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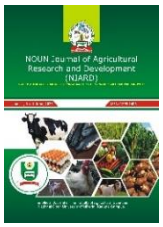

Original Article

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Effects of Lysine-Supplemented Groundnut Cake Diets on Growth Performance and Carcass Quality of *Heterobranchus longifilis fingerlings*

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Abstract

Aquaculture plays a vital role in meeting the increasing demand for high-quality animal protein. However, feed costs account for over 50% of production expenses, limiting profitability and sustainability. Locally available plant protein sources such as groundnut cake offer a cost-effective alternative, but their use is constrained by deficiencies in essential amino acids, particularly lysine. This study evaluated the effects of lysine-supplemented groundnut cake–based diets on the growth performance and carcass quality of Heterobranchus longifilis fingerlings. Fingerlings (mean weight 1.54 g) were stocked at 15 fish per 60-L circular plastic tank and assigned to six experimental diets in triplicate. Diets included a fishmeal-based control (GFM), groundnut cake without lysine (GL0), and four lysine-supplemented diets: GL1 (0.25 g/kg), GL2 (0.5 g/kg), GL3 (0.75 g/kg), and GL4 (1.0 g/kg). All diets contained approximately 35% crude protein and were fed for 8 weeks. Mean weight gain was highest in GL2 (4.05 g), followed by GL3 (3.31 g), GL0 (2.81 g), GL4 (2.67 g), and GL1 (2.25 g). GL2 also recorded the highest specific growth rate (0.63% day^{-1}), best feed conversion ratio (2.15), and protein efficiency ratio (1.48). Carcass protein content was highest in GL3 (56.76%), followed by GL2 (47.95%). Lysine supplementation significantly improved growth, feed utilization, and carcass composition, with 0.5 g/kg (GL2) identified as the optimum inclusion level.

Keywords: Carcass quality, *Heterobranchus longifilis*, Lysine, Groundnut Cake

INTRODUCTION

Aquaculture plays a critical role in meeting the escalating demand for affordable and high-quality animal protein, particularly in developing regions where fish contributes substantially to dietary intake and rural livelihoods (WorldFish, 2018). However, feed cost often accounts for more than 50% of total operating costs, thus limiting profitability and sustainability of culture systems (Yousif, et al., 2020). Traditionally, fishmeal has been the preferred protein source in formulated aquafeeds due to its well-balanced essential amino acid (EAA) profile and high digestibility. However, its escalating price and supply constraints have driven research toward alternative, locally available plant protein sources. Conventional plant protein such as soyabean are also expensive and compete with

human food (Okomoda et al., 2020). Thus, there is the need to search for sustainable alternatives which are locally available and affordable (Yousif, et al*.,* 2020).

Groundnut cake (GNC) is an economically potential ingredient with comparatively high crude protein content, it is available in many tropical countries and is affordable (Olapade & George, 2019; Oyedokun, 2021). (GNC) has the potential to reduce feed costs when substituting more expensive animal based proteins. However, plant protein ingredients possess unbalanced Essential amino acid (EAA) profiles relative to fish requirements, with lysine frequently being the first limiting amino acid (Yousif, et al*.,* 2020; Amir, 2025).

Lysine is essential for protein synthesis, growth, muscle development, absorption of calcium, production of antibodies and enzymes, repairs of tissue fish and effective feed utilization in fish (Etim, et al., 2020; Zhang et al., 2021**).** Deficiency in dietary lysine can result in reduced weight gain, poor feed conversion, and suboptimal carcass quality (Yu et al*.,* 2023). Moreover, the imbalance of EAAs in plant-based diets not only constrains growth performance but can also influence nutrient retention and metabolic efficiency (Amir, 2025). Supplementation of crystalline lysine or lysine-rich ingredients has therefore become a widely recommended strategy to correct amino acid deficiencies and improve the nutritional quality of plant protein-based feeds in aquaculture. (Rachmawati et al., 2022). Empirical evidence from various studies demonstrates the importance of lysine supplementation in enhancing growth performance, feed utilization, and nutrient retention when alternative protein sources are used (Etim, *et al*., 2020; Rachmawati et al., 2022; Abozaid, et al., 2024; Awolumate & Agbo, 2025). The weight gain of Sangkuriang catfish fingerlings improved when lysine was added to their diet (Rachmawati et al., 2022). In *Clarias gariepinus* juvenile, supplementation of lysine has been shown to influence growth performance positively while research involving Hemibagrus wyckioides juveniles illustrated improved growth and digestive enzyme activity with lysine enrichment in plant protein blend feeds (Oyedokun, 2021; Sun et al., 2023). These findings collectively highlight lysine's role in balancing plant protein diets and promoting productive aquaculture.

Despite significant advances in understanding lysine requirements for major cultured fish species, there remains a paucity of species-specific nutritional data and amino acid supplementation of plant proteins for African catfish, particularly *Heterobranchus longifilis*. *H. longifilis* is a species of great cultural and economic importance in Sub-Saharan Africa because of its adaptability to diverse environments (Ogunji & Wuertz, 2023). Existing studies on *H. longifilis* have primarily focused on factors such as dietary lipid source and plant meal inclusion without detailed evaluation of essential amino acid supplementation (Otchoumou 2019; Afe et al 2019; Umanah et al 2019; Aliu & Ademiluyi 2020; Ogah & Ubaka 2025). The response of *H. longifilis* to lysine-supplemented, groundnut cake-based diets, especially with respect to growth performance and carcass quality has not been comprehensively investigated. Understanding how lysine supplementation influences both growth performance and carcass quality is therefore critical for overall nutritional adequacy and consumer acceptance of cultured fish (Rachmawati et al., 2022). This study aims to evaluate the effects of lysine-supplemented groundnut cake diets on the growth performance and carcass quality of *Heterobranchus longifilis* fingerlings. These findings will aid in refining feed formulation strategies to support sustainable and economically viable production of *H. longifilis.*

MATERIALS AND METHODS

Study area

The experiment was carried out at the Fisheries unit of the National Open University of Nigeria, Kaduna, situated in Igabi local government area, between latitude 9º34'N and longitude 8º17'E. It falls within the Guinea Savannah ecological zone in the North-western part of Nigeria.

Experimental Fish and Acclimatization

Two hundred and seventy (270) fingerlings of *Heterobranchus longifilis* with a mean weight (1.54g) were obtained from a reputable hatchery. The fish were acclimatized for 14 days in plastic tanks and fed a commercial diet containing approximately 40% crude protein. The fish were deprived of food for 24 hours before commencing the experiment.

Experimental Design

A completely randomized design was used for the feeding trial. Fish were randomly assigned to six

experimental diets with three replicates per treatment. Six (6) experimental diets were formulated containing graded levels of lysine supplementation. Each dietary treatment was assigned to triplicate tanks, with 15 fish stocked per circular tank of 60-liter capacity. The feeding trial lasted for 8 weeks.

Diet Formulation and Preparation

Groundnut cake served as the major plant protein source in all experimental diets. Synthetic lysine (L-lysine) was supplemented at graded levels. The diets were formulated to have approximately 35% crude protein using Microsoft Excel to balance crude protein and energy based on standard feed formulation principles. All feed ingredients (Fishmeal, vitamin premix, groundnut cake maize, soyabean cake and bonemeal) were finely ground using a hammer mill, weighed according to the formulation, and thoroughly mixed to ensure uniform distribution of lysine. Warm water was added to form a dough, which was pelleted using a manual pelleting machine and sundried. The dried pellets were stored in airtight containers until use. Treatments were GFM (fishmeal-based diet), GL0 (control, 0g/kg Lysine) while GL1, GL2, GL3 and GL4 contain 0.25g/kg, 0.5g/kg, 0.75g/kg and 1g/kg lysine for every 100kg feed respectively. The proximate composition of the experimental feed and carcass analysis was determined as outlined in AOAC (1990). Table 1 shows the ingredient and proximate composition of experimental diets.

Feeding Regime

Fish were fed 5% body weight twice daily for 8 weeks. The ration was recalculated after every two weeks according to the weight increase recorded when the fish were sampled.

Water Quality Management

Water quality parameters including temperature, dissolved oxygen, and pH were monitored biweekly using portable digital oxygen meter (TPB-607), mercury in-glass thermometer, and a pH meter (pH-009) model. Partial water exchange was carried out twice weekly to maintain optimal rearing conditions throughout the experimental period. The mean water quality parameters are presented in Table 2.

Table 1: Composition of experimental diets supplemented with lysine

	Treatments						
Ingredients (g/kg)	GFM	GL0	GL1	GL2	GL3	GL4	
Maize	19.00	7.00	7.20	7.00	7.00	7.00	
G/Cake	61.00	85.00	85.00	85.00	85.00	85.00	
Soya cake	5.00	4.50	4.00	4.00	3.70	3.50	
Fish meal	12.00	0.00	0.00	0.00	0.00	0.00	
Palm oil	1.00	1.00	1.00	1.00	1.00	1.00	
Starch	0.50	1.00	1.00	1.00	1.00	1.00	
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00	
Salt	0.25	0.25	0.25	0.25	0.25	0.25	
Vit Premix	0.25	0.25	0.25	0.25	0.25	0.25	
Lysine	0.00	0.00	0.25	0.50	0.75	1.00	
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	
Proximate composition							SEM
Moisture	11.23	10.94	11.70	10.67	12.51	9.76	0.38
Crude protein	38.21	36.81	34.00	34.05	33.60	34.05	0.78
Crude fibre	2.00	2.11	2.45	2.48	2.51	2.55	0.10
Ether extract	7.08	6.77	6.98	7.01	7.75	8.02	0.20
Ash	10.52	10.16	9.96	10.02	11.31	11.55	0.28
Energy (kcal/kg)	2497.80	2456.10	2417.80	2442.50	2500.20	2502.70	14.64

Table 2**: Mean water quality parameters**

	GFM	GL0	GL1	GL2	GL3	GL4	SEM
Temperature	24.29	24.17	24.30	24.53	24.34	24.48	0.05
DO_2	4.19	3.60	3.09	3.19	3.59	3.17	0.17
pH	7.45	7.03	6.93	6.87	7.39	7.13	0.10

Growth Performance Evaluation

Fish were weighed bi-weekly to assess growth performance. The following parameters were calculated for each treatment to determine the growth response of *Heterobranchus longifilis.*

i. Mean Weight Gain (MWG) = Mean final weight − Mean initial weight

ii. *F*eed Conversion Ratio (FCR) = Total feed consumed/Weight gain by fish

iii. Survival rate=$\frac{Total\ number\ of\ fish\ harvested}{initial\ number\ of\ fish\ stocked} \times 100$

iv. Specific Growth Rate(SGR) = $\frac{lnW1-lnW2}{T2-T1} \times 100$

Where W2 = Final weight, W1 = Initial weight

T2 − T1 = Time interval between W2 and W1 in days

ln = Natural log

v. Protein efficiency ratio (PER) = weight gain by fish/protein intake by fish where protein intake = $\frac{\text{total feed consumed x 100}}{\text{crude protein in feed}}$

Carcass Analysis

At the end of the feeding trial, two fish per tank were randomly sampled and processed for carcass analysis. The carcass composition of the fish was carried out as outlined in AOAC (1990).

Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) to determine the effect of dietary treatments on growth performance and carcass composition of the experimental fish. Additionally, Duncan's multiple range test (DMRT) was employed to identify the means that were significantly different. Differences among treatment means were considered significant at $p < 0.05$. Statistical analysis was carried out using statistical analysis system (SAS) software 2005 version 9.1.

RESULTS

Growth performance, feed utilization, and survival of fish fed the experimental diets are summarized in Table 3. The mean weight gain differed significantly ($p < 0.05$) among dietary treatments. The Mean weight gain (MWG) was higher in GL2 (4.05 g) followed by GL3 (3.31 g) then GL0 (2.81 g), and GL4 (2.67 g). The lowest mean weight gain was observed in fish fed GL1 (2.25 g). Specific growth rate (SGR) followed a similar trend, with the highest SGR observed in GL2 (0.63 % day^{-1}) and fish fed GL1 recorded the lowest SGR (0.41 % day^{-1}).

Feed utilization indices were significantly influenced by dietary treatments ($p < 0.05$). The best feed conversion ratio (FCR) was obtained in GL2 (2.15), indicating superior feed efficiency, while the poorest FCR was recorded in GL4 (4.34). Protein efficiency ratio (PER) showed significant variation among treatments. The highest PER was recorded in GL2 (1.48), followed by GL3 (1.26), while the lowest PER was observed in GL4 (0.67). Survival rate also differed significantly ($p < 0.05$), with the highest survival recorded in GL4 (86.67%) and the lowest in GL3 (42.22%). Overall, diets significantly affected growth performance, feed utilization, and survival of the experimental fish.

Table 3: Growth performance of *Heterobranchus longifilis* fed groundnut cake-based diets supplemented with lysine

Parameters	GFM	GL0	GL1	GL2	GL3	GL4	SEM
Initial Weight (g)	1.77	1.58	1.62	1.40	1.35	1.51	0.06
Final Weight (g)	6.14^{a}	4.38^{cd}	3.87^{e}	5.45^{b}	4.67^{c}	4.18^{d}	0.35
Mean Weight Gain (g)	4.37^{a}	2.81^{c}	2.25^{d}	4.05^{ab}	3.31^{b}	2.67^{c}	0.34

Specific Growth Rate (SGR) (%/day)	0.60^{b}	0.49^{d}	0.41^{e}	0.63^{a}	0.57^{c}	0.49^{d}	0.03
Feed conversion Ratio	2.81^{d}	3.01^{c}	3.84^{b}	2.15^{e}	3.05^{c}	4.34^{a}	0.32
Feed intake	11.85^{a}	8.23^{b}	8.32^{b}	7.82^{c}	7.62^{c}	11.23^{ab}	0.76
Protein efficiency ratio	0.94^{c}	0.93^{c}	0.78^{d}	1.48^{a}	1.26^{b}	0.67^{e}	0.12
Survival rate	63.34^{b}	53.33^{c}	60.00^{b}	53.33^{c}	42.22^{d}	86.67^{a}	6.13

Means in the same row with different superscripts are significantly different (P<0.05); SEM= Standard Error of Mean

Table (4): Carcass composition of *H. longifilis* fed groundnut cake-based diets supplemented with lysine

Parameters (%)	GFM	GL0	GL1	GL2	GL3	GL4	SEM
Moisture	21.60^{e}	23.58^{d}	24.95^{a}	24.46^{b}	20.65^{f}	23.87^{c}	0.37
Ash	3.68^{b}	4.38^{a}	3.68^{b}	3.76^{b}	3.71^{b}	4.38^{a}	0.08
Lipid	18.12^{a}	11.43^{e}	14.85^{c}	12.76^{d}	15.99^{b}	15.53^{b}	0.53
Protein	53.72^{b}	36.58^{e}	35.16^{f}	47.95^{c}	56.76^{a}	37.47^{d}	2.09
Fibre	ND	ND	ND	ND	ND	ND	0.00
NFE	2.88^{e}	24.03^{a}	21.36^{b}	11.07^{d}	2.87^{e}	18.75^{c}	2.06

Means in the same row with different superscripts are significantly different (P<0.05); SEM= Standard Error of Mean ND= not detected

The proximate composition of fish carcass across the experimental dietary treatments is presented in Table 4. Moisture content differed significantly ($p < 0.05$) among treatments, ranging from 20.65% to 24.95%.

Fish fed GL1 recorded the highest moisture content (24.95%), followed by GL2 (24.46%) and GL4 (23.87%), while the lowest value was observed in GL3 (20.65%). Ash content varied significantly ($p < 0.05$) across diets. The highest ash values were recorded in fish fed GL0 and GL4 (4.38%), whereas fish fed GL1, GL2, and GL3 had comparable and lower ash contents, ranging from 3.68% to 3.76%. Lipid content showed significant difference among treatments ($p < 0.05$). Fish fed GL3 (15.99%) and GL4 (15.53%) recorded high lipid content. The lowest lipid content was observed in GL0 (11.43%), while intermediate values were obtained in GL1 and GL2. Crude protein content was significantly influenced by dietary treatments ($p < 0.05$). The highest protein level was observed in fish fed GL3 (56.76%), followed by GL2 (47.95%). Fish fed GL1 recorded the lowest protein content (35.16%), with GL0 and GL4 showing intermediate but significantly lower values. Crude fibre was not detected (ND) in the carcass of fish across all dietary treatments. Nitrogen-free extract (NFE) differed significantly ($p < 0.05$) among treatments, with the highest value recorded in GL0 (24.03%), followed by GL1 (21.36%) and GL4 (18.75%). The lowest NFE values were observed in GL3 (2.87%). Overall, the results indicate that dietary treatments significantly affected the carcass proximate composition of the fish.

DISCUSSION

The results demonstrate that dietary lysine supplementation significantly influenced growth, feed utilization, and carcass composition. This is consistent with reports that balanced essential amino acid profiles are critical for optimal performance in fish species (Sun et al., 2023). The superior growth performance (MWG and SGR) observed in fish fed lysine-enhanced diets compared with plant-based diets lacking adequate lysine reflects the importance of this amino acid in protein accretion and muscle growth. Lysine is often the first limiting essential amino acid in plant protein sources such as groundnut cake, and supplementation improves utilization of dietary protein, as has been demonstrated in other catfish species (*Clarias gariepinus*) where lysine balance enhanced growth performance and feed efficiency (Etim et al., 2020; Oyedokun et al., 2024). The lower (MWG) observed in GL0 and GL1 suggests insufficient lysine in diet led to a depressed growth (Dou et al., 2023). Previous studies have also reported that high lysine content above the optimum requirement disrupts amino acids balance, limits absorption and utilization of other amino acids. Rachmawati et al. (2022) reported

increased MWG and SGR to a peak after which there was a decline when Sangkuriang catfish fingerlings were fed lysine supplemented diets. The relatively low survival rate recorded in this study may be attributed to a combination of factors such as frequent handling for weighing, sampling, or tank cleaning and cannibalism (Hamid et al., 2017). The relative low survival rate was observed in all the diets except GL4 therefore it does not affect the validity of the growth performance data. Feed conversion ratio and protein efficiency ratio in the lysine-supplemented treatments were markedly improved compared to groundnut cake diets without lysine, indicating that amino acid fortification enhances nitrogen retention and reduces waste. This finding aligns with Li et al. (2019) which emphasized that precise essential amino acid fortification can improve protein utilization and reduce feed costs. The finding of the present study on improved feed utilization is also in line with the work of Oyedokun (2024) when 0.6g/100g lysine was incorporated in the diet of *Clarias gariepinus*. The improved feed intake observed in certain lysine-supplemented groups suggests that lysine may also act as a feeding stimulant, as reported previously in other catfish studies, where increasing lysine levels stimulated appetite and intake (Etim et al., 2020). Higher survival rates in some lysine treatments may imply health benefits associated with amino acid balance, potentially through enhanced immune function and physiological resilience. However, it has been reported that survival responses and other growth parameters might be influenced by factors such as diet composition, formulation techniques, size and age of the fish, genetics, feed management, the lysine need among fish species, environmental factors and experimental design (Rachmawati et al., 2022).

Carcass proximate composition reflected significant dietary effects. Increased protein deposition suggests improved nutrient utilization. Higher protein content in lysine-supplemented groups underscores the role of lysine in muscle protein synthesis and lean tissue deposition (Yousif et al 2020). Such shifts in body composition with improved essential amino acid balance have also been documented in fish fed plant protein diets supplemented with limiting amino acids (Oyedokun et al., 2024). For example, Sun *et al*. (2023) observed crude protein content significantly increased when *Hemibagrus wyckioides* juveniles' diet was supplemented with lysine. Similarly, Dou et al. (2023) reported that carcass crude protein content was enhanced with increasing lysine levels in diet of Leopard Coral Grouper.

Groundnut cake as a partial fishmeal substitute possesses advantages in terms of cost and local availability, but its inherent lysine deficiency limits its use in high-performance diets. Previous research underscores that supplementation of plant-based proteins with lysine and other limiting amino acids can enable effective replacement of fishmeal without compromising growth (Yu et al., 2023). However, the form and bioavailability of supplemental lysine remain important considerations, as protein-bound lysine may be utilized more efficiently than free crystalline forms in some contexts (Billah et al 2022). The present findings reinforce the concept that carefully balanced amino acid profiles in diets containing alternative plant protein sources are essential to optimize performance and product quality in fish species like *H. longifilis*. Despite improvements with lysine supplementation, observed variability among treatments suggests that optimal inclusion levels and interactions with other dietary components require further investigation. Future studies should explore the digestibility and bioavailability of lysine from different sources in *H. longifilis*, including the potential benefits of using coated or slow-release formulations to minimize leaching in water. Additionally, research on long-term effects on reproductive performance, fillet quality, and economic returns would support practical feed formulation strategies under commercial conditions.

Conclusion

The findings of this study demonstrate that lysine supplementation of groundnut cake–based diets positively influenced growth performance, feed utilization and carcass composition of *Heterobranchus longifilis*. Fish fed the lysine-supplemented diet at the 0.5g/kg inclusion level (GL2) exhibited superior growth performance indices and feed utilization. These results indicate that adequate lysine supplementation effectively enhanced groundnut cake utilization. Based on growth performance, feed efficiency, and carcass protein quality, the GL2 diet represents the most suitable lysine supplementation level for fingerlings of *H. longifilis*. Adoption of this

feeding strategy could enhance cost-effective and sustainable catfish production, particularly in regions where groundnut cake is readily available.

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