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Amino Acid Digestibility of Catfish Clarias Gariepinus Fed Soyabean Meal Supplemented with Lysine and DL-Methionine

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ABSTRACT

Background and Objective: Soyabean Meal (SBM) could be a suitable replacement for the expensive fishmeal but for its low digestibility in fish. Appropriate dietary supplements of amino acid can improve digestibility of SBM based diets. Therefore, the effect of supplementing lysine and DL-methionine in Solvent Extracted Soyabean Based Diets (SESBD) on performance and amino acid digestibility of *Clarias gariepinus* (*C. gariepinus*) was assessed. **Materials and Methods:** Juvenile fish (n = 360) with the weight of 17.74±0.29 g were fed experimental diet to satiation for 12 weeks. Six diets containing varied combinations of lysine+DL-methionine (g 100 g^{-1}) in SESBD were formulated, S_1 (without supplemental lysine and DL-methionine), S_2 (0+1 g), S_3 (0.25+0.75 g), S_4 (0.5+0.5 g), S_5 (0.75+0.25 g), S_6 (1+0 g). Each treatment was in triplicate. **Results:** Fish fed S_1 (43.35±4.03 g), S_3 (40.80±1.74 g) and S_5 (36.70±5.11 g) were not significantly different (p>0.05) but significantly higher (p<0.05) than those on S_2 (34.77±1.17). Lysine and DL-methionine supplementation had no significant effect (p>0.05) on feed conversion ratio. Supplemental lysine and methionine resulted in an increase (p<0.05) in both apparent (96.34±0.31) and true (96.51±0.31) protein digestibility in diet S_5. The *C. gariepinus* on S_5 (95.75±0.26) had a significantly higher (p<0.05) apparent lysine digestibility than other treatments. The optimum estimates of supplemental lysine and methionine in SESBD for the weight gain of *C. gariepinus* occurred at 0.6 g 100 g^{-1} (R^2 = 0.66) and 0.4 g 100 g^{-1} (R^2 = 0.42), respectively. **Conclusion:** The findings suggest that lysine and DL-methionine supplementation could improve growth performance and amino acid digestibility in *C. gariepinus* fed SESBD.

KEYWORDS

Supplemental lysine and methionine, limiting amino acid, solvent extracted soyabean, *Clarias gariepinus*

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INTRODUCTION

Evaluating the efficiency of feed ingredients could mostly be achieved through digestibility study. Ribeiro *et al.*[1] noted that the nutritional values to be applied in the formulation of nutritionally complex fish feeds could be determined through the evaluation of the digestibility coefficients of feed ingredients. However, the determination of digestibility coefficients is mostly established on fecal measurements, fish

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conditions and methods applied in digestibility trials[2]. Digestibility of individual plant proteins (such as soyabean meal, groundnut cake and sesame cake) in the compound diets is considered as one of the important factors positively affecting the growth of fish[3]. But there is meagre information on the digestibility of plant-based diet most especially in soyabean meal-based diet in *C. gariepinus*.

Soyabean meal has an advantageous nutrient characteristic which makes it an ideal ingredient in aquaculture feed. This is possible because of its well-balanced amino acid profile and low fiber content[4]. However, soyabean meal is constraint with lysine and methionine in fish diet[5], despite it is readily available. These two limiting amino acids are required to improve feed quality, performance, digestibility and profitability of the aquaculture industry. Mai *et al*.[6] reported that lysine is habitually the most required amino acids when replacing fish meal with plant protein sources in fish feed because it is required for normal growth and muscle turnover. Also, its limitation in fish feed affects performance and carcass quality *C. gariepinus*[7]. In many fish diets, the first limiting amino acid is usually methionine especially those having higher inclusion of plant protein sources[8,9].

Nwanna *et al*.[8] posited that DL-methionine supplementation in *Cyprinus carpio* diets significantly improved protein digestibility, weight gain, feed conversion efficiency and carcass quality. Thu *et al*.[10] reported similar observations when lysine utilization efficiency at marginal lysine intake in *Oncorhynchus mykiss* fry was assessed. Ajani *et al*.[9] affirmed that complete substitution of fish meal with soyabean meal is achievable when methionine is supplemented in *Oreochromis niloticus* diet. Ochang *et al*.[11] reported that meeting the amino acid requirement of fish diet could improve digestibility of soyabean based diet when supplemented with dietary lysine and methionine. Therefore, this study investigated the feasibility of improving the utilization and digestibility of soyabean meal-based diet with lysine and DL-methionine supplementation in *C. gariepinus*.

MATERIALS AND METHODS

Study area: The feeding trial was conducted at Aquaculture Research Laboratory, Aquatech College of Aquaculture, Fodacis, Ibadan, Nigeria. The college is situated in the tropical rain forest zone of Nigeria with latitude 7°36"N and longitude 3°86"E with a mean altitude of 275 m above sea level. Temperature range and the average relative humidity of the location were between 20-37°C and 60%, respectively.

Feed ingredients and diet preparation: The experiment was conducted at the onset of the dry season between late September and early December, 2018. Six isonitrogenous chemical composition tested diets were formulated with varying levels of lysine and methionine in Table 1 and their chemical composition was showed in Table 2. The dietary protein level was fixed at 40% crude protein, as reported optimum for the growth of *C. gariepinus*[12]. The ingredients were thoroughly mixed together and each diet mixture was pelleted at 60°C, using 2 mm pellet die to form noodle-like strands, which were crumbled manually into appropriate size for *C. gariepinus* juveniles. The pellets were sundried, packed into labeled transparent bag and stored in a cool dry place to prevent fungal growth. Six dietary treatments of supplemental lysine+DL-methionine (g 100 g^{-1}) combinations in Solvent Extracted Soyabean Based Diets (SESBD) were as follows S_1 (without supplemental lysine and DL-methionine), S_2 (0+1), S_3 (0.25+0.75), S_4 (0.5+0.5), S_5 (0.75+0.25) and S_6 (1+0).

Fish and experimental procedure: The *C. gariepinus* juveniles (n = 360) pieces of aged 2 months weighing 17.74±0.29 g were purchased from a reliable fish farm in Ibadan, Nigeria. The feeding trial was conducted at research laboratory of Aquatech College of Aquaculture, Ibadan, Nigeria using 18 plastic tanks with the dimension measuring 60×45×30 cm. Up to 80% capacity of well water was supplied to each experimental tank and was replaced every 2 days to prevent fouling from feed residues and maintain relatively uniform physico-chemical parameters. There were six dietary treatments were replicated with 20 fish per replicate. The fish were weighed and randomly distributed into experimental tanks after they

Table 1: Gross composition of solvent extracted processed soyabean based diets

Ingredient (g 100 g^{-1})	Control	S_2	S_3	S_4	S_5	S_6
Soyabean meal	70.00	70.00	70.00	70.00	70.00	70.00
Yellow maize	26.00	26.00	26.00	26.00	26.00	26.00
*Vit/min premix	0.25	0.25	0.25	0.25	0.25	0.25
Soyabean oil	1.00	1.00	1.00	1.00	1.00	1.00
$CaCO_3$	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
Lysine	0.00	0.00	0.25	0.50	0.75	1.00
Methionine	0.00	1.00	0.75	0.50	0.25	0.00
Total (%)	99.00	100.00	100.00	100.00	100.00	100.00

Control: Without supplemental lysine and DL-methionine, S_2: 1 g methionine, S_3: 0.75 g methionine, 0.25 g lysine, S_4: 0.5 g methionine, 0.5 g lysine, S_5: 0.25 g methionine, 0.75 g lysine, S_6: 1 g lysine, *1 kg of premix contains Vitamin A: 22,000I.U, Vitamin D3: 5,000I.U, Vitamin E: 300 mg, Vitamin K3: 10 mg, Vitamin B1: 20 mg, Vitamin B2: 25 mg, Vitamin C: 300 mg, Niacin: 120 mg, Calcium pantothenate: 60 J mg, Vitamin B6: 10 mg, Vitamin B12: 0.05 mg, Folic acid: 5 mg, Biotin: 1 mg, Choline chloride: 500 mg, Inositol: 50 mg, Manganese: 30 mg, Iron: 35 mg, Zinc: 45 mg, Copper: 3 mg, Iodine: 5 mg, Cobalt: 2 mg, Lysine: 85 mg, Selenium: 0.15 mg, Anti-oxidant: 80 mg and Methionine: 100 mg

Table 2: Proximate composition of the experimental diets (DM (%))

Ingredient (%)	Control	S_2	S_3	S_4	S_5	S_6
Dry matter	92.29	92.25	92.09	92.31	92.09	92.11
Crude protein	41.59	40.98	41.08	40.75	40.83	40.78
Ash	9.05	7.95	9.30	8.15	7.20	7.80
Ether extract	6.75	6.35	6.55	6.55	7.05	6.70
Crude fibre	5.85	5.15	6.05	5.25	6.70	5.00
Gross energy (kcal g^{-1})	3.99	4.06	3.98	4.11	4.02	3.98

Control: Without supplemental lysine and DL-methionine, S_2: 1 g methionine, S_3: 0.75 g methionine, 0.25 g lysine, S_4: 0.5 g methionine, 0.5 g lysine, S_5: 0.25 g methionine, 0.75 g lysine and S_6: 1 g lysine

have been acclimatized for 14 days. The experiment lasted for 84 days (12 weeks) and the fish were fed to satiation. After the 9 weeks feeding trial, faecal collection was carried out. This procedure was carried out until sufficient volume of faeces for a replication chemical analysis was obtained. The collection of faeces lasted for 3 weeks. During the faecal collection, the faeces were siphoned twice daily (07:00 and 16:00 hrs). Faeces were oven dried at 55°C and stored at -20°C until chemical analysis.

Proximate composition: Proximate composition of the diets and faecal were determined as described by Oyelese *et al.*[13].

Growth studies: Calculations of the growth performance and feed utilization data were according to Gabriel *et al.*[14]:

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

$$\text{Feed Conversion Ratio (FCR) (g)} = \frac{\text{Feed consumed}}{\text{Final weight}}$$

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

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

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

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

Where:

W_1 = Initial weight of fish (g)
W_2 = Final weight of fish (g)
T_2 = Time
T_1 = Time

$$\text{Gross Protein Retention (GPR)} = \frac{\text{Final crude protein of fish} - \text{Initial protein of fish}}{\text{Dry protein fed}}$$

$$\text{Nitrogen Retention Efficiency (NRE)} = \frac{\text{Mean final weight} \times \text{Final body nitrogen} - \text{Initial mean weight} \times \text{Initial body nitrogen}}{\text{Nitrogen consumed}}$$

Survival rate (SR (%)) was calculate as follows:

$$\text{Survival Rate (SR) (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Amino acid analysis: Samples of the diets and feaces were treated with performic acid at 0°C to oxidize methionine and cystine to methionine sulfone and cysteic acid prior to the hydrolysis[15]. The samples were prepared by 6 N HCL hydrolysis for 24 h at 110°C. After which the samples were vaporized in sodium citrate buffer (0.2 mol L^{-1} Na^+, pH 2.2) and the mixture was equalized to a 50 mL volume. The amino acids in the hydrolysate were determined by an AA Analyzer (Biochrom 30.30 Plus, Biochrom Ltd., Cambridge, UK).

Digestibility indices: Chromic oxide in diets and feaces were analyzed[16]. Samples were digested with concentrated nitric acid and oxidation of chromic oxide with 70% prechloric acid. The 50 mg of the samples was put in a Kjeldahl Flask. The 5 mL of concentrated nitric acid was added to the flask and the mixture was gently boiled for 20 min. The boiled sample was cooled at 3 mL of 70% perchloric acid was added to the flask. The resultant mixture was gently heated for another 10 min until the solution changed from green to orange to ensure complete oxidation. The oxidized solution was then put inside a 100 mL volumetric flask and diluted to 100 mL with distilled water. The absorbance of the solution was determined by means of a spectrophotometer at 350 nm. Percentage chromic oxide content in samples and digestibility coefficient was calculated as follows[1]:

$$\text{Weight of chromic oxide in sample} = \text{Absorbance} - \frac{0.0032}{0.2089}$$

$$\text{Chromic oxide (\%)} = \frac{\text{Weight of chromic oxide}}{\text{Weight of the sample}} \times 100$$

Apparent Nutrient Digestibility Coefficient (ANDC):

$$100 - 100 \times \left[\left(\frac{Cr_2O_3 \text{ in diet (\%)}}{Cr_2O_3 \text{ in feaces (\%)}} \right) \times \left(\frac{\text{Nutrient in feaces}}{\text{Nutrient in diet}} \right) \right]$$

True Nutrient Digestibility Coefficient (TNDC):

$$100\times\left\{\frac{\dfrac{\text{Nutrient in diet (\%)}}{Cr_2O_3\text{ in diet (\%)}}-\left(\dfrac{\text{Nutrient in feaces (\%)}}{Cr_2O_3\text{ in feaces (\%)}}-\dfrac{g\dfrac{\text{MFnutrient}}{100\text{ g}}\text{diet}}{Cr_2O_3\text{ in diet (\%)}}\right)}{\dfrac{\text{Nutrient in diet (\%)}}{Cr_2O_3\text{ in diet (\%)}}}\right\}$$

Apparent Amino Acid Digestibility Coefficient (AAADC):

$$100-100\times\left[\left(\frac{(Cr_2O_3\text{ in diet (\%)}}{Cr_2O_3\text{ in feaces (\%)}}\right)\times\left(\frac{\text{Amino acid in feaces (\%)}}{\text{Amino acid in diet (\%)}}\right)\right]$$

True Amino Acid Digestibility Coefficient (TAADC):

$$100\times\left\{\frac{\dfrac{\text{AA in diet (\%)}}{Cr_2O_3\text{ in diet (\%)}}-\left(\dfrac{\text{AA in feaces (\%)}}{Cr_2O_3\text{ in feaces (\%)}}-\dfrac{g\dfrac{\text{MFAA}}{100\text{ g}}\text{diet}}{Cr_2O_3\text{ in diet (\%)}}\right)}{\dfrac{\text{AA in diet (\%)}}{Cr_2O_3\text{ in diet (\%)}}}\right\}$$

Where:
MFAA = Metabolic feaces amino acid (it was determined by feeding protein free diet)
Cr_2O_3 = Chromic oxide
AA = Amino acid

Statistical analysis: The design of the experiment was a completely randomized design. Data were subjected to One-way ANOVA followed by Duncan's Multiple Range Tests were 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 nutrient utilization of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid shown in Table 3. The weight gain of *C. gariepinus* fed diet Control, S_3 and S_5 were similar ($p>0.05$) and were significantly higher from *C. gariepinus* fed diet S_2 (34.77±1.17 g). However, supplementation of lysine and methionine in solvent extracted soyabean based diet had no significant ($p>0.05$) influenced on FCR and GEFC and the values ranged from 2.27±0.32 (S_3)-3.12±0.31 (S_2) and 66.97±7.03 (S_5)-77.97±8.93 (S_3), respectively. Control diet (13.73±1.56) had a significantly higher value in Protein Intake (PI) and least in *C. gariepinus* on diet S_6 (11.24±0.72). Protein efficiency ratio and specific growth rate reduced significantly with amino acid supplementation in solvent extracted soyabean based diet as higher values were in control diet (14.45±1.34 and 0.59±0.08) and least in *C. gariepinus* fed diet S_2 (11.59±0.39 and 0.44±0.02), respectively. Also, gross protein retention increased as methionine decreased and lysine increased in the diet as there was a significant difference in the GPR with the higher value in S_5 (0.79±0.02) and least in diet S_2 (0.66±0.02) (Table 3). Significantly ($p<0.05$) higher nitrogen retention efficiency was in control diet (49.49±6.19) but similar ($p>0.05$) to fish on S_3 (47.64±2.10). *Clarias gariepinus* fed diet S_6 (77.80±0.20) had the least survival rate during the experiment but significantly higher ($p<0.05$) in *C. gariepinus* on diet S_5 (88.90±0.10). Regression of gross protein retention and supplemental lysine and methionine were both quadratic with the optimum dietary inclusion levels 0.6 and 0.4 g 100 g^{-1}, respectively are presented in Fig. 1.

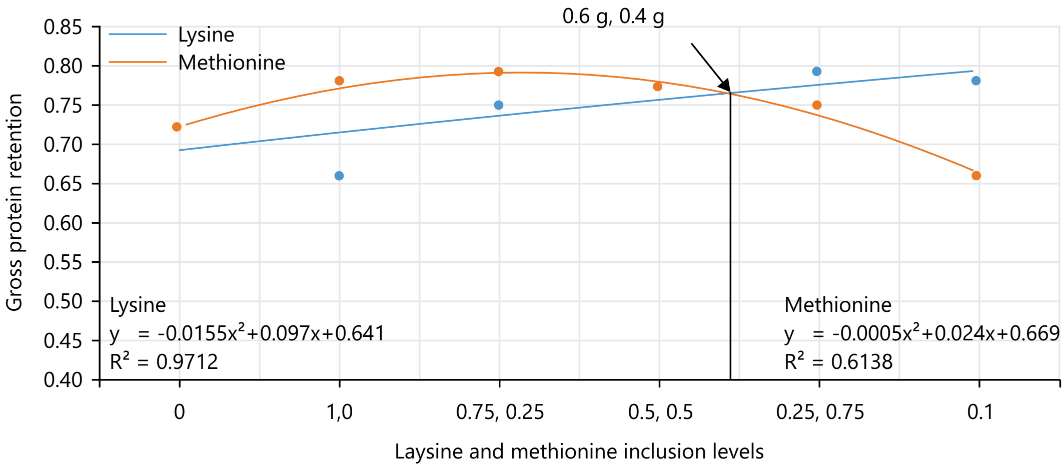

Fig. 1: Relationship between dietary supplement of lysine and methionine in a solvent extracted soyabean based diet and gross protein retention of *Clarias gariepinus*

Table 3: Growth performance and feed utilization of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Parameters	Control	S_2	S_3	S_4	S_5	S_6
IW (g)	17.85±0.49	18.03±0.38	17.50±0.10	17.77±0.32	17.50±0.00	17.80±0.17
WG (g)	43.35±4.03^{b}	34.77±1.17^{a}	40.80±1.74^{ab}	36.13±1.27^{a}	36.70±5.11^{ab}	35.40±4.99^{a}
FCR	2.35±0.15	3.12±0.31	2.27±0.32	2.88±0.26	2.95±0.60	2.88±0.62
GEFC	72.96±1.52	67.25±4.32	77.97±8.93	68.66±4.85	66.97±7.03	72.53±7.51
PI	13.73±1.56^{b}	11.95±0.67^{ab}	12.15±1.05^{ab}	12.17±0.47^{ab}	12.65±1.14^{ab}	11.24±0.72^{a}
PER	14.45±1.34^{b}	11.59±0.39^{a}	13.60±0.58^{ab}	12.04±0.42^{a}	12.23±1.70^{ab}	11.80±1.66^{a}
SGR	0.59±0.08^{c}	0.44±0.02^{a}	0.57±0.03^{bc}	0.47±0.01^{abc}	0.49±0.09^{abc}	0.46±0.09^{ab}
GPR	0.72±0.02^{b}	0.66±0.02^{a}	0.75±0.04^{bc}	0.77±0.03^{cd}	0.79±0.02^{d}	0.78±0.03^{cd}
NRE	49.49±6.19^{b}	35.15±1.42^{a}	47.64±2.10^{b}	41.27±1.66^{ab}	43.17±7.76^{ab}	40.74±7.38^{ab}
SR (%)	87.70±0.20^{d}	87.70±0.20^{d}	82.20±0.20^{b}	85.60±0.20^{c}	88.90±0.10^{e}	77.80±0.20^{a}

Means with different superscripts on the same row are significantly different ($p<0.05$). Control: Without supplemental lysine and DL-methionine, S_2: 1 g methionine, S_3: 0.75 g methionine, 0.25 g lysine, S_4: 0.5 g methionine, 0.5 g lysine, S_5: 0.25 methionine, 0.75 lysine, S_6: 1 g lysine, IW: Initial weight, WG: Final weight gain, TFI: Total feed intake, MWG: Mean weight gain, 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 and SR: Survival rate

Table 4: Apparent nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Parameters (%)	Control	S_2	S_3	S_4	S_5	S_6
Dry matter	74.41±0.09^{e}	49.71±0.05^{a}	69.00±0.22^{c}	51.41±0.02^{b}	80.78±0.05^{f}	71.56±0.03^{d}
Crude protein	94.29±0.04^{c}	91.49±0.07^{b}	94.23±0.11^{c}	90.27±0.14^{a}	96.34±0.31^{e}	95.95±0.02^{d}
Ash	69.58±0.19^{e}	51.62±0.70^{b}	55.70±0.36^{c}	44.51±2.03^{a}	72.54±0.10^{f}	65.89±1.39^{d}
Ether extract	96.45±0.12^{b}	93.70±0.01^{a}	93.54±2.20^{a}	93.56±0.23^{a}	97.22±0.29^{b}	96.12±0.05^{b}
Crude fibre	84.94±1.06^{c}	67.53±0.35^{a}	82.61±1.28^{c}	73.59±0.14^{b}	90.34±2.27^{d}	83.96±0.61^{c}
Energy	85.56±0.03^{e}	68.99±0.01^{a}	82.12±0.30^{c}	71.56±0.04^{b}	89.04±0.00^{f}	82.73±0.04^{d}

Means with different superscripts on the same row are significantly different ($p<0.05$). Control: Without supplemental lysine and DL-methionine, S_2: 1 g methionine, S_3: 0.75 g methionine, 0.25 g lysine, S_4: 0.5 g methionine, 0.5 g lysine, S_5: 0.25 methionine, 0.75 lysine and S_6: 1 g lysine

Apparent nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid is shown in Table 4. Dry matter digestibility values varied significantly ($p>0.05$) among the diets with the higher value in *C. gariepinus* on S_5 (80.78±0.05). Supplemental amino acid significantly ($p<0.05$) improved crude protein digestibility in fish fed diet S_5 (96.34±0.31) compared to other diets. However, ash digestibility significantly reduced with amino acid supplementation except for fish fed diet S_5 that was significantly higher than other treatments. Ether extract digestibility of fish on S_5, control and S_6 were similar ($p>0.05$) but higher than fish on S_2, S_3 and S_4. Also, crude fibre digestibility value was significantly higher in *C. gariepinus* fed diet S_5 (90.34±2.27) while, fish on control, S_3 and S_6 were not significantly different from each other (Table 4). Energy digestibility values varied significantly ($p>0.05$) among the diets with the higher value observed in *C. gariepinus* on S_5 (89.04±0.00).

Table 5: True nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Parameters (%)	Control	S_2	S_3	S_4	S_5	S_6
Dry matter	97.39±0.01^e	91.44±0.06^a	97.26±0.01^d	94.71±0.05^b	98.60±0.01^f	95.58±0.03^c
Crude protein	94.39±0.05^c	91.58±0.07^b	94.33±0.11^c	90.37±0.14^a	96.51±0.31^e	96.05±0.03^d
Ash	69.93±0.19^e	51.96±0.68^b	56.09±0.35^c	44.90±2.05^a	72.94±0.13^f	66.25±1.37^d
Ether extract	96.56±0.12^b	93.77±0.29^a	93.67±2.17^a	93.67±0.23^a	97.34±0.01^b	96.23±0.04^b
Crude fibre	85.36±0.97^c	67.98±2.19^a	83.00±1.30^c	73.96±0.18^b	90.72±0.38^d	84.36±0.64^c

Means with different superscripts on the same row are significantly different ($p<0.05$), Control: Without supplemental lysine and DL-methionine, S_2: 1 g methionine, S_3: 0.75 g methionine, 0.25 g lysine, S_4: 0.5 g methionine, 0.5 g lysine, S_5: 0.25 methionine, 0.75 lysine and S_6: 1 g lysine

Table 6: Apparent amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Parameters	Control	S_2	S_3	S_4	S_5	S_6
Essential amino acid						
Methionine	85.09±0.36^a	96.96±0.13^c	92.33±2.10^b	92.18±0.15^b	96.86±3.68^c	96.55±1.65^c
Lysine	94.01±0.21^c	86.21±0.17^a	94.06±0.25^c	86.08±1.05^a	95.75±0.26^d	92.80±0.14^b
Threonine	97.04±0.21^c	97.67±0.19^{cd}	92.48±0.85^a	96.09±0.71^b	92.69±0.41^a	98.18±0.31^d
Tryptophan	94.88±0.25^d	92.74±0.38^c	97.83±0.67^e	90.12±1.07^b	85.14±0.69^a	92.45±1.38^c
Isoleucine	83.67±0.11^e	86.55±0.53^f	78.42±0.51^d	64.41±0.27^a	67.85±0.79^b	74.93±2.62^c
Leucine	90.38±0.02^e	92.97±0.84^f	78.11±0.39^c	69.66±2.39^a	72.96±0.24^b	83.44±0.26^d
Valine	96.87±0.51^{de}	97.88±1.08^e	89.66±0.88^a	92.55±0.57^b	96.20±0.75^d	94.70±0.78^c
Histidine	84.37±0.83^c	88.40±0.53^e	86.21±0.30^d	66.83±0.58^a	75.87±0.83^b	83.85±0.02^c
Phenylalanine	92.46±0.30^c	96.96±0.18^d	89.80±1.11^b	89.44±0.61^b	86.16±0.67^a	92.99±0.23^c
Arginine	88.74±0.26^e	91.96±0.27^f	87.40±0.29^d	55.38±0.66^a	74.55±65^b	86.18±0.37^c
Non-essential amino acid						
Glycine	93.01±0.06^f	90.71±0.13^e	73.36±0.93^a	76.08±0.11^b	78.00±0.45^c	83.03±0.06^d
Serine	95.77±0.23^c	98.24±0.12^d	94.74±0.31^{bc}	93.06±1.54^a	94.06±0.51^{ab}	97.44±0.10^d
Proline	95.35±0.61^d	93.66±0.06^c	94.03±0.21^{cd}	87.74±0.39^a	94.91±0.33^{cd}	91.36±1.89^b
Alanine	90.43±0.19^e	93.88±0.26^f	79.73±0.04^c	75.62±1.03^b	67.25±0.12^a	83.00±0.87^d
Aspartic	84.55±0.20^d	90.33±0.82^e	82.37±0.62^c	75.18±1.19^b	71.33±0.35^a	83.43±0.30^{cd}
Glutamic	85.09±0.22^e	89.72±0.58^f	77.34±0.06^c	64.37±0.04^a	67.20±0.64^b	82.98±0.34^d
Cysteine	91.56±0.34^c	96.95±0.49^d	84.90±0.36^b	70.92±0.90^a	84.17±0.89^b	90.49±0.36^c
Pyrrolysine	94.55±26^c	97.65±0.25^d	93.96±0.23^{bc}	91.78±1.26^b	85.27±3.34^a	96.13±0.45^{cd}
Tyrosine	96.82±0.21^d	95.25±0.16^d	82.75±0.80^b	72.22±1.89^a	81.08±1.96^b	86.94±0.90^c

Means with different superscripts on the same row are significantly different ($p<0.05$), Control: Without supplemental lysine and DL-methionine, S_2: 1 g methionine, S_3: 0.75 g methionine, 0.25 g lysine, S_4: 0.5 g methionine, 0.5 g lysine, S_5: 0.25 methionine, 0.75 lysine and S_6: 1 g lysine

True nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 5. Crude protein digestibility values varied significantly within the diets with the higher value in *C. gariepinus* on S_5 (96.51±0.31). Amino acid supplementation significantly decreased ash digestibility value except for fish on diet S_5 (72.94±0.13) that was higher significantly ($p>0.05$) than others. Ether extract digestibility values were significantly lower in fish fed diet S_2 (93.77±0.29), S_3 (93.67±2.17) and S_4 (93.67±0.23) than those on S_5, S_6 and control (Table 5). Crude fibre and dry matter digestibility values differs among diets with the higher value in fish on S_5 (90.72±0.38 and 98.60±0.01), respectively.

Methionine composition revealed that *C. gariepinus* on S_2, S_5 and S_6 were similar ($p>0.05$) but were higher ($p<0.05$) than other treatments in Table 6. Lysine digestibility was higher ($p<0.05$) in *C. gariepinus* on S_5 (95.75±0.26) and least value of 96.08±1.05 was in diet S_4. Threonine and valine were least ($p<0.05$) digested when fed diet S_3 (92.48±0.85 and 89.66±0.58) with the higher values in diet S_5 (98.18±0.31 and 96.20±0.75), respectively. Also, significantly ($p<0.05$) least digested values for Tryptophan and Isoleucine were in *C. gariepinus* fed S_5 (85.14±0.69 and S_4 (64.41±0.27) and higher in fish on S_3 (97.83±0.67) and S_2 (86.55±0.53), respectively as shown in Table 6. Leucine had the higher digestible value of 92.97±0.84 in diet S_2. Histidine and Phenylalanine had higher ($p<0.05$) values in *C. gariepinus* on diet S_2 (88.40±0.53 and 96.96±0.18) and least in *C. gariepinus* on diet S_4 (66.83±0.58) and S_5 (86.16±0.67), respectively.

Table 7: True amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Parameters	Control	S_2	S_3	S_4	S_5	S_6
Essential amino acid						
Methionine	$85.51±0.34^a$	$97.32±0.13^c$	$92.70±2.09^b$	$92.62±0.19^b$	$97.25±3.69^c$	$96.90±1.66^c$
Lysine	$94.20±0.21^c$	$86.37±0.17^a$	$94.22±0.25^c$	$86.24±1.04^a$	$95.91±0.26^d$	$92.97±0.14^b$
Threonine	$97.33±0.21^c$	$98.02±0.20^{cd}$	$92.85±0.84^a$	$96.41±0.69^b$	$92.99±0.42^a$	$98.53±0.32^d$
Tryptophan	$95.23±0.24^d$	$93.17±0.38^c$	$98.07±0.67^e$	$90.43±1.07^b$	$85.51±0.68^a$	$92.87±1.38^c$
Isoleucine	$83.75±0.11^e$	$86.63±0.91^f$	$78.49±0.51^d$	$64.48±0.27^a$	$67.92±0.79^b$	$75.02±2.62^c$
Leucine	$90.57±0.02^e$	$93.21±0.84^f$	$78.31±0.39^c$	$69.90±2.39^a$	$73.19±0.24^b$	$83.64±0.26^d$
Valine	$97.07±0.51^{de}$	$98.10±1.07^e$	$89.93±0.87^a$	$92.79±0.58^b$	$96.48±0.75^d$	$94.88±0.78^c$
Histidine	$85.22±0.79^c$	$89.01±0.54^e$	$86.56±0.29^d$	$67.53±0.58^a$	$76.67±0.82^b$	$84.72±0.02^c$
Phenylalanine	$92.66±0.30^c$	$97.13±0.17^d$	$90.00±1.11^b$	$89.61±0.61^b$	$86.33±0.66^a$	$93.18±0.22^c$
Arginine	$89.04±0.26^e$	$92.19±0.26^f$	$87.65±0.29^d$	$55.67±0.66^a$	$74.80±0.64^b$	$86.45±0.37^c$
Non-essential amino acid						
Glycine	$93.39±0.06^f$	$91.16±0.13^e$	$73.69±0.93^a$	$76.57±0.12^b$	$78.50±0.45^c$	$83.51±0.06^d$
Serine	$96.06±0.23^c$	$98.54±0.12^d$	$95.01±0.30^{bc}$	$93.37±1.48^a$	$94.32±0.51^{ab}$	$97.66±0.11^d$
Proline	$95.57±0.60^d$	$93.84±0.06^c$	$94.21±0.21^{cd}$	$87.95±0.39^a$	$95.09±0.34^{cd}$	$91.52±1.89^b$
Alanine	$90.50±0.18^e$	$93.96±0.26^f$	$79.80±0.04^c$	$75.68±1.03^b$	$67.31±0.12^a$	$83.05±0.87^d$
Aspartic	$84.84±0.19^d$	$90.58±0.82^e$	$82.65±0.62^c$	$75.44±1.17^b$	$71.61±0.35^a$	$83.68±0.30^{cd}$
Glutamic	$85.93±0.22^e$	$90.30±0.56^f$	$77.83±0.05^c$	$65.18±0.04^a$	$68.27±0.60^b$	$83.72±0.34^d$
Cysteine	$91.74±0.34^c$	$97.20±0.49^d$	$85.15±0.35^b$	$71.18±0.89^a$	$84.42±0.89^b$	$90.73±0.36^c$
Pyrrolysine	$94.73±0.26^c$	$97.84±0.25^d$	$94.15±0.23^{bc}$	$91.98±1.25^b$	$85.49±3.33^a$	$96.32±0.45^{cd}$
Tyrosine	$97.16±0.20^d$	$95.68±0.17^d$	$83.27±0.78^b$	$72.89±1.87^a$	$81.58±1.96^b$	$87.37±0.90^c$

Means with different superscripts on the same row are significantly different ($p<0.05$), Control: Without supplemental lysine and DL-methionine), S_2: 1 g methionine, S_3: 0.75 g methionine, 0.25 g lysine, S_4: 0.5 g methionine, 0.5 g lysine, S_5: 0.25 methionine, 0.75 lysine and S_6: 1 g lysine

Apparent digestibility values for glycine revealed that fish on S_1 (93.01±0.06) were higher ($p<0.05$) than other treatments (Table 6). Significantly ($p<0.05$) least digested values for serine, proline, glutamic, cysteine, arginine and tyrosine were observed in *C. gariepinus* on S_4 (93.06±1.54, 87.74±0.39, 64.37±0.04, 70.92±0.90, 55.38±0.66 and 72.22±1.89) than other treatments. Furthermore, alanine and aspartic acid had the least ($p<0.05$) value of 67.25±0.12 and 71.33±0.35 in *C. gariepinus* fed S_5 and higher values were observed in diet S_2. Finally, higher ($p<0.05$) value of pyrrolysine was in diet S_2 (95.25±0.16) and least in diet S_5 (85.27±3.34).

Clarias gariepinus fed supplemental amino acid improved ($p<0.05$) methionine digestibility with higher value in diet S_5 (97.25±3.69) as shown in Table 7. Similar trend was also observed in lysine with the higher values in fish fed diet S_5 (95.91±0.26) and least value of 86.24±1.04 was in diet S_4. Higher value was recorded for Threonine from *C. gariepinus* on S_6 (98.53±0.32) but similar ($p>0.05$) to *C. gariepinus* on diet S_2 (98.02±0.20). Tryptophan had a significantly ($p<0.05$) higher value in *C. gariepinus* on S_1 (95.23±0.24) and least in diet S_5 (85.51±0.68). Isoleucine, leucine and histidine had least ($p<0.05$) values in fish on diet S_4 (64.48±0.27, 69.90±2.39 and 67.53±0.58) and higher values of 86.63±0.91, 93.21±0.84 and 89.01±0.54 were in diet S_2, respectively (Table 7). Additionally, supplementation of amino acid altered ($p<0.05$) valine and phenylalanine in *C. gariepinus* on diet S_2 (98.10±1.07 and 97.13±0.17) and least in S_3 (89.93±0.87) and S_2 (97.13±0.17), respectively.

Also, glycine was higher ($p<0.05$) in *C. gariepinus* fed S_1 (93.39±0.06) and the least value in diet S_3 (73.69±0.93). Significantly least ($p<0.05$) values for serine, proline, glutamic, cysteine, arginine and tyrosine were observed in *C. gariepinus* fed S_4 (87.95±0.39, 65.18±0.04, 71.18±0.89, 55.67±0.66 and 72.89±1.87). Serine value was similar in *C. gariepinus* on diet S_4 (93.37±1.48) and S_5 (94.32±0.51) but differed significantly from other treatments. However, alanine, aspartic acid and pyrrolysine had least values in diet S_5 with the higher values of 93.96±0.26, 90.58±0.82 and 97.84±0.25 in diet S_2 (Table 7).

DISCUSSION

Findings from Table 3 indicated that *C. gariepinus* fed soyabean based diet when supplemented with lysine and DL-methionine could improve fish growth performance and utilization of feed. Lu *et al.*[17] attributed it to the fact that, amino acid has a powerful feeding stimulant and it has been good in reducing feed intake while improving the body weights of fish. There have been similar reports of improved performance of fish fed diets supplemented with lysine and DL-methionine[18,19]. The authors noted that supplemental amino acid improved amino acids utilization for protein synthesis which shows that lysine and DL-methionine are essential for the growth of *C. gariepinus*.

Also, Nwanna *et al.*[8] reported that supplementation of DL-methionine in DL-methionine deficient diet significantly increased feed utilization with better feed conversion ratio which resulted in significant improvements in weight gain and feed efficiency of common carp. Similar trends were noted by Gao *et al.*[20], Ren *et al.*[21] and Sardar *et al.*[22] in Juvenile grass carp (*Cyprinus carpio*), juvenile blunt snout bream (*Megalobrama amblycephala*) and Rohu (*Labeo rohita*) fingerlings when fed increasing levels of dietary histidine, isoleucine and Met+Cystine, respectively. Also, the supplementation of lysine and DL-methionine improved feed efficiency and decreased the amount of feed consumption necessary for fish growth which could result in production cost reduction. Yuan *et al.*[23], Nwanna *et al.*[8] and Wang *et al.*[24] reported lower FCR which was due to lysine and DL-methionine supplementation in the diet of *Myxocyprinus asiaticus*, *Cyprinus carpio* and *Pseudobagrusussuriensis*, respectively. These conformed to the result observed in this present study.

Zhou *et al.*[25] observed reduced growth, feed utilization and PER when lysine was supplemented to diets of *Sparus macrocephalus*. It may be due to the negative effects of excessive or insufficient amount of free lysine and DL-methionine and poor palatability. Wang *et al.*[24] suggested that DL-methionine levels used in the diet were not high enough to induce toxic effect. However, the relationship between solvent extracted soyabean based diet and gross protein retention of *C. gariepinus* fed dietary amino acid was shown by the regression model in Fig. 1. From the growth response curve, the estimated optimum level of lysine 0.6 and 0.4 g 100 g^{-1} DL-methionine for gross protein retention.

Supplemental methionine and lysine in solvent extracted soyabean based diet improved crude protein digestion as shown in Table 4. The results observed corroborated with Nwanna *et al.*[8] findings who detected that supplemental methionine and lysine significantly enhanced protein digestibility in *Cyprinus carpio* and *Oreochromis niloticus*, respectively. Also, Ribeiro *et al.*[1] reported similar trend when determining the digestibility of feedstuffs used in tilapia feed. The improvement in protein digestibility suggested that supplemental methionine and lysine in soyabean based diet increased availability of amino acid for maintenance and protein retention as revealed in the regression model of gross protein retention of this study (Fig. 1). Though, the value of protein digestibility observed in this experiment was greater than what Nwanna *et al.*[8] and Ribeiro *et al.*[1] stated. This suggested that combining supplemental methionine and lysine in soyabean based diet is imperative in *C. gariepinus* diets.

Apparent digestibility of the ether extract, crude fibre and dry matter of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with methionine and lysine suggested an increased digestibility though no particular trend were observed. This indicated that supplemental methionine and lysine in soyabean based diet had little influence on the experimental fish. Supplemental methionine and lysine in soyabean based diet fed to *C. gariepinus* further revealed that it supplementation could improve the energy digestibility. This result was in agreement with Sotolu and Faturoti[26] and Nwanna *et al.*[8], who observed improved energy digestibility in *C. gariepinus* and common carp, respectively.

The true digestibility of *C. gariepinus* fed solvent extracted soyabean based diet with supplemental amino acid had few literatures reported, apart from studies of Yamamoto *et al.*[27], with *Cyprinus carpio*, Ribeiro *et al.*[1,28] and Wilson *et al.*[29] with *Ictalurus punctatus* (Table 5). The values observed in true digestibility were higher than what was observed in apparent digestibility of this experiment because the nutrients in the feacal are intact and has not leached away as observed in apparent digestibility. Also, it revealed that study of true digestibility has the potential to correct endogenous losses that do occur in apparent digestibility monitoring. The study showed that true crude protein digestibility value was higher than those revealed by Yamamoto *et al.*[27] and Ribeiro *et al.*[1]. This increase observed could be as a result of supplemental amino acid in soyabean based diets were effectively utilized by the experimental fishes. Also, the dissection method of feacal collection used in the study could have contributed to the increase observed in the study and it was corroborated by Ribeiro *et al.*[1].

The apparent amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid as observed in Table 6. The values observed from the soyabean based diets increased from 85.09±0.36 (S_1)-97.49±0.47 (S_5) for Methionine, lysine values increased from 86.08±1.05 (S_4)-95.75±0.26 (S_5). The increased observed in fish fed diet S_5 are coherent with Yamamoto *et al.*[27] result, who perceived comparative values when several protein sources were fed to fingerlings, red sea beam, rainbow trout and common crap. However, Wilson *et al.*[29] and Ribeiro *et al.*[1] reported relatively lower values when common feedstuffs were fed to channel catfish and tilapia, respectively. The high values noted in this study might be ascribed to the fact that thermal treating of soyabean meal enhanced release the encapsulated protein structure and inactivates the anti-nutritional factors which could have enhanced soyabean based diet with supplemental lysine and methionine to be more digestible by proteolytic enzymes. Higher methionine content presented in the study could be as a result of the higher sulfur amino acids (pancreatic secretions and mucin layer) equated with additional amino acids[30]. Isoleucine and arginine presented the least apparent amino acid digestibility values among essential amino acid for all the studies. Similar trend was observed by Ribeiro *et al.*[28] for soyabean meal. The lease values obtained could be due to the fact that isoleucine is a hydrophobic amino acid situated in protein, deterring the hydrolysis of its peptide bonds, which may explain its low digestibility.

Table 7 showed the true digestibility of *C. gariepinus* fed solvent extracted soyabean based diets with supplemental amino acid. True amino acid digestibility helped to consider the role of endogenous amino acids, quantity of amino acids and values of true digestibility used by fish in more precise, resulting in precise formulation of diets for *C. gariepinus*. Results observed showed a relatively small difference from the apparent digestibility. This could be due to higher levels of digestive enzyme secretions and its inclusion in the feaces from the protein free diets for *C. gariepinus* in this study. Similar trend was observed by Pozza *et al.*[30], Ribeiro *et al.*[28]. The values acquired were within the ranged stated by Yamamoto *et al.*[27] and Pozza *et al.*[30]. Amino acid digestibility values observed in this study revealed that supplemental methionine and lysine in soyabean based diet served as a stimulant which enhance the digestibility of the diet and nutrient utilization as observed in the growth parameter.

CONCLUSION

It can be concluded that *C. gariepinus* are able to utilize solvent extracted soyabean based diet with lysine and DL-methionine supplementation. Supplementation of lysine at 0.6 and 0.4 g 100 g^{-1} of DL-methionine in solvent extracted soyabean based diet when fed to *C. gariepinus* improved growth and feed utilization. However, lysine 0.75 and 0.25 g 100 g^{-1} of methionine in soyabean based diet could improve digestibility of experimental fish. Further studies should, therefore, be conducted on the effect of true digestibility in *C. gariepinus* when fed other plant protein-based diet with or without amino acid supplementation.

SIGNIFICANCE STATEMENT

This study discovered that supplementation of lysine and DL-methionine in soyabean based diet could be utilize by *C. gariepinus* for its optimum growth and improve digestibility. The inclusion levels of 0.6 (lysine) and 0.4 g 100 g^{-1} (DL-methionine) for growth and lysine 0.75 and 0.25 g 100 g^{-1} of methionine in soyabean based diet for digestibility can be beneficial to researchers and fish feed industries for further investigations on how to improve utilization of plant protein-based diets. This study will help the researchers to uncover the critical areas of total replacement of fishmeal that many researchers were not possible able to explore.

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