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## RESEARCH ARTICLE

## ASSESSMENT OF FEEDING TIME ON AMINO ACID COMPOSITION AND MORPHOLOGICAL PARAMETERS IN AFRICAN CATFISH

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## ABSTRACT

An 8-week feeding trial was conducted to investigate the effect of feeding time on growth, morphology, and amino acid composition in juvenile African catfish (*Clarias gariepinus*) in a concrete fish pond. Fish (6.03 ± 0.16 g) was divided into four groups at a stocking density of 10 per pond with different feeding time intervals: group (A) (control) fed 08:00, 13:00, 20:00 h), Group B (08:00 and 13:00), Group C (13:00 and 20:00), and Group D (08:00 and 20:00) in three replicate for 8 weeks. According to the result, Groups B and C obtained body mass index (BMI), and daily growth rate (DGR), lipid gain, and organ indices compared to the control. In addition, whole-body and muscle crude protein were reduced, with an opposite trend observed in nitrogen-free extract in Groups B and C, while Group D was almost similar to the control. This was also similar for the levels of the essential amino acids (threonine, lysine, methionine and phenylalanine) and non-essential amino acids related (serine, glycine) profile. Overall, the findings suggest that timing feeding with the endogenous rhythms of *C. gariepinus* enhances growth, nutrient utilization and health, possibly yielding an economically viable aquaculture production.

## KEYWORDS

feeding time, fish species, circadian rhythms, nutrient supply, amino acid

## 1. INTRODUCTION

Aquaculture plays an important role in Nigeria's national economy, providing employment and income for people and contributing to food security (Ogunji and Wuertz, 2023). Close to 10 million people are engaged in fishing activities, and the industry accounted for approximately 3.24% of the national gross domestic product in the first quarter of 2021 (Ogunji and Wuertz, 2023). Nonetheless, aquaculture development, particularly African catfish production, is impeded by the high cost and irregular supply of high-quality aquafeed (Udo and Umanah, 2017). Since feed makes up the bulk of production costs upscaling feed utilization efficiency rather than increasing feed input is considered a key approach to increase productivity and profitability (El-Sayed et al., 2007).

In this regard, the management of feeding, namely its timing, has become a major yet underexploited determinant of production efficiency (Ragasa et al., 2022). Feeding time has a direct influence on feeding rate, digestion and nutrient utilization, but the extent of its effect is also influenced by the fish's physiological state, defined by the endogenous circadian rhythm of the fish itself (Frøland Steindal and Whitmore, 2019). These rhythms regulate the daily cycles of feeding behaviour, enzyme secretion and metabolism, and they can be synchronized by external factors, including light and temperature. Notably, feeding patterns are species-specific, and they affect the response of fish to feeding schedules (tilapia and rainbow trout are mainly diurnal, while catfish are mainly nocturnal and salmon are repuscated feeders) (Pohlmann et al., 2001). Synchrony of feeding with these activity phases in nature has been demonstrated to enhance

growth and feed utilization (Bolliet et al., 2007). Desynchrony, on the other hand, could compromise digestion, increase stress and decrease nutrient retention (Thoufeek et al., 2025).

Despite these insights, existing research is largely skewed toward growth metrics and feed conversion ratios, with limited integration of underlying biochemical responses. Specifically, knowledge of the impact of feeding time on the amino acid profile remains very limited. This constitutes a limitation, as focusing solely on growth response may conceal inefficiencies in protein utilization; consequently, the effects of feeding regimes may not be fully clarified. Furthermore, there is a lack of information on how feeding time relates to morphological performance in the African catfish. This indicates an evident disconnect between feeding management and mechanistic nutritional insights.

Based on the economic importance and the culture of African catfish *Clarias gariepinus* in Nigeria and its clearly defined nocturnal feeding activity, the present study was conducted to elucidate this gap (Okon et al., 2020). Its physiological plasticity and stable feeding pattern also allow the study of temporal feeding effects on nutrient utilization (Pickel and Sung, 2020). Importantly, tissue amino acid composition serves as a sensitive indicator of dietary protein utilization and metabolic efficiency, while morphological indices such as body weight and condition factor provide integrative measures of growth performance (Adhikari et al., 2025). The simultaneous evaluation of these parameters provides a better understanding of the effect of feeding time on biochemical and phenotypic characteristics.

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Therefore, this study aimed to investigate the effect of feeding time on the amino acid profile and morphometric parameters of African catfish (*Clarias gariepinus*). In linking feeding time to nutrient utilization and growth response, the present work provides mechanisms insights that should enable the development of more precise and cost-effective feeding strategies in aquaculture.

## 2. MATERIAL AND METHOD

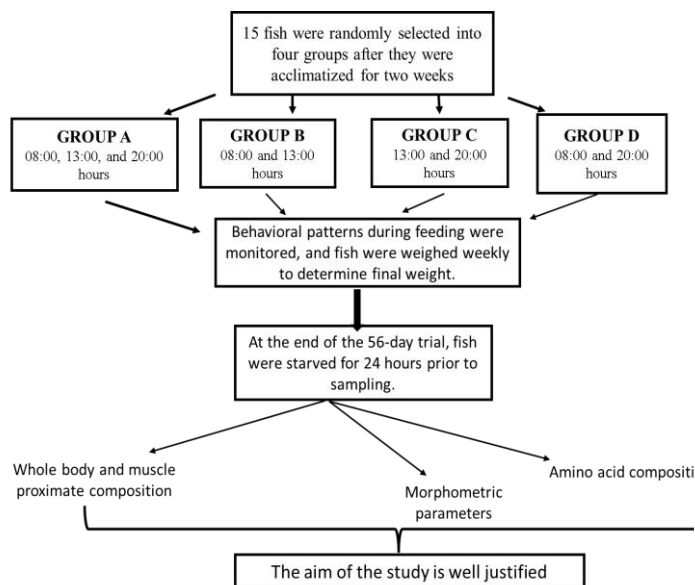
Ethical approval: All experimental trial permission was granted by the Akwa Ibom State University Institutional Review Board, while assurance of quality was overseen by the Department of Fisheries and Aquaculture and the Faculty of Agriculture.

### 2.1 Acquisition Of Experimental Diet

Blue Crown, a commercial formulated feed, was obtained from a reputable aquafeed company in Uyo, the capital city of Akwa Ibom State, Nigeria. The feed was tightly sealed and transported to the feeding facility in the Department of Fisheries and Aquaculture, Akwa Ibom State University, Obio Akpa Campus, under hygienic conditions. The diet proximate composition is as follows; 40 % crude protein, 10 % crude lipid, 4 % crude fibre, 10 % moisture and 5 % ash as per the manufacturer. We also conduct a proximate composition of this diet to confirm the result. However, the result did not differ from that of the manufacturer. The feed was kept in a well-ventilated, dry place and on a cool floor in order to maintain the quality of the feed from the date of preparation until the end of the experiment.

### 2.2 Experimental Design, Fish And Feed Trial

*Clarias gariepinus* fingerlings. were purchased from a fish farm in Uyo, Akwa Ibom State, Nigeria. To reduce the influence of handling stress, the fish were allowed to acclimatize to the experimental conditions for 14 days prior to the commencement of the feeding experiment... During adaptation, the fish were hand-fed to apparent satiation on commercial pellets 3 times daily. At the end of acclimatization, 130 fish having a mean initial body weight of 6.03 ± 0.16 g were randomly distributed at a rate of 10 fish/pond to 12 concrete ponds of 100 × 40 m (length × width) each. This study was conducted using a 4-diet design (Groups A and D, triplicate: n 3) for the test. This consists of: Group A (control) fish were fed thrice daily at 07.00, 13.00 and 20.00 hrs. Permitted to feed at 07:00 and 13:00 (morning and afternoon) were group B, at 13:00 and 20:00 (afternoon and evening) were group C, and at 7:00 and 20:00 (morning and night) were group D. Fish were fed by hand to apparent satiation, and feed consumption was recorded to determine the minimal waste and the most accurate feed conversion ratio (FCR). The experimental design is presented in Figure 1.



**Figure 1:** Experimental Design Showing the Feeding Schedule for *Clarias Gariepinus* Juveniles.

### 2.3 Sampling

At the end of the feeding experiment, a 24 h fast was applied to all fish to empty the gut before sampling. The survival rate and the total biomass were then calculated from the total number of fish and the body weight per cage. The effects of feeding frequencies on fish growth and health were analyzed using the daily gain index, body mass index (BMI), and condition factor (K). From each pond, four fish were randomly selected and immediately frozen at -20 °C to analyze whole body and muscle proximate

composition, as well as amino acid profiles. Then, three more fish per pond chosen at random were dissected to separate and weigh the viscera, liver and intestines; these organs were employed to compute the Viscerosomatic Index (VSI), Hepatosomatic Index (HSI), and Intestinosomatic Index (ISI), indicators of organ development and energy allocation. At last, a separate group of three fish randomly selected per pond was sacrificed for biometric analyses performed by assessing MW (%), DP (%), carcass ratio, head ratio, meat ratio and ratio of non-edible parts (NEBR) and condition factor (CF).

### 2.4 Proximate And Amino Acid Composition

The method of AOAC in 1990 was utilized to determine moisture, crude protein, lipid and ash content in muscle and whole-body fish. Moisture was obtained by constantly drying the specimen to a constant weight at 105 °C. The Kjeldahl method was used in estimating the crude protein (N × 6.25) content after acid digestion method (Kjeltec analyzer (FOSS 2300, Höganäs, Sweden)). The fat content was analyzed by percolation ether extraction using a Soxtec™ 205 System (Foss, Hoganas, Sweden). The ash content was determined by ashing the sample in a muffle furnace (FO610C, Yamato Scientific Co., Ltd., Tokyo, JAPAN) at 55 0 °C for 8 h. The gross energy was measured using a bomb calorimeter (Parr 1281, Parr Instrument Company, Moline, IL, USA).

Fish body and muscle AA profiles were analyzed according to the Cavallarin method using a Beckman Gold system (Beckman Instruments, Palo Alto, CA, USA) with HPLC. Each sample (~60 mg) was hydrolyzed under nitrogen in sealed tubes with 6 mL of 6 M HCl at 110 °C for 24 h. The hydrolysates were reconstituted in sodium citrate buffer (pH 2.2) after being vacuum dried at 40 °C. Amino acids were separated by ion exchange chromatography on a 20 cm Spherogel IEX column, and post-column derivatized with ninhydrin (at 570nm or 440nm for proline) (Abasubong et al., 2019). Amino acids were identified and quantified by means of external standards (17-AA mixture, Beckman Instruments). Tryptophan was excluded because of degradation in acid hydrolysis.

### 2.5 Calculation And Statistical Analysis

- Daily gain index (DGI, %/day) = (Final body weight<sup>1/3</sup> - Initial body weight<sup>1/3</sup>) × 100/days fed.
- Feed efficiency ratio (FER) =  $\frac{\text{Weight gain (g)}}{\text{feed intake (g)}}$
- Relative feed intake =  $\frac{\text{Feed intake (g)}}{[(\text{initial fish weight (g)} + \text{final fish weight (g)}) \times \text{days reared}/2]} \times 100$
- Body mass index (BMI) =  $\frac{\text{Weight of body (kg)}}{\text{body length (m}^2\text{)}}$
- Condition factor (K (%)) =  $\frac{\text{Final weight (g)}}{\text{Length}^3 \text{ (cm)}} \times 100$
- Lipid intake = Feed intake × diet lipid.
- Lipid efficiency ratio (LER) =  $\frac{\text{Weight gain (g)}}{\text{Lipid intake (g)}} \times 100$ .
- Hepatosomatic index (HSI, %) =  $\frac{\text{Liver weight (g)}}{\text{body weight (g)}} \times 100$
- Viscerosomatic index (VSI, %) =  $\frac{\text{Viscera weight (g)}}{\text{body weight (g)}} \times 100$
- Intestinosomatic index (ISI, %) =  $\frac{\text{Intestine weight (g)}}{\text{body weight (g)}} \times 100$
- Dress-out percentage (DP, %) =  $\frac{\text{Dressed Weight (g)}}{\text{body weight (g)}} \times 100$
- Weight-to-Length Ratio (WLR): WLR=  $\frac{\text{Weight (g)}}{\text{Length (cm)}}$
- HI =  $\frac{\text{Total weight (g)} - (\text{head weight (g)} + \text{viscera weight (g)})}{\text{total weight (g)}} \times 100$
- NEPI=  $\frac{[(\text{viscera weight (g)} + \text{head weight (g)} + \text{fins weight (g)} + \text{gills weight (g)})]}{\text{total weight (g)}} \times 100$
- Meat index (MI) =  $\frac{\text{Meat weight (g)}}{\text{total weight (g)}} \times 100$

All data were calculated using a one-way analysis of variance (ANOVA) to test the effect of feeding time on the , using SPSS for Windows (Version 13.0; SPSS Inc., Chicago, IL, USA) to determine the significant difference. When significant differences were found, Duncan's multiple range test was used as a post hoc test to range and compare the means of treatments. All calculated data are shown as mean ± standard error of mean (SEM). p ≤ 0.05 was set as a statistically significant difference among groups.

3. RESULTS

The initial weights were not significantly different among the groups ( $p > 0.05$ ) (Table 1). Both groups B and C had significantly lower values than the control group in body mass index, daily growth, feed efficiency ratio, relative feed intake, lipid productive value, lipid gains, and lipid efficiency ratio ( $p < 0.05$ ). In contrast, these parameters did not differ between groups B, C, and D. Group D did not differ from the control group in daily growth rate, feed efficiency ratio, relative feed intake, and lipid intake. The weight-to-length ratio was not affected by any dietary group.

Table 1: Growth and Feed Utilization Parameters of <i>Clarias Gariepinus</i> at Different Feeding Times				
	Control A	Group B	Group C	Group D
Initial weight (g)	6.21±0.15	6.42±0.08	6.27±0.04	6.31±0.31
Body mass index (kg/m <sup>2</sup> )	20.45±1.45 <sub>a</sub>	16.33±0.88 <sub>b</sub>	17.75±0.43 <sub>b</sub>	18.34±0.58 <sub>a</sub>
Daily growth rate	10.49±0.19 <sub>a</sub>	5.93±0.13 <sub>c</sub>	7.58±0.39 <sub>b</sub>	9.43±0.45 <sub>a</sub>
Weight-to-Length Ratio	1.39±0.13	1.27±0.12	1.17±0.06	1.26±0.07
<b>Feed utilization</b>				
Feed Efficiency Ratio	2.05±0.02 <sub>a</sub>	1.06±0.03 <sub>d</sub>	1.35±0.06 <sub>c</sub>	1.67±0.09 <sub>b</sub>
Relative feed intake (g)	2.33±0.09 <sub>b</sub>	2.76±0.05 <sub>a</sub>	2.78±0.04 <sub>a</sub>	2.92±0.34 <sub>a</sub>
Lipid Productive Value	1.98±0.02 <sub>a</sub>	1.12±0.05 <sub>c</sub>	1.19±0.06 <sub>b</sub>	1.56±0.09 <sub>ab</sub>
Lipid Intake (g/fish)	9.13 ± 0.25 <sub>a</sub>	6.21 ± 0.86 <sub>b</sub>	7.34 ± 0.12 <sub>b</sub>	8.98 ± 0.81 <sub>a</sub>
Lipid gain (g/fish)	12.23±0.34 <sub>a</sub>	8.67±0.65 <sub>c</sub>	9.87±0.67 <sub>bc</sub>	10.56±0.98 <sub>a</sub>
Lipid efficiency ratio	3.76±0.98 <sub>a</sub>	2.67±0.23 <sub>c</sub>	2.98±0.54 <sub>bc</sub>	3.23±0.76 <sub>ab</sub>

Data are expressed as mean ± SEM of 3 replicates. Different superscript letters in a row indicate significant differences between the means ( $p < 0.05$ ).

3.1 Effect Of Feeding Time On Morphological Parameters Of *Clarias Gariepinus* At Different Feeding Times

In Table 2, as compared to the control, HIS, VSI, ISI, CF, MC, DP, inedible parts index (NEPI), and meat index (MI) were significantly decreased ( $p <$

0.05) in groups B and C. However, no such differences were observed in group D. Lastly, there were no significant differences in carcass ratio or headless index among the dietary treatments.

Table 2: Morphological Parameters of <i>Clarias Gariepinus</i> at Different Feeding Times				
	Control A	Group B	Group C	Group D
<i>Biometric parameters</i>				
Hepatosomatic index	5.21±0.22 <sub>a</sub>	4.12±0.12 <sub>c</sub>	4.73±0.21 <sub>b</sub>	4.99±0.32 <sub>a</sub>
Viscerosomatic index	13.51±0.01 <sub>1a</sub>	10.21±0.02 <sub>2c</sub>	11.61±0.01 <sub>1b</sub>	12.63±0.11 <sub>1ab</sub>
Intestinosomatic index	1.77±0.02 <sub>a</sub>	0.78±0.02 <sub>c</sub>	0.81±0.06 <sub>b</sub>	0.91±0.03 <sub>a</sub>
Condition factor	2.12±0.03 <sub>a</sub>	1.35±0.02 <sub>c</sub>	1.78±0.06 <sub>b</sub>	2.01±0.05 <sub>a</sub>
Muscle content (MC, %)	3.23±0.34 <sub>a</sub>	2.17±0.11 <sub>c</sub>	2.76±0.23 <sub>b</sub>	2.90±0.32 <sub>a</sub>
Dress-out percentage (DP, %)	67.6 ± 1.22 <sub>a</sub>	51.71 ± 3.01 <sub>c</sub>	55.87 ± 2.29 <sub>bc</sub>	61.1 ± 0.65 <sub>ab</sub>
Carcass ratio	75.02 ± 0.2	74.24 ± 0.6	74.77 ± 0.4	74.91 ± 0.9
Non-edible parts index (NEPI)	13.99±1.05 <sub>a</sub>	9.32±0.89 <sub>c</sub>	10.93±1.11 <sub>1bc</sub>	12.56±1.06 <sub>6ab</sub>
Headless index	72.46±1.11 <sub>1</sub>	71.73±1.2	72.86±1.62 <sub>2</sub>	72.71±1.34 <sub>4</sub>
Meat index	55.81±0.43 <sub>a</sub>	48.29±0.87 <sub>b</sub>	52.73±0.53 <sub>ab</sub>	52.25±0.87 <sub>a</sub>

Data are expressed as mean ± SEM of 3 replicates. Different superscript letters in a row indicate significant differences between the means ( $p < 0.05$ ).

3.2 Effect Of Feeding Time On Proximate Composition (Whole-Body And Muscle) Of *Clarias Gariepinus* At Different Feeding Times

As presented in Figure 2. The whole body and muscle protein and ash content of group B were significantly lower ( $p < 0.05$ ), with an opposite trend observed in NFE than in the control, but no differences ( $p > 0.05$ ) were attributed to other groups.

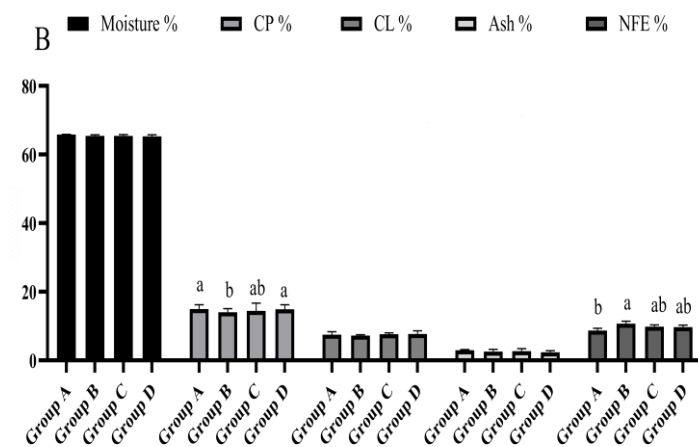


Figure 2: Data are expressed as mean ± SEM of 3 replicates. Different superscript letters in a row indicate significant differences between the means ( $p < 0.05$ ).

3.4 Effect Of Feeding Time On The Amino Acid Profile Of *Clarias Gariepinus* At Different Feeding Times

As presented in Table 3, essential amino acids (threonine, methionine, phenylalanine, lysine, histidine and arginine) were significantly decreased

in Groups B compared to the control ( $p < 0.05$ ), and there were no differences in valine, isoleucine and leucine. For the non-essential amino acids, serine and glycine were markedly reduced in Groups B and C ( $p < 0.05$ ), while aspartate, glutamate, alanine, cystine, tyrosine and proline were unaltered among the groups.

Data are expressed as mean  $\pm$  standard error of 3 replicates. Different superscript letters in a row indicate significant differences between the means ( $p < 0.05$ ). Means in the same row indicated by different superscript letters are significant ( $p < 0.05$ ).

4. DISCUSSION

In this study, fish in groups B and C obtained a significantly lower performance than those in the control. This result indicates that feeding fish in this period did not align with the fish's periods of active metabolism, where enzyme activities, nutrient absorption and energy metabolism attain a higher status of efficiency (Lall and Kaushik, 2021). Therefore, it is possible that this extended fasting period during feeding decreases the availability of metabolites and disrupts metabolic homeostasis, resulting in diminished growth rate and feed efficiency (Adhikari et al., 2025). However, fish in group D had better performance than those fed in more restricted time zones (Groups B and C), suggesting that feeding this fish in the morning and night aligns with the fish's periods of active metabolism, where enzyme activities, nutrient absorption and energy metabolism attain a higher status of efficiency (Lall and Kaushik, 2021). During these periods, the uptake of amino acids and the synthesis of proteins are increased, allowing better growth, feed efficiency, and nutrient supply, resulting in overlapping digestive cycles and in sub-optimal capture of the nutrients, causing a slight decrease in the overall conversion efficiency relative to control. Corresponding results in *Oreochromis niloticus* and *Ictalurus punctatus* have shown that feeding during periods of inherent metabolic activity leads to optimal nutrient conversion, energy equilibrium, and growth (Nasrin et al., 2021). Thus, twice-daily feeding in the morning and evening results in the best synchronization with the fish's circadian rhythms, leading to potential growth enhancement in fish.

In this study, fish in groups B and C had significantly lower biometric content as compared to the control, indicating impaired nutrient utilization, organ dysfunction, reduced energy reserves, and weakened health status, leading to poor growth performance and increased disease susceptibility. This was supported by the fact that biometric parameters are used to assess growth performance, nutritional status, energy storage, and organ health, providing insight into the physiological condition and overall well-being of fish. The significant poor biometric content in this group might be attributed to long fasting intervals, which did not align with fish periods of active metabolism, decreasing the availability of nutrients during digestive and metabolic phases, which negatively influences enzyme activity, energy partitioning and development of organs (Hvas et al., 2024). However, fish in Group D obtained results similar to the control, suggesting that feeding during naturally active metabolic phases promotes better nutrient assimilation, energy use, and organ growth (Pickel and Sung, 2020). This indicated that feeding should be more effective during circadian metabolic periods, among circadian patterns, when digestion, nutrient absorption and energy metabolism are at high flux, fish could use feed for growth most efficiently. A comparable result was obtained in *Ictalurus punctatus* (Noeske-Hallin et al., 1985).

Feeding timing has a large impact on nutrient deposition as they dictate the degree to which feeding is synchronized with the fish's intrinsic metabolic rhythm (Pickel and Sung, 2020). Furthermore, fish in group B exhibited lower whole-body and muscle protein content than the control. This suggests muscle atrophy, defective mineralization, and poor nutritional status and health. This was supported by the fact that an increase in nutritional content (protein, ash, etc.) serves as an indicator of nutritional quality and energy reserves and promotes physiological condition in fish species (Harikrishnan et al., 2003). The significant reduction in protein and ash content might be attributed to the experienced extended fasting between meals, which reduced amino acid availability, impaired enzymatic and hormonal activity, and decreased protein and mineral deposition (Shkorfu et al., 2025). Group D results were comparable to the control, indicating better nutrient utilization. However, feeding during the natural time of activity promotes digestion, amino acid absorption, and hormone regulation of nutrient utilization, which in turn results in higher protein and mineral deposition, as in group A (Boujard and Leatherland, 1992). This effect can be attributed to the fact that feeding aligning with species' active period promotes a continuous supply of amino acids during the active phase thereby enabling uninterrupted digestion, absorption, and protein synthesis (Andersen et al., 2016). It promoted optimal enzyme activity, hormonal status (e.g., insulin and growth hormone), and resultant nitrogen retention and muscle accretion (Bertucci et al., 2019).

Feeding time strongly modifies the amino acid (AA) profile in fish by interacting with their circadian metabolic rhythms. The fish in Group A had the highest EAA level. Continuous nutrient provision sustained digestion, absorption, and protein synthesis during the active phase of metabolism. This constant supply of amino acids enabled enhanced muscle growth and optimal growth (Poortmans et al., 2012). In group B,

**Table 3: Amino acid profile of *Clarias gariepinus* at different feeding times**

	Control (A)	Group B	Group C	Group D
<i>Essential amino acid</i>				
Threonine	8.31 $\pm$ 0.12 <sub>a</sub>	5.44 $\pm$ 0.33 <sub>b</sub>	6.43 $\pm$ 0.43 <sub>b</sub>	7.72 $\pm$ 0.45 <sub>a</sub>
Valine	4.86 $\pm$ 0.22	4.01 $\pm$ 0.08	4.33 $\pm$ 0.15	4.51 $\pm$ 0.31
Methionine	7.91 $\pm$ 0.86 <sub>a</sub>	6.25 $\pm$ 0.23 <sub>b</sub>	7.31 $\pm$ 0.44 <sub>a</sub>	7.59 $\pm$ 0.91 <sub>a</sub>
Isoleucine	1.13 $\pm$ 0.02	1.08 $\pm$ 0.05	1.10 $\pm$ 0.03	1.12 $\pm$ 0.07
Leucine	4.97 $\pm$ 6.21	4.10 $\pm$ 9.68	4.39 $\pm$ 1.76	4.58 $\pm$ 4.42
Phenylalanin <sub>e</sub>	5.87 $\pm$ 0.16 <sub>a</sub>	5.17 $\pm$ 0.13 <sub>b</sub>	5.28 $\pm$ 0.27 <sub>b</sub>	5.72 $\pm$ 0.32 <sub>a</sub>
Lysine	3.51 $\pm$ 0.04 <sub>a</sub>	2.21 $\pm$ 0.08 <sub>b</sub>	2.51 $\pm$ 0.01 <sub>b</sub>	2.93 $\pm$ 0.11 <sub>a</sub>
Histidine	0.97 $\pm$ 0.01 <sub>a</sub>	0.60 $\pm$ 0.02 <sub>b</sub>	0.71 $\pm$ 0.05 <sub>a</sub>	0.82 $\pm$ 0.04 <sub>a</sub>
Arginine	1.81 $\pm$ 0.01 <sub>a</sub>	0.61 $\pm$ 0.01 <sub>b</sub>	1.01 $\pm$ 0.01 <sub>b</sub>	1.43 $\pm$ 0.03 <sub>a</sub>
<i>Non-essential amino acid</i>				
Aspartic acid	6.71 $\pm$ 0.12	6.24 $\pm$ 0.33	6.43 $\pm$ 0.13	6.32 $\pm$ 0.45
Serine	2.98 $\pm$ 0.22 <sub>a</sub>	2.11 $\pm$ 0.12 <sub>a</sub>	2.86 $\pm$ 0.21 <sub>b</sub>	2.91 $\pm$ 0.32 <sub>b</sub>
Glutamic acid	3.51 $\pm$ 0.86	3.25 $\pm$ 0.23	3.61 $\pm$ 0.44	3.69 $\pm$ 0.91
Glycine	1.47 $\pm$ 0.04 <sub>a</sub>	1.28 $\pm$ 0.05 <sub>b</sub>	1.31 $\pm$ 0.01 <sub>b</sub>	1.41 $\pm$ 0.08 <sub>a</sub>
Alanine	3.97 $\pm$ 6.21	3.58 $\pm$ 0.68	3.69 $\pm$ 0.76	3.78 $\pm$ 0.42
Cysteine	4.81 $\pm$ 0.22	4.97 $\pm$ 0.12	4.98 $\pm$ 0.21	4.99 $\pm$ 0.32
Tyrosine	2.61 $\pm$ 0.01	2.41 $\pm$ 0.02	2.50 $\pm$ 0.01	2.53 $\pm$ 0.11
Proline	0.87 $\pm$ 0.02	0.78 $\pm$ 0.02	0.79 $\pm$ 0.06	0.81 $\pm$ 0.03

the threonine, methionine, phenylalanine, lysine, histidine, arginine, serine, and glycine content was significantly decreased compared to the control, implying that extended fasting might degrade the availability of amino acids, thereby interfering with protein synthesis (Ferrando et al., 2023). Similar results have been found in *Salmo trutta*, in which deprivation, the representation of the essential AA (threonine and branched-chain amino acids) and non-essential amino acids fell markedly, an indication that feeding disturbance and fasting for a long period of time impair the amino acid pool required to maintain protein synthesis in fish (Labbé et al., 1999). Notwithstanding, Group D were improved in EAA content as compared with group A, indicating that feeding at species' natural circadian metabolic peaks could promote AA transportation and protein synthesis (Montoya-Mejía et al., 2016). Analogous results were also observed in *Oreochromis niloticus* and *Ictalurus punctatus*, where feeding at circadian metabolic peaks was associated with enhanced EAA availability and muscle protein anabolism (Noeske-Hallin et al., 1985; Andersen et al., 2016). In conclusion, the present data suggest that feeding at the species-specific natural circadian metabolic peak may increase the rates of AA uptake and transport, whereas feeding at an inappropriate time could disrupt metabolism and reduce AA availability. Therefore, future studies are needed.

In conclusion, Groups B and C were characterized by poor morphological parameters and reduced crude protein content of the whole-fish and muscle content, which was associated with poor essential AA (threonine and branched-chain amino acids) and non-essential amino acids profile. The feed schedule morning-evening (Group D) gave more or less the same results as the control, which was consistent with the natural metabolic rhythm of the fish. Hence, two feeding times a day can be opted for if an appropriate feeding time is selected that corresponds to the peaks in the fish's metabolism. This may contribute to a decrease in fish waste, enhance growth, nutrient use, and fish health.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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