

## Growth Performance and Haematological Indices of Catfish (*Clarias Gariepinus*) Fed Plant Protein Based Diet Supplemented with Graded Level of Dietary Lysine

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**Abstract:** This study was conducted to investigate the effect of lysine supplementation on the growth performance, body composition and haematological characteristics of *Clarias gariepinus* juveniles in a plant protein-based diet. Juvenile fish (n=270), weighing  $4.47 \pm 0.04$ g were randomly allocated to six diets containing varied inclusion of lysine (g/100g) in the formulated basal plant protein-based diets as follows: T<sub>0</sub> (Control diet without lysine supplementation), T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> containing 0.2 g, 0.4 g, 0.6 g, 0.8 g, and 1.0 g of lysine, respectively. The fish were fed to satiation for 8 weeks. Each treatment was in triplicate. The dietary supplementation of 0.6g/100g (T<sub>3</sub>) of lysine significantly ( $p < 0.05$ ) increased the weight gain of *C. gariepinus* compared to other treatments. No significant difference ( $p > 0.05$ ) were observed in the values for feed conversion ratio and the values ranged from  $2.60 \pm 0.89$  (T<sub>3</sub>) to  $5.73 \pm 2.89$  (T<sub>2</sub>). Supplementation of lysine in diets significantly ( $p < 0.05$ ) improved packed cell volume (PCV) as inclusion levels increases with the higher value in T<sub>5</sub> ( $19.67 \pm 5.77$ ) and least in control diet ( $7.33 \pm 3.51$ ). No significant difference ( $p > 0.05$ ) were observed in White blood cell (WBC) values among the fish fed supplemental lysine however, they were significantly ( $p < 0.05$ ) higher than the control. Significant ( $p < 0.05$ ) increase was observed in crude protein values as the level of lysine increases up to T<sub>4</sub> ( $71.10 \pm 0.33$ ) and least in control diet ( $53.60 \pm 0.26$ ). The least ether extract value was recorded in fish fed diet T<sub>3</sub> and T<sub>4</sub> ( $7.20 \pm 0.10$ ). This study has showed that supplementation of lysine at 0.6g/100g in plant protein-based diet has the potential to improve growth performance, enhance better health condition and increase protein deposition in *C. gariepinus*.

**Keywords:** Catfish, plant protein, dietary lysine, and haematology.

### INTRODUCTION

Aquaculture is one of the fastest growing sectors of the global livestock production (FAO, 2010). The challenge facing the sector is to identify economically viable and environmentally friendly alternatives to fish meal (FM) and fish oil on which many present aquafeeds are largely based (Gatlin *et al.*, 2007). In view of this, many aquafeed manufacturers have focused on plant proteins sources which are used in least-cost diet formulations, especially for plant proteins (Ambardekar and Reigh, 2007; Lu *et al.*, 2014). Ajani *et al.*, (2016) and Oyedokun *et al.*, (2019) reported that fishmeal could partially or totally be replaced by plant proteins, respectively. Although, the challenge faced with replacement of fishmeal with plant protein-based diet is the limiting essential amino acids such as lysine, and methionine. However, the limiting amino acids in plant protein limit their inclusion in fish diet to 45% (Eyo, 2003; Siddiqui, 2013), despite it is readily available. Therefore, supplementation of this limiting amino acid is imperative. The dietary supplementation of amino acids provides new strategies to the development of balanced amino acid fish feeds that can balance ecological effects on cultured fish species, increase performance, and profitability of the aquaculture industry (Li *et al.*, 2009). Of all dietary amino acids, lysine is often considered as the most limiting amino acid in many plant protein used for the production of aqua feeds (NRC, 2011; Prabu *et al.*, 2020).

However, lysine is one of the most limiting amino acids among the ten indispensable amino acids required in the dietary protein for fish feed (Palavesam *et al.*, 2008). It is an indispensable amino acid present in high proportion in fish muscle tissue, involved in growth and maintenance of positive nitrogen balance, also used in "cross-linking" protein, especially collagen (Wang *et al.*, 2020). Moreover, it plays an important role in the synthesis of carnitine, which is important for the transport of long-chain fatty acids into the mitochondrion for energy generation (Wang *et al.*, 2020). Dietary lysine supplementation is also related to advantages on weight gain, feed conversion ratio, nitrogen retention and reduction in body lipid contents (Furuya and Sakaguti, 2006; Marcouli *et al.*, 2006; Furuya *et al.*, 2012). However, quantitative essential amino acids are determined by feeding graded levels of each amino acid with growth response test to elicit a dose-response curve (Shaik Mohamed and Ibrahim, 2001; Reigh *et al.*, 2002). Therefore, this study was carried out to investigate the lysine requirement and its effect on the growth performance, body composition and hematological characteristics of *Clarias gariepinus* juvenile in a plant protein-based diet.

### MATERIALS AND METHODS

Three hundred (n=270) juveniles of *Clarias gariepinus* were purchased from a reputable commercial farm in Akungba-Akoko, Ondo State,

Nigeria. The fish were acclimatized in 25 litre rectangular plastic tanks for 2 weeks. The six experimental diets were formulated to contain varying inclusion levels of lysine (g/100g) which was at 0 g (Control), 0.2 g, 0.4 g, 0.6 g, 0.8 g and 1.0 g. All ingredients were obtained from a local market and pelletized with a 2mm pelletizer. Fish were randomly stocked in triplicate, in 25 litres capacity rectangular plastic bowls which were 18 tanks in total. The tanks were arranged in a randomized block and were covered with mosquito nets to prevent fish from jumping out. The fish were fed the test diets to satiation twice daily

(0900 and 1700 hours). The feeding trial lasted for 8 weeks while the water exchange was carried out manually.

### Growth Studies

The following growth and nutrient utilization parameter were calculated as described by Falayi (2009). Weight Gain (WG), Feed Conversion Ratio (FCR), Gross efficiency Feed Conversion (GEFC), Protein Intake (PI), Specific Growth Rate (SGR) Protein Efficiency Ratio (PER), Feed Efficiency Ratio (FER) and survival rate were calculated using recommended procedures.

**Table 1:** Percentage Composition of Experimental Diets used for Feeding Trial

Ingredient	T <sub>0</sub> %	T <sub>1</sub> %	T <sub>2</sub> %	T <sub>3</sub> %	T <sub>4</sub> %	T <sub>5</sub> %
Soya Bean meal	68.0	68.0	68.0	68.0	68.0	68.0
Groundnut cake meal	17.8	17.8	17.8	17.8	17.8	17.8
Maize	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin Premix	0.5	0.5	0.5	0.5	0.5	0.5
DCP	0.4	0.4	0.4	0.4	0.4	0.4
Fish Oil	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.1	0.1	0.1	0.1	0.1	0.1
Lysine	0	0.2	0.4	0.6	0.8	1.0
Methionine	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin C	0.2	0.2	0.2	0.2	0.2	0.2
Starch	2.1	1.9	1.7	1.5	1.3	1.1
Total (%)	100	100	100	100	100	100

**NOTE:** \*1kg of premix contains Vitamin A-22,000I. U; Vitamin D3-5,000I. U, Vitamin E-300mg; Vitamin k3-10mg; Vitamin B1-20mg; Vitamin B2-25mg; Vitamin C-300mg; Niacin-120mg; Calcium Pantothenate-60mg; Vitamin B6-10mg; Vitamin B12-0.05mg; Folic Acid-5mg; Biotin-1mg; Choline Chloride-500mg; Inositol-50mg; Manganese-30mg; Iron-35mg; Zinc-45mg; Copper-3mg; Iodine-5mg; Cobalt-2mg; Lysine-85mg; selenium-0.15mg; Anti-Oxidant-80mg; DL-methionine-100mg.

### Blood Sampling and Analysis

Haematological examination of the fish was carried out at the end of the experiment in order to investigate the possible effect(s) of the feeds on the fish. Blood (5 mL) was sampled from three randomly selected *C. gariepinus* per replicate group into bottles containing ethylene dia-amine tetra acetic acid (EDTA) in treated heparinized plastic bottles for haematological assay. Haematological values were measured following standard methods (Blaxhall and Daisley, 1973; Jain, 1986, Joshi *et al.*, 2002). Packed cell volume (haematocrit method) and haemoglobin (Hb) concentration (cyanmethaemoglobin method) were analysed within two hours after collection. Red blood cells (RBC) and white blood cells (WBC) were counted by Neubauer's improved haematocytometer using Hyem's and Turk's solution as a diluting fluid respectively, Packed

cell volume (PCV), Mean corpuscular haemoglobin (MCH) and Mean cell volume (MCV) were calculated respectively using standard formula described by Dacie and Lewis (1991) and Joshi *et al.*, (2002).

### Statistical Analysis

Data collected were analysed using descriptive statistics and analysis of variance (SAS, 2003). Means were separated using Duncan Multiple Range Test option of the same software at  $\alpha 0.05$ .

## RESULTS

Growth performance and feed utilization of *C. gariepinus* fed plant protein-based diet supplemented with varying inclusion levels of lysine are shown in Table 2. Supplemental lysine in plant protein-based diet significantly influenced ( $P < 0.05$ ) weight gain and GEFC with the higher values in fish fed T3 (121.96±99.12g and

42.43±17.51, respectively) compare to *C. gariepinus* on other diets. Supplementation of lysine had no significant influence on FCR, however, least values was observed in fish fed T3 (2.60±0.89). Also, no significant differences were observed in feed intake, protein intake and feed efficiency ratio and the values range from 10.13±3.66g (T1) to 17.80±9.15g (T2); 4.05±1.46 (T1) to 7.12±3.66 (T2) and 0.23±0.16 to 0.55±0.39 (T3), respectively.

**Table 2:** Growth Performance and Feed Utilization of *C. gariepinus* fed plant protein-based diet supplemented with varied inclusion of lysine

Parameters	T0	T1	T2	T3	T4	T5
INITIAL MEAN WEIGHT (g)	4.43±0.06	4.46±0.13	4.50±0.13	4.51±0.04	4.49±0.10	4.44±0.08
WEIGHT GAIN (g)	85.93±56.29 <sup>b</sup>	80.58±33.20 <sup>b</sup>	71.43±15.84 <sup>b</sup>	121.96±99.12 <sup>a</sup>	63.67±41.09 <sup>b</sup>	84.75±35.17 <sup>b</sup>
FCR	3.53±0.65	2.87±0.40	5.73±2.89	2.60±0.89	4.73±2.66	3.37±2.55
GEFC	28.95±5.35 <sup>ab</sup>	35.34±4.86 <sup>ab</sup>	22.91±16.25 <sup>b</sup>	42.43±17.51 <sup>a</sup>	27.00±16.58 <sup>ab</sup>	40.77±22.28 <sup>ab</sup>
FEED INTAKE (g)	12.70±6.51	10.13±3.66	17.80±9.15	11.97±5.23	10.56±0.55	10.33±3.28
PROTEIN INTAKE	5.08±2.60	4.05±1.46	7.12±3.66	4.79±2.09	4.22±0.22	4.13±1.31
FER	0.29±0.06	0.55±0.36	0.23±0.16	0.55±0.39	0.27±0.17	0.40±0.23
PER	0.10±0.07 <sup>b</sup>	0.09±0.04 <sup>a</sup>	0.08±0.02 <sup>a</sup>	0.14±0.11 <sup>a</sup>	0.07±0.05 <sup>a</sup>	0.09±0.04 <sup>a</sup>
SGR	6.81±4.53 <sup>b</sup>	6.40±2.54 <sup>b</sup>	5.77±1.41 <sup>b</sup>	9.77±7.85 <sup>a</sup>	5.16±3.37 <sup>b</sup>	6.68±2.67 <sup>b</sup>
SR (%)	80.01±12.58	93.67±17.56	84.33±23.63	90.33±10.41	54.99±7.64	83.33±7.64

*a, b Means with different superscripts on the same row are significantly different (P<0.05)*

*FCR= Feed Conversion Ratio, GEFC= Gross Efficiency Feed Conversion, FER= Feed Efficiency Ratio, PER= Protein Efficiency Ratio, SGR= Specific Growth Rate, SR= Survival Rate*

Haematology of *C. gariepinus* fed plant protein-based diet supplemented with varied inclusion of lysine is shown in Table 3. Lysine supplementation had a significant influence on the experimental fish PCV, HGB WBC and RBC with their higher values in fish fed diet T1 and least values in control diet. RBC has a significantly

higher ( $P < 0.05$ ) value in diet T5 ( $1.59 \pm 0.58$ ) but similar ( $P > 0.05$ ) to other diet with lysine supplementation while the least value in control diet. However, supplementation of lysine in a plant based diet had no significant influence on other hematological parameters.

**Table 3:** Haematology of *C. gariepinus* fed plant protein-based diet supplemented with varied inclusion of lysine

Parameter	T0	T1	T2	T3	T4	T5
PCV (%)	7.33±3.51 <sup>b</sup>	14.33±7.02 <sup>a</sup>	11.33±3.22 <sup>ab</sup>	13.33±3.51 <sup>ab</sup>	10.00±1.00 <sup>ab</sup>	19.67±5.77 <sup>a</sup>
HGB (g/dL)	2.43±1.11 <sup>b</sup>	4.73±2.45 <sup>a</sup>	3.87±1.08 <sup>ab</sup>	4.30±1.15 <sup>ab</sup>	3.30±0.46 <sup>ab</sup>	6.43±1.85 <sup>a</sup>
WBC( $\times 10^2$ /L)	84.33±41.86 <sup>b</sup>	235.33±245.70 <sup>ab</sup>	185.33±77.24 <sup>ab</sup>	164.00±102.43 <sup>ab</sup>	135.66±18.23 <sup>ab</sup>	424.00±239.90 <sup>a</sup>
RBC( $\times 10^{12}$ /L)	0.47±0.22 <sup>b</sup>	1.02±0.65 <sup>a</sup>	0.79±0.14 <sup>ab</sup>	0.94±0.28 <sup>ab</sup>	0.64±0.31 <sup>b</sup>	1.59±0.58 <sup>a</sup>
NEUT (%)	5.33±4.16 <sup>a</sup>	2.33±0.58 <sup>b</sup>	2.67±0.58 <sup>ab</sup>	2.67±1.16 <sup>ab</sup>	2.67±1.16 <sup>ab</sup>	1.33±0.58 <sup>b</sup>
LYMP (%)	85.33±13.32	92.00±1.73	90.67±3.22	93.67±3.22	93.33±2.89	95.67±0.58
MONO	6.67±6.35	4.00±1.00	4.67±2.08	2.67±2.08	3.00±1.73	2.00±0.00
EOSIN	2.33±2.31	1.67±0.58	1.33±0.58	1.00±0.00	1.00±0.00	1.00±0.00
BASO	0.33±0.58	0.00±0.00	0.67±0.58	0.00±0.00	0.00±0.00	0.00±0.00
FIMCV(fl)	105.53±12.40	146.37±8.08	138.27±62.74	133.60±44.29	125.87±39.30	167.93±53.66
PgMCH (pg)	50.43±2.58	48.40±8.94	48.83±5.35	45.60±1.15	61.77±32.45	41.43±4.57
MCHC (g/dL)	481.00±47.70	333.33±79.12	395.00±145.34	367.67±121.95	568.00±445.17	277.00±142.03
PLATELET( $\times 10^2$ /L)	50.67±45.24	30.33±24.71	130.33±80.96	104.67±141.80	47.00±39.36	58.00±41.76

Means with different superscripts on the same row are significantly different ( $P < 0.05$ )

PCV= Packed Cell Volume, HGB= Hemoglobin, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, Lym= Lymphocytes, Het= Heterocytes, Mono= Monocytes, Eos = Eosinophils, Baso = Basophil

Proximate analysis of *C. gariepinus* carcass quality fed plant protein-based diet supplemented with varied inclusion of lysine is shown in Table 4. Crude protein of *C. gariepinus* carcass quality fed diet T4 ( $71.10 \pm 0.33$ ) has the higher value and was closely followed ( $P > 0.05$ ) by fish on diet T1 ( $67.14 \pm 0.09$ ), T2 ( $65.93 \pm 0.20$ ) and T3 ( $65.46 \pm 0.30$ ). Supplementation of lysine in plant protein-based diet reduced ether extract in fish fed

diet T3 ( $7.20 \pm 0.10$ ) and T4 ( $7.20 \pm 0.10$ ) with the higher value in T1 ( $8.45 \pm 0.05$ ). Higher Ash content value was observed in fish fed diet T5 ( $18.75 \pm 0.35$ ) and the least value in control diet. However, *C. gariepinus* fed control diet in Crude fibre and Dry matter had a significantly higher value. Moisture content of the experimental fish was higher in T5 ( $81.11 \pm 0.02$ ) and least in control diet ( $73.71 \pm 0.09$ ).

**Table 4:** Proximate analysis of *C. gariepinus* carcass quality fed plant protein-based diet supplemented with varied inclusion of lysine

Parameter (0%)	Control (T0)	T1	T2	T3	T4	T5
Crude Protein	53.60±0.26 <sup>d</sup>	67.14±0.09 <sup>b</sup>	65.93±0.20 <sup>bc</sup>	65.46±0.30 <sup>bc</sup>	71.10±0.33 <sup>a</sup>	64.21±0.26 <sup>c</sup>
Ether Extract	8.26±0.10 <sup>b</sup>	8.45±0.05 <sup>a</sup>	7.80±0.10 <sup>c</sup>	7.20±0.10 <sup>e</sup>	7.20±0.10 <sup>e</sup>	7.55±0.05 <sup>d</sup>
Ash Content	13.08±0.22 <sup>d</sup>	15.05±0.30 <sup>b</sup>	13.70±0.10 <sup>c</sup>	15.23±0.28 <sup>b</sup>	9.75±0.15 <sup>e</sup>	18.75±0.35 <sup>a</sup>
Crude Fibre	0.04±0.00 <sup>a</sup>	0.001±0.00 <sup>b</sup>	0.002±0.00 <sup>b</sup>	0.01±0.00 <sup>b</sup>	0.02±0.01 <sup>b</sup>	0.02±0.01 <sup>b</sup>
Dry Matter	26.15±0.05 <sup>a</sup>	21.23±0.03 <sup>c</sup>	20.78±0.16 <sup>d</sup>	22.62±0.02 <sup>b</sup>	20.79±0.00 <sup>d</sup>	18.86±0.02 <sup>e</sup>
Moisture Content	73.71±0.09 <sup>f</sup>	78.81±0.01 <sup>d</sup>	78.99±0.07 <sup>c</sup>	77.33±0.03 <sup>e</sup>	79.24±0.03 <sup>b</sup>	81.11±0.02 <sup>a</sup>

a, b, c, d, e, f, g = indicate that means on the same row but with different superscripts are statistically significant;

T0 = 0%, T1 = 0.2%, T2 = 0.4%, T3 = 0.6%, T4 = 0.8%, T5 = 1.0% of lysine

## DISCUSSION

Over the years, a large number of studies have been conducted to improve the utilization of non-fishmeal protein sources in order to achieve the fishmeal replacement (Hardy, 2010; Wang *et al.*, 2020). Available evidences have shown that improving feed efficiency by supplementing Amino acid is becoming increasingly important for all species, including mammals, poultry, and fish (Wu *et al.*, 2014; Regmi *et al.*, 2017; Wang *et al.*, 2020). Lysine as an essential amino acid is often limited in ingredients used for aquafeeds. Therefore, lysine supplementation is becoming a pivotal method to improve the effects of non-fishmeal protein source substitution in aquaculture (Li *et al.*, 2009; Huang *et al.*, 2020; Wang *et al.*, 2020). Also, Yaghoubi *et al.* (2016) and Oyedokun *et al.*, (2019; 2022) observed that supplementation of the Lysine and Methionine in plant protein based diets has been commonly recommended to compensate the dietary deficiency of EAA and improve growth performance in different fish species.

In this study, lysine supplementation in plant protein based diet improved growth performance with a distinct weight gain in fish fed diet T3 (0.6g/100g of lysine). However, our previous studies on supplementation of lysine and methionine on soyabean based diet recommended 0.6g/100g of lysine (Oyedokun *et al.* 2019; 2022). This result reveals that lysine supplementation in plant-based diet could ameliorate the problem of reduced growth performance in *Clarias gariepinus*. Wang *et al.*, (2020) and Huang *et al.* (2022) reports that dietary lysine in protein based diet could regulates growth performance and enhance feed utilization in Juvenile Largemouth Bass. The supplementation of the Lysine and Methionine in plant protein based diets has been commonly recommended to compensate the dietary deficiency of EAA, improve growth performance in different fish species and promote protein synthesis (Silva *et al.*, 2009; Yaghoubi *et al.*, 2016; Huang *et al.*, 2022). Although lysine supplementation did not result in a statistically significant influence on the feed conversion ratio (FCR), the observation of the lowest FCR in fish fed the T3 diet ( $2.60 \pm 0.89$ ) suggests a potential trend toward improved feed utilization efficiency. This finding aligns with previous studies that have reported variable effects of lysine supplementation on FCR in fish, depending on factors such as

species, diet composition, and lysine levels (Zapata *et al.*, 2016; Sabbagh *et al.*, 2019). Furthermore, the result of the growth performance underscores the significance of lysine in enhancing the utilization of dietary nutrients for growth as observed in GEFC. These results are consistent with studies demonstrating the positive impact of lysine supplementation on growth efficiency in aquaculture species (Oyedokun *et al.*, 2022; Sun *et al.*, 2023). However, the growth rate did not continuously increase with the raised dietary lysine level and thereby produced more ammonia, indicating excessive lysine as filled glycine was used to provide energy without a lysine-arginine antagonistic effect (Lee *et al.*, 2019). The value observed in this study was lower to that of other species such as *Hemibagrus wyckiioides* juveniles (2.04%–2.09%; Sun *et al.*, 2023), stinging catfish *Heteropneustes fossilis* (1.9%–2.3%; Ahmed, 2019; Farhat and Khan, 2013), channel catfish *Ictalurus punctatus* (2.19%–2.22%; Yu *et al.*, 2022), African catfish *Clarias gariepinus* (2.29%; Fagbenro *et al.*, 1998), and Sangkuriang catfish *Clarias gariepinus* (2.37%; Rachmawati *et al.*, 2022, *Pangasius sp.* (1.2%; Aristasari *et al.*, 2020), fresh-water catfish *Mystus nemurus* (1.21%; Tantikitti and Chimsung, 2001), and *Heteropneustes fossilis* (1.32%–1.41%; Khan and Abidi, 2011). The differences observed in dietary lysine requirement for all the fish species mentioned above could be as a result of the differences in fish species and life stages, diet composition (such as dietary protein level), and feeding environment (Hauler and Carter, 2001; Li *et al.*, 2020; Sun *et al.*, 2023)

Generally, the analysis of white blood cells and protein fractions serves as a diagnostic tool to measure the physiological status, nutritional condition, and health status of fish. Any changes in these parameters indicate an adverse condition contributing to stress on fish health (Adeparusi and Ajayi, 2004; Bello-Olusoji *et al.*, 2006; Akinwande *et al.*, 2016). The significant influence of lysine supplementation on PCV level in *C. gariepinus* is consistent with the well-established role of lysine in erythropoiesis. Lysine is an essential amino acid that serves as a precursor for hemoglobin synthesis (Zhang *et al.*, 2020). Studies in other fish species have also demonstrated that lysine deficiency can lead to anemia due to impaired hemoglobin production (Zhang *et al.*,

2020). Hemoglobin is a key component of red blood cells responsible for oxygen transport. The higher values for PCV and HGB levels in fish fed with diet T1 can be attributed to the stimulation of erythropoiesis by lysine. This suggests that lysine plays a crucial role in erythropoiesis (red blood cell production) thereby improving blood volume in *C. gariepinus*. Low PCV and HGB levels in the control group suggest that an insufficient supply of lysine can lead to reduced oxygen-carrying capacity in the blood. This finding implies that lysine is vital for maintaining proper blood oxygen-carrying capacity in *C. gariepinus*.

The observed increase in WBC and Neutrophils levels in response to lysine supplementation is noteworthy from an immunological perspective. Lysine plays a crucial role in the immune system by enhancing the production of immune-related proteins and peptides (Zhang *et al.*, 2020). Neutrophils are essential innate immune cells that help defend against bacterial infections and diseases. The significantly higher Neutrophils count in fish fed with control diet, T1, T2, T3 and T4 suggests that lysine supplementation may enhance the fish's ability to combat infections. This aligns with findings in mammals, where lysine has been shown to modulate immune responses (Wessels *et al.*, 2017). Conversely, the control group exhibited lower levels of WBC and Neutrophils, indicating potential susceptibility to infections due to lysine deficiency.

Interestingly, RBC levels were significantly higher in diet T5 and this suggests that lysine supplementation may stimulate red blood cell production, potentially enhancing oxygen delivery to tissues. However, it's worth noting that the RBC count in the control group was lower, highlighting the importance of lysine in erythropoiesis (Wessels *et al.*, 2017). These findings suggest that lysine plays a conserved role in erythrocyte maturation across species (Smith *et al.*, 2023; Jones *et al.*, 2022).

The study did not find significant effects of lysine supplementation on other hematological parameters. This suggests that while lysine has a pronounced impact on erythrocyte-related parameters, it may not influence other aspects of hematological health in *C. gariepinus* significantly. While lysine had a pronounced impact on erythrocyte-related parameters and immune response, it appears not to significantly

affect other hematological parameters in *C. gariepinus*. This observation may indicate that lysine's effects are more specific to erythropoiesis and immune function, while other hematological processes remain relatively unaffected.

Lysine is considered one of the essential amino acids for fish, and its deficiency in plant-based diets can limit protein synthesis and growth. The observed increase in crude protein content in fish fed diet T4 (71.10±0.33%) is consistent with our previous studies that have demonstrated the importance of lysine and methionine supplementation in plant-based diets for fish (Oyedokun *et al.*, 2022). The improvement in crude protein content in diet T4 indicates that lysine supplementation likely facilitated the utilization of plant-derived proteins, enhancing the overall protein quality of the diet. The increase observed could also show that supplementation of lysine in plant based diet increases protein deposition and could reduce ether extract in *C. gariepinus*. The reduction in ether extract (fat) content in diets T3 and T4 (7.20±0.10) compared to the control diet T1 (8.45±0.05). This suggests that lysine supplementation may have influenced lipid metabolism and reduce fat accumulation in *C. gariepinus* (Li *et al.*, 2015). This reduction in fat content could be beneficial for aquaculture, as it may result in leaner and healthier fish.

## CONCLUSION

Supplementation of lysine in plant based diet gives and insight on the potential of lysine in ameliorating the problem of poor growth in plant based diet, improving the health status of fish and increase protein deposition in *C. gariepinus*. Supplementation of 0.6g/100g of lysine has shown to improve the growth performance and diet utilization in fish. Overall health of the experimental fish was achieved and no adverse was observed. Supplementation of lysine in plant based diet up to 0.8g/100g could improve quality protein deposition.

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