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Comparative Analysis of Amino Acid Composition in the Head, Muscle and Tail of Fresh African Cat Fish (*Clarias gariepinus*)

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Abstract

Consumption of seafood has increased globally over the past 50 years, from an average of 9.9 kg per capital in the 1960s to over 20 kg in 2016. In Nigeria, African catfish (*Clarias gariepinus*) has gained more attention in terms of farming and consumption compared to other seafood. This project was carried out to determine the level of amino acid composition in the head, muscle and tail of catfish so as to enlighten the public about their choice on the part of catfish to be eaten. Eight different sets of fresh catfish with a mean ± standard deviation of 15.6 kg ± 1.89, which were collected from a local fish pound in Ado-Ekiti, Nigeria, were used for the analysis. Extraction and evaluation of the amino acid composition were carried out using standard analytical techniques. The results obtained showed that high values of amino acids were observed in the fresh catfish (g/100g protein): 13.27 - 13.87 (Glu), 8.76 - 10.39 (Asp), 7.20 - 9.15 (Leu), 7.00 - 8.26 (Lys) and a low value in (Cys), 1.27 - 1.38. The total amino acids had a range of 89.16 - 92.12 g/100g. The coefficient of variation percent (CV %) for all the parameters determined were all generally low with values of 0.74 (observed for Ser) and 39.41 (observed for Pro). Total essential amino acid with histidine values ranged from 38.64 - 42.91 g/100g (CV % = 5.66). It was also observed that the concentrations (g/100g) of amino acids in the muscle of fresh catfish (92.12) are higher than that of the tail (91.98) and that of the head (89.16). There is no appreciable variation in the amino composition of the head, muscle and tail of catfish as the values observed were found to be statistically insignificant ($p > 0.05$). Therefore, it could be posited that the amino acid in the muscle, tail and head of African

catfish would therefore be enough to prevent malnutrition in children and in adults who feed solely on any part of this fish as a main source of protein.

Keywords

African Catfish, Amino Acid Composition, Muscle, Head, Tail

1. Introduction

Fish has been reported to be one of the main products consumed in Nigeria in terms of animal protein. The price is generally acceptable with little or no religious bias, which gives it an advantage over pork or beef [1]. About 2.6 billion people consume 20% of their animal protein from fish and over 400 million people in Asia. At least 50 % of Africa's consumption of animal protein is from fish [2]. In Nigeria, fish is eaten fresh or smoked and form a much cherished and delicacy that cuts across socio-economic, age, religion and educational barriers [3]. African catfish (*Clarias gariepinus*) is extremely nutritious, containing a high concentration of unsaturated fatty acids, vitamins, protein and minerals [4]. Fish is commonly consumed due to the higher cost of meat and other sources of animal protein [5].

African catfish (*Clarias gariepinus*) is a typical air-breathing catfish with a scaleless, bony elongated body with long dorsal and anal fins and a helmet like head. Color varies dorsally from dark to light brown and is often mottled with shades of olive and grey, while the underside is pale cream to white. African catfish farming has increased production and has recently gained significant importance in many African countries [6]. It has an almost Pan-African distribution, ranging from the Nile to West Africa and from Algeria to South Africa [7]. There has been a report on people's choice on the part of fish that contain high nutritional value [8]. Thus the objective of the present study was to determine the level of amino acid composition in the head, muscle and tail of an African catfish with the purpose of providing preliminary information about the essential part of fish that is of high nutritional value in terms of amino acid composition.

2. Materials and Methods

2.1. Chemicals (All Reagents and Chemicals Were of Analytical Grade)

Petroleum spirit (Tedia), Dichloromethane (Fisher), Amino acid standards, Sodium carbonate (Ficher), Ethylchloroformate (Fisher), Isooctane (Fisher), potassium hydroxide. (Fisher).

2.2. Sample Collection and Treatment

Eight different samples of African catfish (*Clarias gariepinus*) with an average weight ± standard deviation of 15.6 kg ± 1.89 were collected from a local pound

at Ado-Ekiti, along Iyin road, Ekiti State, Nigeria. After proper identification at the Ekiti State University Zoological laboratory, the catfishes were washed with distilled water and frozen at −4˚C for 24 h ahead of the analysis. The internal organs were discarded and the other separated parts were gutted and dissected into three parts (head, muscle and tail). These three parts were blended separately and iced prior to analysis.

2.3. Extraction and Amino Acid Composition Analysis

Extraction and instrumentation analysis was carried out by following the modified AOAC Methods [9] and Danka's method [10]. Briefly, the prepared three parts of the fish sample were made to be free of water by ensuring constant weight for a period of time in the laboratory. Exactly 0.5 g of each blended and prepared part were weighed separately and defatted with 30 mL of the petroleum spirit three times with a soxhlet extractor. The defatted samples were soaked with 30 mL 1 M potassium hydroxide solution and were incubated for 48 h in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was then neutralized to get pH in the range of 2.5 - 5.0. The solution was purified by cation-exchange solid-phase extraction. The amino acids in the purified solution were derivatised with ethyl chloroformate. The derivatising reagent was scavenged with nitrogen gas for proper mop up of excess reagent. The derivatised amino acids were dissolved in aliquot part of isooctane and analyzed using gas chromatography.

2.4. Chromatographic Conditions

Chromatographic analysis was carried out using gas chromatography coupled with HP 6890 powered with HP Chemstation Rev. A 09.01 [1206] Software. The sample was analyzed under the following Gas chromatographic conditions. Injection Temperature: Split Injection, Split Ratio: 20:1, Carrier Gas: Hydrogen, Flow Rate: 1.0 ml/min, Inlet Temperature: 250˚C, Column Type: EZ, Column Dimensions: 10 m × 0.2 mm × 0.25 µm, Oven Program: Initial at 110˚C, First: Ramp at /min to 320˚C, Second: Constant for 5 mins at 320˚C, Detector: PFPD, Detector Temperature: 320˚C, Hydrogen Pressure: 20 psi Compressed Air: 35 psi [10].

2.5. Statistical Analysis

Data results obtained were subjected to Microsoft excel application for the grand mean, standard deviation (SD) and coefficient of variation (CV %). The student t-test was conducted to determine the level of amino composition variation between the head, muscle and tail. The data were considered statistically significant if p-value was less than or equal to 0.05.

3. Result and Discussion

Eight different samples of African catfish (*Clarias gariepinus*) were used for the study. The mean ± SD of weight is 15.6 kg ± 1.89. After blending and analyzing

the different parts of the fish, the amino acids composition in the head, muscle and tail of the African Catfish g/100g Crude protein (cp) is presented in **Table 1**. The highest concentrated acidic amino acid in the head, muscle and tail was glutamate (Glu) and Aspartate with a value range of 13.27 - 13.87 and 8.76 - 10.39 respectively. A similar observation has been reported [11]. The highest concentrated essential amino acids were Leucine (Leu), with values of 7.20 - 9.15 and Lysine (Lys) with values of 7.00 - 8.26. The total amino acids had a range of 89.16 - 92.12. The coefficient of variation percent (CV %) for all the compositions showed the very closeness of the values obtained for the parts of the African catfish.

Generally, the nutritional properties of fish render them valuable foodstuffs that are beneficial for human health [12]. From the result in **Table 1**, Glutamate (13.27 - 13.87) was observed to be the amino acid composition with the highest value. This indicates that glutamate is the most concentrated amino acid composition in fish. [11]. It is therefore, responsible for the proper functioning of the brain. The brain needs glutamate to form memories; thus a low level of glutamate causes the problem in the brain. Glutamate has been reported as the major excitatory neurotransmitter in the mammalian central nervous system hence, the increase of glutamate levels in the brain improves its functions. [13]. The present Leu values were 7.20 - 9.15 g 100 g^{-1} protein, and therefore considered safe and could be beneficially exploited to prevent pellagra in endemic areas. Cysteine which is a sulphur containing amino acid contributes to the sulfhydryl group in the glutathione molecule. The body requires three amino acids – glutamate, glycine and cysteine in other to manufacture glutathione on its own inside the cell. This means that the level of cysteine is a limiting factor in how fast and how much glutathione and can be produced in ghee body. Only the cysteine of a specific form can actually enter the cell [14]; thus a low amount of cysteine is required, which tallies with the result of cysteine in the study (1.27 - 1.38)g/100g.

The concentration (g/100g) of amino acids in the fresh *Clarias gariepinus* is higher in the muscle (92.12), probably due to the dominance of fish flesh in this section, followed by the tail (91.98) and the least was found in the head (89.16) due probably to the high amount of bone casing in the head. This is to say that the muscle is richer in amino acids than the tail and the head respectively (MF > TF > HF). This agrees with the findings of [15], who reported high higher levels of protein in the middle and tail and least level at the head in *Heterobranchus bidorsalis* and the findings of [16], who reported high higher levels of protein at the middle and tail and least level at the head in pink salmon. The total amino acid ranged from 89.16 - 92.12 g/100g which was higher than the value 777.0 mg·g^{-1} cp reported for *S. africanus africanus* female flesh [17].

The standards (g/100g protein) for pre-school children (2 - 5 years) are: Leu (6.6), Phe + Tyr (6.3), Thr (3.4), Try (1.1), Val (3.5), Ile (2.8), Lys (5.8), Met + Cys (2.5), His (1.9) and total (33.9 with His) and 32.0 (no His) [18]. Based on this information, both head and tail would individually provide adequate Leu, Phe + Tyr, Thr, Val, Ile, Lys, Met + Cys, His and total EAA, whereas muscle satisfied all the requirements except in Thr (3.29 < 3.40g/100g).

Table 1. The amino acid profile in the Head, Muscle and Tail of fresh African catfish, *Clarias gariepinus* in g/100g of Protein (P).

Amino Acids	Head Fresh	Muscle Fresh	Tail Fresh	MEAN	SD	CV%
Gly	4.25	3.27	4.28	3.93	0.57	14.50
Ala	6.82	4.87	6.31	6.00	1.01	16.83
Ser	4.01	4.06	4.06	4.04	0.03	0.74
Pro	3.00	5.90	3.28	4.06	1.60	39.41
Val	4.38	4.87	4.98	4.74	0.32	6.75
Thr	3.92	3.29	4.15	3.79	0.45	11.87
Ile	3.68	3.54	5.01	4.08	0.81	19.85
Leu	7.20	9.15	8.96	8.44	1.08	12.80
Asp	8.72	10.39	8.76	9.29	0.95	10.23
Lys	8.26	7.00	8.08	7.78	0.68	8.74
Met	2.55	2.22	2.92	2.56	0.35	13.67
Glu	13.46	13.87	13.27	13.53	0.31	2.29
Phe	3.60	4.38	4.00	3.99	0.39	9.77
His	4.10	2.07	2.71	2.96	1.04	35.14
Arg	5.80	6.31	4.84	5.65	0.75	13.27
Tyr	2.44	3.43	2.96	2.94	0.50	17.01
Try	1.70	2.12	2.10	1.97	0.24	12.18
Cys	1.27	1.38	1.31	1.32	0.06	4.55
Total	89.16	92.12	91.98	91.09	1.67	1.83

Gly: Glycine, Ala: Alanine, Cys: Cysteine, Ser: Serine, Pro: Proline, Val: Valine, Thr: Threonine Ile: Isoleucine, Leu: Leucine, Asp: Aspartate, Lys: Lysine, Met: Methionine, Glu: Glutamate, Phe: Phenylalanine His: Histidine, Arg: Arginine Tyr: Tyrosine Try: Tryptophan, SD: Standard Deviation, CV%: Coefficient of Variation.

The comparison of the mean concentrations of *Clarias gariepinus* and Turkey-hen samples showed that the values of Lys, His, Arg, Asp, Ser, Pro, Ala, Cys, Val, Met, Ile and Leu are less in Turkey-hen samples than in *Clarias gareipinus*. This is to say that *Clarias gariepinus* is richer in amino acid than Turkey-hen samples [19]. Also, in the evaluation of *Clarias gariepinus* with African giant pouch rat (*Cricetomys gambianus*), it was discovered that the concentrations of Gly (g/100g) in the muscle of *Clarias gariepinus* is low compared to the concentration of Gly in the heart and liver of African giant pouch rat (*Cricetomys gambianus*), but that of the head and tail is higher than the Gly concentration of African giant pouch rat (*Cricetomys gambianus*). Also, the concentration of Lys, Ser, Glu, Ala and Cys in African giant pouch rats (*Cricetomys gambianus*) (Heart and Liver) is less than the concentration of Lys in *Clarias gariepinus*. For Arg, Val, Tyr and Asp composition, the concentration of the heart and liver of

African giant pouch rat (*Cricetomys gambianus*) is greater than that of *Clarias gariepinus*. For Thr, the concentration in *Clarias gariepinus* is greater than that of the heart of African giant pouch rat (*Cricetomys gambianus*) but less than that of the liver of African giant pouch rat (*Cricetomys gambianus*). The concentration of Pro in African giant pouch rat (*Cricetomys gambianus*) is greater than the concentration in the head and tail, but less than the concentration in the muscle of *Clarias gariepinus* [20].

The summary of parameters of amino acid contents (g/100g cp) of the samples is presented in **Table 2**. The total of all the parameters for head, muscle and tail was observed to be 513.32, 529.06 and 541.99 respectively. The tail was observed to be more concentrated with all the parameters than any other parts of the catfish in respect to the total composition of the majored essential amino acid, most especially sulphur containing essential amino acids like (Cysteine, and methionine) which could be responsible for the high amount of the total activity in the tail. This particulate essential amino acids composition is a basic criteria for evaluating the nutritive value of fish [2]. The total amino acid ranges from 89.16 - 92.12 g/100g. However, the total amino acid content of fresh *Clarias gariepinus* (95.03 g/100g) has been reported [21]. The essential amino acid range was 38.64 - 42.91 g/100g with a CV% of 5.66. These values were more than half the average of 56.6 g/100g cp of the egg reference protein [22]. The total sulphur amino acids (TSAA) of the samples range was 3.60 - 4.23 g/100g. These values were close to the 5.8 g/100g cp recommended for infants [18]. The aromatic amino acid range suggested for infant protein (6.8 - 11.8 g/100g cp) [18] can be compared favorably with the present report of 8.57 - 9.40 g/100g cp showing that the samples protein could be used to supplement cereal flowers [17]. The percentage of TEAA to the total AA (TAA) in the samples ranged from 41.96 - 46.65 g/100g. These values were well above the 39% considered adequate for ideal protein food for infants, 26% for children and 11% for adults [18]. The percentage TEAA/TAA for the samples could be favorably compared with other animal protein sources: 46.2% in *Zonocerus variegatus* [23], 43.7% in *Macrotermes bellicossus* [24], 54.8% in *Gymnarchus niloticus* (Tail fish) [25] and 48.1% - 49.9% in brain and eyes of African giant pouch rat [26]. The TEAA in these results were close to the value of 44.4 g/100g cp in soybean [27], melon and gourd oilseeds with respective values of 53.4 g/100g cp and 53.6 g/100g cp [28].

The percentage of total non-essential amino acid (TNEAA) ranged from 53.35 - 58.05, indicating that these formed the bulk of the AA; the percentage of total essential AA –with His (TEAA) ranged from 41.95 - 46.65 which were lower than % TNEAA, while the percentage range in total neutral AA (TNAA) was 41.70 - 45.49 which made them the third largest group among the samples.

Statistical comparison between the head, muscle and tail presented in **Table 3** was carried out to know if the differences in the amino composition present in the parts of the fish are statistically significant. P-value obtained is greater than 0.05 which indicates the values are statistically insignificant.

Table 2. Concentrations of essential, aromatic, non-essential, neutral amino acid (g 100 g^{-1} protein) of the Head, Muscle and Tail of *C. gariepinus*.

Amino Acids	Head	Muscle	Tail	Mean	SD	CV%
TAA	89.16	92.12	91.98	91.09	1.67	1.83
TEAA -with His	39.39	38.64	42.91	40.31	2.28	5.66
-no His	35.29	36.57	40.20	37.35	2.55	6.83
TNEAA	49.77	53.48	49.07	50.77	2.37	4.67
% TNEAA	55.82	58.05	53.35	55.74	2.35	4.22
% TEAA -with His	44.18	41.95	46.65	44.26	2.35	5.31
% TEAA -no His	39.58	39.70	43.71	41.00	2.35	5.73
TEALAA	25.08	28.33	28.54	27.32	1.94	7.10
% TEALAA	28.13	30.75	31.03	29.97	1.60	5.34
TEARAA	9.40	8.57	8.81	8.93	0.43	4.82
% TEARAA	10.54	9.30	9.58	9.81	0.65	6.63
TNAA	37.18	40.32	41. 84	39.78	2.38	5.98
% TNAA	41.70	43.77	45.49	43.65	1.90	4.35
TSAA	3.82	3.60	4.23	3.88	0.32	8.25
% TSAA	4.28	3.91	4.60	4.26	0.35	8.22
Total	513.32	529.06	541.99			

TEAA (Total essential amino acid): His, Iso, Leu, Lys, Met, Phe, Thr, Try, Val; TNEAA (Total non-essential amino acid): Ala, Arg, Asp, Cys, Glu, Gly, Pro, Ser, Tyr; TEALAA (Total essential aliphatic amino acid): Ala, Iso, Leu, Pro, Val; TEARAA (Total essential aromatic amino acid): Phe, Try, His; TNAA (Total neutral amino acid): Try, Phe, Gly, Ala, Val, Iso, Leu, Met, Pro; TSAA (Total sulphur amino acid): Met, Cys.

Table 3. Statistical comparison of the amino compositions in the head, muscle and tail of catfish.

Amino Acid	H + M	H + T	M + T
Total	181.28	181.14	184.10
S.D	3.0926	2.9486	3.0791
P –Value	0.88	0.88	0.99

Values are expressed as H= head, M = Muscle, T = Tail $p > 0.05$.

4. Conclusion

This study has elucidated that, *Clarias gariepinus* is a good source of high-quality protein with adequate essential amino acids in comparison with other protein sources. Concomitantly, the present study also evaluated the amount of amino acid in the head, muscle and tail of African catfish and found it fit for consumption. There is no appreciable variation in the amino composition of the head, muscle and tail of catfish, as the values were found to be statistically insignificant ($p > 0.05$).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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