

RESPONSE OF *Clarias gariepinus* TO DIETARY INCLUSION OF *Cajanus cajan* (L.) HUTH

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ABSTRACT

High feed cost remains a major constraint to fish farmers, with fishmeal being the highest contributor to production costs and sustainability issues in fish farming. This study substituted fishmeal with *Cajanus cajan* (pigeon pea [PP]) in the diet of *Clarias gariepinus*. Antinutritional factors and proximate composition of raw and processed PP and diets were determined. 120 post-juvenile *C. gariepinus* (average initial weight: 77.5 ± 0.12 g) were fed four (4) diets containing 0%, 25%, 50% and 75% autoclaved pigeon pea (APP) as replacement for fishmeal. Fish were fed 5% of their body weight, by broadcasting for 49 days. Growth performance, length-weight relationship (LWR), relative condition factor (Kn), haematology and serum mineral were determined using standard methods. no statistically significant difference ($p > 0.05$) among treatments though highest weight increase was recorded in experimental groups fed 25% and 50% (256.3 ± 104.74 g and 144.7 ± 29.04 g) compared to the control (105.0 ± 31.24 g). The feed conversion ratio was lower in the 25% and 50% (0.9 ± 0.24 and 1.4 ± 0.37) groups than the control (1.9 ± 0.70). Positive allometric growth pattern ($b > 3$) was calculated in fish-fed PP diets. The lowest 'Kn' < 1 (0.7992 ± 0.03) was observed in fish fed 75% PP. Hb, and RBC were significantly ($p > 0.05$) lower in fish-fed diets, with 75% inclusion level more than in the control and other treatments. The highest PCV and Hb values were recorded in 25% and 50% inclusion diets. Serum minerals (sodium, potassium, calcium, magnesium, and phosphorus) significantly increased in the groups fed graded levels of PP compared to the control group. In conclusion, inclusion of pigeon peas (PP) at 25% to 50% increased the weight of experimental fish and reduced the feed conversion ratio. PP did not show any deleterious effect on the blood parameters of the fish. The study recommends 25% and 50% inclusion of PP.

Keywords: Sustainable fish production, *Clarias gariepinus*, produce, growth performance, haematology

INTRODUCTION

The African catfish (*Clarias gariepinus*) is widely farmed in Africa due to its wide acceptance, rapid growth and resistance to adverse weather conditions and handling stress —(Abraham *et al.*, 2018; Dauda *et al.*, 2018). In many African and niche export markets, African catfish (*Clarias gariepinus*) enjoys a favourable market value and strong demand, with both local and international markets—often comparable or higher than commonly-cultured tilapia, particularly when value-added (Oyewole *et al.*, 2023; Pasch and Palm, 2021). The African catfish attains maturity and market size between 5 – 6 months. The omnivorous feeding habit of the African catfish (*Clarias gariepinus*) allows it to utilize a wide range of feedstuffs, making it easier to raise in captivity compared to other aquaculture species such as tilapia or common carp commonly farmed in Nigeria. Among the various feedstuffs used in aquaculture, the production of *Clarias gariepinus* largely depends on the availability of high-quality feed, which represents approximately 70–80% of the overall production cost. Fishmeal, an essential protein source in the diets of *C. gariepinus* (Balogun *et al.*, 2016), can make up to 12 – 60 % of the species' diet depending on the stage of its life cycle (food fish or table size, fingers or fry). Many aquafeeds contain fishmeal and fish oil, which are derivatives of wild fisheries. The value of these ingredients are high and have

high food–feed trade-offs, therefore, further expansion of the aquaculture industry will most likely exert even greater pressure on ocean fisheries. In fish feed production, fishmeal and fish oil are expensive primarily because they are derived from limited wild fish resources and face strong global demand. The dependence of omnivorous and carnivorous species on these ingredients further drives up their use and market value (Caruso, 2015); thus, alternatives are needed. An alternative source of protein to fishmeal in fish feed is important not only because fish is a finite resource but because alternatives will reduce the cost of aquaculture management/production and environmental pollution, including reducing carbon footprints and burden on wild resources.

The aquaculture sector is reported to be the fastest-growing agricultural sub-sector, and this has put a notable load on forage fish resources. Moreover, feeding fish as feed or as food poses a kind of tension and trade-offs between alternative uses of the product. With the growth recorded in the sub-sector, continued reliance on these finite, environmental, and economically unsustainable wild fish resources will harm the teeming world population more than good. In line with this, several kinds of research have been conducted on the partial and complete replacement of fishmeal in fish feed with various

non-conventional feed ingredients, including aquatic plants (Ahmed et al., 2023; Ali & Kaviraj, 2018), terrestrial plants – (Cai et al., 2022; Daniel, 2018; Dorothy et al., 2018; Egerton et al., 2020), insect meal (Gougbedji et al., 2022; Prakoso et al., 2022; Terova et al., 2021), and poultry by-product meal – (Djissou et al., 2016; Qiu et al., 2023; X. Wang et al., 2023; Yu et al., 2023). Of these alternative protein sources, plant-based sources remain the preferred option for substituting fishmeal in fish feed (Mahmud et al., 2025). Limited research has been executed on substitution of fishmeal with pigeon pea (*Cajanus cajan*) seed in *Clarias gariepinus* diets (Yagoub et al., 2023), although pigeon pea has been investigated as a substitute for soybean meal in the diets of *Oreochromis niloticus* and other fish species. (Ganzon-Naret, 2014; Jamabo et al., 2020; Obasa et al., 2003; Solomon et al., 2017; Hamed et al., 2013).

Pigeon pea is of marginal use as a primary livestock feed component due to anti-nutritional factors (ANFs), low nutrient digestibility in its raw form, and deficiencies in key amino acids Gwata et al. (2025). It is a widely grown tropical and subtropical legume for its edible seeds. Pigeon pea is a fast-growing, hardy, and drought-tolerant crop that is readily available and less competitive with human food crops, making it a promising alternative protein source for livestock and aquaculture feed. (Sarkar et al., 2020). Despite its potential value, the presence of antinutritional factors such as saponins, phytates, and trypsin in the seed limit its nutritive value. However, Samtiya et al. (2020) reported that processing of this crop reduces the levels of these antinutrients, thereby increasing nutrient digestibility. This leguminous seed is a good source of plant protein, and its amino acid profile is well-balanced. It may thus be a substitute for fishmeal in fish diets. The raw pigeon pea seeds contain 26.25% crude protein. Nsa et al., (2011) documented boiling and soaking of the seeds in water to increase their crude protein content and reduce antinutritional content – (Adeleke et al., 2017).

In light of the above, substituting fishmeal with pigeon pea or other plant-based proteins can contribute to reducing fish production costs, lowering carbon emissions, and mitigating environmental pollution such as eutrophication. as fishmeal production depends on removal of fish from the wild resulting in possible overfishing and production of nutrient-rich effluent (Naylor et al., 2021). Pigeon pea cultivation on the other hand, is less resource intensive and contribute to soil fertility. Though, processing of the pigeon pea seed plays a key role in nutrient availability of the seed. In addition, a reduction in the cost of fish production will bring about an increased supply of the much-needed animal protein, which has been documented to be low compared to demand (Animashahun et al., 2018). The present study investigated the use of pigeon pea (*Cajanus cajan*) seed meal as an underexploited plant protein source as substitute for fishmeal in *Clarias gariepinus* feed formulations.

MATERIALS AND METHODS

Ethical approval statement

The study adopted methods for handling experimental fish that are in line with international and local regulations guiding the use of animal subjects during experimentation. The Landmark University Ethics Committee gave approval with approval number LMUIREC/ACSC/002/2021.

Collection and processing of selected plant

Cajanus cajan was sourced from the local market in Omu-Aran, Kwara State, Nigeria. Plant seeds were processed by autoclaving and soaking. Two Hundred and Fifty grams of raw *Cajanus cajan* was autoclaved. Raw PP seeds were placed in the autoclave tray, pressure-cooked at 121°C for 15 minutes, and allowed to cool at room temperature. The same quantity was percolated in a calibrated bowl with 1.5 litres of tap water for 3, 6, and 9 days. The seeds were rinsed in clean tap water, drained, air dried and milled into a fine flour using an electronic blender (model: BLG-402-1.5 L). The soaked and autoclaved samples were tested for antinutrients and the treatment with reduced or eliminated values was selected for the feeding trial.

Chemical evaluation of processed seeds and experimental diets

Determination of proximate composition

Processed seeds were taken to the laboratory for determination of proximate composition using the methods described by AOAC (1990) and phytochemical assay (AOAC, 2005; Lin & Tang, 2007)

The proximate composition of the samples (raw pigeon pea [PPR], autoclaved pigeon pea [PPA], 3-day soaked pigeon pea [PPS3], 6-day soaked pigeon pea [PPS6], 9-day soaked pigeon pea [PPS9], fish meal [FM], and the experimental diets) was determined through standard analytical procedures assessing moisture, ash, ether extract, crude protein, crude fibre, and fibre fractions, alongside phytochemical assays. Moisture content was measured by oven-drying samples at 105°C and calculating weight loss, while ash content was obtained after incineration at 550°C. Fat content was determined using the Soxhlet extraction method with n-hexane as the solvent, and crude protein was analyzed via the Kjeldahl method, converting total nitrogen to protein using a 6.25 factor.

Determination of crude fibre and fibre fractions

Crude fibre was measured by sequential acid and alkali digestion followed by ashing, whereas fibre fractions such as ADF, cellulose, lignin, and hemicellulose were determined using acid and neutral detergent solutions.

Qualitative Phytochemical screening

Phytochemical assays, including tests for alkaloids, tannins, flavonoids, saponins, phenols, steroids, and terpenoids, were conducted on extracts from raw, soaked, and autoclaved pigeon peas, using qualitative indicators like color change or precipitate formation to confirm the



presence of each compound..

Quantitative Phytochemical assay

The total phenolic and tannin contents were determined using the Folin–Ciocalteu method, with gallic acid as the standard. For calibration, gallic acid solutions (6.25–50 µg/mL) were prepared in 50% methanol, reacted with 1.5 mL of Folin–Ciocalteu reagent, followed by 4 mL of 20% Na₂CO₃, and made up to 25 mL with distilled water. After 30 minutes of incubation at room temperature, absorbance was measured at 765 nm using a UV/VIS spectrophotometer against distilled water as a blank. For sample analysis, 1 g of methanolic extract was macerated with 50% methanol, filtered, and diluted to 100 mL. The same reagent and incubation procedures were followed, and absorbance was recorded at 765 nm for phenolics and 740 nm for tannins, using distilled water as the blank.

Experimental diets

After the chemical evaluation, the autoclaved pigeon pea was selected and used as the test ingredient to compound the experimental diet in partial replacement for fish meal with amino acid supplementation. The choice of the

reference ingredient was because autoclaving totally eliminated the presence of saponin content in the pigeon pea in this study and as reported by Liu *et al.*, (2020). Also, autoclaving increases the digestibility of legumes, as reported by Drulyte & Orlien (2019)..

Four isonitrogenous diets (36% crude protein) were formulated using Pearson square method with autoclaved pigeon pea (APP) included as one of the protein ingredients at 0% (Control diet CD), 25% (APPD 25%), 50% (APPD 50%), and 75% (APPD 75%) levels (Adesina *et al.*, 2017). The basal diet formulation contained fish meal and soybean meal (defatted) only as dietary protein sources. All dry diet ingredients (Table 1) were mixed thoroughly before adding vitamin premix. Oil was incorporated into the dry ingredients, followed by the addition of bread flour that had been cooked in 600 mL of water and cooled. The resulting feed mixture was pelletized, air-dried, and subsequently stored in plastic bags at the feed mill until use. Nutrients in experimental diets were analysed for use (AOAC, 1990).

Table 1: Overall constitution of experimental feeds

INGREDIENTS %	RD	APPD (25%)	APPD (50%)	APPD (75%)
FM	20.9	16.80	12.66	7.28
SBM	40.18	44.82	50.64	58.21
PP	0	5.60	12.66	21.83
M	15.46	12.39	8.02	2.35
WB	15.46	12.39	8.02	2.35
BM	2.5	2.5	2.5	2.5
Fish premix*	2.0	2.0	2.0	2.0
Lysine	1.0	1.0	1.0	1.0
Methionine	1.0	1.0	1.0	1.0
Salt	0.25	0.25	0.25	0.25
Groundnut oil	1.0	1.0	1.0	1.0
Chromic oxide	0.1	0.1	0.1	0.1
Toxin binder	0.15	0.15	0.15	0.15
Total	100	100	100	100.02

FM= Fishmeal, SBM= Soya bean meal, PP= Pigeon pea, M= Maize, WB= Wheat bran, BM= Bone meal, RD= Reference diet, APPD= Autoclaved pigeon pea. Mean ± SE on the same row with different superscript are significantly different (p < 0.05).

***Fish premix composition: Vitamin:** Vitamin A 4000 000 IU, Vitamin E 40 000 mg, Vitamin D3 400 000 IU, Vitamin B1 3000 mg, Vitamin B2 4000 mg, Vitamin B6 3000 mg, Vitamin B12 8000 µg, , Vitamin K3 1200 mg, Nicotinic acid 30 000 mg, ca-pantothenate 10 000 mg, p-amino benzoic 20 000 mg, folic acid 1000 mg, Biotin 300 000 µg, Vitamin C 50 000 mg. **Minerals:** Iron 25 000 mg, Copper 1200 mg, Manganese 4000 mg, Zinc 6000 mg, Iodine 400 mg, Selenium 20 mg, Cobalt 200 mg.

Collection of experimental fish and experimental design

A total of 120 healthy *Clarias gariepinus* post-juveniles, with an initial mean weight of 77.5 ± 0.12 g, were obtained from a reputable fish farm in Omu-Aran. The fish were fed twice daily—morning and evening—with the experimental diets. Borehole water served as the water source, with 40 L supplied to each aquarium through a

connected piping system. Growth performance assessments were conducted at commencement and on a weekly basis while blood parameters were analyzed at the start of the study and bi-monthly. The experiment followed a Completely Randomized Design (CRD), with fish randomly assigned to four dietary treatments of 30 fish each, and each treatment replicated three times.

Data collected

Investigation of Growth Performance

The growth performance of study animals was evaluated by the use of growth indices (Falaye *et al.*, 2015).

Mean weight gain (MWG): The total weight gain of fish was calculated using the formula

$$MWG = W_2 - W_1$$

Where,

W2 = Final weight

W1 = Weight at the commencement of the feeding trial



Average weight gain (AWG): AWG was calculated using the formula;

$$AWG = \frac{\text{mean weight gained}}{\text{days of feeding}}$$

Percentage Weight Gain (PWG): PWG was calculated using the formula;

$$PWG = \frac{\text{Mean weight gain}}{\text{Initial weight}} \times 100$$

Relative growth rate (RGR): RGR was estimated using the equation:

$$RGR = \frac{W_2 - W_1}{T_2 - T_1}$$

Where

W₂ – W₁ is Weight gain at time T₂

T₂ – T₁ is the Number of experimental days

Specific growth rate (SGR): SGR was calculated as follows;

$$SGR = \frac{\log W_2 - \log W_1}{T_2 - T_1}$$

Where

W₂ = Final weight at time T₂ by days

W₁ = Initial weight at time T₁ by days

T₂ - T₁ = Number of experimental days

Loge = Natural logarithm to base e.

Feed conversion ratio (FCR): The feed conversion ratio is calculated as

$$FCR = \frac{\text{Weight of feed fed}}{W_2 - W_1}$$

Where:

W₂ – W₁ is weight gain for the time period

Gross Feed Conversion Efficiency (GFCE): GFCE was calculated using the formula;

$$GFCE = \frac{1}{FCR}$$

Length-Weight Relationship (LWR) Determination

Length Weight Relationship (LWR) of study fish was appraised using the equation described by Abdul *et al.*, (2016):

$$W = aL^b$$

Where:

W = fish weight (g),

L = fish total length (TL) (cm),

a = constant

b = length exponent

Linearisation of the equation by logarithmic transformation was as described by Oliva-Paterna *et al.*, (2009) to give:

$$\log W = \log a + b \log L$$

“a” and “b” values were obtained from the linear regression of the LWRs of the study fish. The extent of reliability, or squared correlation (r²), was enumerated.

Relative condition factor

The relative condition factor (Kn) was estimated using the formula described by (Cren, 1951).

$$Kn = \frac{W}{aL^b}$$

Where

Kn = Relative condition factor

W = Observed weight of fish (g)

aL^b = Calculated weight of fish (g)

Haematological assay of experimental fish

Blood samples were collected as described by Lawrence *et al.* (2018) and haematological assay carried out according to Okeniyi *et al.* (2020) The period between fish removals from the culture environment to completion of blood sampling was ≤ 3 minutes. The blood sample was estimated for Packed Cell Volume (PCV), Haemoglobin (HB), Red and White Blood Cell (WBC) and WBC differential (heterophils, lymphocytes, monocytes, eosinophils and basophils).

Serum chemistry

One millilitre of whole blood sample was centrifuged at 1100 rpm for 5 min, and the serum was separated. The serum was then used to analyse for levels of potassium, chloride, sodium, phosphorus, manganese, calcium, and magnesium (Barham & Trinder, 1972; Maruna, 1957; Skeggs *et al.*, 1964; Tietz, 1976; Trinder, 1951) using the colourimetric method as described in the Teco kit (Teco Diagnostics, California, USA).

Statistical Analysis

Proximate composition, growth data, condition factor, and blood and serum indices were subject to the One-way analysis of variance procedures using the Statistical Package for Social Sciences (SPSS) version 20. The length-weight relationship and water parameters were analysed using Microsoft Excel. Significant means separation was achieved by using the least significant difference test and Dunnett's test of the same package at 95% probability.

RESULTS

Proximate composition

The result of the proximate composition of the raw and processed pigeon pea (PP) is shown in Table 2. Crude protein (CP) content was highest in the processed pigeon pea (PP) soaked for six days (PPS6), followed by the autoclaved PP. Crude protein content was generally very low in the test ingredients compared to the CP content of fishmeal. There was a significant difference at the 95% probability in the CP content between raw PP and autoclaved, sixth and ninth-day soaked PP. Ether extract (EE) and crude fibre (CF) were significantly (p < 0.05) higher in the raw PP than the processed PP. The proximate composition of the experimental diets is shown in Table 3. Crude protein (CP) decreased with increased inclusion of the test ingredient. However, there was no significant difference (p > 0.05) between the control and 25% inclusion diets. No significant difference was observed at the 95% probability in moisture, ash and EE.



Table 2: Proximate components of test and reference ingredients

Parameters	Sample					
	PPR	PPA	PPS3	PPS6	PPS9	FM
Moisture (%)	9.0 ± 0.58 ^a	12.5 ± 1.15 ^{bc}	11.0 ± 0.00 ^b	10.6 ± 0.00 ^{ab}	13.6 ± 0.14 ^c	7.8 ± 0.14 ^a
Crude Protein (%)	13.4 ± 0.11 ^a	16.6 ± 0.41 ^c	12.6 ± 0.09 ^a	17.0 ± 0.36 ^c	15.1 ± 0.20 ^b	66.4 ± 0.86 ^d
Ether Extract (%)	18.5 ± 0.58 ^d	8.0 ± 0.29 ^a	8.0 ± 0.29 ^a	8.5 ± 0.00 ^a	9.5 ± 0.29 ^b	11.1 ± 0.08 ^c
Crude Fibre (%)	9.5 ± 0.72 ^c	6.9 ± 0.55 ^b	6.7 ± 0.68 ^b	7.4 ± 0.29 ^b	6.5 ± 0.00 ^b	0.00 ± 0.00 ^a
Ash (%)	4.8 ± 0.43 ^a	4.0 ± 0.58 ^a	3.5 ± 0.29 ^a	3.2 ± 0.72 ^a	4.8 ± 0.14 ^a	14.8 ± 0.72 ^b
Nitrogen Free Extract (%)	44.0 ± 0.40 ^b	52.0 ± 1.06 ^{cd}	58.3 ± 0.19 ^d	53.4 ± 0.79 ^d	50.4 ± 0.20 ^c	0.00 ± 0.00 ^a

PPR= Raw pigeon pea, PPA= Autoclaved pigeon pea, PPS3= Pigeon pea soaked for 3 days, PPS6= Pigeon pea soaked for 6 days, PPS9= Pigeon pea soaked for 9 days. Mean ± SE on the same row with different superscript are significantly different ($p < 0.05$).

Table 3: Proximate composition of experimental feeds

INGREDIENTS %	CD	APPD (25%)	APPD (50%)	APPD (75%)
Moisture (%)	12.8 ± 1.59	12.5 ± 1.44	17.3 ± 1.30	12.5 ± 1.44
Crude Protein (%)	36.0 ± 0.00 b	35.6 ± 0.14 b	34.7 ± 0.15 a	34.1 ± 0.27 a
Ether Extract (%)	20.8 ± 0.14	21.3 ± 0.43	19.0 ± 1.44	20.3 ± 0.14
Crude Fibre (%)	3.5 ± 0.20 a	4.7 ± 0.03 a	7.2 ± 0.38 b	7.0 ± 0.46 b
Ash (%)	11.8 ± 0.72	12.3 ± 1.01	10.5 ± 1.44	10.3 ± 1.01
Nitrogen Free Extract (%)	15.3 ± 2.25	13.8 ± 2.19	11.4 ± 1.82	15.9 ± 3.33

Table 4, which showed the fibre fractions of the raw and processed PP, revealed that cellulose was significantly ($p < 0.05$) higher in the raw PP than processed PP while the reverse was observed for lignin content.

Table 4: Fiber fractions of test ingredient

Fractions	Samples				
	PPR	PPA	PPS3	PPS6	PPS9
Cellulose	7.0 ± 0.29 ^c	2.0 ± 0.00 ^b	2.0 ± 0.00 ^b	2.5 ± 0.29 ^{ab}	1.5 ± 0.29 ^a
Lignin	2.8 ± 0.14 ^a	9.0 ± 0.29 ^b	9.3 ± 0.14 ^b	13.8 ± 0.14 ^c	11.5 ± 0.87 ^c
Hemicellulose	6.0 ± 0.00 ^c	2.00 ± 0.00 ^a	4.0 ± 0.29 ^b	3.8 ± 0.72 ^b	8.0 ± 0.29 ^d

PPR= Raw pigeon pea, PPA= Autoclaved pigeon pea, PPS3= Pigeon pea soaked for 3 days, PPS6= Pigeon pea soaked for 6 days, PPS9= Pigeon pea soaked for 9 days. Mean ± SE on the same row with different superscript are significantly different ($p < 0.05$).

Phytochemical composition

The qualitative and quantitative screening of PP is shown in Tables 5 and 6. For qualitative screening, total phenols and tannin are present in the raw and processed PP. PP9 showed the presence of all phytochemicals except for flavonoids. Saponin was absent in PPA. Quantitative analysis showed that total phenols and tannin were significantly ($p < 0.05$) lower in the autoclaved PP.

Table 5: Qualitative phytochemical composition of test ingredients

Phytochemicals	Samples				
	PPR	PPA	PPS3	PPS6	PPS9
Saponins	++	-	++	+	+
Phenolic	+	+	+	+	+
Tannin	+	+	+	+	+
Steroid	-	-	-	-	++
Terpenoid	-	-	-	-	++
Alkaloid	-	-	-	-	+
Flavonoid	-	-	-	-	-

PPA= Autoclaved pigeon pea, PPR= Raw pigeon pea, PPS3= Pigeon pea soaked for 3 days, PPS6= Pigeon pea soaked for 6 days, PPS9= Pigeon pea soaked for 9 days.

+ / ++ Light & heavy presence of chemical

- Absence of chemical

Table 6: Quantitative composition of phenols and tannin in raw and processed Pigeon pea

Phytochemicals	Samples				
	PPR	PPA	PPS3	PPS6	PPS9
Phenol (µl/ml)	8.6 ± 0.5 ^b	3.2 ± 0.78 ^a	8.7 ± 0.95 ^b	9.6 ± 0.58 ^b	9.4 ± 0.17 ^b
Tannin (µl/ml)	8.5 ± 0.89 ^b	6.5 ± 0.40 ^a	10.0 ± 0.14 ^b	10.1 ± 0.06 ^b	10.0 ± 0.09 ^b



PPA= Autoclaved pigeon pea, PPR= Raw pigeon pea, PPS3= Pigeon pea soaked for 3 days, PPS6= Pigeon pea soaked for 6 days, PPS9= Pigeon pea soaked for 9 days. Mean ± SE on the same row with different superscript are significantly different (p < 0.05).

Growth assessment

The growth capability of *C. gariepinus* fed experimental feeds is summarised in Table 7. There was no significant difference (p > 0.05) between the treatment effects. Weight gain was observed to decrease with an increase in the PP inclusion. Though not statistically different, a numerical increase was observed in mean weight gain in the 25% and 50% inclusion diets. The lowest growth rate was observed in the 75% inclusion diet. The reversed trend was noticed in the FCR and GCFE of the treatments.

Table 7: Growth capability of *Clarias gariepinus* post juveniles fed experimental feeds

Growth parameters	Feeds			
	CD (0%)	APPD (25%)	APPD (50%)	APPD (75%)
Experimental period (Days)	49	49	49	49
Fish stocked	30	30	30	30
Initial weight (g)	720.0 ± 47.26	683.3 ± 16.66	750.0 ± 50.00	795.0 ± 29.30
Final weight (g)	825.0 ± 37.00	939.7 ± 116.35	894.7 ± 22.58	883.7 ± 41.53
Mean Weight Gain (g)	105.0 ± 31.24	256.3 ± 104.74	144.7 ± 29.04	88.7 ± 14.42
Percent Weight Gain (%)	15.0 ± 4.76	37.1 ± 14.68	20.0 ± 4.87	11.1 ± 1.48
Relative Growth Rate (g)	2.1 ± 0.64	5.2 ± 2.14	3.0 ± 0.59	1.8 ± 0.30
Specific Growth Rate (g)	2.9 ± 0.20	2.9 ± 0.05	2.9 ± 0.01	2.9 ± 0.02
Feed Conversion Ratio	1.9 ± 0.70	0.9 ± 0.24	1.4 ± 0.37	2.1 ± 0.27
Gross Feed Conversion Efficiency (%)	64.6 ± 18.92	136.1 ± 42.45	83.9 ± 18.32	50.2 ± 6.47
Survival (%)	86.7	86.7	86.7	90

CD= Control diet, APPD= Autoclaved pigeon pea Diet. Mean ± SE on the same row without superscript are not significantly different (p > 0.05)

Length-weight relationship

The length-weight relationship and condition factor of the experimental fish are represented in Figure. The highest and lowest LWR parameter (b) values were recorded in the 75% inclusion diet (5.5165) and control diets (2.7729), respectively. The reverse was the case for 'a' (0.000002 and 0.0151 respectively). The control group

showed a negative allometric growth pattern ('b' < 3). Fish in the PP-included diets showed positive allometric growth patterns ('b' > 3). Relative condition factor (Kn) values approximately or > 1 in all treatments indicate that fish were in good health condition and compared well with fish fed the control diets.

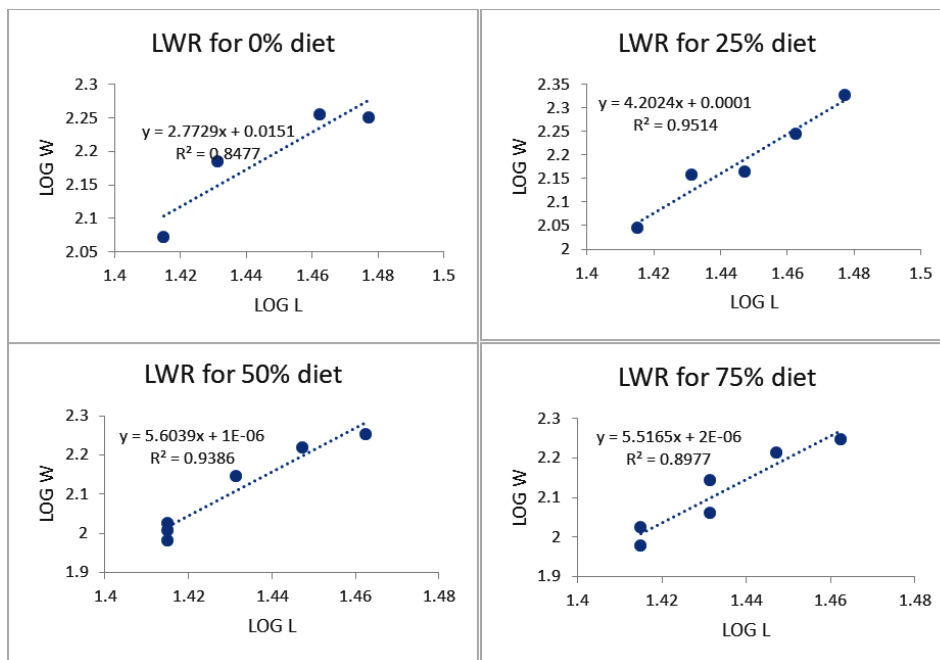


Fig 1: Length-Weight Relationship of *Clarias gariepinus* fed graded levels of autoclaved PP



Haematology and Serum Mineral Assay

The full blood count of *C. gariepinus* fed graded levels of pigeon peas is recorded in Table 8. Hb, and RBC were significantly ($p > 0.05$) lower in fish-fed diets, with a 75% inclusion level more than in the control and other treatments. The highest PCV and Hb values were recorded in 25% and 50% inclusion diets. The RBC indices

recorded significantly high and low volumes for mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in 25% fed fish. WBC count was recorded to be greater in fish fed 25% and 50% inclusion diets. The highest and significantly low lymphocyte and neutrophil counts were recorded in the control group.

Table 8: Haematological parameters of *Clarias gariepinus* fed experimental diet

Parameters	Reference interval	Diets			
		CD (0%)	APPD (25%)	APPD (50%)	APPD (75%)
Packed cell volume (%)	27.00 – 45.10	28.0 ± 0.58 ^a	34.5 ± 0.29 ^b	33.5 ± 1.44 ^b	26.5 ± 0.8 ^a
Haemoglobin (mg/l)	9.00 – 18.64	35.5 ± 0.29 ^b	36.0 ± 1.73 ^b	34.5 ± 0.29 ^b	30.5 ± 0.87 ^a
Red blood cell (x10 ¹² /l)	2.38 – 5.72	2.7 ± 0.06 ^b	2.6 ± 0.00 ^b	2.9 ± 0.00 ^b	2.2 ± 0.06 ^a
Mean Corpuscular volume (fl)	58.51 – 152.54	103.7 ± 0.02 ^a	132.7 ± 0.11 ^b	115.5 ± 0.30 ^a	121.0 ± 1.01 ^a
Mean Corpuscular Haemoglobin (pg)	13.15 – 70.58	13.2 ± 0.01	13.9 ± 0.42	11.9 ± 0.30	13.9 ± 0.71
Mean Corpuscular Haemoglobin concentration (g/dl)	21.23 – 55.45	12.7 ± 0.37 ^b	10.4 ± 0.10 ^a	10.4 ± 0.25 ^a	11.5 ± 0.50 ^b
White blood cell (x10 ⁹ /l)	13.19 – 17.62	10.9 ± 0.46 ^a	11.6 ± 0.06 ^a	12.7 ± 0.12 ^b	10.2 ± 0.00 ^a
Lymphocytes (%)		34.0 ± 0.58	31.0 ± 1.15	31.5 ± 0.29	32.0 ± 1.15
Neutrophils (%)		61.5 ± 0.87 ^a	66.0 ± 0.00 ^b	67.0 ± 0.00 ^b	64.0 ± 0.29 ^b
Monocytes (%)		1.5 ± 0.87	1.0 ± 0.58	0.00 ± 0.00	1.5 ± 0.29
Eosinophils (%)		2.0 ± 1.15	1.5 ± 0.29	1.5 ± 0.29	1.5 ± 0.87
Basophils (%)		1.5 ± 0.87	0.0 ± 0.00	0.0 ± 0.00	0.5 ± 0.29

APPD= Autoclaved pigeon pea diet, CD= Control diet. Mean ± SE on the same row with different superscript are significantly different ($p < 0.05$).

Source of Reference interval: Adeyemo et al., (2014); Jamabo *et al.*, (2020), and Ogunji *et al.* (2008)

Serum minerals showed a significant ($p > 0.05$) increase in sodium, potassium, calcium, magnesium and phosphorus levels in fish-fed graded levels of PP diets. The reverse was the case in the levels of chloride and manganese, with the highest values recorded in the control group (Table 9).

Table 9: Serum minerals of *Clarias gariepinus* fed experiments diets

Minerals	Diets			
	CD (0%)	APPD (25%)	APPD (50%)	APPD (75%)
Sodium (MEq/l)	105.0 ± 0.87 ^a	121.0 ± 6.35 ^b	133.0 ± 2.31 ^b	139.5 ± 0.87 ^b
Potassium (mmol/l)	4.2 ± 0.06 ^a	4.5 ± 0.06 ^a	5.4 ± 0.36 ^b	6.1 ± 0.30 ^b
Calcium (mg/dl)	8.4 ± 0.04 ^a	8.5 ± 0.05 ^a	8.9 ± 0.07 ^b	9.5 ± 0.06 ^b
Magnesium (mg/dl)	3.5 ± 0.02 ^a	3.6 ± 0.07 ^a	5.0 ± 0.12 ^b	5.4 ± 0.11 ^b
Phosphorus (mg/dl)	2.6 ± 0.00 ^a	3.3 ± 0.05 ^b	4.0 ± 0.17 ^b	4.0 ± 0.02 ^b
Chloride (MEq/l)	71.2 ± 1.61 ^b	65.2 ± 0.04 ^a	64.3 ± 0.42 ^a	58.5 ± 1.33 ^a
Manganese (µg/dl)	6.7 ± 0.20 ^b	6.1 ± 0.17 ^b	5.2 ± 0.25 ^a	4.3 ± 0.01 ^a

APPD= average pigeon pea diet, CD= Control diet. Mean ± SE on the same row with different superscript are significantly different ($p < 0.05$).

DISCUSSION

Increasing 'b' value recorded in the LWR indicated a faster increase in weight than the length in experimental fish-fed graded levels of PP. The marked increase observed in the weight of fish fed at 25% and 50% means that the feed, especially at 25%, was well utilised by the experimental fish, which could be attributed to the fibre composition of the diets – (Rani *et al.*, 2021). According to Halpin (2019), low levels of fibre in the fish diet, though not digestible,

aid in the digestion of other feed nutrients by aiding the release of digestive microbes and enzymes. It could also be due to the protein-sparing function of other ingredients (Kangombe *et al.*, 2007), and the blend of amino acids was adequate for the experimental fish growth. Studies have proven that an optimal blend of amino acid profiles enhances growth and survival (Wu *et al.*, 2014). This was not the case; however, fish in the 75% group which showed a marked reduction in weight gain. The reason



could be the excess of fibre in the diet and presence of ANFs in PP, reducing feed intake and nutrient availability for the experimental fish (Rani *et al.*, 2021; Ishiwu *et al.*, 2020). Studies have shown that a fish diet high in fibre and ash decreases other ingredients' digestibility, resulting in poor fish growth (Etim, 2014; Hilton *et al.*, 1983). It could also be because of the limited amino acids in plant proteins, such as methionine, which reduces feed intake (L. Wang *et al.*, 2023).

This response was also confirmed in the calculated FCR, which was highest in the group fed with a 75% inclusion level of PP, indicating less feed converted to flesh by the experimental fish. This decrease in the weight at a 75% inclusion level did not, however, reflect in the LWR of this group, which also showed an increase in the weight of the fish than its length ($b > 3$, positive allometry) and was comparatively greater than observed for the control group. The growth response observed in this study agreed with previous studies on graded levels of PP as a partial and complete replacement for soya bean (Ganzon-Naret, 2014; Solomon *et al.*, 2017) and substitution of fish meal in the diets of *C. gariepinus* (Yagoub *et al.*, 2023). However, the findings of this study did not align with the studies of Hammed *et al.* (2013), Jamabo *et al.*, (2020), and Obasa *et al.* (2003), who observed a higher weight gain with higher inclusion of pigeon peas as partial to complete replacement for soya bean in the diet of *O. niloticus*. The observed increase in FCR with higher levels of PP inclusion aligns with the findings of Ganzon-Naret (2014). However, this trend contrasts with the results of Obasa *et al.* (2003), who recorded the highest FCR in *O. niloticus* fed a 20% inclusion diet, and with Jamabo *et al.* (2020), who similarly reported increased FCR at 25% PP inclusion. This disparity could be because PP was used as a replacement for soya beans in the diet of *O. niloticus* in both studies.

Blood indices are suitable indicators for evaluating the health condition of an animal (Akinrotimi *et al.*, 2011; Opoku-Agyemang *et al.*, 2019). Not all blood parameters were markedly affected by the diets, although variations were observed among the different treatments. The increased level of PCV noticed in experimental fish fed with 25% and 50% PP diets could indicate shrunken red blood cells, which could reflect abnormal RBC production. Nonetheless, the PCV for all treatments were within the reference values documented for this species (Adeyemo *et al.*, 2014; Jamabo *et al.*, 2020). Moreover, the increase in PCV did not reflect in the fish level of haemoglobin in all the treatments, as a low level of haemoglobin observed in fish fed the control and PP diets could imply a low supply of oxygen and protein to various parts of the fish body. However, not much research have been conducted on blood parameters of *C. gariepinus*-fed experimental diets. However, RBC and MCV were within the reference range documented for the species by Ogunji *et al.*, (2008). Variations in the Hb, MCHC, and MCH levels, which were within the reference range in some

treatments, could be due to environmental stress conditions and not necessarily because of the dietary intake (Adeyemo *et al.*, 2014). The findings of this study did not align with the documentation by Jamabo *et al.* (2020), who reported an increase in RBC, PCV, haemoglobin, WBC and platelet count of *O. niloticus* with increasing inclusion levels of PP. Similarly, the study was not in alignment with the study of Prabhu (2015), who reported decreased blood parameter in *C. gariepinus* fed diet containing differently processed PP. The differences in the blood parameters of the current study and the studies mentioned above could be attributed to the soil quality from which the PP was obtained, the processing methods, and the feed formulation processes adopted in the studies.

One major challenge in substituting fishmeal with plant-based protein in fish diet is the effect of anti-nutrients such as oxalate, saponin, lectin, tannin, and so on which impair mineral absorption and utilisation in fish, thereby limiting their availability to the fish (Mohan *et al.*, 2016). However, This was not the case in this study as serum minerals (sodium, potassium, calcium, magnesium and phosphorus) significantly increased in the groups fed graded levels of PP diets without supplementation of minerals. This trend was, however, not the case for serum chloride and manganese, which decreased with increasing substitution of PP. There is, however, a paucity of data to further substantiate these findings.

CONCLUSION

In conclusion, pigeon peas at 25% to 50% markedly increased the weight of experimental fish and reduced feed conversion ratio. Pigeon peas increased the weight of *C. gariepinus* faster, as shown in the LWR. The inclusion of pigeon peas in the diet of post-juvenile *C. gariepinus* did not show any deleterious effect on the blood parameters of the fish. The inclusion of the pigeon pea markedly improved the level of serum macrominerals. This study recommends that 25% to 50% of dietary inclusion of PP in the diet of *C. gariepinus* produces a better yield.

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Authors Contribution

OMR and SAB designed the study; ESN and OPO experimented and collected the data. ESN analysed the data and wrote the draft of the manuscript. OMR and SAB corrected first draft of the manuscript. All authors approved the manuscript for submission.

REFERENCES

- Abdul, W. O., Omoniyi, I. T., Adekoya, E. O., Adeosun, F. I., Odulate, O. O., Idowu, A. A., Olajide, A. E., and Olowe, O. S. (2016). Length-Weight Relationship and Condition Factor of Some Commercial Fish Species In Ogun State Coastal Estuary, Nigeria. *Ifè*



- Journal of Agriculture*, 28(1): 1- 10. DOI: 10.13140/RG.2.2.13139.07200
- Abraham, T. J., Mallick, P. K. and Paul, P. (2018). African catfish *Clarias gariepinus* farming practices in North and South 24 Parganas districts of West Bengal, India. *Journal of Fisheries*, 6(1): 579-586. <https://doi.org/10.17017/jfish.v6i1.2018.280>
- Adeleke, O., Adiamo, O. Q., Fawale, O. S. and Olamiti, G. (2017). Effect of Soaking and Boiling on Anti-nutritional Factors, Oligosaccharide Contents and Protein Digestibility of Newly Developed Bambara Groundnut Cultivars. *Turkish Journal of Agriculture - Food Science and Technology*, 5(9): 1 0 0 6 - 1 0 1 4 . <https://doi.org/10.24925/turjaf.v5i9.1006-1014.949>
- Adesina, S. A., Falaye, A. E. and Ajani, E. K. (2017). Evaluation of haematological and serum biochemical changes in *Clarias gariepinus* juveniles fed graded dietary levels of boiled sunflower (*Helianthus annuus*) seed meal replacing soybean meal. *Ife Journal of Science*, 19(1): 51-68. <https://doi.org/10.4314/ijfs.v19i1.7>
- Adeyemo, B. T., Obande, R. A. and Solomon, S. G. (2014). Haematological reference ranges of cultured *Clarias gariepinus* in the Lower Benue River Basin, Nigeria. *Comparative Clinical Pathology*, 23 : 3 6 1 - 3 6 6 . <https://doi.org/10.1007/s00580-012-1624-1>
- Ahmed, I., Khan, Y. M., Lateef, A., Jan, K., Majeed, A. and Shah, M. A. (2023). Effect of fish meal replacement by Azolla meal on growth performance, hemato-biochemical and serum parameters in the diet of scale carp, *Cyprinus carpio* var. *communis*. *Journal of the World Aquaculture Society*, 54 (5) : 1 3 0 1 - 1 3 1 6 . <https://doi.org/10.1111/jwas.12995>
- Akinrotimi, O. A., Bekibebe, D. O. and Orokotan, O. O. (2011). Select Hematological Values of the African Catfish (*Clarias gariepinus*) Raised in a Water Recirculating Aquaculture System. *International Journal of Recirculating Aquaculture*, 12(1): 1-12. <https://doi.org/10.21061/ijra.v12i1.1351>
- Ali, S. and Kaviraj, A. (2018). Aquatic weed *Ipomoea aquatica* as feed ingredient for rearing Rohu, *Labeo rohita* (Hamilton). *Egyptian Journal of Aquatic Research*, 44 (4) : 3 2 1 - 3 2 5 . <https://doi.org/10.1016/j.ejar.2018.09.004>
- Animashahun, R. A., Omoikhoje, S. O., Alabi, O. O., Shoyombo, A. J. and Olawoye, S. O. (2018). Influence of graded levels of instant noodle waste in the diets on the performance, carcass traits and haematology of broiler chickens. *Agrosearch*, 18 (1) : 4 0 - 5 2 . <https://doi.org/10.4314/agrosh.v18i1.4>
- AOAC. (1990). Official methods of analysis (AOAC) Official method 923.03. In Association of Official Analytical Chemists International.
- AOAC. (2005). Official Method of Analysis. 18th Edition. *AOAC Press, Maryland, USA*.
- Balogun, B. I., Oniye, S. J., Auta, J., Lakpini, C. A. M. and Abeke, F. O. (2016). Growth Performance and Feed Utilization of *Clarias gariepinus* (Teugels) Fed Different Dietary Levels of Soaked Bauhinia Monandra (Kutz) Seed Meal. *Journal of Food Research*, 5 (1) : 9 7 - 1 0 6 . <https://doi.org/10.5539/jfr.v5n1p97>
- Barham, D. and Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *The Analyst*, 97(151): 142-145. <https://doi.org/10.1039/an9729700142>
- Cai, W., Liu, H., Han, D., Zhu, X., Jin, J., Yang, Y. and Xie, S. (2022). Complete Replacement of Fishmeal With Plant Protein Ingredients in Gibel Carp (*Carassius auratus gibelio*) Diets by Supplementation With Essential Amino Acids Without Negative Impact on Growth Performance and Muscle Growth-Related Biomarkers. *Frontiers in Marine Science*, 8 : 7 5 9 0 8 6 . <https://doi.org/10.3389/fmars.2021.759086>
- Caruso G. (2015). Use of Plant Products as Candidate Fish Meal Substitutes: An Emerging Issue in Aquaculture Productions. *Fisheries and Aquaculture Journal*, 6 (3) : 1 - 3 . <https://doi.org/10.4172/2150-3508.1000e123>
- Daniel, N. (2018). A review on replacing fish meal in aqua feeds using plant protein sources. *International Journal of Fisheries and Aquatic Studies*, 6(2): 164-179.
- Dauda, A. B., Natrah, I., Karim, M., Kamarudin, M. S. and Bichi, A. u H. (2018). African Catfish Aquaculture in Malaysia and Nigeria: Status, Trends and Prospects. *Fisheries and Aquaculture Journal*, 9(1): 1-5. <https://doi.org/10.4172/2150-3508.1000237>
- Djissou, A. S. M., Adjahouinou, D. C., Koshio, S. and Fiogbe, E. D. (2016). Complete replacement of fish meal by other animal protein sources on growth performance of *Clarias gariepinus* fingerlings. *International Aquatic Research*, 8: 333-341. <https://doi.org/10.1007/s40071-016-0146-x>
- Dorothy, M. S., Raman, S., Nautiyal, V., Singh, K., Yogananda, T. and Kamei, M. (2018). Use of Potential Plant Leaves as Ingredient in Fish Feed-A Review. *International Journal of Current Microbiology and Applied Sciences*, 7(7): 112-125. <https://doi.org/10.20546/ijcmas.2018.707.014>
- Drulyte, D. and Orlien, V. (2019). The effect of processing on digestion of legume proteins. *Foods*, 8(6): 224-232. <https://doi.org/10.3390/foods8060224>
- Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Busca, K., Egan, F., Muller, N., Whooley, J., McGinnity, P., Culloty, S., Ross, R. P. and Stanton, C. (2020). Replacing fishmeal with plant protein in Atlantic salmon (*Salmo salar*) diets by supplementation with fish protein hydrolysate. *Scientific Reports*, 10 : 4 1 9 4 . <https://doi.org/10.1038/s41598-020-60325-7>
- Etim, N. N. (2014). Haematological Parameters and Factors Affecting Their Values. *Agricultural Science*, 2 (1) : 3 7 - 4 7 . <https://doi.org/10.12735/as.v2i1p37>
- Falaye, A.E., Omoike, A. and Adesina, S.B. (2015). Growth Performance and Nutrient Utilization of Catfish *Clarias Gariepinus* Fed Varying Inclusion



- Level Of Fermented Unsieved Yellow Maize. *Continental Journal of Biological Sciences*, 8(1): 14–23. doi:10.5707/cjbiolsci.2015.8.1.14.23.
- Ganzon-Naret, E.S. (2014). Evaluation of graded levels of cooked pigeon pea seed meal (*Cajanus cajan*) on the performance and carcass composition of Asian sea bass (*Lates calcarifer*). *ABAH Bioflux*, 6(1). 1-9 pp. <http://www.abah.bioflux.com.ro>.
- Gougbedji, A., Detilleux, J., Lalèyè, P. A., Francis, F. and Megido, R. C. (2022). Can Insect Meal Replace Fishmeal? A Meta-Analysis of the Effects of Black Soldier Fly on Fish Growth Performances and Nutritional Values. *Animals*, 12(13), 1700. <https://doi.org/10.3390/ani12131700>
- Gwata, E. T., Mashifane, D. C. and Maphosa, T. M. (2025). *Leveraging underutilized food plants for food and nutrition security: Selected nutritional attributes in pigeonpea (Cajanus cajan) seed. In Leveraging Underutilized Food Plants for Food and Nutrition Security* (pp. 401–419). CRC Press. <https://www.taylorfrancis.com/chapters/edit/10.1201/9781003469766-22/>
- Halpin, C. (2019). Lignin engineering to improve saccharification and digestibility in grasses. In *Current Opinion in Biotechnology*, 56:223-229. <https://doi.org/10.1016/j.copbio.2019.02.013>
- Hammed, A.M., Amosu, A.O. and Fashina-Bombata, H.A. (2013). Effect of partial and total replacement of soybean meal with pigeon pea (*Cajanus cajan*) as alternative plant protein source in the diet of juveniles African Mudcatfish *Clarias gariepinus* (Burchell, 1822). *The Journal of Food Technology*, 105: 139-145.
- Hilton, J. W., Atkinson, J. L. and Slinger, S. J. (1983). Effect of Increased Dietary Fiber on the Growth of Rainbow Trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences*, 40(1): <https://doi.org/10.1139/f83-012>
- Ishiwu, C., Opara, . A., Obiegbuna, . J. and Okeke, P. (2020). Assessment of growth performance of African catfish (*Clarias gariepinus*) fed with feed produced from blend of pigeon pea (*Cajanus cajan*) bambara groundnut (*Vigna subterranea*) and fish meal. *Global Journal of Agricultural Sciences*, 19(1), 17–26. <https://doi.org/10.4314/gjass.v19i1.3>
- Jamabo, N.A., Legborsi, F.B. and Lamidi, A.A. (2020). Growth performance and haematological responses of *Oreochromis niloticus* (Linnaeus) Fingerlings fed Pigeon Pea (*Cajanus cajan* (L.) Millsp). *Global Journal of Fisheries and Aquaculture*, 8(2): 541-547.
- Kang'ombe, J., Likongwe, J. S., Eda, H. and Mtimuni, J. P. (2007). Effect of varying dietary energy level on feed intake, feed conversion, whole-body composition and growth of Malawian tilapia, *Oreochromis shiranus* - Boulenger. *Aquaculture Research*, 38(4): 373–380. <https://doi.org/10.1111/j.1365-2109.2007.01676.x>
- Lawrence, M. J., Jain-Schlaepfer, S., Zolderdo, A. J., Algera, D. A., Gilmour, K. M., Gallagher, A. J. and Cooke, S. J. (2018). Are 3 minutes good enough for obtaining baseline physiological samples from teleost fish? *In Canadian Journal of Zoology*, 96: 7. <https://doi.org/10.1139/cjz-2017-0093>
- Le Cren, E.D. (1951). The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in the Perch (*Perca fluviatilis*). *Journal of Animal Ecology*, 20(2): 201–219. <https://doi.org/10.2307/1540>
- Lin J.Y. and Tang, C. Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chemistry*. 101(1): 140–147. <https://doi.org/10.1016/j.foodchem.2006.01.014>
- Liu, Y. J., Lai, Y. J., Wang, R., Lo, Y. C. and Chiu, C. H. (2020). The Effect of Thermal Processing on the Saponin Profiles of *Momordica charantia* L. *Journal of Food Quality*, Article ID: 8862020. 7 pages. <https://doi.org/10.1155/2020/8862020>
- Mahmud, M. N., Ritu, F. Y., Ansary, A. A. and Haque, M. M. (2025). Exploring Protein-Based Fishmeal Alternatives for Aquaculture Feeds in Bangladesh. *Aquaculture nutrition*, 2025, 3198303. <https://doi.org/10.1155/anu/3198303>
- Maruna, R. F. L. (1957). Beitrag zur Serum-Natrium-Bestimmung. Eine kritische studie über kolorimetrische bestimmungen und angabe einer einfachen fotometrischen methode. *Clinica Chimica Acta*, 2(6): 581–585. [https://doi.org/10.1016/0009-8981\(57\)90064-5](https://doi.org/10.1016/0009-8981(57)90064-5)
- Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E. and Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, 591: 551–563. <https://doi.org/10.1038/s41586-021-03917-1>
- Nsa, E. E., Ukachukwu, S. N., Isika, M. A. and Ozung, P. O. (2011). Effect of Boiling and Soaking Durations on the Proximate Composition, Ricin and Mineral Contents of Undecorticated Castor Oil Seeds (*Ricinus Communis*). *Intenational Journal of Plant, Animal, and Environmental Sciences*, 1(3): 244-252.
- Obasa, S. O., Dada, A. A. and Alegbeleye, W. O. (2003). Evaluation of pigeon pea (*Cajanus cajan*) as a substitute for soya bean meal in the diet of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Nigerian Journal of Animal Production*, 30(2): 265-270. <https://doi.org/10.4314/njap.v30i2.3305>
- Ogunji, J. O., Osuigwe, D. I., Okogwu, O. and Uwadiogwu, N. (2008). Response of African catfish, *Clarias gariepinus* (Burchell, 1822), to diets of pigeon pea, *Cajanus cajan*, subjected to different processing methods. *Journal of the World Aquaculture Society*, 39(2): 215–224. <https://doi.org/10.1111/j.1749-7345.2008.00158.x>
- Okeniyi, F.A., Olawoye, S.O., Adeyonu, A.G., Animashahun, R.A., Onwuka, C.F.I., Aina, A.B.J. and Sowande, O.S. (2020). Performance, haematology and serum biochemical indices of West African dwarf bucks fed elephant grass (*pennisetum purpureum*) Supplemented with urea-maize stover diets. *Coast Journal of the Faculty of*

- Science*, 2 (2): 469 - 481
- Oliva-Paterna, F. J., Torralva, M. and Carvalho, E. D. (2009). Length-weight relationships for 20 species collected in the Jurumirim reservoir (Paranapanema Basin, Brazil). *Journal of Applied Ichthyology*, 25(2009): 360-361. <https://doi.org/10.1111/j.1439-0426.2009.01194.x>
- Opoku-Agyemang, T., Oppong, C., Asamoah Sakyi, S., Amemor, E., Sia, D.D. and Emikpe, B.O. (2019). Baseline Haematological parameters reference ranges of Dogs in the Ashanti Region of Ghana. *Animal Research International*, 16(3): 3529 – 3535.
- Oyewole, B. O., Ibitoye, S. J., Adejo, P. E. and Oyibo, F. O. (2023). Resource Use Efficiency and Profitability Analysis of Catfish (*Clarias gariepinus*) Production in Kogi State, Nigeria. *Asian Journal of Agricultural Extension, Economics & Sociology*, 41(9), 103 – 116. DOI:10.9734/ajaees/2023/v41i92021.
- Prabhu, A. J. (2015). Minerals in fish: does the source matter? In PhD Thesis. Wageningen University and Research Center, Wageningen University - 9789462574809. <https://edepot.wur.nl/358026.267p>.
- Prakoso, V. A., Irawan, A., Iswantari, A., Maulana, F., Samsudin, R. and Jayanegara, A. (2022). Evaluation of dietary inclusion of black soldier fly (*Hermetia illucens*) larvae on fish production performance: a meta-analysis. *Journal of Insects as Food and Feed*, 8(11), 1373 - 1384. <https://doi.org/10.3920/JIFF2021.0159>
- Qiu, Z., Xu, Q., Xie, D., Zhao, J., Yamamoto, F. Y., Xu, H. and Zhao, J. (2023). Effects of the Replacement of Dietary Fish Meal with Poultry By-Product Meal on Growth and Intestinal Health of Chinese Soft-Shell Turtle (*Pelodiscus sinensis*). *Animals*, 13(5):865. <https://doi.org/10.3390/ani13050865>
- Rani, S., Sahoo, S., Srivastava, P. P., Kumar, M., Gupta, S., Munilkumar, S., Gupta, G. and Krishna, G. (2021). Influence of inclusion of pigeon pea (*Cajanus cajan*) leaf meal on growth, physio-metabolic and immune parameters, and expression of IGF-1, IGF-1R and IGFBP-1 genes in Labeo rohita (Hamilton, 1822) fingerlings. *Aquaculture Research*, 52(5), 1977 – 1994. <https://doi.org/10.1111/are.15046>
- Samtiya, M., Aluko, R. E. and Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*, 2(6): 1-14. <https://doi.org/10.1186/s43014-020-0020-5>
- Sarkar, S., Panda, S., Yadav, K.K. and Kandasamy, P. (2020). Pigeon pea (*Cajanus cajan*) an important food legume in indian scenario – a review. In *Legume Research*, 43(5): 601 - 610. <https://doi.org/10.18805/LR-4021>
- Skeggs, L.T. and Hochstrasser, H.C. (1964). Thiocyanate (colometric) Method of Chloride Estimation. *Journal of Clinical Chemistry*, 10: 918.
- Solomon, S. G., Okomoda, V. T. and Oda, S. O. (2017). Nutritional value of toasted pigeon pea, *Cajanus cajan* seed and its utilization in the diet of *Clarias gariepinus* (Burchell, 1822) fingerlings. *Aquaculture Reports*, 7: 34 - 39. <https://doi.org/10.1016/j.aqrep.2017.05.005>
- Terova, G., Gini, E., Gasco, L., Moroni, F., Antonini, M. and Rimoldi, S. (2021). Effects of full replacement of dietary fishmeal with insect meal from *Tenebrio molitor* on rainbow trout gut and skin microbiota. *Journal of Animal Science and Biotechnology*, 12, 30. <https://doi.org/10.1186/s40104-021-00551-9>
- Tietz, N.W. (1976). *Fundamental of Clinical Chemistry*, 2nd Edition; Philadelphia (USA): W.B. Saunders Co. Pp874.
- Trinder, P. (1951). A rapid method for the determination of sodium in serum. *The Analyst*, 76: 596-599. <https://doi.org/10.1039/AN9517600596>
- Wang, L., Gao, C., Wang, B., Wang, C., Sagada, G. and Yan, Y. (2023). Methionine in fish health and nutrition: Potential mechanisms, affecting factors, and future perspectives. *In Aquaculture*, 568: 7393 - 7399. <https://doi.org/10.1016/j.aquaculture.2023.739310>
- Wang, X., Luo, H., Zheng, Y., Wang, D., Wang, Y., Zhang, W., Chen, Z., Chen, X. and Shao, J. (2023). Effects of poultry by-product meal replacing fish meal on growth performance, feed utilization, intestinal morphology and microbiota communities in juvenile large yellow croaker (*Larimichthys crocea*). *Aquaculture Reports*, 30: 101547. <https://doi.org/10.1016/j.aqrep.2023.101547>
- Wu, G., Bazer, F. W., Dai, Z., Li, D., Wang, J. and Wu, Z. (2014). Amino acid nutrition in animals: Protein synthesis and beyond. *Annual Review of Animal Biosciences*, 2: 387 - 417. <https://doi.org/10.1146/annurev-animal-022513-114113>
- Yagoub, H.M., Ali, A.N.S., Mohammed, R.M. and Ali, M.M. (2023). Effect of adding different levels of soaked pigeon pea seeds (*Cajanus cajan* L) on the Growth and Feed Utilization Efficiency of Nile tilapia (*Oreochromis niloticus*) fingerlings. *International Journal of Aquaculture Research and Development*, 1(3):24-33. <https://doi.org/10.14302/issn.2691-6622.ijar-23-4637>
- Yu, H., Li, M., Yu, L., Ma, X., Wang, S., Yuan, Z. and Li, L. (2023). Partial Replacement of Fishmeal with Poultry By-Product Meal in Diets for Coho Salmon (*Oncorhynchus kisutch*) Post-Smolts. *Animals*, 13(17):2789. <https://doi.org/10.3390/ani13172789>

