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Nutrient Retention and Feed Utilization Efficiency in *Clarias gariepinus*: The Role of Lysine and Methionine in Enhancing Protein Deposition and Reducing Nitrogen Waste

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Abstract: *The increasing shift towards plant-based protein sources in aquafeeds has necessitated optimizing nutrient retention and feed utilization efficiency to ensure sustainable aquaculture practices. This study evaluated the effects of lysine and methionine supplementation on protein deposition, feed efficiency, and nitrogen waste reduction in Clarias gariepinus. A completely randomized design (CRD) was used to assign 525 juvenile fish to six dietary treatments containing graded levels of lysine and methionine, with each treatment replicated three times. Fish were fed at 5% of their body weight thrice daily for eight weeks, after which growth performance, nutrient retention efficiency, and nitrogen waste excretion were analyzed. The results revealed that fish fed the highest lysine and methionine supplementation (1.00 g/kg each) exhibited significantly higher ($P < 0.05$) final weight (11.58 g), weight gain (5.27 g), and specific growth rate (0.29%/day) compared to the unsupplemented control (GL0: final weight = 4.67 g, weight gain = 1.27 g, SGR = 0.17%/day). Feed utilization was significantly enhanced, as indicated by an improved feed conversion ratio (FCR: 4.02) and protein efficiency ratio (PER: 0.74) in supplemented groups compared to the control (FCR: 7.28, PER: 0.54). Additionally, nitrogen retention efficiency (NRE) improved from 45.2% in GL0 to 74.5% in GL4, while total ammonia nitrogen (TAN) concentration decreased from 4.5 mg/L to 1.9 mg/L, reflecting better nitrogen metabolism and reduced environmental nitrogen loading. These findings demonstrate that lysine and methionine supplementation in plant-based diets significantly enhances nutrient retention, protein deposition, and feed efficiency while mitigating nitrogen waste accumulation. This study underscores the potential of amino acid supplementation in formulating sustainable, cost-effective, and environmentally friendly aquafeeds. Future research should explore long-term impacts on fish health, immune function, and environmental nitrogen dynamics in intensive aquaculture systems.*

Keywords*: Clarias gariepinus, lysine, methionine, feed efficiency, nitrogen retention, sustainable aquaculture*

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1.0 Introduction

The increasing demand for sustainable aquaculture has intensified the search for efficient and cost-effective feed formulations that optimize nutrient utilization while minimizing environmental impact (Gatlin et al., 2021). In aquaculture nutrition, protein is the most expensive dietary component, and its efficient utilization is essential for promoting fish growth and reducing nitrogen waste emissions (Furuya et al., 2022). *Clarias*

gariepinus, an economically important freshwater species, has gained widespread attention due to its adaptability, high growth rate, and resilience in intensive culture systems (Tiamiyu et al., 2023). However, the shift towards plant-based protein sources in fish diets presents challenges related to essential amino acid deficiencies, particularly lysine and methionine, which are critical for protein synthesis and metabolic balance (Abdel-Tawwab et al., 2021).

Lysine and methionine are indispensable amino acids required for efficient protein metabolism, muscle accretion, and immune function in fish (Espe et al., 2023). Deficiencies in these amino acids can lead to suboptimal growth, poor feed conversion efficiency, and increased nitrogen excretion, which not only affects aquaculture profitability but also contributes to water pollution through excessive ammonia release (Li et al., 2022). Research has shown that supplementing lysine and methionine in plant-based diets improves protein deposition, enhances feed utilization efficiency, and reduces nitrogen waste output by optimizing nitrogen retention and minimizing protein catabolism (Ahmed et al., 2021).

Feed conversion ratio (FCR), protein efficiency ratio (PER), and nutrient digestibility are key parameters for evaluating the efficiency of feed utilization in fish farming (Sarker et al., 2022). Optimized amino acid supplementation has been reported to enhance nitrogen retention, thereby improving protein deposition while decreasing nitrogenous waste production (Hossain et al., 2023). Therefore, understanding the impact of lysine and methionine on nutrient retention in *Clarias gariepinus* is critical for developing sustainable feeding strategies that balance growth performance with environmental conservation.

This study investigated the role of lysine and methionine supplementation in enhancing nutrient retention and feed utilization efficiency in *Clarias gariepinus*. Specifically, it assessed how amino acid optimization influenced protein deposition, feed conversion efficiency, and nitrogen waste reduction. The findings provided valuable insights into the formulation of cost-effective, nutritionally balanced, and environmentally sustainable diets for the commercial production of *Clarias gariepinus*.

2.0 Materials and Methods

2.1 Study Location and Experimental Conditions

This study was conducted at the National Open University of Nigeria Teaching and Research Farm, Igabi, Kaduna, Nigeria (10° 37' 57" N, 7° 28' 22" E). The farm is situated in a tropical climate region, with annual temperatures ranging between 21°C and 36°C and relative humidity levels of 30% to 80%. Borehole-sourced water was used to maintain stable aquaculture conditions throughout the experimental period.

2.2 Experimental Fish and Design

A total of 525 juvenile *Clarias gariepinus* with an initial mean weight of 5.71 ± 0.45 g were procured from a commercial hatchery and acclimatized for two weeks in holding tanks before the experiment. Fish were then randomly distributed into six dietary treatment groups using a Completely Randomized Design (CRD), with each treatment replicated three times. Each replicate consisted of 15 fish per tank, housed in circular tanks (100 L capacity) with a continuous aeration system.

2.3 Diet Formulation and Experimental Treatments

The experimental diets were formulated to be **isonitrogenous** (35% crude protein) and **isoenergetic**, with groundnut cake serving as the primary protein source. Lysine and methionine were supplemented at varying levels to evaluate their effects on fish growth performance.

Dietary Treatments

The six dietary treatments consisted of incremental levels of methionine and lysine supplementation, as detailed in Table 1. This table presents the levels of methionine and lysine supplementation (g/kg diet) in each

dietary treatment. The control diet (T1) contained no additional amino acid supplementation, while T2–T5 received increasing concentrations of methionine and lysine.

Table 1: Composition of Experimental Diets with Incremental Methionine and Lysine Supplementation

Treatment	Methionine (g/kg diet)	Lysine (g/kg diet)
T1 (Control)	0.00	0.00
T2	0.25	0.25
T3	0.50	0.50
T4	0.75	0.75
T5	1.00	1.00

Fish were fed at 5% of their body weight three times daily (07:00 hrs, 13:00 hrs, and 18:00 hrs). The feeding rate was adjusted weekly based on observed weight changes. Feed was pelleted (2 mm diameter), dried, and stored in airtight containers before use.

2.4 Water Quality Monitoring

Water quality parameters were closely monitored throughout the study to maintain optimal conditions for fish growth and health. The parameters measured included:

- **Temperature (°C):** Measured daily using a mercury-in-glass thermometer.
- **Dissolved Oxygen (DO_2) (mg/L):** Assessed using a dissolved oxygen meter.
- **pH:** Measured with a digital pH meter.
- **Ammonia (NH_3) (mg/L):** Determined using a spectrophotometer.
- **Nitrate (NO_3^-) and Nitrite (NO_2^-) levels:** Measured using ion chromatography.

To prevent the accumulation of nitrogenous waste, 80–90% of the water was renewed daily to maintain water quality at optimal levels.

2.5 Growth Performance and Feed Utilization Parameters

At the end of the eight-week feeding trial, growth performance and feed utilization parameters were evaluated using the following indices:

Mean Weight Gain (MWG) (g):

$$MWG = \text{Final Weight} - \text{Initial Weight} \quad (1)$$

Specific Growth Rate (SGR) (%/day):

$$SGR = \frac{\text{In(Final Weight) - In(Initial Weight)}}{\text{Day}} \times 100 \quad (2)$$

Feed Conversion Ratio (FCR):

$$FCR = \frac{\text{Total Feed Intake (g)}}{\text{Weight Gain (g)}} \quad (3)$$

Protein Efficiency Ratio (PER):

$$PER = \frac{\text{Weight Gain (g)}}{\text{Protein Intake (g)}} \quad (4)$$

Survival Rate (%):

$$SR = \left(\frac{\text{Number of fish at the end}}{\text{Number of fish at the start}}\right) \times 100 \quad (5)$$

2.6 Nutrient Retention and Nitrogen Waste Analysis

To assess protein deposition and nutrient retention efficiency, fish carcass samples were analyzed at the beginning and end of the experiment for the following parameters:

- Crude Protein (%): Determined using the Kjeldahl method
- Lipid Content (%): Measured using the Soxhlet extraction method
- Ash Content (%): Assessed via furnace incineration at 550°C
- Moisture Content (%): Measured using oven drying at 105°C

Nutrient Retention Efficiency (NRE) (%)

$$NRE = \left(\frac{\text{Final body nitrogen} - \text{Initial body nitrogen}}{\text{Total nitrogen intake}}\right) \times 100 \quad (6)$$

Protein Retention Efficiency (PRE) (%)

$$PRE = \left(\frac{\text{Protein gain in fish}}{\text{Protein intake}}\right) \times 100 \quad (7)$$

Energy Retention Efficiency (ERE) (%)

$$ERE = \left(\frac{\text{Final body nitrogen} - \text{Initial body nitrogen}}{\text{Total nitrogen intake}}\right) \times 100 \quad (8)$$

To evaluate nitrogen waste excretion, water samples were collected from each treatment tank at 7-day intervals, and the following nitrogenous compounds were analyzed:

- Total Ammonia Nitrogen (TAN) (mg/L): Measured via spectrophotometry
- Nitrate (NO_3^-) and Nitrite (NO_2^-) (mg/L): Measured using ion chromatography

2.7 Digestibility Analysis

To determine feed utilization efficiency, apparent digestibility coefficients (ADC) for protein and energy were calculated using the chromic oxide marker technique:

Apparent Digestibility Coefficient for Protein (ADC_p) (%)

$$ADC_{protein} = 100 - \left(\frac{\%\text{ marker in feed}}{\%\text{ marker in feces}} \times \frac{\%\text{ protein in feces}}{\%\text{ protein in feed}}\right) \times 100 \quad (9)$$

Fecal nitrogen analysis: Nitrogen loss in feces was measured to determine digestible nitrogen and nitrogen loss.

2.8 Haematological Analysis

At the end of the trial, blood samples were collected from three randomly selected fish per replicate via the caudal vein, using sterile syringes containing EDTA as an anticoagulant. The following haematological indices were analyzed:

- Packed Cell Volume (PCV) (%): Microhematocrit method
- Haemoglobin (HGB) (g/dL): Cyanmethemoglobin method
- Total White Blood Cell Count (TWBC) ($\times 10^9$/L): Neubauer hemocytometer
- Total Red Blood Cell Count (TRBC) ($\times 10^{12}$/L): Hemocytometer method
- Differential Leukocyte Count: Heterophils and lymphocytes

2.9 Statistical Analysis

All data were analyzed using one-way Analysis of Variance (ANOVA) to determine significant differences among treatments. Duncan's Multiple Range Test (DMRT) was used for post hoc comparisons where significance was observed ($P < 0.05$). Statistical analyses were conducted using SPSS Version 26.0 (IBM Corp., USA).

3.0 Results and Discussion

The growth performance and feed utilization efficiency of *Clarias gariepinus* fed varying levels of lysine and methionine supplementation in plant-based diets are presented in Table 2. The results demonstrate significant variations ($P < 0.05$) among dietary treatments in terms of final weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate. These findings highlight the crucial role of amino acid supplementation in enhancing fish growth and nutrient utilization efficiency.

The highest final weight (12.40 g) and mean weight gain (MWG: 6.69 g) were recorded in fish fed the GFM (fishmeal-based control diet). Among the plant-based diets, fish in the GL4 group (highest lysine and methionine supplementation) exhibited significantly higher ($P < 0.05$) weight gain (5.27 g) compared to those in the GL0 (unsupplemented plant-based diet), which recorded the lowest MWG (1.27 g).

The reduced growth performance in GL0 can be attributed to the deficiency of essential amino acids, particularly lysine and methionine, which are limiting in plant-based protein sources (Abdel-Tawwab et al., 2021). The observed improvement in GL4 suggests that adequate supplementation of these amino acids mitigated growth-limiting deficiencies, aligning with previous reports indicating that dietary lysine and methionine enhance protein synthesis, nitrogen retention, and overall growth performance in *Clarias* species (Espe et al., 2023; Furuya et al., 2022).

The SGR (%) was significantly higher in GFM (0.34%/day) compared to GL0 (0.17%/day), demonstrating the negative impact of amino acid deficiencies on growth efficiency. Among the plant-based diets, the highest SGR (0.29%/day) was observed in GL4, further confirming the growth-enhancing effects of lysine and methionine supplementation.

These findings corroborate previous research indicating that fish require optimal levels of essential amino acids to support muscle accretion and metabolic efficiency (Ahmed et al., 2021). Moreover, the relatively lower SGR in GL1-GL3 suggests that moderate supplementation may not fully compensate for amino acid deficiencies, which is consistent with reports by Li et al. (2022), who emphasized the importance of precise amino acid balancing in formulating plant-based aquafeeds.

The lowest FCR (2.84) was recorded in GFM, indicating superior feed utilization. Among the plant-based diets, GL4 exhibited the best FCR (4.02), whereas GL3 had the highest FCR (9.57), reflecting inefficient feed conversion. High FCR values indicate increased feed wastage and poor protein assimilation, which can be linked to imbalanced amino acid profiles (Sarker et al., 2022).

The PER values further support this trend, with GL4 showing improved PER (0.74) compared to GL0 (0.54) and GL3 (0.45). The increase in PER with higher amino acid supplementation suggests improved protein utilization efficiency, in line with previous studies demonstrating that lysine and methionine enhance dietary nitrogen retention and protein deposition in *Clarias gariepinus* (Hossain et al., 2023).

The highest feed intake (FI: 24.27 g) was observed in GL1, followed by GL2 (22.10 g), while the lowest was recorded in GL0 (11.00 g). The increased FI in GL1 and GL2 could be indicative of compensatory feeding behaviour, wherein fish consume more feed to offset amino acid deficiencies (Kaushik et al., 2021).

However, improved FCR and PER in GL4 suggest that optimal lysine and methionine supplementation enhanced nutrient absorption, reducing excessive feed consumption while maximizing protein retention. These findings align with studies by Rahman et al. (2022), who reported that optimal amino acid supplementation enhances feed efficiency and reduces protein wastage in fish diets.

The highest survival rate (35.56%) was recorded in GL2, followed by GL1 (31.11%) and GL4 (26.56%), whereas GL0 exhibited the lowest survival (17.78%).

The improved survival rates in amino acid-supplemented groups suggest that lysine and methionine play a vital role in enhancing immune function and metabolic resilience. Similar findings have been reported by Abdel-Tawwab et al. (2021), who demonstrated that dietary amino acid balance significantly improves disease resistance and stress tolerance in *Clarias gariepinus*. Conversely, the low survival in GL0 highlights the detrimental effects of essential amino acid deficiencies, further reinforcing the need for amino acid-balanced diets in aquaculture (Nguyen et al., 2021).

Table 2: Growth Performance and Feed Utilization Parameters

Parameter	GFM	GL0	GL1	GL2	GL3	GL4
Initial Weight (g)	5.71	3.40	6.24	5.98	5.02	6.31
Final Weight (g)	12.40	4.67	10.48	8.82	6.42	11.58
Mean Weight Gain (MWG) (g)	6.69	1.27	4.24	3.21	1.40	5.27
Specific Growth Rate (SGR) (%/day)	0.34	0.17	0.25	0.26	0.17	0.29
Feed Conversion Ratio (FCR)	2.84	7.28	6.34	5.49	9.57	4.02
Feed Intake (FI) (g)	19.38	11.00	24.27	22.10	16.40	20.88
Protein Efficiency Ratio (PER)	0.93	0.54	0.50	1.11	0.45	0.74
Survival Rate (%)	26.66	17.78	31.11	35.56	31.11	26.56

Table 3 showed the nutrient retention efficiency parameters, including Nitrogen Retention Efficiency (NRE), Protein Retention Efficiency (PRE), and Energy Retention Efficiency (ERE), exhibited significant variations ($P < 0.05$) among different dietary treatments in *Clarias gariepinus*. Nitrogen retention efficiency (NRE) was highest in the GFM (fishmeal-based diet; 78.4%), followed closely by GL4 (74.5%), whereas the lowest NRE was observed in GL0 (45.2%). The GL4 diet, which contained the highest lysine and methionine supplementation, significantly ($P < 0.05$) improved nitrogen retention compared to lower supplementation levels (GL1–GL3).

The low NRE observed in GL0 suggests poor nitrogen assimilation and increased nitrogen excretion, which is consistent with previous findings indicating that essential amino acid deficiencies lead to increased catabolism of dietary protein for energy rather than tissue growth (Espe et al., 2023). Lysine and methionine are known to play a crucial role in protein synthesis and nitrogen balance, and their supplementation in plant-based diets helps mitigate the inefficiencies associated with non-fishmeal protein sources (Kaushik & Seiliez, 2021).

The improvement in NRE with higher lysine and methionine supplementation (GL4: 74.5%) aligns with studies by Li et al. (2022), which demonstrated that balanced amino acid profiles enhance nitrogen utilization, reducing ammonia excretion and improving overall feed efficiency. Moreover, diets deficient in essential amino acids often result in the deamination of excess protein, leading to increased ammonia release into the aquatic environment, thus contributing to eutrophication and poor water quality (Hossain et al., 2023).

A similar trend was observed in Protein Retention Efficiency (PRE), with GFM (62.1%) showing the highest value, followed closely by GL4 (61.7%) and GL2 (59.4%), whereas GL0 exhibited the lowest PRE (38.5%). The enhanced PRE in GL4 suggests superior protein utilization and deposition as a result of optimal amino acid supplementation.

The significant decline in PRE in GL0 (38.5%) and GL3 (40.1%) highlights the impact of an imbalanced amino acid profile, which limits protein synthesis efficiency and increases protein catabolism (Furuya et al., 2022). Research has demonstrated that lysine is essential for muscle growth, and methionine plays a key role in methylation reactions, which regulate protein turnover and metabolism (Ahmed et al., 2021).

The findings in this study corroborate the results by Abdel-Tawwab et al. (2021), who reported that optimal lysine and methionine levels in plant-based diets enhance protein retention and muscle accretion while reducing metabolic losses. Additionally, diets with poor amino acid balance often lead to elevated urea cycle activity, as excess nitrogen from inefficient protein utilization must be excreted (Rahman et al., 2022). This process not only reduces the energy available for growth but also increases ammonia excretion, leading to higher nitrogenous waste output in aquaculture systems (Nguyen et al., 2021).

Energy retention efficiency (ERE) followed a pattern similar to NRE and PRE, with GL4 (57.3%) exhibiting significantly ($P < 0.05$) higher values compared to GL0 (35.6%) and GL3 (38.0%). The enhanced ERE in GL4 and GL2 indicates that higher amino acid supplementation improved energy utilization, reducing reliance on protein catabolism for energy.

The lower ERE in GL0 (35.6%) suggests that fish were unable to effectively utilize dietary protein and lipids for growth, leading to increased energy losses through excretion and inefficient metabolic pathways (Li et al., 2022). In contrast, GL4 exhibited a 60% improvement in ERE compared to GL0, demonstrating the metabolic benefits of amino acid supplementation in plant-based diets.

Previous studies have shown that methionine plays a key role in lipid metabolism, particularly in the synthesis of S-adenosylmethionine (SAM), a precursor in

fatty acid metabolism (Sarker et al., 2022). Moreover, lysine has been reported to enhance muscle protein synthesis and reduce metabolic inefficiencies associated with plant-based protein sources (Kaushik & Seiliez, 2021).

Furthermore, the decreased ERE in GL0 and GL3 aligns with findings by Furuya et al. (2022), who reported that fish consuming low-quality plant-based diets exhibit higher metabolic energy losses due to increased excretion of nitrogenous waste. This suggests that an optimal balance of essential amino acids is required to maximize energy retention and minimize nutrient wastage.

The observed differences in NRE, PRE, and ERE among dietary treatments emphasize the importance of balancing essential amino acids in plant-based aquafeeds. The findings indicate that GL4 (1.00 g/kg lysine + 1.00 g/kg methionine) provided the best nutrient retention efficiency, comparable to fishmeal-based diets (GFM). This suggests that with proper amino acid supplementation, plant-based diets can replace fishmeal-based diets while maintaining high nutrient retention and reducing environmental impact.

Moreover, the significantly lower NRE, PRE, and ERE in GL0 highlight the negative consequences of feeding diets deficient in essential amino acids, which lead to increased nitrogenous waste production. Given that excessive nitrogen release contributes to aquatic pollution, eutrophication, and water quality degradation, the optimization of amino acid profiles in fish feeds is crucial for sustainable aquaculture practices (Hossain et al., 2023).

These results support the implementation of precision-formulated aquafeeds that incorporate ideal protein ratios, reducing the need for excess dietary protein while maximizing nutrient retention and minimizing nitrogen excretion. Future research should explore the long-term effects of amino acid supplementation on fish health, immune response, and gut microbiota modulation to further optimize sustainable plant-based diets in aquaculture.

Table 3: Nutrient Retention Efficiency

Parameter	GFM	GL0	GL1	GL2	GL3
Nitrogen Retention Efficiency (NRE) (%)	78.4	45.2	68.9	72.1	49.8
Protein Retention Efficiency (PRE) (%)	62.1	38.5	54.3	59.4	40.1
Energy Retention Efficiency (ERE) (%)	58.7	35.6	50.2	55.8	38.0

Table 4 showed that efficient nitrogen utilization and reduced nitrogen waste excretion are essential for sustainable aquaculture, as excessive nitrogen discharge into aquatic environments contributes to eutrophication, water quality deterioration, and increased production costs due to frequent water exchange requirements (Wang et al., 2023). The nitrogen waste analysis in Table 3 evaluates Total Ammonia Nitrogen (TAN), Nitrate (NO_3^-), Nitrite (NO_2^-), and Nitrogen Excretion Rate to determine the impact of lysine and methionine supplementation on nitrogen metabolism and waste output in *Clarias gariepinus*.

The Total Ammonia Nitrogen (TAN) levels were significantly ($P < 0.05$) higher in GL0 (4.5 mg/L), whereas the lowest levels were recorded in GFM (1.2 mg/L) and GL4 (1.9 mg/L). The elevated TAN levels in GL0 indicate excessive protein catabolism, likely due to an imbalance in essential amino acids, leading to increased deamination of surplus nitrogen and its subsequent excretion (Zhou et al., 2023).

The observed reduction in TAN levels in GL4 suggests that optimal lysine and methionine supplementation facilitated improved nitrogen retention and protein synthesis efficiency, thereby minimizing nitrogenous waste production. These findings align with studies by Xie et al. (2022), who demonstrated that supplementing limiting amino acids significantly reduces TAN accumulation by enhancing nitrogen

assimilation and reducing excess amino acid catabolism for energy production.

Additionally, the increased TAN concentrations in GL3 (3.7 mg/L) suggest that moderate amino acid supplementation may not be sufficient to fully optimize nitrogen retention, further emphasizing the importance of precise amino acid balancing in aquafeeds (Gong et al., 2023).

Nitrate (NO_3^-) and Nitrite (NO_2^-) levels followed similar trends, with the highest nitrate concentration observed in GL4 (5.6 mg/L), indicating improved nitrification efficiency and lower nitrogen excretion into the culture water. The low nitrate levels in GL0 (3.2 mg/L) and GL3 (3.8 mg/L) suggest inefficient nitrogen metabolism, leading to increased ammonia excretion rather than its conversion into nitrate through microbial nitrification processes (Zhang et al., 2023).

Nitrite (NO_2^-) levels were significantly higher ($P < 0.05$) in GL0 (2.1 mg/L) compared to GL4 (0.7 mg/L) and GFM (0.6 mg/L). Elevated NO_2^- concentrations in GL0 indicate incomplete nitrification due to excess ammonia excretion, potentially posing toxic effects on fish health (Chen et al., 2023). The reduction in NO_2^- in GL4 aligns with findings from Liu et al. (2023), who reported that amino acid-balanced diets enhance protein deposition and metabolic efficiency, reducing toxic nitrogen waste output.

Moreover, NO_2^- accumulation in aquaculture systems is a key indicator of excessive dietary protein metabolism and suboptimal microbial nitrification activity, further supporting the necessity for efficient protein utilization through targeted amino acid supplementation (Sun et al., 2023).

The Nitrogen Excretion Rate (%) was significantly lower in GL4 (28.9%) compared to GL0 (48.6%), demonstrating the positive impact of amino acid supplementation on nitrogen efficiency. The highest nitrogen excretion observed in GL0 and GL3 (42.5%) indicates inefficient dietary protein utilization, resulting in increased ammonia release and environmental nitrogen loading (Zhou et al., 2023).

The enhanced nitrogen retention efficiency in GL4, leading to lower nitrogen excretion rates, aligns with recent research by Zhang et al. (2023), who found that precise amino acid supplementation significantly enhances nitrogen metabolism and reduces nitrogen losses in fish-fed plant-based diets. Additionally, protein degradation and nitrogen excretion are closely linked to dietary amino acid composition, further emphasizing the importance of optimizing lysine and methionine levels for sustainable feed efficiency (Wang et al., 2023).

Reduced nitrogen excretion in GL4 also suggests improved hepatic nitrogen metabolism, as methionine is a key precursor in the urea cycle, facilitating efficient detoxification of excess nitrogen (Liu et al., 2023). Consequently, formulating diets with optimized amino acid profiles can substantially reduce the environmental footprint of aquaculture systems, making plant-based diets a viable alternative to fishmeal-based formulations (Sun et al., 2023).

Table 4: Nitrogen Waste Analysis

Parameter	GFM	GL0	GL1	GL2	GL3	GL4
Total Ammonia Nitrogen (TAN) (mg/L)	1.2	4.5	2.8	2.3	3.7	1.9
Nitrate (NO_3^-) (mg/L)	5.4	3.2	4.8	5.1	3.8	5.6
Nitrite (NO_2^-) (mg/L)	0.6	2.1	1.2	0.9	1.7	0.7
Nitrogen Excretion Rate (%)	22.3	48.6	35.1	30.4	42.5	28.9

4.0 Conclusion

This study demonstrates that lysine and methionine supplementation in plant-based diets significantly enhances growth performance, feed efficiency, and nitrogen retention in Clarias gariepinus. Fish-fed diets supplemented with 1.00 g/kg lysine and 1.00 g/kg methionine exhibited the highest weight gain, improved feed conversion ratio (FCR = 4.02), and superior protein efficiency ratio

(PER = 0.74). Nitrogen retention efficiency increased from 45.2% (control) to 74.5%, while total ammonia nitrogen (TAN) excretion decreased from 4.5 mg/L to 1.9 mg/L, indicating improved nitrogen metabolism and reduced environmental impact.

These findings highlight the critical role of amino acid supplementation in optimizing nutrient utilization and reducing nitrogen waste in plant-based aquafeeds. Implementing precision-formulated diets could enhance sustainable aquaculture by reducing reliance on fishmeal, lowering production costs, and minimizing environmental pollution.

1. Optimal Feed Formulation: Fish feed manufacturers should incorporate lysine and methionine at 1.00 g/kg each in plant-based diets to enhance protein utilization and growth performance while reducing nitrogen excretion.
2. Environmental Management: The significant reduction in TAN suggests that amino acid-balanced diets should be widely adopted to mitigate aquaculture-induced water pollution.
3. Future Research: Further studies should explore the long-term effects of lysine and methionine supplementation on fish health, immunity, and disease resistance.
4. Policy Support: Policymakers should promote sustainable aquafeeds through incentives, subsidies, and capacity-building programs for farmers and feed manufacturers.

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Compliance with Ethical Standards Declaration

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The authors declare that they have no known competing financial interests

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Authors' Contribution

Both authors contributed to the benchwork and the manuscript writing. SA designed the work and while ANA participated in all other aspects of the work.