



# Growth performance and proximate composition of *Clarias gariepinus* (Burchell, 1822) fed roasted soyabean-based diets with supplemental amino acids

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## Abstract

The effects of supplementing roasted soyabean-based diets with the first two limiting amino acids (L-lysine and DL-methionine) on growth performance and proximate composition of *Clarias gariepinus* were investigated in this study. Juvenile fish (n=360), weighing 19.60±0.10g were randomly allocated to six diets containing varied supplemental combinations of lysine + DL-methionine (g/100g) in the formulated basal roasted soyabean-based diets (RSBD): R<sub>1</sub> (without supplemental lysine and DL-methionine), R<sub>2</sub> (0+1g), R<sub>3</sub> (0.25g+0.75g), R<sub>4</sub> (0.5g+0.5g), R<sub>5</sub> (0.75g+0.25g), R<sub>6</sub> (1g+0) of supplemental L-lysine + DL-methionine, respectively. The fish were fed to satiation with the diets for 12 weeks. Each treatment was in triplicate. Results revealed that the diets had no significant influence (P>0.05) on fish final weight and feed conversion ratio. Gross protein retention of fish on R<sub>3</sub> (1.01±0.04) and R<sub>5</sub> (0.97±0.04) were similar. The crude protein composition of whole-body *C. gariepinus* on R<sub>5</sub> (70.03±1.60) and R<sub>3</sub> (68.39±1.46) were not significantly different (p>0.05) but significantly higher than those on other diets. Supplemental amino acids significantly increased the methionine composition (P<0.05) with the highest value of 2.42±0.04 in fish on R<sub>4</sub> and least value of 2.12±0.02 in those on R<sub>2</sub>. Regression of crude protein of fish whole body and supplemental lysine and methionine were both quadratic with the optimum dietary inclusion levels of 0.6g/100g (R<sup>2</sup>= 0.79) and 0.4g/100g (R<sup>2</sup>=0.76), respectively. In conclusion, dietary supplement of lysine and methionine in roasted soyabean-based diets had no impact on growth performance but enhanced the body composition of *C. gariepinus*.

## INTRODUCTION

Soyabean cake is the most widely used plant protein source in animal feeds

globally, especially in Nigeria (Oyedokun *et al.*, 2019). Soyabean demands is high due to its usage in industries (Silva-Carrillo *et*

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*al.*, 2012). Soyabean cake has been extensively used to substitute fishmeal in fish diets due to their agreeable flavour which makes it highly palatable (Silva-Carrillo *et al.*, 2012). Presence of anti-nutritional factors makes soyabean not easily digested and utilized by fish when compared with animal protein sources (Ogbonna *et al.*, 2014). Improvement of its digestibility, therefore, requires various processing methods like solvent extraction, roasting and micronization which have been shown to inactivate the anti-nutritional factors (Oyedokun *et al.*, 2018). However, the improvement in protein and energy digestibility for the optimum performance of fish species remained relatively low compared to fish meal (Al-Kenawy *et al.*, 2008). Despite these limitations, research priority in aquaculture nutrition has been geared towards possible replacement of fish meal with plant or grain by-products in developing low cost fish feeds (Al-Kenawy *et al.*, 2008). Although, total fishmeal replacement with various plant proteins in fish diet has been achieved (Al-Kenawy *et al.*, 2008; Ajani *et al.*, 2016; Cai *et al.*, 2017), information on the effect of total replacement of fish meal with soyabean meal in *C. gariepinus* diets still

remained very scanty.

Soyabean cake, despite being the best plant protein source due to favourable amino acid profile is, however, deficient in methionine and have low cysteine (Ogbonna *et al.*, 2014). This deficiency limited its inclusion in fish diet to 45%, despite its ready availability. The limiting amino acids in the soyabean-based diets could be improved when supplemented with dietary lysine and methionine to enhance the growth performances of cultured fish species (Ochang *et al.*, 2007). Therefore, supplementation of lysine and methionine in RSBM on the growth performance and proximate composition of *C. gariepinus* was investigated.

## MATERIALS AND METHODS

### Experimental site and diets

The feeding trial was conducted at the Research Laboratory, Aquatech College of Aquaculture, Fodacis, Ibadan, Nigeria. Three hundred and sixty *C. gariepinus* juveniles aged eight weeks, weighing  $19.60 \pm 0.10$ g were purchased from a reputable fish farm in Ibadan, Nigeria. Six isonitrogenous diets were formulated with varied supplemental levels of L-lysine and

**Table 1. Gross composition (g/100gDM) of experimental roasted soyabean meal-based diets fed to fish**

Ingredient	R <sub>1</sub> (Control)	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>
Soyabean meal	81.60	81.60	81.60	81.60	81.60	81.60
Yellow maize	14.40	14.40	14.40	14.40	14.40	14.40
*Vit/min premix	0.5	0.5	0.5	0.5	0.5	0.5
Fish oil	1	1	1	1	1	1
CaCO <sub>3</sub>	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
<b>Total</b>	<b>99</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

\*vitamin/mineral premix

R<sub>1</sub> (without lysine and DL-methionine), R<sub>2</sub> (0+1), R<sub>3</sub> (0.25+0.75), R<sub>4</sub> (0.5+0.5), R<sub>5</sub> (0.75+0.25), R<sub>6</sub> (1+0) supplemental L-lysine+DL-methionine

**Table 2. Chemical composition of the experimental diets**

Chemical composition	R <sub>1</sub> (Control)	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	Roasted soyabean
Crude protein (%)	40.90	39.98	40.96	40.88	40.68	41.53	46.01
Ash (%)	5.68	5.30	6.45	5.00	5.90	6.15	5.10
Ether extract (%)	6.60	6.80	6.50	6.95	6.60	7.15	19.20
Crude fiber (%)	4.05	3.60	4.75	4.15	4.75	4.90	5.5
Gross energy (kcal/g)	4.122	4.019	4.027	4.107	4.009	4.129	4.127

R<sub>1</sub> (without lysine and DL-methionine), R<sub>2</sub> (0+1), R<sub>3</sub> (0.25+0.75), R<sub>4</sub> (0.5+0.5), R<sub>5</sub> (0.75+0.25), R<sub>6</sub> (1+0) supplemental L-lysine+DL-methionine

DL-methionine (Table 1). The diet was fixed at 40% crude protein level (Table 2), for the optimum growth of the experimental fish (Faturoti et al., 1986). *The ingredients were thoroughly mixed together and each diet mixture was pelleted at 60 °C, using 2mm pellet die. The pellet was sundried (two days), then crumbled manually into sizes suitable for the experimental fish and stored in transparent bags placed in a cool place to inhibit the fungal growth. Six dietary treatments of supplemental lysine+DL-methionine (g/100g) in basal RSBD were formulated as follows: R<sub>1</sub> (without lysine and DL-methionine), R<sub>2</sub> (0+1g), R<sub>3</sub> (0.25g+0.75g), R<sub>4</sub> (0.5g+0.5g), R<sub>5</sub> (0.75g+0.25g), R<sub>6</sub> (1g+0) supplemental L-lysine+DL-methionine, respectively.*

### Fish feeding trial

The experiment was conducted using 18 plastic tanks each measuring 60 cm × 45 cm × 30 cm for 12 weeks. Every three days, the tanks were provided with well water to about 80% capacity. The water was changed to prevent fouling from feed residues and to maintain relatively uniform physico-chemical parameters. The dietary treatments were six and each was in triplicate comprising 20 fish each. The fish were weighed and randomly allocated to experimental tanks. They were thereafter acclimatized for 14 days with experimental diets. Weight changes were documented biweekly and the fish were fed to satiation for 84 days.

### Growth studies

Calculations of the growth performance and nutrient utilization was according to Falayi, 2009.

$$\text{Weight gained (WG)} = \text{Final Weight} - \text{Initial weight}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed Consumed in g}}{\text{Weight gained in g}}$$

$$\text{Gross Efficiency of Feed Conversion (GEFC)} = \frac{1}{\text{FCR}} \times 100$$

$$\text{Protein Intake (PI)} = \frac{\text{Total feed consumed} \times \text{percentage protein}}{100}$$

Feed Intake (FI): This was obtained by summing up the amount of feed taken per week for each of the treatments for the 12 weeks duration of the experimental period.

$$\text{Specific Growth Rate (SGR)} (\%) = \frac{\text{Log}_e W_2 - \text{Log}_e W_1 \times 100}{T_2 - T_1}$$

Where W<sub>1</sub> = Initial weight of fish (gm), W<sub>2</sub> = Final weight of fish (gm), T<sub>2</sub> = Final Time, T<sub>1</sub> = initial Time

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Net Final weight}}{\text{Protein intake}}$$

Gross Protein Retention (GPR) =  

$$\frac{\text{Final crude protein of fish} - \text{Initial protein of fish}}{\text{Dry protein fed}}$$

Nitrogen Retention Efficiency (NRE) =  

$$\frac{(\text{FMW} \times \text{FBN}) - (\text{IMW} \times \text{IBN})}{\text{Nitrogen consumed}}$$

Where; FMW = Final mean weight; FBN = Final body nitrogen; IMW = Initial mean weight; IBN = Initial body nitrogen

Survival rate (SR%) was calculated as follows:

$$\frac{\text{Final number of fish} \times 100}{\text{Initial number of fish}}$$

### Chemical composition

Feed ingredients, diets and the whole body of fish were analysed chemically (AOAC, 2005). All the determinations were in triplicates.

### Amino acids analysis

Samples of oven dried whole body of fish was treated with performic acid at 0°C to oxidise methionine and cystine to methionine sulphone and cysteic acid prior to the hydrolysis (Moore and Stein, 1948). The samples were prepared by 6 N HCL hydrolysis for 24 h at 110 °C. After which the samples were vapourised in sodium citrate buffer (0.2 mol.<sup>1</sup> Na<sup>+</sup>, pH 2.2) and the mixture was equalized to a 50 mL volume. The amino acids in the hydrolysate were determined by an AA analyser (Biochrom 30. 30 plus, Biochrom Ltd, Cambridge, UK).

### Statistical analysis

The design of the experiment was a completely randomized design. Data were subjected to one-way ANOVA. Duncan Multiple Range Test was used to compare differences among individual means and polynomial regression. All statistics were

performed using SPSS 20.0 (SPSS, Chicago, IL, USA).

### RESULTS

Growth performance and feed utilization by *C. gariepinus* fed roasted soyabean-based diets supplemented with amino acids are as shown in Table 3. Supplemental lysine and methionine in roasted soyabean-based diets fed to fish had no significant influence ( $p > 0.05$ ) on weight gain, feed conversion ratio, feed intake, specific growth rate and protein intake. However, gross protein retention of fish on R<sub>3</sub> (1.01±0.04) was similar ( $p > 0.05$ ) to those on R<sub>5</sub> but were superior to those on other diets. Nitrogen retention efficiency increased with amino acid supplementation and least values was in fish on control diet (33.78±4.11). The survival rate of fish on R<sub>5</sub> (85.60±0.60%) was significantly higher ( $p < 0.05$ ) than those on control, R<sub>2</sub> and R<sub>6</sub>. However, fish fed R<sub>3</sub> and R<sub>4</sub> were intermediate to control, R<sub>2</sub> and R<sub>6</sub>.

The crude protein of fish fed R<sub>5</sub> (70.03±1.60%) and R<sub>3</sub> (68.39±1.46%) were similar ( $p < 0.05$ ) though significantly higher ( $P < 0.05$ ) than other diets (Table 4). Ash content significantly differ ( $P < 0.05$ ) among diets and the values ranged from 3.53±0.22% in R<sub>6</sub> to 5.65±0.21 in R<sub>5</sub>. Dietary supplementation of amino acids significantly ( $P < 0.05$ ) increased the ether extract values and least value in initial fish. However, the dry matter of the experimental fish varied significantly among diets with the higher value in fish on R<sub>2</sub> (28.31±1.17%) and least in initial fish (22.52±0.96%) dry matter composition. Regression of the crude protein of fish and supplemental lysine and methionine were both quadratic with the optimum dietary inclusion levels 0.6g/100g and 0.4g/100g, respectively are as presented in Figure 1.

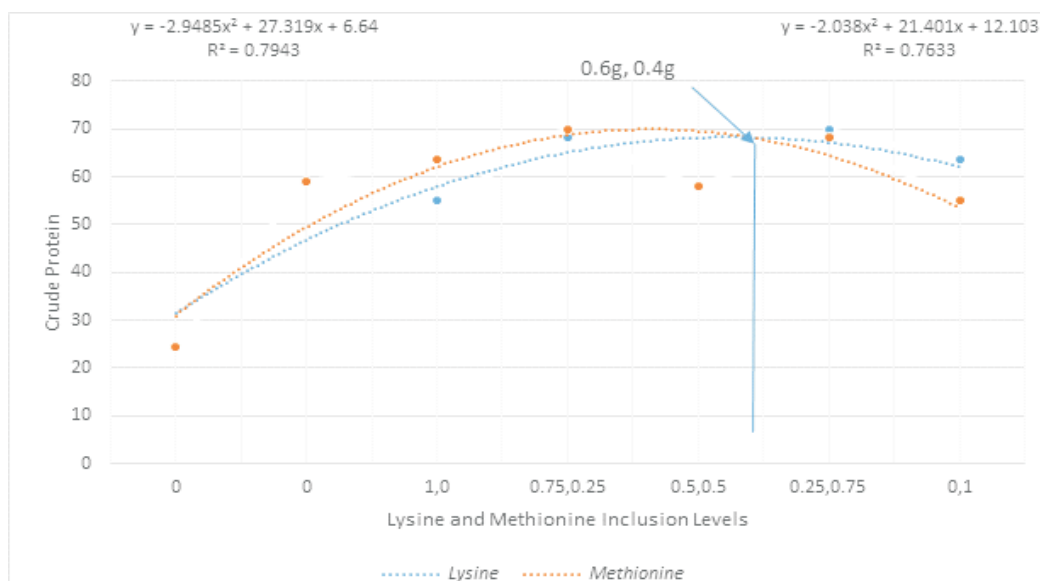
**Table 3. Growth performance and feed utilisation by *C. gariepinus* fed roasted soyabean-based diets supplemented with amino acids**

Parameter	Control	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>
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
WG (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 <sup>b</sup>	0.64±0.01 <sup>a</sup>	1.01±0.04 <sup>d</sup>	0.71±0.03 <sup>b</sup>	0.97±0.04 <sup>d</sup>	0.86±0.03 <sup>c</sup>
NRE	33.78±4.11 <sup>a</sup>	34.12±3.71 <sup>a</sup>	47.41±7.82 <sup>b</sup>	36.27±4.57 <sup>ab</sup>	47.12±8.86 <sup>b</sup>	37.52±5.23 <sup>ab</sup>
SR %	70.00±2.00 <sup>a</sup>	71.00±1.00 <sup>a</sup>	82.27±0.31 <sup>b</sup>	82.20±0.10 <sup>b</sup>	85.60±0.60 <sup>c</sup>	69.40±0.40 <sup>a</sup>

abc: Means with same letter in row are not significantly different (P>0.05); R1 (without lysine and DL-methionine), R2 (0+1), R3 (0.25+0.75), R4 (0.5+0.5), R5 (0.75+0.25), R6 (1+0) supplemental L-lysine+DL-methionine; IW = Initial Weight, WG= Weight Gained, 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

$y = -2.038x^2 + 21.401x + 12.103 \dots R^2 = 0.7633 \dots 1$  (lysine)  
 $y = -2.9485x^2 + 27.319x + 6.64 \dots R^2 = 0.7943 \dots 2$  (Methionine)  
 Methionine contents were significantly (P<0.05) influenced in fish fed diets with amino acid supplementation with fish on R<sub>4</sub> recording the highest value (2.42±0.04) and least value of 2.12±0.02 recorded in fish fed

R<sub>2</sub> (Table 5). Fish fed R<sub>5</sub> had higher (P<0.05) lysine value of 10.69±0.01 while the least value of 6.94±0.04 was recorded in fish fed the control diet. Also, a higher (P<0.05) threonine value of 3.02±0.02 was recorded in fish fed the control diet and fish



**Figure 1:** Relationship between dietary supplement of lysine and methionine in roasted soyabean-based diets and *Clarias gariepinus* crude protein.



**Table 4. Proximate composition of *C. gariepinus* fed roasted soyabean-based diets supplemented with amino acids**

Parameter (%)	Initial	Control	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>
Crude protein	24.50±0.00 <sup>a</sup>	59.01±1.22 <sup>c</sup>	55.01±0.23 <sup>b</sup>	68.39±1.46 <sup>e</sup>	57.95±1.39 <sup>c</sup>	70.03±1.60 <sup>e</sup>	63.75±1.11 <sup>d</sup>
Ash content	3.80±0.18 <sup>ab</sup>	3.93±0.13 <sup>bc</sup>	3.73±0.10 <sup>ab</sup>	5.68±0.31 <sup>d</sup>	4.16±0.19 <sup>c</sup>	5.65±0.21 <sup>d</sup>	3.53±0.22 <sup>a</sup>
Ether extract	7.15±0.21 <sup>a</sup>	8.10±0.24 <sup>bc</sup>	8.33±0.17 <sup>c</sup>	8.35±0.21 <sup>c</sup>	8.88±0.25 <sup>d</sup>	7.90±0.36 <sup>b</sup>	8.03±0.25 <sup>bc</sup>
Crude fibre	0.04±0.01 <sup>b</sup>	0.03±0.01 <sup>ab</sup>	0.03±0.01 <sup>a</sup>	0.03±0.01 <sup>ab</sup>	0.04±0.01 <sup>ab</sup>	0.03±0.01 <sup>ab</sup>	0.03±0.01 <sup>ab</sup>
Dry matter	22.52±0.96 <sup>a</sup>	25.56±0.50 <sup>b</sup>	28.31±1.17 <sup>c</sup>	27.49±0.41 <sup>de</sup>	26.14±0.71 <sup>bc</sup>	27.18±0.24 <sup>cd</sup>	26.92±0.31 <sup>cd</sup>

abcde: Means with same letter in row are not significantly different (P>0.05); R1 (without lysine and DL-methionine), R2 (0+1), R3 (0.25+0.75), R4 (0.5+0.5), R5 (0.75+0.25), R6 (1+0) supplemental L-lysine+DL-methionine.

fed diets R<sub>3</sub> and R<sub>4</sub> had similar threonine values. *Clarias gariepinus* fed diet R<sub>3</sub> had higher (P<0.05) tryptophan value of 2.60±0.10 than other treatments.

Fish fed diet R<sub>2</sub> had higher (P<0.05) isoleucine and leucine values of 8.93±0.03

arginine with the least values in fish fed diet R<sub>6</sub> (3.32±0.02, 3.20±0.20, 3.12±0.10, 1.30±0.10, 2.73±0.03) and the higher values of 8.68±0.02, 9.04±0.04, 8.63±0.03, 3.65±0.05 7.29±0.01 was recorded in fish fed the control diet, respectively.

**Table 5. Amino acid composition of whole body of *C. gariepinus* fed roasted soyabean-based diets supplemented with amino acids**

Parameter	Control	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>
<b>Essential Amino Acids</b>						
Methionine	2.30±0.10 <sup>cd</sup>	2.12±0.02 <sup>a</sup>	2.24±0.04 <sup>bc</sup>	2.42±0.02 <sup>e</sup>	2.18±0.02 <sup>ab</sup>	2.35±0.05 <sup>de</sup>
Lysine	6.94±0.04 <sup>a</sup>	7.91±0.01 <sup>b</sup>	9.28±0.02 <sup>d</sup>	9.89±0.01 <sup>e</sup>	10.69±0.01 <sup>f</sup>	8.52±0.02 <sup>c</sup>
Threonine	3.02±0.02 <sup>e</sup>	2.57±0.03 <sup>d</sup>	2.23±0.03 <sup>b</sup>	2.18±0.02 <sup>b</sup>	2.40±0.10 <sup>c</sup>	2.05±0.05 <sup>a</sup>
Tryptophan	2.41±0.10 <sup>c</sup>	1.91±0.01 <sup>a</sup>	2.60±0.10 <sup>d</sup>	2.44±0.04 <sup>c</sup>	2.18±0.02 <sup>b</sup>	3.01±0.01 <sup>e</sup>
Isoleucine	1.22±0.02 <sup>a</sup>	8.93±0.03 <sup>f</sup>	7.42±0.02 <sup>d</sup>	7.06±0.03 <sup>c</sup>	7.92±0.02 <sup>e</sup>	3.64±0.02 <sup>b</sup>
Leucine	1.19±0.01 <sup>a</sup>	8.84±0.02 <sup>e</sup>	7.34±0.02 <sup>cd</sup>	6.98±0.02 <sup>c</sup>	7.57±0.50 <sup>d</sup>	3.76±0.02 <sup>b</sup>
Valine	2.38±0.02 <sup>b</sup>	2.26±0.02 <sup>a</sup>	2.30±0.10 <sup>ab</sup>	2.94±0.10 <sup>d</sup>	2.25±0.05 <sup>a</sup>	2.51±0.01 <sup>c</sup>
Histidine	8.13±0.03 <sup>f</sup>	7.47±0.03 <sup>e</sup>	6.00±0.20 <sup>c</sup>	5.71±0.01 <sup>b</sup>	6.69±0.01 <sup>d</sup>	3.20±0.20 <sup>a</sup>
Phenylalanine	2.25±0.05 <sup>e</sup>	1.87±0.02 <sup>a</sup>	2.17±0.03 <sup>d</sup>	2.07±0.01 <sup>c</sup>	1.97±0.03 <sup>b</sup>	2.73±0.03 <sup>e</sup>
Arginine	7.29±0.01 <sup>f</sup>	6.62±0.02 <sup>e</sup>	5.42±0.02 <sup>c</sup>	5.07±0.07 <sup>b</sup>	5.85±0.05 <sup>d</sup>	2.73±0.03 <sup>a</sup>
<b>Non-Essential Amino Acids</b>						
Glycine	8.68±0.02 <sup>f</sup>	7.78±0.02 <sup>e</sup>	6.34±0.04 <sup>c</sup>	6.08±0.02 <sup>b</sup>	6.98±0.02 <sup>d</sup>	3.32±0.02 <sup>a</sup>
Serine	2.73±0.03 <sup>d</sup>	2.11±0.01 <sup>a</sup>	2.38±0.01 <sup>b</sup>	2.52±0.02 <sup>c</sup>	2.40±0.10 <sup>b</sup>	2.49±0.01 <sup>c</sup>
Proline	2.32±0.02 <sup>a</sup>	2.31±0.01 <sup>a</sup>	2.29±0.01 <sup>a</sup>	2.96±0.04 <sup>c</sup>	3.01±0.01 <sup>d</sup>	2.48±0.02 <sup>b</sup>
Alanine	9.04±0.04 <sup>f</sup>	7.85±0.05 <sup>e</sup>	6.45±0.05 <sup>c</sup>	6.25±0.05 <sup>b</sup>	6.91±0.01 <sup>d</sup>	3.20±0.20 <sup>a</sup>
Aspartic	6.09±0.03 <sup>e</sup>	4.83±0.03 <sup>d</sup>	1.85±0.05 <sup>b</sup>	1.38±0.02 <sup>a</sup>	3.26±0.01 <sup>c</sup>	6.28±0.02 <sup>f</sup>
Glutamic	8.63±0.03 <sup>f</sup>	7.61±0.01 <sup>e</sup>	6.31±0.01 <sup>c</sup>	5.96±0.04 <sup>b</sup>	6.76±0.06 <sup>d</sup>	3.12±0.02 <sup>a</sup>
Cysteine	3.65±0.05 <sup>e</sup>	3.18±0.02 <sup>d</sup>	2.67±0.02 <sup>b</sup>	2.56±0.06 <sup>b</sup>	2.80±0.10 <sup>c</sup>	1.30±0.10 <sup>a</sup>
Ornithine	2.64±0.04 <sup>d</sup>	2.30±0.20 <sup>c</sup>	1.99±0.01 <sup>b</sup>	1.82±0.02 <sup>a</sup>	2.09±0.03 <sup>b</sup>	1.97±0.03 <sup>ab</sup>
Pyrrolysine	1.94±0.04 <sup>a</sup>	2.46±0.04 <sup>c</sup>	1.97±0.03 <sup>a</sup>	2.16±0.04 <sup>b</sup>	2.17±0.02 <sup>b</sup>	1.90±0.10 <sup>a</sup>
Tyrosine	4.00±0.10 <sup>e</sup>	3.87±0.02 <sup>d</sup>	3.98±0.02 <sup>e</sup>	2.74±0.04 <sup>a</sup>	3.61±0.01 <sup>c</sup>	3.23±0.03 <sup>b</sup>

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

and 8.84±0.02 while the least values of 1.22±0.02 and 1.19±0.01 was recorded in fish fed diet R<sub>1</sub>, respectively. Dietary supplementation of lysine and methionine significantly (P<0.05) decreases the values of glycine, alanine, glutamic, cysteine and

## DISCUSSION

For optimal growth of fishes, selection of feed ingredients and balancing amino acid composition through appropriate supplementation are important. Some researchers (Khan and Abidi, 2011; Lu et

al., 2014) noted that several amino acids or their metabolites are imperative regulators of main metabolic pathways which are essential for growth responses, feed utilization and whole-body quality. Lee (2015) reported that, balancing the Essential Amino Acid (EAA) composition of fish feed requires the supplementation of limiting amino acids to the diets containing plant protein mix. Likewise, the addition of EAAs with other protein sources in fish diet when fishmeal is substituted or completely replaced with plant protein ingredients helps to meet the EAA requirement of fish (NRC, 2011).

Supplemental dietary amino acid in this study had no significant effect on growth performance and feed utilization of *C. gariepinus* fed roasted soyabean-based diet as shown in Table 3. Conversely, Mokrani et al. (2020) reported a significant improvement in growth performance when fishmeal was totally substituted by plant protein ingredients with supplemental dietary essential amino acids in juvenile blunt snout bream. Other authors observed significant improvement in fish growth such as *Solea senegalensis* (Silva et al., 2009); *Carassius auratus gibeilo* (Ren et al., 2016), and *C. gariepinus* (Oyedokun et al., 2019) when fed diets supplemented with amino acids. Also, Li and Robinson (1998) reported that EAA supplementation in diets did not enhance weight gain of channel catfish (*Ictalurus punctatus*). Also, similar decrease in weight gained were reported in *Psetta maxima* (Regost et al., 1999), *Paralichthys olivaceus* (Kikuchi, 1999), *Dicentrarchus labrax* (Dias et al., 2005) and *Gadhus morhua* L. (Hansen et al., 2007).

Contrary to improvement observed by

authors cited, the growth of *C. gariepinus* were not influenced by lysine and methionine supplementation in roasted soyabean-based diets in this study. However, Lu et al. (2014) noted that growth performance was not influenced when supplied with lysine and methionine individually in fish. Furthermore, Cai et al. (2017) observed that replacement of fish meal with rice protein concentrate with lysine supplementation had no significant impact on growth performance of blunt snout bream (*Megalobrama amblycephala*), which supports the current results. With or without supplementation of amino acid, replacement of fishmeal with plant protein ingredients had no significant effect on the final weight gain of *Oreochromis niloticus* (Abdel-Warith et al., 2019). No significant effect of amino acid supplementation on the growth performance observed in this study could be as a result of several factors such as, the absence of fish meal in the experimental diets which could also serve as attractant, existence of anti-nutritional factors in the plant protein ingredient thereby inhibiting the effective utilization of the encapsulated nutrients, processing method, feed palatability and environmental conditions.

The GPR and NRE values of *C. gariepinus* on diet R<sub>3</sub> were significantly higher than in those on the control diet. This shows that supplementation of lysine and methionine improved the protein deposition in the experimental fish. Supplementation of crystalline amino acids (lysine, methionine and threonine) in pea protein concentrate improved the protein synthesis in rainbow trout (Rolland et al., 2015). Gomes et al. (1993) and Cheng et al. (2003) reported an increase in protein retention with improved nitrogen retention and reduced ammonia

excretion in fish fed co-extruded plant protein (rapeseed and peas). Likewise, reduced ammonia excretion rates were observed in tilapia fed palm kernel meal (Obirikorang *et al.*, 2015). Also, gilthead sea bream fed the combination of wheat gluten, extruded peas, rapeseed meal and corn gluten meal with EAA had better protein deposition than fish meal-based diet (Gómez-Requeni *et al.*, 2005). Nutrient deposition and protein utilization in Nile tilapia were improved with phytase supplementation (Liebert and Portz, 2005). However, supplemental fatty acid and citric acid in the plant-based diets significantly increased nitrogen retention and reduced nitrogen excretion in fish (Sarker *et al.*, 2012). Zheng *et al.* (2014) noted that Japanese flounder fed high plant protein diets had improved protein retention.

*Clarias gariepinus* fed diet R<sub>5</sub> had the least Ether extract value compared to roasted soyabean based diets supplemented with lysine and amino acids (Table 3). This shows that lysine and methionine supplementation in the plant protein-based diet significantly improves protein deposition and could reduce lipid deposition in the whole body of *C. gariepinus*. The increase in protein deposition could be as a result of balanced amino acid profile which assisted to enhance protein synthesis rates in the tissue while amino acid levels above those needed for growth does not improve deposition likely due to increased rate of protein catabolism (Lu *et al.*, 2014). Zhou *et al.* (2010, 2011) reported that supplementation of coated lysine and methionine in juvenile black sea bream diets increased the protein deposition in dorsal muscle than those on fish meal diet. Nwanna *et al.* (2012) also reported that common carp (*Cyprinus*

*carpio L.*) fed DL-Methionine-deficient diet had lower protein content compared to DL-Met supplemented diet. Increased protein deposition could be as a result of balanced amino acid profile which enhanced protein synthesis rates in muscle (Furuya *et al.*, 2004). Increases in protein deposition, muscle gain and reduction in carcass fat in response to required dietary lysine intake were reported for various cultivable fish species (Luo *et al.*, 2006; Farhat and Khan, 2011). However, reduced body fat has been reported in Atlantic salmon, *S. salar* (Ji *et al.*, 1996), Rohu, *Labeo rohita* (Keshavanath and Renuka, 1998), rainbow trout, *O. mykiss* (Rodehutsord *et al.*, 2000), and juvenile grouper, *E. coioides* (Luo *et al.*, 2006) fed dietary L-carnitine or L-lysine supplements.

The regression of the crude protein of *C. gariepinus* whole body (Table 4) representing the optimum levels of lysine and methionine inclusion in roasted soyabean based diet as shown in Figure 1. It showed that the optimum levels of lysine and methionine inclusion in roasted soyabean-based diets were 0.6g/100g and 0.4g/100g. The result was in agreement with the findings of Fagbenro *et al.* (1998, 1999), which confirmed that *C. gariepinus* required more lysine than methionine in fish diet.

## CONCLUSION

The supplementation of dietary lysine and methionine in a roasted soyabean-based diets fed to *C. gariepinus* had no proven enhancement on the growth performance of *C. gariepinus* but it could improve the retention of protein in *C. gariepinus*. Also, the supplementation of dietary lysine and methionine in *C. gariepinus* improves the



deposition protein in the whole-body fish while reducing the ether extract content in the fish. Supplemental lysine at 0.6g/100g and methionine at 0.4g/100g in roasted soyabean-based diets improved whole body crude protein composition in *C. gariepinus*. However, further studies should investigate on how the growth performance of *C. gariepinus* fed plant protein-based diets could be improved and utilized.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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