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Effect of Dietary Lysine and DL-Methionine Supplementation in Roasted Soyabean-Based Diet on the Growth Performance and Blood Profiles of *Clarias gariepinus*

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

This study was designed to investigating the growth performance, hematology, and serum biochemistry of *Clarias gariepinus* nourished with roasted soyabean based diets with varied inclusion of dietary amino acid. A total of 360 *Clarias gariepinus* juveniles, aged eight weeks and weighing 19.0±0.4 grams, were randomly assigned to six different diets with varying inclusion levels of lysine and DL-methionine (g/100g). The diets were as follows: RS1 (Control) without lysine or DL-methionine supplementation; RS 2 - 0g + 1g; RS 3 - 0.25g + 0.75g; RS 4 - 0.5g + 0.5g; RS 5 - 0.75g + 0.25g; and RS 6 - 1g + 0g of Lysine and DL-methionine. For 84 days, the fish were fed until they were completely satisfied. Each treatment was in triplicate. Supplementing lysine and methionine in a diet based on roasted soybeans did not significantly (P>0.05) effect on the growth parameter such as final weight, feed conversion ratio, and/or specific growth rate in fish. A

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significantly higher survival rate was observed in fish on diet RS 5 (85.60±0.60) and was closely followed by fish fed diet RS 3 and RS 4. Fish fed diets RS 4 and RS 5 exhibited significantly higher ($P<0.05$) packed cell volume (PCV %) and hemoglobin levels. While, White blood cell counts (x10^9/L) varied significantly among the different diets. The addition of supplemental amino acids did not significantly ($P>0.05$) affect total protein (g/L), and the values ranges from 6.60±0.52 (RS 6) to 7.87±0.55 (RS 4). 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.

1. 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 [1]. 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 [2,3]. 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 [1].

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 [4]. 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 [5,6]. 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 [7]. Additionally, the roasting process enhances the palatability of soybean, making it more acceptable to fish [8,9,2].

The incorporation of roasted soybean with lysine and methionine supplementation into the diets of *Clarias gariepinus* has been extensively studied [8,2,10]. Oyedokun et al. [11] 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 [11]. 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 [12].

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 [13-16]. 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 [16]. 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 [17]. Zhang et al. [18] 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 [19-21]. Elesho et al. [1] noted that diets for African catfish enriched with crystalline methionine led to improved growth when fed plant-protein based diets. The synergistic effects of supplemental amino acid in soyabean-based diets could therefore lead to significant enhancements in growth performance, nutrient digestibility, and overall health status in *Clarias gariepinus*. Such dietary optimization could provide substantial benefits to the aquaculture industry by improving the health and productivity of *Clarias gariepinus*. This study aims to examine the impact of a roasted soybean-based diet with dietary lysine and DL-methionine supplementation on the growth performance and blood profile of *Clarias gariepinus*.

2. MATERIALS AND METHODS

The Aquatech College of Aquaculture, located in Fodacis, Ibadan, Nigeria, hosted the feeding study at its Aquaculture Research Laboratory. An established fish farm in Ibadan provided 360 juvenile *Clarias gariepinus* fish, which were eight weeks old and weighed 19.0±0.4 grams. With varying amounts of lysine and DL-methionine, six isonitrogenous diets were created. (Table 1). Protein content of the diets was maintained at 40% crude protein (Table 2), as this level is considered optimal for the growth of *Clarias gariepinus* according to previous studies [22]. The main ingredients of the diets were soybean, yellow maize, soy oil, salt, and a vitamin/mineral premix. After mixing all the ingredients well, the diet mixes were made into pellets at 60°C with a 2mm pellet die to create strands that resembled noodles. These were then crushed by hand into pieces that were about the right size for the juveniles. In order to prevent fungal contamination, the pellets were sun-dried, sealed into clear bags, and kept in a cool, dry location. The six dietary treatments (g/100g) were as follows: RS1 (Control) = 0g lysine + 0g DL-methionine; RS 2 = 0g lysine+ 1g DL-methionine; RS 3 = 0.25g lysine + 0.75g DL-methionine; RS 4 = 0.5g lysine +0.5g DL-methionine; RS 5 = 0.75g lysine + 0.25g DL-methionine; RS 6= 1g lysine + 0g DL-methionine.

2.1 Fish Feeding Trial

The experiment was carried out using eighteen plastic tanks with 60 cm × 45 cm × 30 cm dimension for 84 days 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 three replicates of each of the six dietary treatments, each containing twenty fish. After being weighed, the fish were arranged in experimental tanks at random. For two weeks, the fish were given commercial foods to acclimate them. 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.

2.2 Determination of Proximate Composition

The proximate composition of the diets was assessed following the methods described in AOAC [23].

2.3 Assessment of Growth

Growth performance and feed utilization were evaluated based on the calculations described by Falayi [24]. Various parameters including weight gain (WG), feed conversion ratio (FCR), feed intake (FI), protein intake (PI), specific growth rate (SGR), protein efficiency ratio (PER), nitrogen retention efficiency (NRE), gross protein retention (GPR), survival rate (SR) were recorded every 2 week during the experimental study.

Table 1. Gross composition of roasted soyabean based diets fed to *Clarias gariepinus*

Ingredient (g/100g)	Control	RS 2	RS 3	RS 4	RS 5	RS 6
Soyabean meal	81.6	81.6	81.6	81.6	81.6	81.6
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

Ingredient (%)	Control	RS 2	RS 3	RS 4	RS 5	RS 6
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
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
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}
Crude fat	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}
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}
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}
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}
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}
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)

2.4 Blood Sampling and Analysis

For each replication group, three randomly chosen *Clarias gariepinus* individuals had their blood samples (5 mL) collected. For a subsequent haematological investigation, the samples were stored in treated heparinized plastic bottles containing ethylene diamine tetra acetic acid (EDTA). Parameters including packed cell volume (PCV), haemoglobin concentration, red blood cell count (RBC), and white blood cell count (WBC) were determined following the procedures outlined by Blaxhall and Daisley [25].

2.4.1 Mean Corpuscular Volume (MCV)

The model presented by Feldman et al. [26] was used to estimate MCV:

MCV = (volume of red blood cell (in mL per 100mL blood)) / (Number of red blood cells oer 100mL blood) ×100

2.4.2 Mean Corpuscular Haemoglobin (MCH)

MCH was estimated using the model described by Stoskopf, [27]

MCH = (Haemoglobin (g/100ml))/(Number of red blood cells (millions/L blood)) ×100

2.4.3 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC was estimated using the model as described by Stoskopf, [27]

MCHC = (Haemoglobin concentration) / (Packed cell volume) ×100

2.5 Serum Biochemical Analysis

Two milliliters of blood were drawn into heparinized flasks devoid of anticoagulant from three randomly selected *C. gariepinus* individuals per replication group in order to perform serum biochemical analysis. A Hawsley minor bench centrifuge (P spectrum, Centromix number 231254 CD7000549, Spain) was used to centrifuge the samples after the blood had been allowed to coagulate for five minutes at 3000 rpm. Upon extraction, the serum was kept cold—-20°C. As stated by Soedjak [28], the Biuret method was used to determine the levels of total protein and albumin. The difference between the two amounts was used to compute the levels of globulin. Soedjak [28] provided the spectrophotometric method for determining the albumin:globulin ratio as well as the serum enzyme activity of alkaline phosphatase, alanine amino transaminase, and aspartate amino transaminase. Blood urea nitrogen and creatinine levels were measured using the techniques outlined by Harrison [29].

2.6 Analytical Statistics

Analysis of variance (ANOVA), polynomial regression, and descriptive statistics were used to analyze the data as described by Statistical Analysis Software [30]. Mean differences were determined using Duncan's multiple range test at a significance level of α = 0.05, utilizing the same software.

3. RESULTS AND DISCUSSION

Table 3 presents the growth performance and feed utilization of *C. gariepinus* fed a roasted soybean-based diet supplemented with varying levels of dietary amino acids. The addition of lysine and methionine to the roasted soybean-based diet did not significantly affect (P>0.05) the final weight, feed conversion ratio, feed intake, specific growth rate, or protein intake of the fish. The gross protein retention in fish fed the RS 3 diet (1.01±0.04) had a significantly (P>0.05) higher value followed by fish those fed RS 5 diet. Nitrogen retention efficiency improved with amino acid supplementation, with the lowest value observed in the control diet (33.78±4.11). The survival rate of fish fed the RS 5 diet (85.60±0.60) was significantly higher than those fed the control, RS 2, and RS 6 diets, while the survival rates of fish on the RS 3 and RS 4 diets were intermediate. Regression equations 1 and 2 illustrate the relationship between the inclusion of lysine and methionine and the final weight gain of *C. gariepinus*.

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

Table 3. Growth performance and feed utilization of *C. gariepinus* fed roasted soybean-based diets supplemented with amino acids

Parameter	Control	RS 2	RS 3	RS 4	RS 5	RS 6
Initial Weight (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
Final Weight (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
Feed Conversion Ratio	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
Protein Intake	11.20±0.78	11.10±0.23	11.47±0.63	11.07±0.86	11.04±1.01	10.25±0.27
Feed Intake	0.75±0.05	0.74±0.02	0.76±0.04	0.74±0.07	0.74±0.06	0.68±0.02
Protein Efficiency Ratio	10.92±0.97	11.80±0.97	11.76±1.66	11.68±1.05	12.12±1.53	10.87±1.20
Specific Growth Rate	0.34±0.04	0.39±0.06	0.39±0.09	0.39±0.06	0.40±0.08	0.34±0.07
Gross Protein Retention	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}
Nitrogen Retention Efficiency	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}
Survival Rate %	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), GEFC= Gross Efficiency Feed Conversion.

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

Parameter	Control	RS 2	RS 3	RS 4	RS 5	RS 6
Packed Cell Volume (%)	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}
Red Blood Cell (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
White Blood Cell (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}
Hemoglobin(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}
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}
Mean Cell Volume (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
Mean Cell Hemoglobin (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
Heterocytes (%)	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}
Lymphocytes (%)	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}
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}
Monocytes (%)	3.00±1.00	2.67±2.08	2.33±1.15	3.67±0.58	3.67±1.15	3.33±1.53
Basophils (%)	0.33±0.58	0.00±0.00	0.33±0.58	0.33±0.58	0.33±0.58	0.33±0.58
Eoinophilss (%)	4.67±1.53	3.67±0.58	4.33±2.52	3.67±1.53	3.67±1.53	2.00±3.46

The means that have the same letter in a row do not differ significantly. (P>0.05), MCHC= Mean Cell Hemoglobin Concentration

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Fig. 1 illustrate that the optimal inclusion levels of lysine and methionine in a soybean-based diet were determined to be 6.5 g/kg and 3.5 g/kg, respectively, according to the equations.

The haematological parameters of *C. gariepinus* fed a diet based on roasted soybeans supplemented with varying levels of dietary amino acids are presented in Table 4. Fish fed diets RS 4 and RS 5 exhibited significantly higher levels of PCV (%) and hemoglobin compared to those fed diets RS 2, RS 3, and RS 6, although these differences were not significant. However, there were significant differences in white blood cell count (x10^9/L) among the diets. No significant difference was observed in red blood cell count (x10^12/L), MCV (fl), MCH (pg), and MCHC (g/dL). Fish fed the control diet had a significantly higher platelet counts than those of the other treatments, despite the fact that the roasted soybean-based diet supplemented with amino acids had a lowered platelet count (x10^9/L). Diets RS 5, RS 6, and the control group exhibited notably elevated lymphocyte (%) values with Lysine and DL-methionine inclusion. Furthermore, the minimum value of 27.00±2.00 in RS 5 was less than the heterocyte (%) count in RS 3, which was greater (P<0.05) at 39.00±4.58. Across the treatments, there were substantial differences in the lymphocyte to heterozyte ratios. The percentages of monocytes, eosinophils, and basophils were unaffected by additional amino acids (P>0.05).

The serum biochemical parameters of fish fed diets based on roasted soybeans supplemented with dietary amino acids are summarized in Table 5. Supplementation of amino acids did not have a significant (P>0.05) effect on total protein (g/L), albumin (g/L), globulin (g/L), Albumin-Globulin ratio, and Aspartate Transaminase (IU/L). However, alanine transaminase (IU/L) and blood urea nitrogen (µmol/L) levels were significantly (P<0.05) higher diets RS 2 and RS RS@4, respectively and their lowest value observed in control diet. Although, they were much higher than those in fish fed the RS 2 diet, alkaline phosphatase (IU/L) did not reveal significant differences (P<0.05) among fish fed diets RS 3, RS 5, and RS 6. Furthermore, creatinine (µmol/L) levels increased considerably with amino acid supplementation; diet RS 2 had the highest value (0.77±0.56), whereas the control diet had the lowest value (0.53±0.06).

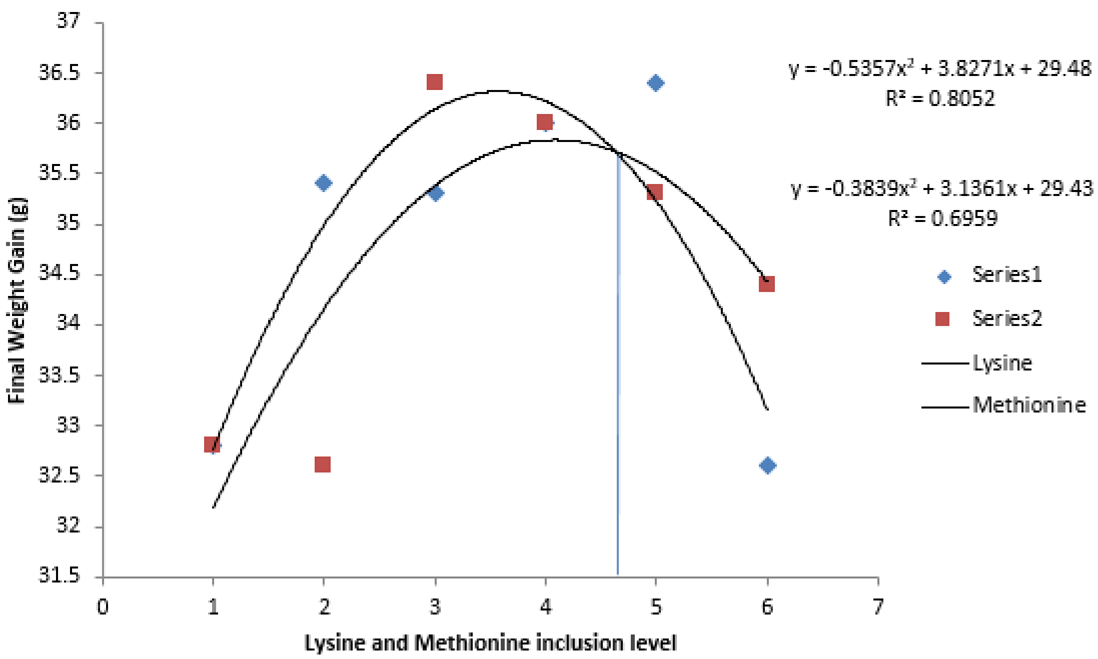

Fig. 1. Relationship between dietary supplemental lysine and methionine of a roasted soyabean based diet and final weight gain of *Clarias gariepinus*

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

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

Parameter	Control	RS 2	RS 3	RS 4	RS 5	RS 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
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
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
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
Aspartate Transaminase (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
Alanine Transaminase (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}
Alkaline Phosphatase (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}
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}
Blood Urea Nitrogen (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}

The means that have the same letter in a row do not differ significantly. (P>0.05), A-G Ratio = Albumin-Globulin Ratio.

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 [31,1]. These nutrient demands are not static and must be provided in correct proportions for optimal growth [32]. 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 intake, Protein intake, Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), and. 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. [16] 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 [33].

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 Fig. 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. [24]. 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 [20,33,34]. Fish fed the RS 5 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. [35].

Further, diets RS 4 and RS 5, 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 [36,32]. Red blood cell (RBC) counts were notably higher in fish on the RS 5 diet, improving oxygen supply to tissues and supporting metabolic functions [37]. White blood cell (WBC) count variability among the diets suggested different immune responses, potentially influenced by amino acid profiles in the diets [38].

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 [39]. 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 [40]. Lymphocyte percentages were higher in diets RS 5, RS 6, and the control, suggesting an enhanced specific immune response, consistent with findings that dietary amino acids can modulate lymphocyte activity [41]. However, another type of white blood cells, heterocytes, exhibited a different trend, with the highest counts in RS 3, possibly reflecting a stress response or shift in immune strategy, depending on the dietary composition [42].

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 [36,43,44]. 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 [45,46]. Additionally, creatinine levels, significantly higher in diet RS 2 compared to the control, could indicate enhanced muscle metabolism or slightly impaired kidney function due to higher metabolic loads associated with amino acid processing [41], warranting further investigation.

4. CONCLUSION

Significant improvements were observed in growth performance, haematology and serum biochemistry of *Clarias gariepinus* fed diets based on roasted soybean supplemented with dietary lysine and DL-methionine. The regression analysis model in this study shows a strong correlation between dietary amino acid and final weigh gain and predicted that addition of dietary lysine and DL-methionine at 6.5g/kg (R^2 = 0.805) and 3.5g/kg (R^2 = 0.696), respectively in roasted soybean based diet could improve growth and utilization of diet. Also, *C. gariepinus* fed soybean-based diet with supplemental lysine and DL-methionine in fish feed could enhance fish health and immune response.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Elesho FE, Kröckel S, Ciavoni E, Sutter DAH, Verreth JAJ, Schrama JW. 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. 2021;281: 115098.
   Available:https://doi.org/10.1016/j.anifeedsci.2021.115098
2. Oyedokun JO. 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; 2019.
   Available:http://repository.pgcollegeui.com:8080/xmlui/handle/123456789/896
3. National Research Council. Nutrient Requirements of Fish and Shrimp. Washington, DC: The National Academies Press; 2011.
4. Olude OO, Sahu NP, Sardar P, Nuzaiba PM. Utilization of valorized cassava leaf meal as an alternative feedstuff to defatted soybean meal in feed for Rohu, *Labeo rohita* fingerlings. Bioresource Technology Reports. 2023;22:101400
5. Pettigrew JE, Soltwedel KT, Miguel JC, Palacios MF. Fact sheet - Soyabean Use - Swine. Soyabean Meal Information Center. 2008.
6. Heuzé V, Tran G, Kaushik S, Soybean meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO; 2020.
   Available:https://www.feedipedia.org/node/674.
7. Oyedokun JO, Oyelese OA, Ogunwole O. Effect of soybean processing on chemical composition and digestibility of soybean-based diet by *Clarias gariepinus* (Burchell, 1822). African Journal of Fisheries and Aquatic Resources Management. 2018;3: 1-10.
8. Fapohunda, Olawumi. Evaluation of processed soybean meal in the feeding of *Clarias gariepinus* fingerlings. Journal of Animal Science Advances. 2012;2:244-249.
9. Agume Aurelie, Njintang Nicolas and Mbofung Carl. Effect of soaking and roasting on the physicochemical and pasting properties of soybean flour. Foods. 2017;6:12.
   DOI: 10.3390/foods6020012
10. Azubuike Adams. Effect of raw and toasted soya beans diets on growth of *Clarias gariepinus*. Global Science Research Journal. 2021;9(1):1-12.
11. Oyedokun JO, Ogunwole OA, Oyelese OA, Adene IC, Sule SO, Folorunso T. 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. 2022;2(1):76-86.
12. Hu Xuecai , Bin Huo, Jiameng Yang, Kun Wang, Lingjie Huang, Lianqiang Che. Effects of dietary lysine levels on growth performance, nutrient digestibility, serum metabolites, and meat quality of baqing pigs. Animal (Basel). 2022;12(15): 1884.
    DOI: 10.3390/ani12151884

13. Lu J, Hua Y, Fu WZ, Zhou F, Yang BB, Xiao JX, Shao QJ. 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. 2014;14(3):633-642.
14. Kokou F, Sarropoulou E, Cotou E, Kentouri M, Alexis M, and Rigos G. 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. 2017;64:111-121. Available:https://doi.org/10.1016/j.fsi.2017.03.017
15. Su YN, Wu P, Feng L. The improved growth performance and enhanced immune function by DL-methionyl-DL-methionine are associated with NF-κB and TOR signalling in intestine of juvenile grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immunol. 2018;74:101–118.
16. Guo Tian-Yu, Zhao Wei, He Ju-Yun, Liao Shi-Yu, Xie Jiajun, Xie Shiwei, *et al*. 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. 2019; 123:1-23.
DOI:10.1017/S0007114519002289.
17. Ebeneezara S, Vijayagopal P, Srivastava PP, Gupta S, Sikendrakumar S, Varghese T. Dietary lysine requirement of juvenile Silver pompano, *Trachinotus blochii* (Lacepede, 1801). Aquaculture. 2019;511: 734234.
Available:https://doi.org/10.1016/j.aquaculture.2019.734234
18. Zhang Jiatu, Shunju Geng, Yahao Zhu, Lan Li, Lihong Zhao, Qiugang Ma, and Shimeng Huang. Effects of dietary methionine supplementation on the growth performance, immune responses, antioxidant capacity, and subsequent development of layer chicks. Poultry Science. 2024;103(3):103382.
DOI: 10.1016/j.psj.2023.103382
19. Wang Y, Zhou J, Wang G, Cai S, Zeng X, Qiao S. Advances in low-protein diets for swine. Journal of Animal Science and Biotechnology. 2018;9:60.
DOI:10.1186/s40104-018-0276-7
20. Oyedokun JO, Ogunwole OA, Oyelese OA. Growth performance and blood profile of *Clarias gariepinus* fed processed soybean-based diets supplemented with amino acids. International Journal of Scientific and Technology Research. 2019;8(10): 1569-1575.
21. Wang Wennan, Lisha Ye, Xingtang Dou, Haiying Liu and Di Han. Effects of rumen-protected methionine supplementation on growth performance, nutrient digestion, nitrogen utilisation and plasma amino acid profiles of liaoning cashmere goats. Animals*. 2023;*13(19):2995.
Available:https://doi.org/10.3390/ani13192995
22. Faturoti EO., Balogun AM, Ugwu LLC. Feed utilization and growth responses of *Clarias gariepinus* fed different dietary protein levels. Nigerian Journal of Applied Fish and Hydrobiology. 1986;1:41-45.
23. AOAC. Official Methods of Analysis of the Association of Analytical Chemists International, 18th ed. Gathersburg, MD U.S.A Official methods, 2005;08.
24. Falayi BA. 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; 2009. ISBN 978-978-48686-9-3.
25. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. Journal of Fish Biology. 1973;5: 771-781.
26. Feldman BF, Zinkl JG, Jain NC. Schalm's veterinary hematology. 5.ed. Lippincott Williams and Wilkins. 2000;787.
27. Stoskopf MK. Fish Medicine. W. B. Saunders Company, Harcourt Braces Jovanovich Inc. Philadephis, London. 1992;125.
28. Soedjak HS. Colorimetric micromethod for protein determination with erythrosin-B. Anal. Biochem. 1994;220: 142-148.

29. Harrison P. 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. 2006;186-207.
30. SAS. Statistical Analysis Software (SAS) systems for Windows, SAS Inc., Cary. NC, USA. 2005.
31. Aderolu Ademola Zaid, Lawal Muyideen Owonire, Awobajo, Funmileyi Olubajo, Olaniyan, Stephen and Bello, Yetunde. 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. 2018;15(3):3090 – 3100.
32. Lu J, Hua Y, Fu WZ, Zhou F, Yang BB, Xiao JX, Shao QJ. Effects of supplementation coated lysine and 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. 2014;14(3):633-642.
33. Garg CK, Sardar P, Sahu NP, Maiti MK, Shamna N, Varghese T, Deo AD, Harikrishna V. Dietary lysine requirement of genetically improved farmed tilapia (GIFT) juvenile reared in inland saline water of 10 ppt salinity. Aquaculture. 2022; 555:738223
34. Khalil Hala, Momoh Taofik, Al-Kenawy Diaa, Yossa Rodrigue, Badreldin Alaa, Roem Arjen, Schrama Johan, Verdegem Marc. Nitrogen retention, nutrient digestibility and growth efficiency of Nile tilapia (*Oreochromis niloticus*) fed dietary lysine and reared in fertilized ponds. Aquaculture Nutrition. 2021;1-13. DOI:10.1111/anu.13365.
35. Oliva-Teles A, Enes P, Peres H. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. Feed Science and Technology. 2015;239: 34-48.

    DOI:10.1016/j.anifeedsci.2014.11.008
36. Fazio F. Protein metabolism in marine species: Using blood parameters as health indicators in fish. Marine Biology Research. 2019;15(5):380-392.

    DOI: 10.1080/17451000.2019.1621054
37. Wessels I, Maywald M, Rink L. Zinc as a gatekeeper of immune function. *Nutrients.* 2017;9:1286.

    DOI: 10.3390/nu9121286
38. Sardar P, Abid M, Randhawa H, Prabhakar S. Effect of dietary lysine and methionine supplementation on growth, feed utilization, carcass compositions and haemato-biochemical status in Indian Major Carp, Rohu (*Labeo rohita H*.) fed soy protein-based diet. Aquaculture Nutrition. 2009;15:339–346.
39. Kuhn Viktoria, Lukas Diederich, T.C. Stevenson Keller, Christian M. Kramer, Wiebke Lückstädt, Christina Panknin. Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. 2017; 26(13):718–742. DOI: 10.1089/ars.2016.6954
40. Moha Esmaeili. Blood Performance: A new formula for fish growth and health, biology *(Basel)*. 2021;10(12):1236.

    DOI: 10.3390/biology10121236
41. Wu G. Dietary protein intake and human health. Food and Function. 2018;9(3): 1236-1259.

    DOI:10.1039/C7FO01782A
42. Abo-Al-Ela HG, Seham El-Kassas, Karima El-Naggar, Safaa E. Abdo, Ali Raza Jahejo, and Rasha A. Al Wakeel. Stress and immunity in poultry: light management and nanotechnology as effective immune enhancers to fight stress, Cell Stress Chaperones. 2021;26(3):457–472.

    DOI: 10.1007/s12192-021-01204-6
43. Teles A, Boltaña S, Reyes-López F, Santos MA., MacKenzie S, Tort L. Role of dietary amino acids in immune response and stress resilience in European sea bass. Aquaculture Reports. 2021;20: 100600.

    DOI:10.1016/j.aqrep.2021.100600
44. Martins CF, Jesus G., Gallani SU, Roubach R. Soyabean meal as a protein source in aquafeeds: Effects on growth and health of fish. Journal of Animal Science and Technology. 2018;60(1):12. DOI: 10.1186/s40781-018-0183-0
45. Cengiz EI, Unlu E, Satar A. The role of dietary enzymes in enhancing gut health in fish aquaculture. Aquaculture Nutrition. 2019;25(2):456-464.

DOI: 10.1111/anu.12869

46. Yun Biao, Kangsen Mai, Wenbing Zhang, Wei Xu. Effects of dietary cholesterol on growth performance, feed intake and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets. Aquaculture. 2011;319(1–2): 105-110.

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