

Effect of Roasted Soyabean Based Diets supplemented with Dietary Lysine and DL-methionine on Growth performance and blood profile of *Clarias gariepinus*

ABSTRACT

This study was aimed at investigating the effect of roasted soyabean based diets supplemented with dietary amino acid on growth performance, hematology and serum biochemistry of *Clarias gariepinus*. *Clarias gariepinus* juveniles (n=360) aged eight weeks, weighing 19.0 ± 0.4 grams were randomly allocated to six diets containing varied inclusion of Lysine and DL-methionine (g/100g) were as follows: RS1 (Control) = no supplemental lysine and DL-methionine; RS2 = 0g lysine + 1g DL-methionine; RS3 = 0.25g lysine + 0.75g DL-methionine; RS4 = 0.5g lysine + 0.5g DL-methionine; RS5 = 0.75g lysine + 0.25g DL-methionine; RS6 = 1g lysine + 0g DL-methionine. The fish were fed to satiation for 84 days. Each treatment was in triplicate. Lysine and methionine supplementation in roasted soyabean based diet fed to fish had no significant influence ($P > 0.05$) on final weight, feed conversion ratio, feed intake, specific growth rate and protein intake. The survival rate of fish fed diet RS5 (85.60 ± 0.60) was significantly higher than those fed control, RS2 and RS6, however, fish fed RS3 and RS4 were intermediate to them. Significantly ($P < 0.05$) higher PCV (%) and hemoglobin volume were observed in fish fed diet RS4 and RS5. white blood cell ($\times 10^9/L$) differ significantly among diets. Supplemental amino acid had no influence ($P > 0.05$) on total protein (g/L), Globulin (g/L), Albumin (g/L), A-G ratio and AST (IU/L). This study showed that supplementation of lysine and DL-methionine in Roasted Soyabean based diet could improve growth performance of fish with no adverse effect on fish health.

Keywords: Amino acid, Serum Biochemistry, Growth Performance, *Clarias gariepinus*, Growth performance

INTRODUCTION

The development of economically viable and nutritionally comprehensive diets for aquaculture species, such as *Clarias gariepinus*, is crucial for the advancement of sustainable aquaculture operations. In response to economic and environmental challenges associated with traditional fishmeal-based diets, the global aquaculture sector is increasingly turning towards plant-based protein sources, notably soybean. These plant-derived proteins, however, contain anti-nutritional factors that can interfere with digestion and metabolism (Eleshoet *al.*, 2021). To compensate for the nutritional gaps in plant-based feeds, it is often necessary to fortify them with essential amino

acids to satisfy the specific dietary requirements of different aquaculture species (Furuya *et al.*, 2020; National Research Council, 2011). Among these essential amino acids, lysine and methionine are particularly important due to their limited availability in plant proteins and their critical roles in the growth and metabolic functions of fish. This has led to the widespread acceptance of plant-based protein sources, particularly roasted soybean, as viable alternatives in aquafeeds (Eleshoet *et al.*, 2021).

Roasted soybean has been identified as a preferable substitute for raw soybean in aquafeeds due to its enhanced digestibility and reduced content of anti-nutritional factors (Oludeet *et al.*, 2023). Notably, roasted soybean meal exhibits high digestibility of amino acids, especially lysine and DL-methionine, which supports the formulation of nutritionally adequate diets for monogastric animals (Pettigrew *et al.*, 2008; Heuzéet *et al.*, 2020). The thermal processing involved in roasting effectively deactivates most trypsin inhibitors present in raw soybeans, which are known to impair protein digestion and utilization in fish (Oyedokunet *et al.*, 2018). Additionally, the roasting process enhances the palatability of soybean, making it more acceptable to fish (Fapohunda, 2012; Akumeet *et al.*, 2017; Oyedokun, 2019).

The incorporation of roasted soybean supplemented with lysine and methionine into the diets of *Clarias gariepinus* has been extensively studied (Fapohunda, 2012; Oyedokun, 2019; Azubuike, 2021). Oyedokunet *al.* (2022) reported significant improvements in growth performance of *C. gariepinus* fed diets enriched with these supplements compared to those without. These findings indicate that the blood profile, reflecting the physiological and nutritional status of fish, corroborates the dietary adequacy and balance (Oyedokunet *al.*, 2022). However, research involving silvery-black porgy juveniles suggests that despite lysine and methionine supplementation, growth performance was not significantly enhanced, likely due to the persistent presence of anti-nutritional factors in soy protein products (Hu *et al.*, 2022).

Supplementing plant protein-based diets with DL-methionine and lysine is essential to meet the dietary requirements of fish, facilitating protein synthesis, growth, immune response, and overall health (Lu *et al.*, 2014; Guo *et al.*, 2019; Kokou *et al.*, 2017; Su *et al.*, 2018). The roles of lysine and methionine extend beyond simple growth promotion to involve complex metabolic processes, including taurine synthesis, which is crucial for fish health (Hays *et al.*, 2020). The requirement for lysine varies not only among different fish species but also across different growth stages within the same species, with insufficient dietary lysine leading to feed inefficiency (Ebenezara *et al.*, 2019). Zhang *et al.* (2024) highlighted the beneficial effects of lysine supplementation on the growth performance across various animal species and noted its significant role in enhancing the digestibility of essential amino acids.

Further, the inclusion of DL-methionine in diets complements lysine supplementation by improving the amino acid profile, which is associated with enhanced growth rates and nutrient utilization (Wang *et al.*, 2018; Oyedokunet *al.*, 2019; Souza *et al.*, 2023; Wang *et al.*, 2023). Eleshoet *al.* (2021) noted that diets for African catfish enriched with crystalline methionine led

*Vit/min premix	0.25	0.25	0.25	0.25	0.25	0.25
Soyabean oil	1	1	1	1	1	1
Calcium carbonate	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Chromic Oxide	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0	0	0.25	0.5	0.75	1
Methionine	0	1	0.75	0.5	0.25	0
Total (%)	99	100	100	100	100	100

Table 2: Nutrient composition of roasted soyabean based test diets

Ingredient (%)	Control	Diet (RS)				
		2	3	4	5	6
Crude protein	40.90±0.28	39.98±0.46	40.96±1.27	40.88±1.24	40.68±1.10	41.53±0.60
Ash	5.68±0.04 ^{bc}	5.30±0.14 ^{ab}	6.45±0.70 ^b	5.00±0.14 ^a	5.90±0.28 ^{cd}	6.15±0.70 ^d
Ether extract	6.60±0.14 ^a	6.80±1.14 ^a	6.50±1.14 ^a	6.95±0.14 ^a	6.60±0.14 ^a	7.15±0.07 ^b
Crude fibre	4.05±0.21 ^{bc}	3.60±0.14 ^a	4.75±0.70 ^{ab}	4.15±0.70 ^c	4.75±0.21 ^{ab}	7.90±0.14 ^{abc}
Dry Matter	92.19±0.17	91.36±0.33	92.07±0.04	92.88±0.39	92.67±0.16	92.24±0.08
Gross energy (kcal/g)	4.12±0.00	4.02±0.00	4.03±0.00	4.11±0.00	4.01±0.00	4.13±0.00
Calcium	1.29±0.00 ^b	1.86±0.00 ^e	1.77±0.00 ^d	1.28±0.00 ^a	1.63±0.00 ^c	1.29±0.00 ^{ab}
Phosphorus	0.59±0.00 ^b	0.83±0.00 ^e	0.81±0.00 ^d	0.57±0.00 ^a	0.75±0.00 ^c	0.60±0.00 ^b
Potassium	0.82±0.00 ^a	0.97±0.00 ^d	0.97±0.00 ^d	0.82±0.00 ^b	0.95±0.00 ^c	0.81±0.00 ^{ab}
Sodium	0.28±0.00 ^c	0.38±0.00 ^e	0.38±0.00 ^e	0.27±0.00 ^b	0.36±0.00 ^d	0.27±0.00 ^a

Means with same letter in row are not significantly different (P>0.05)

Proximate composition

Proximate composition of the diets was determined according to AOAC, (2005).

Growth studies

Calculations on growth performance and feed utilization were according to Falayi, (2009). Data on weight gain (WG), feed conversion ratio (FCR), protein intake (PI), feed intake (FI), protein efficiency ratio (PER), specific growth rate (SGR), gross protein retention (GPR), nitrogen retention efficiency (NRE) and survival rate (SR) were taken bi-weekly throughout the duration of the study.

Blood sampling and analysis

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. The blood samples were analysed for packed cell volume (PCV), haemoglobin concentration, red blood cell (RBC), white blood cell (WBC) as outlined by Blaxhall and Daisley, (1973).

Mean Corpuscular Volume (MCV)

MCV was estimated using the model described by Feldman *et al.* (2000):

$$\text{MCV} = \frac{\text{volume of red blood cell (in mL per 100mL blood)}}{\text{Number of red blood cells oer 100mL blood}} \times 100$$

Mean Corpuscular Haemoglobin (MCH)

MCH was estimated using the model described by Stoskopf, (1992)

$$\text{MCH} = \frac{\text{Haemoglobin } (\frac{g}{100mL})}{\text{Number of red blood cells } (\frac{\text{millions}}{L} \text{ blood})} \times 100$$

Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC was estimated using the model as described by Stoskopf, (1992)

$$\text{MCHC} = \frac{\text{Haemoglobin concentration}}{\text{Packed cell volume}} \times 100$$

Serum Biochemical Analysis

Blood (2 mL) was sampled from three randomly selected *C. gariepinus* per replicate group into heparinized flask without anticoagulant for serum biochemical analysis. The blood was allowed to clot and samples was then centrifuged at 3000rpm for five minutes with Hawsley minor bench centrifuge (P spectra, Centromix no 231254 CD7000549, Spain). The blood serum was harvested and stored at -20 °C. Total protein and albumin were determined by Biuret method according to Reinhold,(1953), globulin was estimated as the difference between total protein and albumin. Albumin:globulin ratio, serum enzymes: aspartate amino transaminase, alanine amino transaminase and alkaline phosphatase activities were determined spectrophotometrically according to Henry *et al.* (1974). Blood urea nitrogen and creatinine were determined by the techniques of Harrison, (2006).

Statistical Analysis

Data were analyzed using descriptive statistics, polynomial regression 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 utilisation of *C. gariepinus* fed roasted soyabean based diet supplemented with varying inclusion of dietary amino acid is shown in Table 3. Lysine and methionine supplementation in roasted soyabean based diet fed to fish had no significant influence ($P>0.05$) on final weight, feed conversion ratio, feed intake, specific growth rate and protein intake. Gross protein retention of fish fed diet RS3 (1.01 ± 0.04) was not significantly difference ($P>0.05$) from that of RS5 diet. Nitrogen retention efficiency increased with amino

acid supplementation and least values was in diet control (33.78±4.11). The survival rate of fish fed diet RS5 (85.60±0.60) was significantly higher than those fed control, RS2 and RS6, however, fish fed RS3 and RS4 were intermediate to them. The relationship between lysine and methionine inclusion and final weight gain of *C. gariepinus* are represented by the regression equations 1, 2, respectively.

For Final weigh gain

$$y = -0.5357x^2 + 3.8271x + 29.48 \dots \dots \dots R^2 = 0.8052 \dots \dots \dots 1$$

$$y = -0.3839x^2 + 3.1361x + 29.43 \dots \dots \dots R^2 = 0.6959 \dots \dots \dots 2$$

From the graphs (Figures 1) it could be depicted that 6.5 and 3.5 g/kg inclusion of lysine and methionine was observed at the equations for it optimum inclusion in soyabean based diet.

Table 3: Growth performance and feed utilisation by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Parameter	Control	Diet (RS)				
		2	3	4	5	6
IW (g)	19.70±0.42	19.70±0.20	19.50±0.20	19.63±0.12	19.90±0.20	19.53±0.25
FW (g)	32.75±2.90	35.40±2.90	35.30±4.98	35.03±3.16	36.37±4.59	32.60±3.59
FCR	3.76±0.46	3.13±0.55	3.37±1.14	3.19±0.56	3.03±0.67	3.54±0.78
GEFC	67.44±1.30	73.56±4.82	71.06±9.37	73.23±7.34	75.87±3.70	73.28±6.41
PI	11.20±0.78	11.10±0.23	11.47±0.63	11.07±0.86	11.04±1.01	10.25±0.27
FI	0.75±0.05	0.74±0.02	0.76±0.04	0.74±0.07	0.74±0.06	0.68±0.02
PER	10.92±0.97	11.80±0.97	11.76±1.66	11.68±1.05	12.12±1.53	10.87±1.20
SGR	0.34±0.04	0.39±0.06	0.39±0.09	0.39±0.06	0.40±0.08	0.34±0.07
GPR	0.74±0.03 ^b	0.64±0.01 ^a	1.01±0.04 ^d	0.71±0.03 ^b	0.97±0.04 ^d	0.86±0.03 ^c
NRE	33.78±4.11 ^a	34.12±3.71 ^a	47.41±7.82 ^b	36.27±4.57 ^{ab}	47.12±8.86 ^b	37.52±5.23 ^{ab}
SR %	70.00±2.00 ^a	71.00±1.00 ^a	82.27±0.31 ^b	82.20±0.10 ^b	85.60±0.60 ^c	69.40±0.40 ^a

Means with same letter in row are not significantly different (P>0.05)

IW = Initial Weight, FW= Final weight, FCR= Feed Conversion Ratio, GEFC= Gross Efficiency Feed Conversion, PI= Protein Intake, FI= Feed Intake, PER= Protein Efficiency Ratio, SGR= Specific Growth Rate, GPR= Gross Protein Retention, NRE= Nitrogen Retention Efficiency, SR= Survival Rate

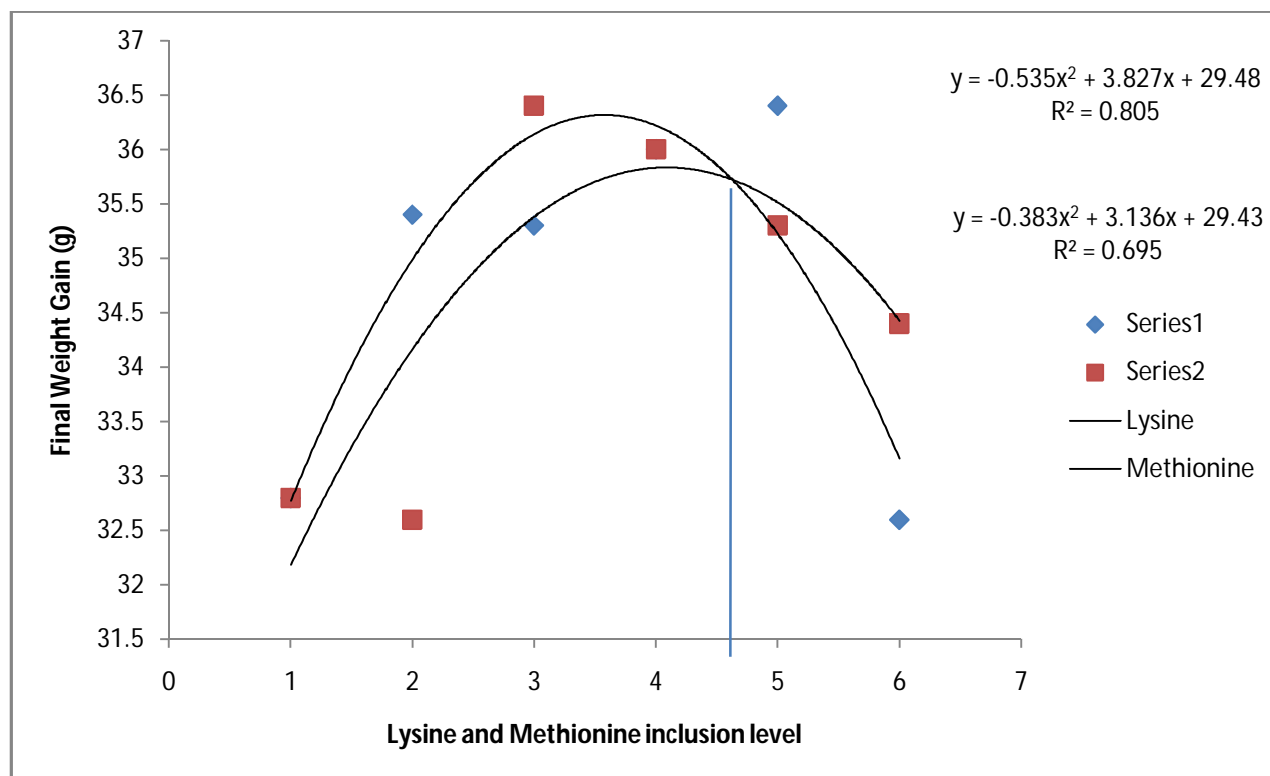


Figure 1: Relationship between dietary supplemental lysine and methionine of a roasted soyabean based diet and final weight gain of *Clarias gariepinus*

1 – Control **2** - 1gram (g) Methionine; **3** - 0.75g Methionine, 0.25g Lysine; **4** - 0.5g Methionine, 0.5g Lysine; **5**- 0.25 Methionine, 0.75 Lysine; **6** - 1g Lysine

Haematological parameters of *C. gariepinus* fed roasted soyabean based diet supplemented with varying inclusion of dietary amino acid is shown in Table 4. Significantly ($P < 0.05$) higher PCV (%) and hemoglobin volume were observed in fish fed diet RS4 and RS5 while RS2, RS3 and RS6 did not differ significantly. Red blood cell ($\times 10^{12}/L$) value ranged from 1.43 ± 0.05 (control) to 2.75 ± 0.92 (RS5). However, white blood cell ($\times 10^9/L$) differ significantly among diets. MCV (fl), MCH (pg) and MCHC (g/dL) values ranged from 105.35 ± 31.76 (RS5) to 146.76 ± 8.93 (control); 33.11 ± 10.29 (RS5) to 47.30 ± 0.87 (control) and 31.17 ± 0.14 (RS2) to 33.16 ± 1.82 (RS4).

Supplementation of amino acid in roasted soyabean based diet significantly ($P < 0.05$) reduced platelet ($\times 10^9/L$) value and platelet value of fish fed control diet was significantly higher than other treatment. Also, a significantly higher lymphocytes (%) values was observed in RS5, RS6 and control diet. Moreso, heterocytes (%) count was higher ($P < 0.05$) in RS3 (39.00 ± 4.58) and least value of 27.00 ± 2.00 in RS5. Lymphocytes: heterocytes ratio values varies significantly between the treatments. Supplemental amino acid had no influences ($P > 0.05$) on Monocytes (%), Eosinophils (%) and basophils (%).

Table 4: Haematological parameters of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Parameter	Control	Diet (RS)				
		2	3	4	5	6
PCV (%)	21.00±1.73 ^a	24.33±2.52 ^{ab}	24.00±1.00 ^{ab}	26.00±1.73 ^b	27.00±2.65 ^b	24.67±2.08 ^{ab}
HB(g/dL)	6.77±0.38 ^a	7.77±0.80 ^{ab}	7.73±0.70 ^{ab}	8.60±0.10 ^b	8.47±0.76 ^b	8.00±1.00 ^{ab}
RBC(x10 ¹² /L)	1.43±0.05	2.19±0.88	1.64±0.10	2.28±0.98	2.75±0.92	2.23±0.93
WBC(x10 ⁹ /L)	12.82±0.33 ^a	15.43±0.98 ^{abc}	18.13±0.49 ^c	16.05±0.59 ^{bc}	15.00±2.96 ^{ab}	15.42±1.38 ^{abc}
MCV (fl)	146.76±8.93	119.35±30.41	146.49±5.54	124.96±37.46	105.35±31.76	120.04±33.54
MCH (pg)	47.30±1.58	38.11±9.78	47.14±3.06	41.87±14.25	33.11±10.29	38.72±10.60
MCHC(g/dL)	32.27±0.87	31.17±0.14	32.18±1.61	33.16±1.82	31.38±0.93	32.36±1.67
Platelet (x 10 ⁹ /L)	32.83±6.48 ^b	19.03±9.46 ^a	15.30±3.54 ^a	12.00±2.80 ^a	15.13±3.53 ^a	12.60±3.80 ^a
Lym (%)	63.33±3.21 ^b	56.33±4.16 ^a	53.33±3.79 ^a	59.00±5.00 ^a	65.33±2.08 ^b	63.67±3.51 ^b
Het (%)	28.67±2.52 ^a	37.33±3.51 ^{bc}	39.00±4.58 ^c	33.33±2.52 ^{abc}	27.00±2.00 ^a	30.67±5.86 ^{ab}
Lym:Het Ratio	0.46±0.06 ^a	0.67±0.11 ^{bc}	0.74±0.14 ^c	0.57±0.10 ^{abc}	0.41±0.05 ^a	0.49±0.11 ^{ab}
Mono (%)	3.00±1.00	2.67±2.08	2.33±1.15	3.67±0.58	3.67±1.15	3.33±1.53
Eos (%)	4.67±1.53	3.67±0.58	4.33±2.52	3.67±1.53	3.67±1.53	2.00±3.46
Baso (%)	0.33±0.58	0.00±0.00	0.33±0.58	0.33±0.58	0.33±0.58	0.33±0.58

Means with same letter in row are not significantly different (P>0.05)

PCV= Packed Cell Volume, HB= 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 = Basophils.

Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 5. Supplemental amino acid had no influence ($P>0.05$) on total protein (g/L), Globulin (g/L), Albumin (g/L), A-G ratio and AST (IU/L). Alanine Transaminase (IU/L) and Blood Urea Nitrogen ($\mu\text{mol/L}$) were significantly higher ($P<0.05$) in fish fed diet RS2, RS4, RS5 and RS6 while least value was in *C. gariepinus* fed control diet (19.33 ± 5.03). Also, Alkaline Phosphatase (IU/L) was not significantly ($P<0.05$) different among the fish fed diet RS3, RS5 and RS6, however, these were significantly superior than fish fed RS2 diet. Furthermore, Creatinine ($\mu\text{mol/L}$) significantly increased with the supplementation of amino acid. Higher value was observed in diet RS2 (0.77 ± 0.56) and least value in control diet (0.53 ± 0.06).

Table 5: Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Parameter	Diet (RS)					
	Control	2	3	4	5	6
Total protein(g/L)	6.67±0.29	7.33±1.26	6.83±0.76	7.87±0.55	7.07±0.50	6.60±0.52
Albumin(g/L)	1.87±0.38	2.43±0.90	1.77±0.40	2.60±0.53	2.13±0.67	1.67±0.15
Globulin(g/L)	4.80±0.10	4.90±0.36	5.07±0.40	5.27±0.06	4.93±0.25	4.93±0.38
A-G ratio	0.33±0.06	0.47±0.15	0.30±0.10	0.47±0.12	0.37±0.15	0.30±0.00
AST (IU/L)	185.33±3.06	189.33±4.04	182.67±5.13	197.67±21.39	188.33±7.57	186.00±5.29
ALT (IU/L)	19.33±5.03 ^a	32.33±3.21 ^b	21.33±2.31 ^a	24.33±7.51 ^{ab}	26.67±3.79 ^{ab}	30.67±4.04 ^b
ALP (IU/L)	208.67±29.96 ^{ab}	144.67±18.50 ^a	289.33±57.74 ^b	231.67±121.33 ^{ab}	289.67±24.21 ^b	291.67±13.80 ^b
BUN($\mu\text{mol/L}$)	8.57±0.74 ^a	9.40±1.11 ^{ab}	8.80±0.62 ^a	10.47±0.70 ^b	9.27±0.50 ^{ab}	9.10±0.66 ^{ab}
Creatinine ($\mu\text{mol/L}$)	0.53±0.06 ^a	0.77±0.56 ^c	0.60±0.10 ^{ab}	0.70±0.10 ^{bc}	0.60±0.10 ^{ab}	0.67±0.06 ^{abc}

Means with same letter in row are not significantly different ($P>0.05$)

A-G Ratio = Albumin-Globulin Ratio, AST = Aspartate Transaminase, ALT = Alanine Transaminase, ALP = Alkaline Phosphatase, BUN = Blood Urea Nitrogen.

DISCUSSION

The nutritional adequacy of dietary energy, protein quality and quantity, and their balance are critical for meeting the growth requirements of aquaculture species at specific life stages, as emphasized in previous research (Aderoluet *al.*, 2018; Eleshoet *al.*, 2021). These nutrient demands are not static and must be provided in correct proportions for optimal growth (Wilson, 1994; Lu *et al.*, 2014). In this study, *Clarias gariepinus* were provided with diets comprising roasted soybean enhanced with different concentrations of lysine and methionine. The results

demonstrated that these amino acid additions did not significantly affect growth performance indices including Final weight, Feed Conversion Ratio (FCR), feed intake, Specific Growth Rate (SGR), and Protein intake. This suggests that the existing levels of these amino acids in roasted soybean may satisfy the minimal requirements of *C. gariepinus* for these indices, or that the benefits of supplementation may not be evident under the tested conditions. Guo *et al.* (2019) noted that although lysine and methionine are essential for optimal fish growth, their effectiveness may reach a plateau once baseline nutritional needs are met, supporting the protein sparing hypothesis which posits that once essential amino acid needs are met, further supplementation does not necessarily boost growth but could enhance other physiological or metabolic functions (Garg *et al.*, 2022).

Additionally, an optimal growth response curve and regression model predicted optimal inclusion levels of 6.5g/kg lysine and 3.5g/kg methionine, as demonstrated in figure 1. Enhanced nitrogen retention efficiency was observed with amino acid supplementation, though this did not translate into improved growth performance, indicating potential enhancements in protein metabolism or utilization not directly reflected in growth metrics, as noted by Khalil *et al.* (2021). The physiological or health status of the fish might have been positively influenced by the dietary enhancements, reflecting the broader objectives of dietary optimization in aquaculture which include growth, feed efficiency, and health enhancement (Oyedokun *et al.*, 2019; Garg *et al.*, 2022). Fish fed the RS5 diet also showed a notably higher survival rate, potentially due to optimal nutrient balance and effective handling of anti-nutritional factors through roasting and amino acid supplementation, a finding aligned with that reported by Oliva-Teles *et al.* (2015).

Further, diets RS4 and RS5, with higher levels of lysine and DL-methionine supplementation, resulted in significantly higher packed cell volume (PCV) and hemoglobin concentrations, indicative of enhanced physiological health as these metrics are essential for the oxygen-carrying capacity of blood, critical for metabolic activities (Fazio, 2019; Lu *et al.*, 2014). Red blood cell (RBC) counts were notably higher in fish on the RS5 diet, improving oxygen supply to tissues and supporting metabolic functions (Wessels *et al.*, 2017). White blood cell (WBC) count variability among the diets suggested different immune responses, potentially influenced by the amino acid profiles of the diets (Sardar *et al.*, 2008).

The study showed significant variations in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) across treatments, indicating potential changes in red blood cell functionality and developmental stages due to dietary treatments (Kuhn *et al.*, 2017). A significant reduction in platelet count in amino acid-supplemented groups, except the control, could suggest a modulation of hemostasis and thrombotic activities by dietary components, critical for fish health management (Moha *et al.*, 2021). Lymphocyte percentages were higher in diets RS5, RS6, and the control, suggesting an enhanced specific immune response, consistent with findings that dietary amino acids can modulate lymphocyte activity (Li *et al.*, 2019). However, another type of white blood cells, heterocytes, exhibited a different trend, with the highest counts in RS3, possibly reflecting a

stress response or shift in immune strategy, depending on the dietary composition (Abo-Al-Ela *et al.*, 2021).

Overall, the levels of total protein, globulin, and albumin were within normal ranges across all dietary treatments, indicating that the baseline nutritional requirements for these proteins are likely met by the roasted soybean-based diets, whether supplemented with lysine and DL-methionine or not (Fazio, 2019; Oliva-Teles *et al.*, 2015; Martins *et al.*, 2018). The stability in the A-G ratio and AST levels across all dietary treatments further supports liver function not being adversely affected by the diets. However, significant elevations in alanine transaminase (ALT) and blood urea nitrogen (BUN) in certain diets suggest shifts in amino acid catabolism or increased protein turnover, while significant differences in alkaline phosphatase (ALP) could indicate variances in bone metabolism or intestinal health statuses (Cengiz *et al.*, 2019; Yun *et al.*, 2011). Additionally, creatinine levels, significantly higher in diet RS2 compared to the control, could indicate enhanced muscle metabolism or slightly impaired kidney function due to higher metabolic loads associated with amino acid processing (Wu, 2018), warranting further investigation.

Conclusion

The addition of lysine and methionine to the roasted soybean-based diets for *C. gariepinus* did not significantly alter traditional growth parameters such as final weight, feed conversion ratio (FCR), feed intake, and specific growth rate (SGR), and protein intake. Although, the regression analysis model reveals a strong correlation between dietary amino acid and final weight gain at $R^2 = 0.805$ (lysine) and 0.696 (DL-methionine). However, the best expressed by optimum growth response curve and the regression model was predicted at 6.5g/kg lysine and 3.5g/kg for methionine inclusion level and recommended

Also, this study confirms that dietary supplementation with amino acids in roasted soybean-based diets influences various hematological parameters in *C. gariepinus*. These findings concluded that, the potential of diet manipulation in aquaculture to enhance fish health and immune response.

Overall, the results of this study suggest that supplementing roasted soybean-based diets with varying levels of amino acids can influence the serum biochemical profile of *C. gariepinus*. While the non-significant effects of supplemental amino acids on total protein, globulin, albumin, albumin-globulin (A-G) ratio, and aspartate transaminase (AST) levels suggest that these nutritional modifications do not compromise the basic protein metabolism and liver function under the experimental conditions.

References

- Abo-Al-Ela, H. G., Seham El-Kassas, Karima El-Naggar, Safaa E. Abdo, Ali Raza Jahejo, and Rasha A. Al Wakeel (2021) Stress and immunity in poultry: light management and nanotechnology as effective immune enhancers to fight stress, *Cell Stress Chaperones*, 26(3): 457–472. doi: 10.1007/s12192-021-01204-6.

- Aderolu, Ademola Zaid, Lawal, MuyideenOwonire, Awobajo, FunmileyiOlubajo, Olaniyan, Stephen and Bello, Yetunde (2018). Dietary Energy Requirement of *Clarias gariepinus* Juvenile at Fixed Crude Protein and its Effects on Growth, Nutrient Performance, Haematology and Biochemical Indices. *Animal Research International* 15(3): 3090 – 3100.
- Agume Aurelie, Njintang Nicolas and Mbofung Carl. (2017). Effect of Soaking and Roasting on the Physicochemical and Pasting Properties of Soybean Flour. *Foods*. 6. 12. 10.3390/foods6020012.
- AOAC. (2005). Official Methods of Analysis of the Association of Analytical Chemists International, 18th ed. Gathersburg, MD U.S.A Official methods, 2005.08.
- Azubuike Adams. (2021). Effect of Raw and Toasted Soya Beans Diets on Growth of *Clarias gariepinus*. *Global Science Research Journal*, 9(1), 1-12.
- Bickerdike, R., Evans, M., Tsoupras, A., and Wright, P. A. (2017). Evaluating the impact of dietary amino acids on liver function and urea production in fish. *Journal of Fish Biology*, 91(3), 728-739. doi:10.1111/jfb.12458
- Blaxhall P. C and Daisley K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5:771-781.
- Cengiz, E. I., Unlu, E., and Satar, A. (2019). The role of dietary enzymes in enhancing gut health in fish aquaculture. *Aquaculture Nutrition*, 25(2), 456-464. doi:10.1111/anu.12869
- Ebeneezara, S., Vijayagopal, P., Srivastava, P. P., Gupta, S., Sikendrakumar, S., Varghese, T., Prabua, D. L., Chandrasekar, S., Varghese, E., Sayooj, P., Tejpal, C. S., and Wilson L. (2019). Dietary lysine requirement of juvenile Silver pompano, *Trachinotus blochii* (Lacepede, 1801). *Aquaculture*, 511, 734234. <https://doi.org/10.1016/j.aquaculture.2019.734234>
- Elesho, F. E., Kröckel, S., Ciavoni, E., Sutter, D. A. H., Verreth, J. A. J., and Schrama, J. W. (2021). Effect of feeding frequency on performance, nutrient digestibility, energy and nitrogen balances in juvenile African catfish (*Clarias gariepinus*) fed diets with two levels of crystalline methionine. *Animal Feed Science and Technology*, 281, 115098. <https://doi.org/10.1016/j.anifeedsci.2021.115098>
- Falayi B. A. (2009). Feed formulation, manufacture and quality appraisal for fish and livestock. A guide in nutrition technology Book Series 4. First edition 2009, Published by B.A. Falayi, National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa. ISBN 978-978-48686-9-3.

- Fapohunda, Olawumi. (2012). Evaluation of processed soybean meal in the feeding of *Clarias gariepinus* fingerlings. *Journal of Animal Science Advances*. 2. 244-249.
- Faturoti, E. O., Balogun, A. M. and Ugwu, L. L. C. (1986). Feed utilization and growth responses of *Clarias gariepinus* fed different dietary protein levels. *Nigerian Journal of Applied Fish and Hydrobiology*, 1: 41-45.
- Fazio, F. (2019). Protein metabolism in marine species: Using blood parameters as health indicators in fish. *Marine Biology Research*, 15(5), 380-392. doi:10.1080/17451000.2019.1621054
- Feldman, B. F., Zinkl, J. G., and Jain, N. C. (2000). Schalm's Veterinary Hematology. 5.ed. Lippincott Williams and Wilkins, 787p.
- Furné, M., Morales, A. E., Trenzado, C. E., García-Gallego, M., and Domezain, A. (2014). Markers of nutritional status and metabolism in fish: Implications for aquaculture production. *Aquaculture International*, 22(5), 1707-1723. doi:10.1007/s10499-014-9780-1
- Garg C.K., Sardar P., Sahu N.P., Maiti M.K., Shamna N., Varghese T., Deo A.D. and Harikrishna V. (2022), Dietary lysine requirement of genetically improved farmed tilapia (GIFT) juvenile reared in inland saline water of 10 ppt salinity. *Aquaculture*, 555, 738223
- Guo Tian-Yu, Zhao Wei, He Ju-Yun, Liao Shi-Yu, Xie Jiajun, Xie Shiwei, Masagounder Karthik, Liu Yong-Jian, Tian Li-Xia and Niu Jin. (2019). Dietary DL-Methionyl-DL-Methionine (Met-Met) supplementation increased growth performance, antioxidant ability, the content of essential amino acids and improved the diversity of intestinal microbiota in Nile tilapia (*Oreochromis niloticus*). *British Journal of Nutrition*. 123. 1-23. 10.1017/S0007114519002289.
- Harrison, P. (2006) Integrated Development Plans and Third Way Politics. In: Pillay, U., Tomlinson, R. and du Toit, J., Eds., Democracy and Delivery: Urban Policy in South Africa, HSRC Press, Cape Town, 186-207.
- Henry G. H., Dreher B. and Bishop P. O. (1974). Orientation specificity of cells in cat striate cortex. *Journal of Neuro-physiology*. 37, 1394-1409.
- Heuzé V., Tran G., Kaushik S., (2020). Soybean meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. <https://www.feedipedia.org/node/674>.
- Hu Xuecai, Bin Huo, Jiameng Yang, Kun Wang, Lingjie Huang, Lianqiang Che, Bin Feng, Yan Lin, Shengyu Xu, Yong Zhuo, Caimei Wu, De Wu, and Zhengfeng Fang. (2022). Effects of Dietary Lysine Levels on Growth Performance, Nutrient Digestibility, Serum

Metabolites, and Meat Quality of Baqing Pigs. *Animal (Basel)*. 12(15): 1884. doi: 10.3390/ani12151884

- Khalil Hala, Momoh Taofik, Al-KenawyDiaa, Yossa Rodrigue, Badreldin Alaa, Roem Arjen, Schrama Johan and Verdegem Marc. (2021). Nitrogenretention, nutrient digestibility and growth efficiency of Nile tilapia (*Oreochromis niloticus*) fed dietary lysine and reared in fertilized ponds. *Aquaculture Nutrition*. 1-13. 10.1111/anu.13365.
- Kokou, F., Sarropoulou, E., Cotou, E., Kentouri, M., Alexis, M., and Rigos, G. (2017). Effects of graded dietary levels of soy protein concentrate supplemented with methionine and phosphate on the immune and antioxidant responses of gilthead sea bream (*Sparus aurata* L.). *Fish and Shellfish Immunology*, 64, 111-121. <https://doi.org/10.1016/j.fsi.2017.03.017>
- Krogdahl, Å., Hemre, G. I., and Mommsen, T. P. (2010). Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition*, 16(5), 499-506. doi:10.1111/j.1365-2095.2009.00727.x
- Kuhn Viktoria, Lukas Diederich, T.C. Stevenson Keller, Christian M. Kramer, Wiebke Lückstädt, Christina Panknin, Tatsiana Suvorava, Brant E. Isakson, Malte Kelm, and Miriam M. Cortese-Krott (2017). Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, *Anemia*. 26(13): 718–742. doi: 10.1089/ars.2016.6954
- Lu, J., Hua, Y., Fu, W. Z., Zhou, F., Yang, B. B., Xiao, J. X. and Shao, Q. J. (2014). Effects of Supplementation Coated Lysine and DL-methionine in Mixture Protein Diets on Growth Performance, Digestibility and Serum Biochemical Indices of Juvenile Black Sea Bream. *Turkish Journal of Fisheries and Aquatic Sciences*, 14(3): 633-642.
- Martins, C. F., Jesus, G., Gallani, S. U., and Roubach, R. (2018). Soyabean meal as a protein source in aquafeeds: Effects on growth and health of fish. *Journal of Animal Science and Technology*, 60(1), 12. doi:10.1186/s40781-018-0183-0
- Moha Esmaeili. (2021) Blood Performance: A New Formula for Fish Growth and Health, *Biology (Basel)* 10(12): 1236. doi: 10.3390/biology10121236
- National Research Council (2011). Nutrient Requirements of Fish and Shrimp. Washington, DC: The National Academies Press.
- Oliva-Teles, A., Enes, P., and Peres, H. (2015). Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. *Feed Science and Technology*, 239, 34-48. doi:10.1016/j.anifeedsci.2014.11.008

- Olude O.O., N.P. Sahu, P. Sardar, P.M. Nuzaiba (2023) Utilization of valorized cassava leaf meal as an alternative feedstuff to defatted soybean meal in feed for Rohu, *Labeorohita fingerlings*. *Bioresource Technology Reports*. 22, 101400
- Oyedokun J. O, Ogunwole O. A, and Oyelese O. A. (2019); Growth performance and blood profile of *Clarias gariepinus* fed processed soybean-based diets supplemented with amino acids. *International Journal of Scientific and Technology Research*, Vol 8 (10), 1569-1575.
- Oyedokun J. O. (2019), Growth performance and protein digestibility in *Clarias gariepinus*, (burchell, 1822) fed soyabean meal based diets supplemented with amino acid and protease. (PhD Thesis, University of Ibadan). University of Ibadan Postgraduate School repository. <http://repository.pgcollegeui.com:8080/xmlui/handle/123456789/896>
- Oyedokun J.O., O. A. Ogunwole , O. A. Oyelese , I. C. Adene , S. O. Sule , and T. Folorunso. (2022). Growth performance and proximate composition of *Clarias gariepinus* (Burchell, 1822) fed roasted soyabean-based diets with supplemental amino acids. *Annal of Animal and Biological Research*. 2(1): 76-86.
- Pettigrew, J. E., Soltwedel, K. T., Miguel, J. C., Palacios, M. F. (2008). Fact sheet - Soyabean Use - Swine. Soyabean Meal Information Center.
- SAS, (2005). Statistical Analysis Software (SAS) systems for Windows, SAS Inc., Cary. NC, USA.
- Stoskopf, M. K. (1992). Fish Medicine. W. B. Saunders Company, Harcourt Braces Jovanovich Inc. Philadephis, London. 125 pp.
- Su Y. N., Wu P., Feng L, *et al.* (2018) The improved growth performance and enhanced immune function by DL-methionyl-DL-methionine are associated with NF- κ B and TOR signalling in intestine of juvenile grass carp (*Ctenopharyngodonidella*). *FishShellfish Immunol* 74, 101–118.
- Teles, A., Boltaña, S., Reyes-López, F., Santos, M. A., MacKenzie, S., and Tort, L. (2021). Role of dietary amino acids in immune response and stress resilience in European sea bass. *Aquaculture Reports*, 20, 100600. doi:10.1016/j.aqrep.2021.100600
- Wang Wennan, Lisha Ye, Xingtang Dou, Haiying Liu and Di Han. (2023). Effects of Rumen-Protected Methionine Supplementation on Growth Performance, Nutrient Digestion, Nitrogen Utilisation and Plasma Amino Acid Profiles of Liaoning Cashmere Goats *Animals*, 13(19), 2995; <https://doi.org/10.3390/ani13192995>
- Wessels I., Maywald M., and Rink L. (2017) Zinc as a gatekeeper of immune function. *Nutrients*. 9:1286. doi: 10.3390/nu9121286.

- Wu, G. (2018). Dietary protein intake and human health. *Food and Function*, 9(3), 1236-1259. doi:10.1039/C7FO01782A
- Y. Wang, J. Zhou, G. Wang, S. Cai, X. Zeng, S. Qiao (2018) Advances in low-protein diets for swine *Journal of Animal Science and Biotechnology*, 9: 60, 10.1186/s40104-018-0276-7
- Yun Biao, Kangsen Mai, Wenbing Zhang, Wei Xu (2011). Effects of dietary cholesterol on growth performance, feed intake and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets. *Aquaculture*, 319 (1–2), 105-110
- Zhang Jiayu, Shunju Geng, Yahao Zhu, Lan Li, Lihong Zhao, Qiugang Ma, and Shimeng Huang. (2024). Effects of dietary methionine supplementation on the growth performance, immune responses, antioxidant capacity, and subsequent development of layer chicks. *Poultry Science*. 103(3): 103382. doi: 10.1016/j.psj.2023.103382