

DIETARY EFFECTS OF *PARQUETINA NIGRESCENS* INCLUSION ON THE GROWTH PERFORMANCE AND HAEMATOLOGY OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

Christianah Oludayo OLANIYI, Olaolu Olalekan FAWOLE*

Department of Fisheries and Aquaculture, Ladoke Akintola University of Technology, Ogbomosho, Nigeria

ABSTRACT

The continuous rise in cost of fish feed in the sub-Sahara Africa necessitates the need to look inward for cheaper and viable alternative feed ingredients. A nutritional study of *Parquetina nigrescens* leaf meal was undertaken to determine their effect on growth performance, physiology and health of African catfish (*Clarias gariepinus*). One hundred and fifty (150) juvenile African catfish were randomly selected, divided into 5 dietary treatments and stocked at 10 juveniles per tank (120 litres) and with three replicates each. Five experimental diets were formulated with 0 g (T1), 0.02 g (T2), 0.03 g (T3), 0.04 g (T4) and 0.05 g (T5) per 100 g of dietary inclusion of *P. nigrescens* leaf meal (PNLM) respectively. The fish were fed on experimental diet to satiation twice daily for 12 weeks. At the end of the feeding trials, data collected on growth performance and haematology were subjected to one-way analysis of variance (ANOVA) using completely randomized design (CRD). Result showed significant effect ($p < 0.05$) of *P. nigrescens* in final mean weight, feed conversion ratio and protein efficiency ratio of *Clarias gariepinus* in diet T3. Haematological parameters: Packed Cell Volume (PCV), Red Blood Cell (RBC), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell (WBC), Lymphocytes and Heterocytes were also significantly different ($p < 0.05$). Based on these findings, it can be confirmed that the inclusion of *P. nigrescens* leaf meal (PNLM) at 0.03 g as additive is the most suitable for optimizing performance of African catfish.

Key words: African catfish; *Parquetina nigrescens*; blood parameter; growth performance; haematology

INTRODUCTION

Aquaculture industry is facing challenges of the high cost of fish feed, and therefore it is very crucial to explore the alternative raw materials for feed formulation at the lowest cost. There is also the need to increase fish production and as such, need for fish farmers to source for feeding that will supply high quality protein to improve the growth of fish (FAO, 2018). Previous studies including that of Ali-Emmanuel *et al.* (2003) have already evaluated the efficiency of various plant-based ingredients as alternative protein sources in aqua-feed production. Plant sources of feed according to Ayoola and Bamiro (2017) contain appre-

ciable crude protein content for maximum productivity. This had attracted a lot of trial experiments by fish nutritionists around the world on the use of leaf meal as a possible fish meal substitute with the aim of reducing the cost of fish feed (Bairagi *et al.*, 2004). *P. nigrescens* popularly called African *Parquetina* in English, Kwakwani by the Hausas, Mgbidingbe by the Igbos and Ewe Ogbo among the Yorubas is a shrub commonly found growing in equatorial West Africa; not cultivated but as a secondary forest and around villages (Oluwafemi and Debiri, 2008; Ayoola *et al.*, 2011; Awoniyi *et al.*, 2022). Previous phytochemical studies of the plant revealed that the ethanol extract of the leaves, roots, stem bark, and latex contained

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***Correspondence:** E-mail: oofawole78@lautech.edu.ng

Olaolu Olalekan Fawole, Department of Fisheries and Aquaculture,
Ladoke Akintola University of Technology, Ogbomosho, 210214, Nigeria
Tel.: +2348035002060

Received: June 13, 2025
Accepted: September 4, 2025



phytochemical compounds such as phenols, tannins, alkaloids, flavonoids, reducing sugars, phlobatannins, terpenoids, saponins, cardiac glycosides, steroids, and coumarin (Ayoola *et al.*, 2011; Sopeyin and Ajayi 2016; Airaodion *et al.*, 2019; Ajayi *et al.*, 2021). Haematological analysis of the peripheral blood is a very useful tool for evaluation of fish physiological status and health (Grant, 2015; Docan *et al.*, 2018; Fazio, 2019; Witeska *et al.*, 2022). There has not been any documented report on the effect of *P. nigrescens* in fish despite its usage for treating human ailments in Nigeria. This study therefore determined the effects of *P. nigrescens* on growth performance and haematology of African catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

The research followed the guidelines for use of fish in research study as recommended by Jenkins *et al.* (2014).

Collection and treatment of test ingredients

Fresh leaves of *Parquetina nigrescens* samples were obtained within Ladoko Akintola University of Technology, Ogbomoso, Oyo State and Ogbomoso metropolis. Identification and authentication of the leaves were carried out at the Crop Production and Soil Science Department of the University. The leaves were

washed, air-dried at room temperature for 7-days and grounded into fine particles using mortar, pestle and grinding machine to make *Parquetina nigrescens* leaf meal (PNLM); then stored in a plastic container to avoid contamination prior to analysis.

Feed ingredients were purchased from a reputable feed mill in Ogbomoso and the ingredients used are maize, wheat offal, groundnut cake, fishmeal, soybean meal, vegetable oil, oyster shell, bone meal, salt, methionine and lysine. Five diets (35 % crude protein, CP) containing varying levels *P. nigrescens* leaf meal was prepared as shown in Table 1. The control diet (T1) has *P. nigrescens* leaf meal inclusion of 0 g, while diet T2, T3, T4 and T5 were 0.02 g, 0.03 g, 0.04 g and 0.05 g inclusion per 100 g feed, respectively. The feed was pelletized and packaged in polythene bag to avoid contamination wastage and mould invasion.

Experimental procedure

Two hundred (200) juvenile African catfish, (*Clarias gariepinus*) were procured from a reputable farm. The fish were acclimatized in a water temperature averaging 27 °C for two weeks during which they were fed extruded feed (2 mm size) twice daily. The water used was well aerated while waste and faeces in all the tanks were siphoned every day to prevent pollution. After acclimatization period, a total of one hundred and fifty (150) juveniles were randomly selected and separated into five groups of treatments and then

Table 1. Gross composition of experimental diets

Ingredients	T1 (Control)	T2 (0.02 g)	T3 (0.03 g)	T4 (0.04 g)	T5 (0.05 g)
Maize	20.60	20.60	20.60	20.60	20.60
W/offal	10.30	10.30	10.30	10.30	10.30
GNC	22.20	22.20	22.20	22.20	22.20
SBM	33.30	33.30	33.30	33.30	33.30
Fish meal	11.10	11.10	11.10	11.10	11.10
Bone meal	0.50	0.50	0.50	0.50	0.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
V/Premix	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.25	0.23	0.22	0.21	0.20
Salt	0.25	0.25	0.25	0.25	0.25
PNLM		0.02	0.03	0.04	0.05
Qty (g)	100	100	100	100	100

GNC = Groundnut cake, SBM = Soya bean meal, V/premix = Vitamin premix, PNLM = *Parquetina nigrescens* leaf meal, Qty = Quantity, D = Diet

distributed into plastic tanks (120 liters) at the rate of 10 catfish per tank having 6 females to 4 males. The fishes were fed to satiation twice daily in the morning (8:00 am) and evening (4:00 pm) while feeding was adjusted every two weeks. The fishes were weighed fortnightly, including record of the feed consumption, for the period of twelve weeks using an electronic digital weighing scale (Camry Model EK5350 of 5 kg capacity, manufactured by SENSSUN Weighing).

Data collection on growth performance

Fish weight and feed intake data were collected throughout the experiment period from which the weight gain (WG), mean weight gain (MWG), percentage weight gain (PWG), specific growth rate (SGR), feed conversion ratio (FCR), protein intake (PI) and protein efficiency ratio (PER) parameters were calculated.

$$(1) \text{ Weight gain (g) = Final weight (g) - Initial weight (g)}$$

$$(2) \text{ Mean weight gain (MWG) (g) = } \frac{\text{Weight gain (g)}}{\text{Number of fish}}$$

$$(3) \text{ Specific growth rate (SGR) (\%/day) = } \frac{(\text{Ln } W_2 - \text{Ln } W_1) \times 100}{\text{Number of culture days}}$$

where: W_1 = Initial weight, W_2 = Final weight, Ln = Natural logarithms

$$(4) \text{ Feed conversion ratio (FCR) = } \frac{\text{Dry weight of feed consumed (g)}}{\text{Wet weight gain (g)}}$$

$$(5) \text{ Protein Intake (PI) (g) = \% } \frac{\text{Protein content of feed} \times \text{Total feed intake}}{\text{Total feed intake}}$$

$$(6) \text{ Protein efficiency ratio (PER) = } \frac{\text{Wet weight gain (g)}}{\text{Protein intake (g)}}$$

Haematology analysis

At the end of the experiment, four fish samples were randomly selected from each of the treatment, two male and two female totaling twenty fish samples. They were dissected and blood was then collected through puncture of caudal blood vessel with a syringe. The blood collected was kept in clean EDTA bottle before conducting the analysis using an automated haematological analyzer systemex KX-21 (Japan) as described by Dacie and Lewis (2015). The haematological parameters measured include: Packed cell volume (PCV), Red blood cells (RBC) also known as Erythrocytes, White blood cells (WBC), Haemoglobin (Hb), Mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC), monocytes, lymphocytes and Platelets (Thrombocytes).

Chemical analysis

The proximate composition of the *P. nigrescens* experimental diet was determined using the method of Association of Analytical Chemistry (AOAC, 2006).

Statistical analysis

All data collected were subjected to one way analysis of variance (ANOVA) using a completely randomized design (CRD) and means were separated by Duncan's Multiple Range Test.

RESULTS

The proximate composition of *P. nigrescens* leaf meal is shown in Table 2. Moisture Content 7.50 %, Dry matter content 92.50 %, Ash content 13.50 %, Crude fat content 3.10 %, Crude protein 23.10 %, Crude fibre 12.20 %.

Table 2. Proximate composition of *Parquetina nigrescens* leaf meal (Test ingredients)

Parameters	Moisture Content	Dry Matter	Ash Content	Crude Fat	Crude Protein	Crude Fibre
%	7.50	92.50	13.50	3.10	23.10	12.20

Growth performance of African catfish (*Clarias gariepinus*) fed with varying inclusion levels of *Parquetina nigrescens* leaf meal

The growth performance of *Clarias gariepinus* fed with *Parquetina nigrescens* at different levels of inclusion are as shown in Table 3. The highest final mean weight-FMW (395.51 g), mean weight gained-MWG (157.94 g), percentage mean weight gain-PMWG (66.48 g), specific growth rate-SGR (0.26 %) were recorded in T3 (0.03 g inclusion level) while the lowest value of FMW (284.43 g), MWG (47.86 g), PMWG (20.15 g), SGR (0.09 %) were recorded in T4 (0.04 g). The values of T3 in FMW and MWG were however significantly different ($p < 0.05$) from other treatments. The SGR for T4 was also significantly different ($p < 0.05$) across the treatments. Although, fish fed T3 had the least value of feed conversion ratio-FCR (1.94) revealing it to be the best performing diet while T4 had the highest value of FCR (4.67). The highest feed intake-FI (332.48 g) and protein intake-PI (116.37 g) were recorded in control diet (T1) while the lowest FI (223.34 g) and PI (78.27 g) were recorded in T4. The highest PER (1.43 %) obtained in T3 is significantly different ($p < 0.05$) from other treatments as shown in Table 3. However, there is lower FCR and higher PER values in *P. nigrescens* supplemented diets compared to the control diet (T1).

Haematology of African catfish (*Clarias gariepinus*) fed varying inclusion levels of *Parquetina nigrescens* leaf meal

The result for PCV (35.5 %), RBC ($3.90 \times 10^{12}/L$), Haemoglobin (11.75 g/dL) and Heterocytes (21.00 %) showed highest value for control (T1) which is significantly different ($p < 0.05$) from the values of T2, T3, T4 and T5. The value of T3 was highest in MCHC (34.02 g/dl) and significantly different ($p < 0.05$) from other treatments. WBC (241.00 mm^{-3}) was also highest in T3 but not significantly different ($p > 0.05$). MCV (92.13 fl) and lymphocytes (76.00 %) results were highest in T4 and were significantly different ($p < 0.05$) from other treatments. The lowest values of 89.14 fl in T3 and 68.00 % in T1 were however respectively recorded for MCV and Lymphocytes. The highest value for MCH was 31.11 pg in T5 and the lowest (30.14 pg) was recorded in T1. Platelet result shows that there is no significant difference ($p < 0.05$) across the treatments and the highest value ($172.50 \times 10^9/L$) was recorded in T1, while the lowest value ($161.00 \times 10^9/L$) was in T4. The result for Monocytes shows that the highest value (3.00 %) was recorded in T1 and T3, while the lowest value (2.00 %) was in T2 and T5 and there is no significant difference across the treatments. Overall, the values of PVC, RBC, Hb, Heterocytes, Monocytes and Platelets were lower

Table 3. Growth performance of the African catfish (*Clarias gariepinus*) fed with varying inclusion levels of *Parquetina nigrescens* leaf meal

Blood parameters	T1 (Control)	T2 (0.02 g)	T3 (0.03 g)	T4 (0.04 g)	T5 (0.05 g)	SEM
IMW (g)	237.57	237.57	237.57	237.57	237.57	3.27
FMW(g)	361.92 ^b	347.71 ^b	395.51 ^a	285.43 ^c	322.88 ^c	11.45
MWG (g)	124.35 ^{ab}	110.14 ^b	157.94 ^a	47.86 ^c	85.31 ^b ^c	9.42
PMWG (%)	52.34 ^a	46.36 ^a	66.48 ^a	20.15 ^b	35.91 ^a	3.64
SGR (%)	0.22 ^a	0.20 ^a	0.26 ^a	0.09 ^b	0.16 ^a	0.19
FI (g)	332.48 ^a	296.03 ^c	305.92 ^b	223.34 ^e	261.12 ^d	7.17
FCR	2.67 ^b	2.69 ^b	1.94 ^b	4.67 ^a	3.06 ^b	0.19
PI (g)	116.37 ^a	103.61 ^c	107.07 ^b	78.27 ^e	91.39 ^d	2.50
PER (%)	1.07 ^{ab}	1.07 ^{ab}	1.43 ^a	0.61 ^c	0.93 ^b ^c	0.07

All the parameters with different superscripts are significantly different ($p < 0.05$)

IMW = Initial Mean Weight; FMW = Final Mean Weight; MWG = Mean Weight Gain; PMWG = Percentage Mean Weight Gain; SGR = Specific Growth Rate; FCR = Feed Conversion Ratio; FI = Feed Intake; PI = Protein Intake; PER = Protein Efficiency Ratio; SEM = Standard Error of Mean.

Table 4. Haematology of African catfish (*Clarias gariepinus*) fed varying inclusion levels of *Parquetina nigrescens* leaf meal

Parameters	T1 (Control)	T2 (0.02 g)	T3 (0.03 g)	T4 (0.04 g)	T5 (0.05 g)	SEM
PCV (%)	35.50 ^a	32.00 ^b	29.00 ^b	29.00 ^b	29.00 ^b	0.63
RBC (x 10 ¹² /L)	3.90 ^a	3.50 ^b	3.25 ^b	3.15 ^b	3.15 ^b	0.07
Hb (g/dL)	11.75 ^a	10.55 ^b	9.85 ^b	9.80 ^b	9.70 ^b	0.20
MCH (pg)	30.14 ^c	30.18 ^c	30.31 ^c	30.79 ^b	31.11 ^a	0.08
MCV (fl)	91.06 ^a	91.61 ^a	89.14 ^b	92.13 ^a	92.12 ^a	0.30
MCHC (g/dL)	33.10 ^c	32.95 ^c	34.02 ^a	33.42 ^{bc}	33.77 ^{ab}	0.10
WBC (mm ⁻³)	210.00 ^b	239.00 ^a	241.00 ^a	163.00 ^c	173.00 ^c	6.59
HETEROCYTES (%)	21.00 ^a	18.50 ^{ab}	17.50 ^b	16.50 ^b	19.50 ^{ab}	0.50
LYMPHOCYTES (%)	68.00 ^b	73.50 ^a	72.50 ^a	76.00 ^a	73.00 ^a	0.72
MONOCYTES (%)	3.00 ^a	2.00 ^a	3.00 ^a	2.50 ^a	2.00 ^a	0.19
PLATELET (x 10 ⁹ /L)	172.50 ^a	171.50 ^a	167.50 ^a	161.00 ^a		2.21

All the parameters with different superscripts are significantly different ($p < 0.05$)

PCV = Packed cell volume, RBC = Red blood cell, Hb = Haemoglobin, MCH = Mean corpuscular haemoglobin, MCV = Mean corpuscular volume, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood cell.

across treatments than control diet (T1) while MCH, MCV, MCHC and Lymphocytes were of higher values than T1 as shown in Table 4.

DISCUSSIONS

The proximate composition of the experimental diet in the study revealed that Crude Protein (35 %) is slightly higher than the value of 20.6 ± 0.25 % and 18.0 ± 0.25 % recorded by Adjatin *et al.*, (2013). The protein contents, is however, around the normal reported range of protein level found in green leafy vegetables (20.48 – 41.66 %) on dry weight basis as noted by Hussain *et al.*, (2010). Recent research by Abubakar *et al.*, (2015) and Mwangi *et al.*, (2018) reported as low as 20 % crude protein in the diet of African catfish without negative consequence.

On growth performance and feed utilization, highest protein efficiency ratio (PER) recorded in treatment 3 (1.43) implies that the sample fish gained highest protein from the feed. The results revealed T3 to be the best for *Clarias gariepinus* for better feed utilization by converting the feed to flesh. As observed by Davies *et al.*, (2006), protein efficiency ratio, is a measure of how well the protein sources in a diet could provide the essential amino acid requirement of

the fish. The best feed conversion ratio (FCR) was in T3 (1.94). This is in agreement with De Silva (2001), who posited that the best feed conversion ratio is between 1.2 – 1.95 for fish fed carefully prepared diets, and the result from the present study falls within this range. FCR decreases and PER increases when the quality of protein in the diet improves which indicate increased feed utilization efficiency of fish and reduction of production cost (Zahan *et al.*, 2024). Therefore, this study indicates that treatment 3 shows the best performance in *Clarias gariepinus* with regards to FCR, weight gain, and PER.

Haematological parameters showed that control diet (T1) is significantly different from other treatments while other treatments were however within the recommended limit for African catfish according to Akinrotimi *et al.*, (2011). Infused extracts of *P. nigrescens* in feed possess haematopoietic properties (Awoniyi *et al.*, 2022). This was corroborated by Adase *et al.*, (2022), who posited that *P. nigrescens* is rich in phytochemical constituents and possesses pharmacological properties and might be considered as a remedy for different diseases such as anaemia, diabetes, ulcers, inflammation, asthma, and typhoid. This further indicates that addition of *P. nigrescens* leaf meal at the various inclusion levels have no detrimental effect on physiology and well-being of African catfish.

CONCLUSION

It can be concluded that T3 at 0.03 g inclusion level of *Parquetina nigrescens* leaf meal had the best results in terms of growth performance and blood profile (haematology) of African catfish (*Clarias gariepinus*).

ACKNOWLEDGEMENTS

The efforts of Adu Funmilayo, Baba-Yusuf Sheu Ibrahim and Ayanleke Tunmishe Olayemi of the Department of Fisheries and Aquaculture, Ladoko Akintola University of Technology, Ogbomoso, at making this research a success cannot be over emphasized. This research however received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

AUTHOR'S CONTRIBUTIONS

Conceptualization: OLANIYI, C., FAWOLE, O.

Methodology: OLANIYI, C., FAWOLE, O.

Writing-original draft preparation: OLANIYI, C., FAWOLE, O.

Writing-review and editing: FAWOLE, O.

Project administration: OLANIYI, C., FAWOLE, O.

All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this research.

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