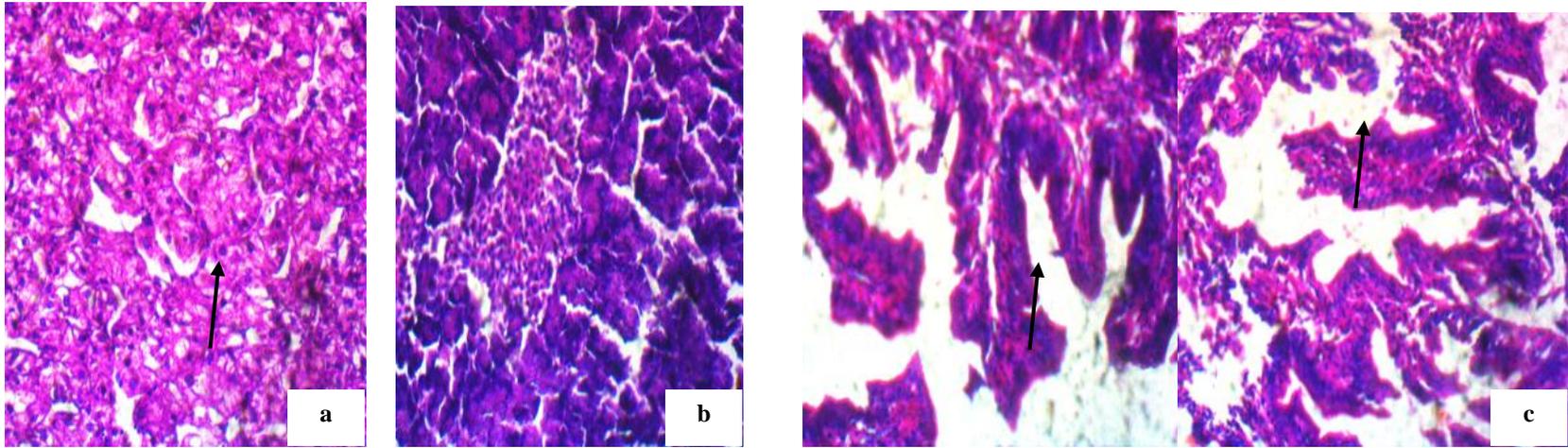


Plate 3: Micrograph organs of BMD 30% *C. gariepinus* juveniles fed bambara diets (a) Liver-Multiple foci of hepatocellular degeneration (b) Kidney-No observable lesion (c) Intestine- Moderate atrophy of villi. HE x400

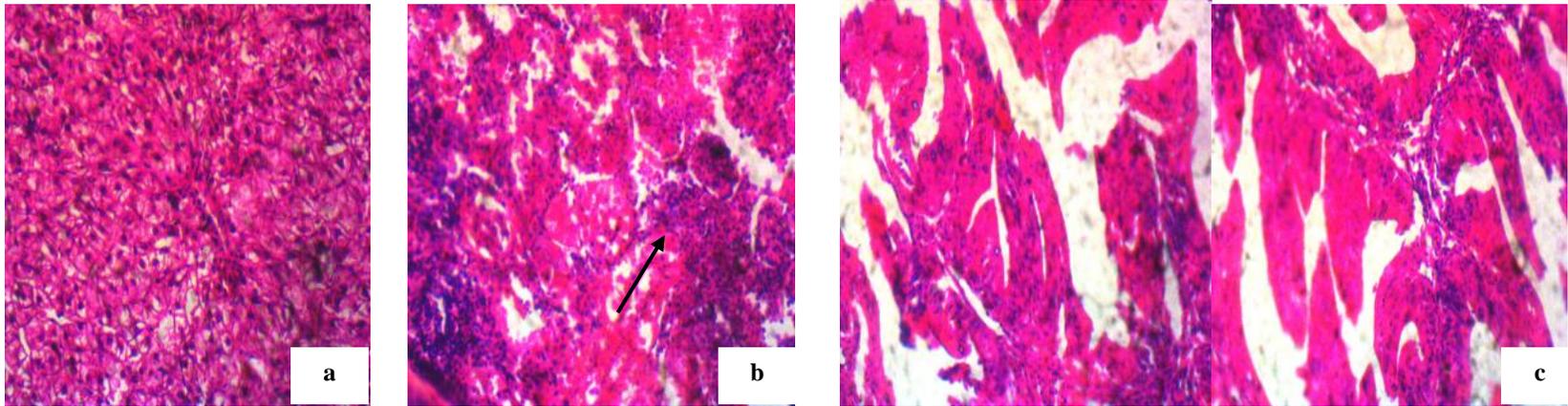


Plate 4: Micrograph organs of BMD 45% *C. gariepinus* juveniles fed bambara diets (a) Liver- No observable lesion (b) Kidney- Patchy tubular epithelial degeneration (c) Intestine- No observable lesion. HE x400

Discussion

Analyzing the nutritional content of feedstuff is a major step in the knowledge of utilization of ingredients. Roasted Bambara seed was evaluated and the levels of ANF was high in the sample and this corroborated the assertion of Bamishaye *et al.* (2011) that even after heat treatment ANF activity still remain high in processed samples of Bambara nut. Fasoyiro *et al.* (2012) reported that roasting is a form of processing which increases the nutritive value of Bambara. Enyidi and Mgbenka (2015) reported crude protein of Bambara seed 21.92%, fat 7.16%, fibre 3.78% and phytate 0.84kg. Orire *et al.*, (2015) reported a ranged in protein of 14% - 24%, CHO 60%, fat 6% - 12% and noted that the seed contains ANF. Lower protein 15.3%, ash 0.38% and fibre 0.34% was reported by Katya *et al.* (2017). Also, Adewunmi and Odeyemi (2018) reported low protein 16% with high fat 9%, fibre 4% and ash 3.8%. According to Aliu and Egwemi (2022) when bambara seed was toasted for 10 minutes, protein 31.5%, fat 9.25%, fibre 4.54%, ash 10.34% higher than for this study and moisture content was similar. Mohammed *et al.*, (2024) noted low protein of Bambara 18%, high CHO65%, and similar fat 6.5% content. Ukwe *et al.* (2025) also reported a lower protein content for Bambara seed meal. The use of biotechnology to advance science was explored by Olude *et al.* (2025) with Bambara concentrate protein 77%, while raw Bambara seed 21% was reported. From the various authors it is clearly defined that processing method affects the proximate and ant-nutritional factors of the seed meal which either qualified it as a protein or carbohydrate source in the experiments.

The nutritional requirement of fish species needs to be met within a stipulated range for optimal fish growth at different life stages. Hence, the diet needs to be formulated to take into cognizance the species and life stages if appropriate recommendation is to be made for feedstuff utilization in fish feed. Also, the substitution and complementarity effect of feedstuff need be considered, with respect to plant or animal protein replacement in the formulated feed. As well as the regime of feeding the diet in question either at a lower or higher rate than the body weight. Tiamiyu and Solomom (2008) formulated a 40% diet of Bambara meal to replace fishmeal at 5% body weight feed. This diet composition was similar to our study. However, Enyidi and Mgbenka (2015) reported nutritional content of the feed increased with increase inclusion of fishmeal from 17.9% to 40.4%. This clearly indicate that fishmeal in diet can affect the substitution effect leading to a good growth response to some experimental treatments over the others. Also, similar trend was observed by Orire *et al.*, (2015) as dietary protein ranged 47% -51% due to FM inclusion. Furthermore, Enyidi *et al.* (2017) noted that inclusion of fishmeal at 50% diet increased diet protein.

Laboratory analysed diet of our study maintained 40% - 42% range of protein, while that of Aliu and Egwemi (2022) ranged 28% - 43%. CF in our study with respect to treatment diet was within the range of treatment diet for Aliu and Egwemi (2022) 3.15% - 3.95%, while fat and ash were higher than our study. This can be adduced to the corresponding content in the toasted BNM ingredient. Salisu *et al.*, (2024) formulated a 40% protein diet with Bambara meal up to 20% inclusion level. With a lower protein requirement for *O. niloticus* Mohammed *et al.* (2024) fed a Bambara meal 25% protein diet formulated at 25%, 50%, 75%, 100%. This revealed clearly that different species, and life stages require different nutritional requirements.

The performance of fish on ingredient testing is noticed visibly in terms of growth performance and utilization of the nutrients in the diet. This goes a long way in making justifiable recommendation which leads to profit for farmers while fish survival in terms of total harvest is brought to the fore with minimal feed cost reduction. Tiamiyu and Solomom (2008) experimented toasted bambara diet included at 10%, 20%, 30%, 40% for catfish fingerlings up to 8weeks period. FCR and WG follow normal convention for 20% and WG decreased at 40% level. Reason for this compared to our study may be as a result of the small size of the fish, which may not allow for the proper digestion of the diet at this stage of life. Likewise, Enyidi (2012) reported reduced growth rate and FCR with increase inclusion of Bambara in the diet which was adduced to inability of catfish to use high carbohydrate present in the diet at the initial stages of life. The post larvae feeding to fingerlings lasted 22 days experiment, while the WG in this study exceeds that reported for the PLF-Fingerling stage (Enyidi 2012), when SBM and Bambara meal were varied with combination of 5 differences. The FCR in F3 (0.71), FCR in F4 (0.72) and FCR in F2 (0.73) reported by Enyidi (2012), were also reported in our study of 15% BMD, 30% BMD and 45% BMD which were similar respectively. While the final weight of Enyidi (2012) was within the range reported for this study (61.94 - 72.51). This showed that reproducibility of experiments using similar ingredients in varied levels is proven.

Oso *et al.* (2013) noted that at 5% body weight feed rate, 75% inclusion Bambara meal showed improved growth and utilisation for catfish, while at 100% WG was drastically decreased and this is similar to Enyidi and Mgbenka (2015); Enyidi *et al.* (2017) when diet was whole bambara ingredient compounded feed. Enyidi and Mgbenka (2015) reported that weight was not improved with BMD at high level 94%. This point out that larval catfish are unable to digest feed without a source of animal protein. However, it was found out that 74%, 59% and 39% showed improved WG and FCR, when 20%FM, 35%FM and 55%FM was included in larvae diet which improved larval growth. Orire *et al.* (2015) reported that catfish fingerlings fed 50% BMD inclusion was

best diet and utilized up to 75% while total inclusion of 100% leads to decrease response. FCR 1.12 - 1.29 range indicated the efficiency of feed conversion to body muscle. However, this were not consistent with the weight reported for the diet as best WG should reveal least FCR. Katya et al. (2017) fed *O. niloticus* in 8 weeks trial with improved weight and SGR up to 25% at best, it can still be included >50% but not to 100%.

Enyidi et al. (2017) reported that BMD can partly substitute Fishmeal and completely replace soybean meal. WG (13g) was least at total inclusion of BMD in diet, when BMD and FM (at equal proportion of 50% each) WG was highest. The SGR (3.94) when BMD10+SBM10+FM40 was fed, similar to BMD 30% BMD 45% in this study which showed that almost 40% FM was in the treatment diet and also FCR (0.82) which was close to this study. While WG of 72g for feed BMD10+SBM10+FM40 corresponds to BMD30% and BMD45% respectively. Adewunmi and Odeyemi (2018) noted growth rate was enhanced at 50% BMD inclusion among treatment and compared significantly with control. This result can be compared to 45% BMD as significant difference was not noticed with respect to weight gain at 15%, 30% and 45% BMD in our study.

Enyidi and Onyenakazi (2019) stated that 45% inclusion of Bambara nut meal resulted in best growth rate and FCR among dietary treatments when replaced with fishmeal followed by 25%, while at 65% growth performance was depressed. Another processing method was employed by Enyidi and Etim (2020) in which fermented BMD and non-fermented BMD replaced fishmeal in a 90days period on fingerlings. F6 non-fermented compared with 20% fermented BMD which is similar to the range reported in our trial BMD15% and BMD30% weight gain. Weight gain is paramount to farmers in achieving the best profit from production which also asserts the findings of Salisu et al. (2024). This indicated that fermented (20% and nonfermented can improve growth. However, fermented 5% showed the best result which iterates the importance of processing on BMD with respect to solid state fermentation. With respect to cost implication/daily feed intake F6 nonfermented need to be considered as farmers may not be able to go through the process of fermentation when it is evident that cost is reduced with non-fermentation.

In a further research, Ishiwu et al. (2020) blended pigeon pea+BMD+Fishmeal and fed catfish while soaking the bambara as a form of processing. The blend of diet with BMD at 20g improved performance followed by at 30g which corroborated the performance at BMD15% and BMD30% in this study. In a study on another catfish breed, Aliu and Egwemi (2022) fed *H. bidorsalis* fingerlings with BMD. Survival rate in treatments was similar to our study. FCR ranged 1.61 - 2.68 in treatment diet which was also similar to Oire et al. (2015) in not following the principle of conversion for feed. The PWG in control, 10%BMD similar to our study for control and BMD15% diet. Feed intake in our study followed a decreasing trend while with Aliu and Egwemi (2022) it revealed mixed variation even when diets were fed at 2% more to this study for catfish. Up to 20%BMD in *H. bidorsalis* gives best result.

In another trial with *O. niloticus* Mohammed et al. (2024) revealed that inclusion of BMD at 50% showed superiority with least FCR and increased WG, while with BMD45% in this study it showed the least performance this may be due to the ability of *O. niloticus* to utilize plant protein better than catfish. Survival of *O. niloticus* fed treatment diet ranged 92-97% similar to 90-95% for this study. It was also noted that growth depression was reported at 100% BMD even for an herbivore species like *O. niloticus* thus it is imperative to further probe the utilization with other processing methods to reduce the intrinsic factors preventing this complete usage in fish diet.

In 12 weeks, experimental period Salisu et al. (2024) fed BMD at 3% body weight replacement for fishmeal at 5%, 10%, 15%, 20%. Final WG was best in control diet and followed a decrease trend to 20% while within the treatment, 5% was best in WG. However, net profit from sold fish and profit index with least incidence cost was reported at 20 g while cost of feed and investment cost was reduced at 20 g. Olude et al. (2025) probed further with the use of concentrate with higher nutritional value. Bambara meal concentrate fed at 0%, 33%, 66% and 100% as fishmeal replacer, it was evident that FCR was low at 33% with the best growth performance among treatment while at 100% decrease was noticed at higher inclusion. It was reported in this 45-day trial, that concentrate of BMD can be used up to 66% with respect to weight gain. The body weight, feeding rate, life stage, species and experimental duration might be responsible for the variation in various experimental literature, as in this study. It can thus be inferred that more factors are responsible for the lack of complete use of the BMD in fish despite the processing methods employed. Saidu et al. (2025) fed BMD up to 50% with highest WG at 20% and least at 50%, this was in line with the findings in this study. Ukwe et al. (2025) reported that WG was highest at total replacement of soybean with bambara meal and least in control soybean diet. Reason for this may be adduced to the sub-adult catfish used as well as the complementarity effect of groundnut cake meal in the diet, while the sub-adult fish have developed digestive enzymes capable of utilizing BMD at higher inclusion levels.

Utilization of nutrients in feed need to culminate in muscle protein deposition as evidenced with weight gain. This will boost the interest of researchers in finding the best feed that can translate into weight gain as well as nutritional quality of the farmed fish, while assuring consumers of the quality of the product in terms of nutritional availability of fish fed from farm-raised fish. Tiamiyu and Solomom (2008) reported that the carcass

analysis protein of treatment fish fed BMD was increased above initial and control which was in line with our findings. Oriri *et al.* (2015) also noted highest protein of carcass in 50% and 75% (61.31%) similar to BMD 45% inclusion in our study indicating that at higher level BMD in diet protein deposition is possible. Katya *et al.* (2017) also revealed carcass protein at 25% and 100% was higher than other treatment, which further brings out the fact that BMD increase carcass protein at 100%. While, Adewunmi and Odeyemi (2018) showed carcass protein was enhanced at 50% (68.2%) similar to high value in 45% of this study. Aliu and Egwemi (2022) stated carcass analysis showed that treatment fish deposited more protein, whilst Olude *et al.* (2025) confirmed increased muscle protein at 33% and 66%. These findings noted that even with reduced growth rate, improved flesh protein can be achieved for consumer's satisfaction which asserts our result.

Body fluid of fish can be used to infer the health response of fish to diet with importance on specific ingredient inclusion in diet. This will go a long way to ascertain the implication of the diet on survival and health parameters of the fish. Blood parameters are indicative of the wellbeing of fish, response to stress and immunity level. Jimoh *et al.* (2020) fed catfish with *Luffa cylindrica* seedmeal as soybean replacement. The RBC, Hb in BMD30% and BMD45% was within range reported, with RBC in BMD30% similar with D515T (2.73) and PCV in control, BMD15%, BMD30% within range, while PCV in BMD45% higher than Jimoh *et al.* (2020). While the blood chemistry parameters in this study (globulin, cholesterol, glucose, total protein) were higher than values of Jimoh *et al.* (2020). The BMD was shown to have varied parameters which was checked with the findings of Olude *et al.* (2025). The blood parameters lymphocyte at BMD30% and BMD45% similar to Olude *et al.* (2025) when concentrate BNM 66% and 100%, eosinophils was significantly compared in both studies. The PCV of BMD45% vs 66% concentrate BNM (31.5); BMD30% vs 100% concentrate BNM (26.5), Hb of BMD 45% vs 66% concentrate BNM (10.47). TP in concentrate BNM100% compared with the dietary treatments of this study. Globulin in concentrate BNM lower to this study while albumin was constant in this study and lower to concentrate BNM. Higher glucose was recorded in this study to lower values in concentrate BNM, while AST, ALT is lower to BNM concentrate. The reduced blood and serum activity in this study to others can be attributed to the high ANF in dietary ingredient. However, fish survival was not significantly different among treatment, but different from the control treatment.

Digestive enzymes serve catalytic role in the catabolism and final metabolism of diet in fish, whilst uptake of nutrient is envisaged through the intestinal morphological organs responsible for nutrient absorption. Enyidi (2012) also noted the inability of catfish to digest carbohydrate rich feed, which is evident in the carbohydrate content as reported by various scientist. Also, Enyidi and Mgbenka (2015) reported that larval catfish find it difficult to digest BMD diet with little or no animal protein source in diet. And that the ANF especially phytate content in BMD and low-fat content may hamper growth. Olude *et al.* (2025) reported amylase activity was highest at concentrate BNM 33% and fall within the range of the same activity in BMD30% and BMD45% of this study. Reason for this compared to our study can be as a result of the not too well-formed digestive organs which can assist in secretion of the enzymes.

Nutrient absorption is carried out by the intestinal structures in fish, after the digestion is completed and assimilation into the blood stream is imminent for normal physiological growth. The structures in the intestine allows for absorption to take place via the villi height or width, and cryptal depth or width. In Table 9 there was increase in cryptal depth, cryptal width and villi width in control diet, this account for the increased WG and reduced FCR, while there is significant difference ($P < 0.05$) compared to dietary inclusion levels.

The histology report as evident in Table 10 and Plate 1 - Plate 4 revealed that inclusion of BMD treatment in catfish diet showed a form of alteration to liver, kidney and intestine. According to Rastovic *et al.* (2011) new feed of plant origin can impart negatively on fish digestive system, stating that the liver and intestine are the key organs of digestion and absorption of nutrients in feed. They further stated that histopathological changes in the intestine may vary depending on the species and feed used in the experiments. Histological changes in the liver are easily recognized if the food used is not adequate. The liver is considered as a good indicator of nutritional pathology due to its function in metabolizing products. Rastovic *et al.* (2011) evaluated effects of different fish feed on production traits of the common carp, and did not observe any intestinal changes or other nutritional pathologies, noting that carp has a better tolerance to the presence of nutritive ingredients in food, and this claim of tolerance is specific for the species. This was in line with the control diet of this trial as the treatment showed no visible distortion of organs. The inclusion of fishmeal above 30% showed no intestinal distortion in control, BMD15% and BMD45% aligned with Rastovic *et al.* (2011) who noted in the experiment with Egyptian Sole that 30% fishmeal replaced with soybean meal had no pathological changes in intestines when observed. In a similar trend Jimoh *et al.* (2020) observed the liver and kidney to show various degree of alterations when catfish was fed *L. cylindrica* seed meal. This study noted that the inability of catfish to digest above certain limit of BMD diet may be responsible for the organ responses to varied alterations and coupled with the phytochemicals in the test ingredient even after processing which may be the cause of the disorder noting that growth decrease was noticed also with authors cited.

Conclusion

The utilization of roasted Bambara seed meal can be included in diet of catfish up to 45% inclusion level especially with consideration of percentage weight gain which will enhance profitability as well as nutritional quality of the fish when consumer preference and acceptability is a factor of marketing the harvested fish.

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