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

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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 *Clariasgariepinus*. *Clariasgariepinus* juveniles (n=360) aged eight weeks, weighing 19.0±0.4grams 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 ($x10^9$/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, *Clariasgariepinus*, Growth performance

INTRODUCTION

The development of economically viable and nutritionally comprehensive diets for aquaculture species, such as *Clariasgariepinus*, 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

Comment [A1]: use number 1

Comment [A2]: Clariasgariepinus is an omnivorous fish and tends to be carnivorous, so it needs a stronger explanation regarding the use of vegetable protein. This is related to the length of the digestive process even though it is fortified with amino acids. This will later be related to overall growth performance

Comment [A3]: [1]

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 (Elesho*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 (Olude*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; Heuzé*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 (Oyedokun*et al*., 2018). Additionally, the roasting process enhances the palatability of soybean, making it more acceptable to fish (Fapohunda, 2012; Akume*et al*., 2017; Oyedokun, 2019).

The incorporation of roasted soybean supplemented with lysine and methionine into the diets of *Clariasgariepinus* has been extensively studied (Fapohunda, 2012; Oyedokun, 2019; Azubuike, 2021). Oyedokun*et 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 (Oyedokun*et 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 (Ebeneezara *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; Oyedokun*et al*., 2019; Souza *et al*., 2023; Wang *et al*., 2023).

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Comment [A6]: [4]

Comment [A7]: [5]

Comment [A8]: [6] etc

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Elesho*et al*. (2021) noted that diets for African catfish enriched with crystalline methionine led to improved growth when fed plant-protein based diets. The synergistic effects of lysine and DL-methionine supplementation in soybean-based diets could therefore lead to significant enhancements in growth performance, nutrient digestibility, and overall health status in *Clariasgariepinus*. Such dietary optimization could provide substantial benefits to the aquaculture industry by improving the health and productivity of *Clariasgariepinus*. This study aims to examine the impact of a roasted soybean-based diet supplemented with dietary lysine and DL-methionine on the growth performance and blood profile of *Clariasgariepinus*.

Comment [A9]: This research wants to prove the truth of the results of research conducted by previous researchers

Materials and Methods

Comment [A10]: Use number 2

The feeding trial was conducted at the Aquaculture Research Laboratory, Aquatech College of Aquaculture, Fodacis, Ibadan, Nigeria. *Clariasgariepinus* juveniles (n=360) aged eight weeks, weighing 19.0±0.4grams were purchased from a reputable fish farm in Ibadan, Nigeria. Six isonitrogenous diets were formulated with varying levels of lysine and DL-methionine (Table 1). The dietary protein level was fixed at 40% crude protein (Table 2), reported optimum (Faturoti*et al*., 1986) for the growth of *Clariasgariepinus*and mainly based on soyabean, yellow maize, vitamin/mineral premix, soya oil and salt. The ingredients were thoroughly mixed together and each diet mixture was pelleted at 60 ^{o}C, using 2mm pellet die to form noodle-like strands, which were manually crumbled into a suitable size for the *Clariasgariepinus* juveniles. The pellets were sundried, packed into transparent bags and stored in a cool dry place to prevent fungal growth. The six dietary treatments (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

Fish feeding trial

Comment [A11]: Use number 2.1

The experiment was carried out using eighteen plastic tanks of dimension 60 cm × 45 cm × 30 cm for 12 weeks (February-April, 2017) at the Research Laboratory. Each tank was supplied with well water up to 80% capacity which was replaced every three days to maintain relatively uniform physico-chemical parameters and prevent fouling from feed residues. There were six dietary treatments and each had three replicates with 20 fish per replicate. The fish were weighed and randomly allocated to experimental tanks. They were thereafter allowed to acclimatize for 14 days with commercial diets. The experiment lasted for 84 days during which the fish were fed to satiation. Weight changes were recorded weekly and feeding rate properly adjusted to the new body weight.

Comment [A12]: 84 days

TABLE 1: Gross composition of roasted soyabean based diets fed to *Clariasgariepinus*.

Ingredient (g/100g)	Control	RS2	RS3	RS4	RS5	RS6
Soyabean meal	81.6	81.6	81.6	81.6	81.6	81.6

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Yellow maize	14.4	14.4	14.4	14.4	14.4	14.4
*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

Comment [A13]: Was this tested before the study was conducted? If yes, then there is no problem. However, if it is from other people's research, it is best to include references

		Diet (RS)				
Ingredient (%)	Control	2	3	4	5	6
Crude protein	40.90±0.28	39.98±0.46	40.96±1.27	40.88±1.24	40.681.10	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

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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):

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

Mean Corpuscular Haemoglobin (MCH)

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

$$\text{MCH} = \frac{Haemoglobin\ (\frac{g}{100ml})}{Numberofredbloodcells\ (\frac{millions}{L}blood)} \times 100$$

Mean Corpuscular Haemoglobin Concentration (MCHC)

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

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

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 ^{o}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

Comment [A14]: Use number 3
Results and discussion are combined

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

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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

$y = -0.5357x^2 + 3.8271x + 29.48$………………. $R^2 = 0.8052$…………………… 1

$y = -0.3839x^2 + 3.1361x + 29.43$………………. $R^2 = 0.6959$…………………… 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

		Diet (RS)				
Parameter	Control	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

Comment [A15]: This is the optimal condition, this should be explained in the discussion

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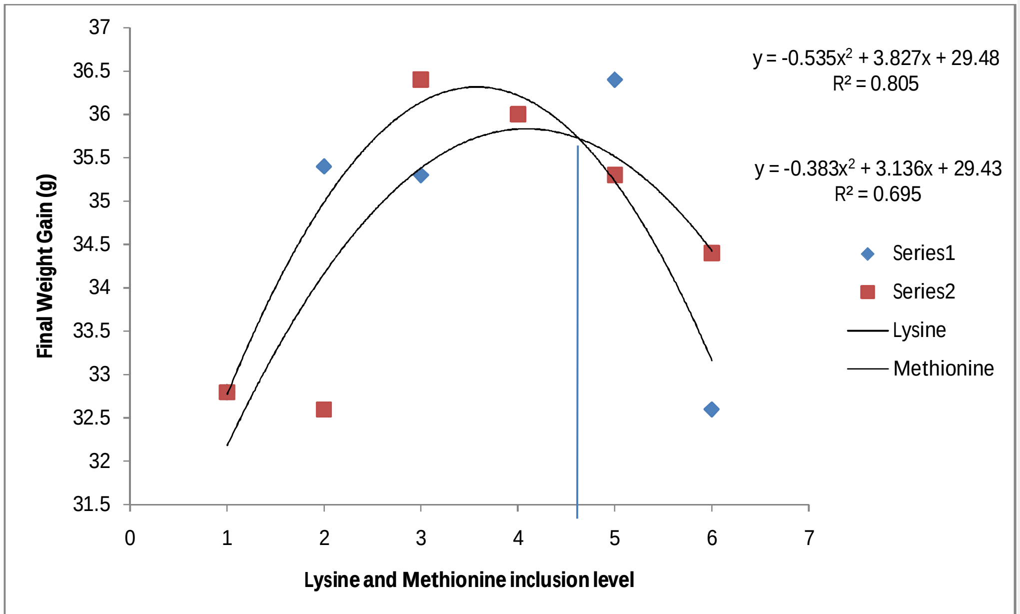

Figure 1: **Relationship between dietary supplemental lysine and methionine of a roasted soyabean based diet and final weight gain of *Clariasgariepinus***

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

Comment [A16]: What should series 1 and 2 in the picture show? Shouldn't dots of the same color be connected to each other? need to check again

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 (x10^{12}/L) value ranged from 1.43±0.05 (control) to 2.75±0.92 (RS5). However, white blood cell (x10^{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 (x10^{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

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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.

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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 (µmol/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 (µmol/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

		Diet (RS)				
Parameter	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(µmol/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 (µmol/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

Comment [A17]: combine it with the research results, then organize and arrange them according to the research results and objectives

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 (Aderolu*et al.*, 2018; Elesho*et 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, *Clariasgariepinus* were provided with diets comprising roasted soybean enhanced with different concentrations of lysine and methionine. The results

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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 di*et al*so 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

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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

Comment [A18]: It should be short, concise and clear and answer the research objectives

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 weigh gain at R2 = 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

Comment [A19]: Sort and adapt to the citation order in the manuscript.

Comment [A20]: Adjust to AJFR journal guidelines

Comment [A21]: You should pay attention to the placement and adjust it according to the guide

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