

# Performance of pacu (*Colossoma macropomum*) fed varying dietary inclusion levels of fish visceral and duckweed (*Lemna minor*) as replacement for fish and soybean meals

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## Abstract

The performance of *Colossoma macropomum* subjected to a six-month feeding trial using duckweed (*Lemna minor*) and fish visceral as partial replacements for soybean meal and fishmeal respectively was evaluated in this study. A total of one thousand five hundred (1500) nearly same sizes (average size 45 g) juveniles of *C. macropomum* were sourced from the nearby research man-made lake and subjected to proper acclimatisation for two weeks prior to the commencement of the feeding trial. One hundred (100) juveniles per experimental pond (EP) were stocked in triplicate with EP0 %<sub>i-iii</sub> (control), EP25 %<sub>i-iii</sub>, EP50 %<sub>i-iii</sub>, EP75 %<sub>i-iii</sub>, and EP100 %<sub>i-iii</sub>. The formulated diets with varying dietary inclusion levels (0 %, 25 %, 50 %, 75 %, and 100 %) of duckweed as a substitute for soybean meal, and at the same levels, fish visceral meal was used as a substitute for fishmeal. The formulated diets were fed to the experimental fishes on a twice-daily schedule (7 am and 5:30 pm). The control diet contained the conventional protein sources (fishmeal and soybean) while the other diets had fish visceral and duckweed as dietary replacements for fishmeal and soybean, respectively. A biweekly sampling of growth parameters such as body weight was carried out on twenty (20) randomly selected juveniles from each EP and data collected from the feeding trial was subjected to analysis of variance (ANOVA) (SPSS version 26) while Duncan's multiple ranges test distinguished the means at  $p < 0.05$  significant level. The highest value for mean body weight gain at 6 months was observed at a 50 % dietary inclusion level of the test ingredients (1411.7 g), while at a 100 % inclusion level, the lowest value was recorded (1213.6 g). Water quality parameters sampled were within the acceptable standards indicating no adverse effect of the test ingredients on the culture medium. In general, no adverse effect was recorded across treatments. Conclusively, *C. macropomum* culture in Nigeria using the test diets at a 50 % dietary inclusion level to replace the conventional protein sources is feasible and can help improve fish availability and sustainability through species diversification and reduced production cost since the test ingredients are obtained at minimal or zero cost from fish traders (fish visceral) and fallow earthen ponds (duckweed).

**Keywords:** aquaculture, fish feed, fish nutrition, juveniles

## 1 Introduction

Aquaculture as a sector have contributed immensely to the GDPs of some African countries including Nigeria (Irabor *et*

*al.*, 2023a). It is also considered the main source of affordable and healthy protein, as fish and other aquatic products are relatively cheap. Considering the importance of aquaculture, the number of fish farmers has continued to increase in recent decades to meet the constantly increasing demand for fish (Benjamin *et al.*, 2022). Nevertheless, even with the

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increasing number of fish farmers, the gap between fish demand and supply is widening.

The inability to meet the fish demand in Nigeria has been attributed to the mono-species culture system being practiced since over 98 % of fish farms in Nigeria produce only catfish (*Clarias* spp, *Heterobranchus* spp, and their hybrids) (Adeleke et al., 2020; Eyayu et al., 2023; Nwachi et al., 2023). In addition, catfish farming is known to be capital-intensive due to the high feed costs, which contributes significantly to the low production capacity of most farms, which in turn has a negative impact on the availability and sustainability of the fish.

Numerous studies have suggested ways to improve the production capacity of fish farms in Nigeria, such as species diversification, increased stocking density, and polyculture (Iruo et al., 2018; Obiero et al., 2019; Tran et al., 2020; bin Mohd Khatib & Mat Jais 2021; Oboh 2022). Species diversity has been considered a priority approach, which led to the adoption and culture of tilapia species, although still on a small scale. Due to the challenges associated with tilapia fish species (*Oreochromis niloticus*), mainly because of its small size, most farmers have not adopted the farming of this species. With this shortcoming in the proposed species, there is still a gap between supply and demand.

Pacu (*C. macropomum*) is native to the Amazonian basin, although a few populations have been found in the lower river Niger (Alho et al., 2015). It is considered a favourable commercial species cultured mainly in cages in most countries where it is farmed. The advantages of this species include the year-round availability of fingerlings, high acceptance of formulated feeds, and excellent tolerance to environmental and physiological stress. Some studies have shown that under standard culture conditions, pacu attains marketable size within an average of 4 to 6 months when fed diets containing 35 - 40 % crude protein and other essential nutrients (Marchão et al., 2020; Nascimento et al., 2020; Bussons et al., 2021; Silalahi et al., 2021; Silver et al., 2022).

Considering the quest to cut down production costs, and since pacu has been reported to easily adapt to formulated feed, it allows the replacement of expensive feed ingredients using affordable, available, and nutrient-rich ingredients such as moringa leaf meal, duckweed meal, sweet potato leaf meal, pawpaw seed meal, and fish visceral. These alternative plant sources have been explored at varying inclusion levels by numerous researchers in the culture of some fish species such as *C. gariepinus*, *O. niloticus*, and *Machrobranchium rosenbergii* without adverse effect (Hutabarat et al., 2019; Huang et al., 2020; Islam et al., 2020; Wanderi & Olendi,

2020; Irabor et al., 2021a; 2022a; 2023b; Ekelemu et al., 2023).

To ensure a productive and sustainable aquaculture sector in Nigeria, there is a need to constantly make available healthy and affordable fish for the steadily growing population. Hence, a need to explore other culturable fish species such as pacu using a cost-effective feed (locally formulated diets). Therefore, this research aimed to evaluate the performance of pacu (*C. macropomum*) fed diets with varying dietary inclusion levels of fish visceral and duckweed as replacements for fish meal and soybean meal, respectively.

## 2 Materials and methods

### 2.1 Study area

The study site was the Research Farm and Research Laboratory of the Fisheries and Aquaculture Department, Faculty of Agriculture, Dennis Osadebay University, Anwai, Asaba, Delta State, Nigeria.

### 2.2 Feed ingredients

The conventional feed ingredients for this study were procured from the local market (Akwa-aza Market) close to the university premises. The fish visceral were collected from fish traders, adequately dried, and ground into granules. Duckweed was identified from some designated disease-free earthen ponds, collected, dried at ambient temperature, grounded into granules, and kept in a moisture-free container. Prior to the compounding of the ingredients to formulate the test diets, a proximate analysis was carried out on the test ingredients to ascertain their nutrient composition. The components were appropriately combined and pelletised into dissolvable 2 mm pellets at the specified ratio (Table 1) using a locally constructed pelletiser.

### 2.3 Sample procurement and experimental design

In this study, a total of 1500 healthy juveniles of *C. macropomum* were collected from the wild (man-made lake) by skilled fishermen. The fish samples were measured individually and an initial average weight and length of 4.9 g and 5.5 cm, respectively were ensured to avoid bias. These fingerlings were carefully treated with a 1.5 ppm sodium permanganate solution for 10 minutes prior to stocking to reduce the impact of physiological stress. Following that, they were placed in a suitably constructed earthen pond measuring 6 m × 6 m × 3 m, and subjected to a twice-daily (7 am and 5:30 pm) schedule of feeding to satiation (5 % body weight) using purchased commercial feed (2 mm) for a week prior to

**Table 1:** Diets composition.

Ingredient (%)	EP treatments				
	0%	25%	50%	75%	100%
Fishmeal	26.0	19.5	13.0	6.50	0.0
Soybean meal	17.5	13.12	8.75	4.38	0.00
GNC	8.50	8.50	8.50	8.50	8.50
Fish visceral*	0.0	6.50	13.0	19.5	26.0
Duckweed meal*	0.0	4.38	8.75	13.12	17.5
Yellow maize	18.0	18.0	18.0	18.0	18.0
Wheat bran	12.5	12.5	12.5	12.5	12.5
Rice bran	8.00	8.00	8.00	8.00	8.00
Vit C	1.00	1.00	1.00	1.00	1.00
Premix	1.00	1.00	1.00	1.00	1.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Lysine	2.00	2.00	2.00	2.00	2.00
Methionine	2.00	2.00	2.00	2.00	2.00
NaCl	0.50	0.50	0.50	0.50	0.50
Starch	0.50	0.50	0.50	0.50	0.50

EP for experimental pond, GNC for groundnut cake. \*test ingredients.

the start of the six-months feeding trial. The fish were fed by pouring feed directly onto the water's surface.

At the start of the experiment, the fingerlings were distributed in triplicate to the five treatments: EP0%<sub>i-iii</sub>, EP25%<sub>i-iii</sub>, EP50%<sub>i-iii</sub>, EP75%<sub>i-iii</sub>, and EP100%<sub>i-iii</sub>. In this way, each clearly labelled experimental earthen pond of 10 m × 6 m × 1.3 m contained 100 juvenile *C. macropomum*. The feeding schedule was kept the same as during acclimatisation, and proper adherence to routine management and inspection of water parameters was ensured.

A biweekly measurement of the growth indices was carried out. Thirty (30) fish samples per treatment were randomly collected using a scope net. A finely calibrated meter rule and sensitive scale (Presica 30 kg Electronic Scales Weighing Scale LCD Gram Balance, model: TS500) were used to measure the length (cm) and weight (g), respectively. The method suggested by Irabor *et al.* (2021b) was employed during the measurement to reduce stress on the fish samples.

This research was carried out within a period of 6 months (October 2022 to April 2023). The protocols provided by Irabor *et al.* (2022b) were used to determine parameters including daily and total feed intake, feed conversion, and mean weight gain (MWG), while Limbu (2020) procedure was used to determine the mortality rate. Using the methods outlined by Muna *et al.* (2021), certain relevant water quality parameters such as dissolved oxygen, temperature, and pH were determined bi-weekly.

## 2.4 Statistical analysis

The collected data were statistically analysed using analysis of variance from SPSS version 26, while Duncan's multiple ranges test distinguished the means at  $p < 0.05$  significant level.

## 3 Results

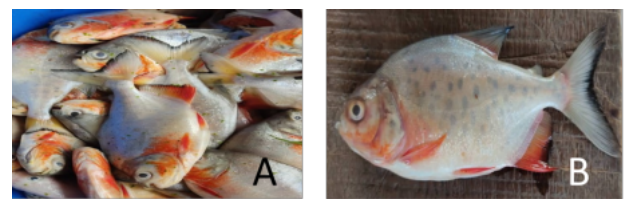
### 3.1 Proximate composition of the experimental ingredients and diets

As presented in Table 2, duckweed and fish visceral were found to have crude protein values of 40.9% and 52.2%, respectively. A maximum crude protein (CP) level (41.4%) was found in EP 50% with fish visceral and duckweed meals (50% and 50% dietary inclusion levels, respectively), while the lowest CP level (33.9%) was found in EP 100% with 0% fish visceral and 100% duckweed meals (Table 3).

**Table 2:** Proximate composition of the test diet ingredients duckweed (*Lemna minor*) and fish visceral meals.

Properties	Duckweed meal (%)	Fish visceral meal (%)
Moisture	3.2	2.1
Crude protein	40.9	52.2
Crude fibre	28.1	0.6
Ether extract	1.2	24.0
Ash	9.0	17.6
NFE	17.4	3.2

NFE: Nitrogen free extract

**Fig. 1:** A: *Colossoma macropomum* harvested from the experimental ponds; B: *C. macropomum* fed EP 50% inclusion level of the test diets (duckweed and fish visceral meals).

### 3.2 Growth performance

The growth performance indices as displayed in Table 4 revealed that optimum mean values for weight gained, feed conversion ratio, specific growth rate, and survival rate (1411.7 g, 1.00, 3.11%, and 96%, respectively) of *C. macropomum*, were observed in EP 50%, while the lowest mean

**Table 3:** Proximate composition of the diets with varying dietary inclusion levels of the test ingredients.

Parameters (%)	EP treatments*					SEM	P
	0 %	25 %	50 %	75 %	100 %		
Crude protein	39.5 <sup>c</sup>	39.7 <sup>b</sup>	41.4 <sup>a</sup>	35.5 <sup>d</sup>	33.9 <sup>e</sup>	1.21	0.02
Ether extract	2.37 <sup>e</sup>	2.42 <sup>d</sup>	2.89 <sup>c</sup>	3.75 <sup>b</sup>	4.37 <sup>a</sup>	2.06	0.04
Crude fibre	2.11 <sup>e</sup>	2.59 <sup>d</sup>	3.38 <sup>c</sup>	4.62 <sup>b</sup>	5.69 <sup>a</sup>	1.70	0.03
Moisture	3.35 <sup>e</sup>	3.47 <sup>d</sup>	4.29 <sup>c</sup>	4.88 <sup>b</sup>	4.98 <sup>a</sup>	1.91	0.01
Total ash	7.61 <sup>e</sup>	8.52 <sup>d</sup>	9.02 <sup>c</sup>	11.9 <sup>b</sup>	12.7 <sup>a</sup>	3.63	0.03
Nitrogen-free extract (NFE)	42.4 <sup>a</sup>	41.2 <sup>b</sup>	37.0 <sup>c</sup>	36.7 <sup>d</sup>	35.9 <sup>e</sup>	2.67	0.02

\*See table 1 for detailed diet ingredients. <sup>a,b,c,d,e</sup> are used to separate the means at  $p < 0.05$ . EP: experimental pond; SEM: significant error mean.

**Table 4:** Growth performance indices and nutrient utilisation of *Colossoma macropomum* fed test diets for six months (186 days).

Parameters (%)	EP treatments*					SEM	P
	0 %	25 %	50 %	75 %	100 %		
IW (g)	34.3	34.4	34.3	34.4	34.4	0.00	0.07
FW (g)	1312 <sup>b</sup>	1307 <sup>bc</sup>	1416 <sup>a</sup>	1267 <sup>c</sup>	1218 <sup>d</sup>	3.74	0.03
BWG (g)	1307.7 <sup>b</sup>	1302.6 <sup>bc</sup>	1411.7 <sup>a</sup>	1262.6 <sup>c</sup>	1213.6 <sup>d</sup>	1.62	0.01
FI/Day/Fish (g)	7.73 <sup>a</sup>	7.69 <sup>ab</sup>	7.62 <sup>b</sup>	7.38 <sup>c</sup>	7.35 <sup>d</sup>	2.01	0.02
FI/186Days/Fish (g)	1437.8 <sup>a</sup>	1430.3 <sup>ab</sup>	1417.3 <sup>b</sup>	1372.7 <sup>bc</sup>	1367.1 <sup>c</sup>	4.23	0.04
FCR	1.10 <sup>c</sup>	1.10 <sup>c</sup>	1.00 <sup>d</sup>	1.12 <sup>b</sup>	1.13 <sup>a</sup>	0.02	0.01
SGR (%)	3.08 <sup>b</sup>	3.06 <sup>bc</sup>	3.11 <sup>a</sup>	3.04 <sup>c</sup>	3.02 <sup>d</sup>	0.48	0.03
SR (%)	92 <sup>c</sup>	88 <sup>b</sup>	96 <sup>d</sup>	85 <sup>c</sup>	74 <sup>a</sup>	2.02	0.02

\*See table 1 for detailed diet ingredients. <sup>a,b,c,d,e</sup> are used to separate the means at  $p < 0.05$ .

EP: experimental pond; SEM: significant error mean; IW: initial weight; FW: final weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; SGR: specific growth rate; SR: survival rate.

values (1213.6 g, 1.13, 3.02 % and 74 %), were observed in EP 100 %.

As shown in Table 5, several water quality indices evaluated were all within acceptable standards, even though the mean values fluctuated slightly as dietary inclusion levels of the test ingredients increased across treatments. The EP 100 % recorded the highest mean values for pH, temperature, ammonia, TOM, and BOD respectively, whereas the EP 50 % had the highest mean value for DO.

#### 4 Discussion

Diversification of the fish species farmed in Nigeria is of utmost importance to meet the ever-increasing demand for fish. Therefore, the utilisation of fish species such as *C. macropomum*, which has a profitable and cost-efficient production potential, is of paramount importance.

The results of the proximate analyses of the test ingredients (fish visceral and duckweed meals) revealed that the nutritional profile of both ingredients was within the acceptable range for crude protein content (40 % and above) which

was also in line with the findings of Irabor *et al.* (2022c) in a study carried out on African catfish (*Clarias gariepinus*) fingerlings. This further demonstrated the test ingredients' potential as protein source for fish when compared to other alternative sources such as mulberry and sweet potato leaf (Rohela *et al.*, 2020). According to Silver *et al.* (2022), the dietary crude protein level requirement of *C. macropomum* ranges from 35 - 42 %, which is as reported in this study. However, as inclusion level of the test ingredients increased, a significant difference ( $p < 0.05$ ) was observed across diets. This finding is in line with those of Marchão *et al.* (2020) and Nascimento *et al.* (2020) where variations in the crude protein levels was recorded as the dietary inclusion level of diluted lysine increased in the diets of *C. macropomum*. Ekokotu *et al.*, (2018) in the same vein, reported significant reduction in the crude protein levels of test diets as varying dietary inclusion levels of pawpaw seed meal increased in the diets fed to *C. gariepinus* fingerlings reared in indoor tanks.

The growth parameters and feed utilisation indicated a continuous decline across treatments as dietary inclusion

**Table 5:** Summary of physicochemical parameters of the water of the experimental ponds.

Parameters (%)	EP treatments*					SEM	P
	0 %	25 %	50 %	75 %	100 %		
pH	6.84 <sup>c</sup>	6.92 <sup>b</sup>	6.86 <sup>c</sup>	6.98 <sup>a</sup>	6.99 <sup>a</sup>	1.01	0.08
DO (mg/L)	6.22 <sup>d</sup>	6.36 <sup>b</sup>	6.47 <sup>a</sup>	6.34 <sup>c</sup>	6.39 <sup>b</sup>	1.44	0.14
Temperature (°C)	31.3 <sup>c</sup>	31.8 <sup>a</sup>	30.1 <sup>d</sup>	31.7 <sup>b</sup>	31.8 <sup>a</sup>	2.00	1.32
Ammonia (mg L <sup>-1</sup> )	0.07 <sup>b</sup>	0.07 <sup>b</sup>	0.06 <sup>c</sup>	0.08 <sup>a</sup>	0.09 <sup>a</sup>	1.66	0.19
TOM (%)	1.73 <sup>c</sup>	1.74 <sup>c</sup>	1.67 <sup>d</sup>	1.76 <sup>b</sup>	1.78 <sup>a</sup>	0.27	0.73
BOD (mg L <sup>-1</sup> )	3.58 <sup>c</sup>	3.59 <sup>c</sup>	3.53 <sup>d</sup>	3.62 <sup>b</sup>	3.64 <sup>a</sup>	1.09	1.00

\*See table 1 for detailed diet ingredients. <sup>a,b,c,d</sup> are used to separate the means at  $p < 0.05$ . EP: experimental pond; SEM: significant error mean; DO: dissolved oxygen; TOM: total organic matter; BOD: biological oxygen demand.

levels of the test ingredients increased above 50 %. However, an optimum increase in weight gain was observed at the 50 % dietary inclusion level of the test ingredients. The test ingredients were considered to have contributed to the significant weight gain because of their high nutritional properties. This is consistent with the findings of Hutabarat *et al.* (2019) and Irabor *et al.* (2021b; 2022c), who noted that *O. niloticus* weight gain increased significantly as the amount of duckweed meal in the diets increased this could be attributed to the high crude protein content in the diet due to the increased presence of duckweed. Although at a dietary inclusion level above 50 %, a decline in weight gain was observed, this was linked to the poor palatability and digestibility of the feed which further led to poor acceptance by the sampled fish. Bussons *et al.* (2021) reported similar findings, noting that when the dietary inclusion level of the test ingredient (glycerin) as an alternative protein source was raised over 50 % in the diets, the growth indices of *C. macropomum* reduced significantly.

The result recorded for feed intake revealed a proportionate decline in consumption rate as dietary inclusion levels of the test ingredients increased although a more significant level of decline was observed as dietary inclusion level increased above 50 %. This finding affirms that of Irabor *et al.* (2023b) who recorded a steady decline in the feed intake of *C. gariepinus* juveniles as the dietary inclusion levels of duckweed and fish visceral increased above 50 %. Also, Wanderi & Olendi (2020) recorded a significant decline in feed intake as inclusion levels of duckweed meal increased above 50 % in the diets of *O. niloticus*. In the same vein, a decline in feed intake was reported in *Litopenaeus vannamei* fed diets with increased dietary inclusion levels of fermented duckweed meal (Flores-Miranda *et al.*, 2015).

The water quality parameters evaluated were all within acceptable range indicating that the test ingredients had no adverse effects on the water (culture medium). This also

revealed no toxic characteristics of the test ingredients and the reason for the optimal growth performance observed in the sampled fish. This confirms the results of Huong *et al.* (2020) & Irabor *et al.* (2022d; 2023b) were optimum growth performance of clown knife fish and Nile tilapia fed diets with varying inclusion levels of duckweed and fish visceral at a temperature range of 29–31.9 °C. A similar result was observed in *C. gariepinus* juveniles cultured in a concrete tank with temperatures fluctuating between 28.5–31.9 °C (Nwachi & Irabor, 2015; Nwachi *et al.*, 2023; Irabor *et al.*, 2023a). However, in European bass fed diets with varying dietary inclusion levels of *M. oleifera* leaf meal, a contrary finding was recorded (Islam *et al.*, 2020). The temperature was reported to be below acceptable range (below 24.7 °C) although this was linked to increased turbidity of the water thereby resulting to a low feed acceptability rate as expressed by the sampled fish.

## 5 Conclusion

It was found that *C. macropomum* can be cultured in earthen ponds in Nigeria and optimum growth performance is achieved when up to 50 % of the conventional protein sources soybean and fish meal are replaced by duckweed and fish visceral meals, respectively. With most local fish feed producers still suffering from ever increasing prices and scarcity of the major conventional feed ingredients (fishmeal and soybean), the use of feeds produced from cheap alternative ingredients sourced locally is cost-effective. This will in turn help to significantly reduce production costs without compromising fish performance and water quality parameters, thus ensuring the production and sustainability of the fish farming and aquaculture sector in Nigeria.

### Conflict of interest

The authors declare that they have no conflict of interest.

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