Amino acid profile of different size grades of farmed African catfish, C*lariasgariepinus*Burchell, 1822.

Osho F. E.*, Ajani E. K., Ndoh O. N. and Ekwerike A. O.
Department of Aquaculture and Fisheries Management, University of Ibadan
**Corresponding author: oshofriday@yahoo.com*

Abstract

Clariasgariepinus obtained from a reputable fish farm in Ibadan were sorted into four groups based on sizes: small (150-250 g), medium (251-450 g), large (451-650 g), very large (651-1000 g). Amino acid profiles were determined using the Applied Biosystems PTH Amino Acid Analyzer. Protein quality was estimated using the amino acid score formula, while statistical analysis was carried out using one-way ANOVA and Tukey's test was used to test for significant differences (p>0.05) in the amino acid composition of the four treatments. All the size grades contained eighteen amino acids, including all the nine essential amino acids*considered essential for humans*. There was no significant difference in the concentrations of leucine, isoleucine, tryptophan, methionine, histidine, tyrosine, alanine, and serine among the four treatments studied. However, total amino acid concentration (g/100 g protein) were significantly higher (p<0.05) in small (89.74 ±0.10) and medium sized groups (89.01±0.08), compared tothe large (80.10± 0.09) and very large ones (82.36±0.09). Concentrations of isoleucine, valine, threonine, histidine, cysteine, proline, serine, and aspartic acid decreased significantly (p<0.05) with increasing size of fish from small to large, then increased in group very large. The amino acid score of leucine, lysine, isoleucine, valine, methionine + cysteine, and threonine were significantly different (p<0.05) among the four groups of fish. Leucine, isoleucine, valine, and threonine all had high amino acid scores in the small sized fish group with 1.18, 1.13, 0.83 and 0.99, respectively while methionine + cysteine, tryptophan, phenylalanine + tyrosine and lysine had highest values recorded in the medium sized group. From this study, it could therefore be deduced that of *C.gariepinusthat should be consumed for* optimal protein quality is 150 to 450 g, corresponding to the small and medium sized fishes.

Keywords: Amino acid score, *Clariasgariepinus*, Essential amino acid, Protein quality

Introduction

As the world's population increases rapidly against the limitations of land and water resources, it is increasingly important to accurately define, the quantity and quality of proteins required to meet human food need and appropriately define the protein obtained from food ingredients and whole foods (FAO, 2013). Fish is an important component of menus globally, essential for global ecological balance, and serves as a crucial source of livelihood worldwide (FAO, 2018). In Nigeria, fish constitutes 40% of protein intake (Ajani and Osho, 2019), sometimes contributing more than 60% of total animal protein intake in rural areas (Adekoya and Miller, 2004). The nutritional values of fish are predominantly derived from its high protein content (Taiwo *et al*., 2014). Fish proteins contain all the important amino acids needed for a healthy human nutrition. The amino acid composition is one of the most important nutritional qualities of protein, and the amino acid score is used to evaluate protein quality worldwide (FAO/WHO/UNU, 2002). Certain amino acids like the aspartic acid, glycine and glutamic acid are known to playessential roles in the process of wound healing. Aside from their high nutritive value, amino acids also provide several health benefits, such as reduction of blood cholesterol (Gibbs *et al.,* 2004). Some amino acids like tyrosine, methionine, histidine, lysine and tryptophan act as antioxidants (Saito *et al*., 2003) while aspartic acid, glutamine, proline, glycine and leucine have strong cytotoxicity against carcinogenic cells (Dutta *et al*., 2013)

Due to a decline in wild fish populations and thegrowing human population, aquaculture is expected to fill the gap in supplies of food fish and raw materials for industries, as demand continues to increase (Osho *et al.,* 2016). *Clariasgariepinus*is the most cultured fish species in Nigeria and sub-Saharan Africa and consequently the most consumed farm-raised fish in the sub-region (FAO, 2016). It is wildly cultured for its good meat quality, high growth rate irrespective of high stocking densities as well as its ability to tolerate adverse culture water quality (Taiwo *et al*., 2014). The role of this fish in attaining protein food security, amelioration of malnutrition and job creation has therefore been gaining increased attention (FAO, 2016). Increasing demand has led to the harvest of different life stages of this fish to satisfy market demands. Several studies have been carried out on the proximate composition of *C. gariepinus* (Osibona*et al.,* 2009; Funmilayo, 2016) as well as the comparative analysis of the amino acid profiles of the wild and farmed *C. gariepinus* (Taiwo *et al*., 2014; Ibhadon*et al*., 2015). However, additional information on the amino acid composition of different sizes of farmed*C. gariepinus*harvested for food is vital for food security and nutrition decisions. Therefore, this research work sought to document and compare the amino acid profiles and estimate the quality of dietary protein contained in the different size grades of farmed *C. gariepinus*.

Materials and Methods

Experimental fish

Clariasgariepinus samples were obtained from a reputable private fish farm in Ibadan, South-West, Nigeria. Sample sizes ranged from 150 g to 1000 g. The sizes were graded as shown in Table 1. Each sample group was regarded as a treatment, with each treatment containing three samples. All analyses were conducted in triplicates for each treatment (Usydus*et al*., 2009).

Table 1: Size Grades of Experimental Fish

Group	N	Weight (g)
Small	9	150 – 250
Medium	9	251 – 450
Large	9	451 – 650
Very large	9	651 – 1000

Sample Preparation

In order to achieve homogeneity, the fish samples were filleted, oven-dried and blended. Oven drying was carried out at 105 °C for 24 hours using a laboratory oven, before blending.

Amino Acid Determination

The amino acid profiles were determined using methods described by Benitez (1989). The samples were dried to constant weight, defatted and hydrolyzed using 6N Hydrochloric acid (HCl), except for tryptophan. Tryptophan detection is by alkaline hydrolysis. This was carried out using 4.2N Sodium hydroxide (NaOH).

Hydrolysates were then evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Model 120A Analyzer for amino acid determination. An integrator, Model C-R43, attached to the Analyzer calculated the peak area proportional to the concentration of each amino acids, which were identified using available standards. The quality of dietary protein was measured by finding the ratio of available amino acids in the protein concentrate, compared with needs expressed as a ratio (Audu and Aremu, 2011). Amino acid score (AAS) was then estimated by applying the FAO/WHO (1991) formula:

$$AAS = \frac{mg\ of\ amino\ acid\ in\ 1g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1g\ of\ reference\ protein}\ x\ \frac{100}{}$$

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) while means were separated using Tukey's test at 5% significant level (Sayad *et al*., 2016). All statistical analyses of data were performed using SPSS 22.0 (2013) software and the data were reported as mean values ± standard deviation (SD).

Results

Amino acid profile of different size grades of farmed*Clariasgariepinus*

Eighteen different amino acids were obtained in the four sampled groups. The nine essential amino acids; leucine, lysine, isoleucine, phenyalanine, tryptophan, valine, methionine, histidine, and threonine were all present in the four sampled groups. Cystine, tyrosine, arginine, alanine, glutamic acid, glycine, proline, serine, aspartic acid were the non-essential amino acids obtained from the four sample groups (Table 2).

Amino acid composition of different size grades of *Clariasgariepinus*

The total amino acid composition in g/100 g protein for the fish in the small, medium, large and very large categories were 89.74, 89.01, 80.10, and 82.36 g/100 g protein, respectively, with the small sized fish having the highest total amino acid composition (89.74 g/100g protein). The predominant essential amino acid in all the four groups was leucine, with concentrations of 8.21, 7.94, 7.37, and 7.28 g/100 g protein, respectively while the major non-essential amino acid (NEAA) was glutamic acid 15.14, 14.53, 13.89, 13.48 g/100 g protein for sample groups small, medium, large, and very large, respectively. The concentration of leucine was not significantly different ($p>0.05$) but glutamic acid content differed significantly among the treatments ($p<0.05$). Cysteine was the least concentrated essential amino acid in all the groups, except small sized fish, which had tryptophan as the least concentrated amino acid.

Lysine, phenylalanine, tryptophan, methionine, arginine, alanine and glycine all had high values recorded in the medium sized fish (251-450 g) compared to the other size categories of *C. gariepinus* (Table 2). Small sized *fish* had the highest concentrations of cysteine, isoleucine, glutamic acid, proline, serine, while the very large sized fish had the highest concentration of tyrosine. Large sized fish had the least concentration of all the amino acids, except glutamic acid, leucine and lysine which were least concentrated in the very large sized group. Lysine, phenylalanine, valine, cysteine, arginine, glutamic acid, glycine, proline, aspartic acid concentrations were significantly different (p<0.05) among the various size groups of *C. gariepinus*. Lysine, phenylalanine, threonine, arginine, glycine, and proline varied significantly between the medium and large sized fish groups, and medium and very large sized groups, while concentrations of aspartic acid and arginine were significantly different (p<0.05) between the large sized and very large fish groups. However, there were no significant differences in the concentration of leucine, isoleucine, tryptophan, methionine, histidine, tyrosine, alanine, and serine among the four treatments. Concentrations of isoleucine, valine, threonine, histidine, cysteine, proline, serine, and aspartic acid decreased as the size of fish increased from the small sequentially to the very large size categories.

However, there were fluctuations in the concentration of tryptophan, methionine, arginine, alanine, glycine, lysine, tyrosine, and phenylalanine as the size of fish increased. Most amino acids in these later group had their peak concentrations in the medium sized stage when the fish were between 251-450 g in weight.

Amino Acid Score

The amino acid score of leucine, lysine, isoleucine, valine, methionine + cysteine, and threonine were significantly different (p>0.05) among the four size groups of fish. Leucine, isoleucine, valine, and threonine all had the high scores in the size group A with 1.18 ± 0.01, 1.13 ± 0.01, 0.83 ± 0.04 and 0.99 ± 0.02, respectively while methionine + cystine, tryptophan, phenylalanine + tyrosine

Table 2: Amino acid composition (g/100g protein) of different size grades of *Clariasgariepinus*

AMINO ACIDS	Small	Medium	Large	Very large
		Essential Amino Acids		
Leucine	8.21 ± 0.04[a]	7.94 ± 0.08[a]	7.37 ± 0.18[a]	7.28 ± 0.44[a]
Lysine	6.71 ± 0.18[b]	7.21 ± 0.07[a]	6.39 ± 0.04[bc]	5.97 ± 0.11[c]
Isoleucine	4.52 ± 0.04[a]	4.31 ± 0.06[a]	3.82 ± 0.12[a]	3.96 ± 0.04[a]
Phenyalanine	4.17 ± 0.13[ab]	4.48 ± 0.06[a]	3.86 ± 0.19[b]	3.86 ± 0.06[b]
Tryptophan	1.24 ± 0.04[a]	1.26 ± 0.07[a]	1.13 ± 0.04[a]	1.22 ± 0.01[a]
Valine	4.16 ± 0.21[a]	3.92 ± 0.04[ab]	3.67 ± 0.06[b]	3.85 ± 0.06[ab]
Methionine	2.59 ± 0.04[a]	2.79 ± 0.06[a]	2.22 ± 0.04[a]	2.33 ± 0.04[a]
Histidine	2.37 ± 0.09[a]	2.27 ± 0.04[a]	2.27 ± 0.04[a]	2.33 ± 0.04[a]
Threonine	3.95 ± 0.08[a]	3.64 ± 0.04[b]	3.23 ± 0.06[c]	3.39 ± 0.04[c]
		Non-Essential Amino Acids		
Cystine	1.36 ± 0.04[a]	1.21 ± 0.00[ab]	1.03 ± 0.08[b]	1.18 ± 0.04[ab]
Tyrosine	3.10 ± 0.00[a]	3.27 ± 0.00[a]	3.10 ± 0.00[a]	3.44 ± 0.00[a]

Arginine	**6.41 ± 0.18[b]**	**7.06 ± 0.12[a]**	**5.33 ± 0.00[c]**	**5.94 ± 0.12[b]**
Alanine	4.23 ± 0.08[a]	4.53 ± 0.08[a]	4.08 ± 0.19[a]	4.46 ± 0.03[a]
Glutamic Acid	15.14 ± 0.21[a]	14.53 ± 0.21[ab]	13.89 ± 0.16[bc]	13.48 ± 0.22[c]
Glycine	4.63 ± 0.10[ab]	5.06 ± 0.17[a]	4.04 ± 0.07[c]	4.29 ± 0.12[bc]
Proline	3.91 ± 0.07[a]	3.60 ± 0.07[b]	3.25 ± 0.00[c]	3.35 ± 0.00[c]
Serine	3.55 ± 0.06[a]	3.18 ± 0.09[a]	3.01 ± 0.10[a]	3.10 ± 0.13[a]
Aspartic Acid	9.49 ± 0.17[a]	8.75 ± 0.09[bc]	8.41 ± 0.13[c]	8.93 ± 0.08[b]
Total Amino Acids	89.74 ±0.10[a]	89.01±0.08[a]	80.10± 0.09[b]	82.36±0.09[b]

Values with the same letters across rows are not significantly different (P > 0.05)

and lysine had highest values recorded in the group B sizes. Most of these sets of amino acids were least in the very large size class. However, tryptophan and phenylalanine + tyrosine did not differ significantly (p>0.05) in their amino acid scores among the four groups (Table 3).

Table 3: Amino acid score of the four size grades of farmed *Clariasgariepinus*

Amino acids	**Fish groups**			
	Small	medium	Large	very large
Leucine	1.18 ± 0.01[a]	1.14 ± 0.01[ab]	1.05 ± 0.03[ab]	1.04 ± 0.06[b]
Lysine	1.22 ± 0.03[b]	1.31 ± 0.01[a]	1.17 ± 0.01[b]	1.09 ± 0.02[c]
Isoleucine	1.13 ± 0.01[a]	1.08 ± 0.01[a]	0.96 ± 0.04[b]	0.99 ± 0.01[b]
Phe + Tyr	1.22 ± 0.02[a]	1.29 ± 0.02[a]	1.23 ± 0.13[a]	1.22 ± 0.01[a]
Tryptophan	1.24 ± 0.04[a]	1.26 ± 0.07[a]	1.13 ± 0.04[a]	1.22 ± 0.01[a]
Valine	0.83 ± 0.04[a]	0.79 ± 0.01[ab]	0.73 ± 0.01[ab]	0.77 ± 0.01[b]
Meth + Cyst	1.13 ± 0.03[ab]	1.23 ± 0.11[a]	0.93 ± 0.01[b]	1.00 ± 0.00[ab]
Threonine	0.99 ± 0.02[a]	0.91 ± 0.01[b]	0.81 ± 0.01[c]	0.85 ± 0.01[c]

Values with the same superscripts across rows are not significantly different (P > 0.05) Keys: Phe + Tyr = Phenyalanine + Tryosine; Meth + Cyst = Methionine + Cystine

Discussion

Total amino acids detected and quantified in the studied *Clariasgariepinus* were 18, comprising nine essential and nine non-essential amino acids. The presence of all the essential amino acids needed in human diet in *C. gariepinus* makes it an excellent choice for food and nutrition security because failure to take enough of any one of the essential amino acids may lead to the degradation of the proteins in the muscles. Arginine, histidine and serine are classified as semi-essential amino acids because they also have to be supplied under extreme conditions such as stress, intensive growth and certain disease (Usydys*et al*., 2009). According to Hryniewiecki (2000), the quality of a protein is determined by factors such as the contents of its essential and nonessential amino acids as well as the mutual proportions of specific amino acids. The more similar it is to the human body, the better. The present study has shown that catfish possesses high quality protein. The amino acid profile and composition obtained in this study were like the results obtained for *C. gariepinus* and *Coptodonzilli* by Osibona*et al*. (2009), but higher than the amino acid profiles of *Parachanna obscura, Hepsetusodoe* and *C. gariepinus* documented by Funmilayo (2016).

The dominance of glutamic acid in all four treatments agreed strongly with studies carried out by Funmilayo (2016) on *P. obscura*, *Hepsetusodoe*and *C. gariepinus* and *Coptodonzilli;* Ovie and Ovie, (2007) on *Heterobranchuslongifilis*; Sayad *et al*., (2016) on *Lates niloticus*; and Osibona (2011) on *C. gariepinus, C. zilli, Pentanemusquinquarius*and*Pseudolithus typus. Glutamic acid plays* important roles in amino acid metabolism, including transamination reactions and the synthesis of key molecules and it is also *one of the factors responsible for the* umami taste of food fish (Andersen *et al*., 2016). Glutamic acid is also the most versatile amino acid in the human body, reputed for enhancing memory and focus, improving the immune system, supporting prostate health, detoxifying the body, improving athletic performance and immensely promoting digestive health (Cruzat *et al*., 2018).

Lysine is an EAA which is extensively required for optimal growth. Useful for preventing and treating cold sores (Chen *et al*., 2003). A deficiency in lysine in animal diet would automatically lead to immunodeficiency (Chen *et al.,* 2003). Arginine has been documented to play important role for proper growth in children. It plays an important role in cell division, wound healing, ammonia removal, immune function, and hormone release. Leucine is the only dietary amino acid that can stimulate muscle protein synthesis and it also performs therapeutic role when an organism is under stress conditions (Brandt *et al*., 2009). Tryptophan is used by the brain to produce serotonin, a relaxing neurotransmitter. It can reduce pain sensitivity, work as an anti-depressant, reduce anxiety and tension as well as induce sleep (Richard *et al*., 2009). The predominant essential amino acid observed in this study was leucine. However, in a study carried out by Ovie and Ovie (2007) on *Heterobranchuslongifilis,* arginine was the highest essential amino acid found in fingerlings and adults while leucine was the highest in fry. Osibona*et al*. (2009) also observed lysine to be the most concentrated essential amino acid in *C. gariepinus* and *C. zilli*.Amino acid variation among stocks and species may also be influenced by environmental factors such as feed (Ibhadon*et al.,* 2015). Leucine is one of the branched amino acids that help to upregulate the production of protein or breakdown of excess protein, thereby maintaining muscle health (Andersen *et al*., 2016).

In the current study, different amino acids varied significantly in concentration across the four treatment groups, except leucine, isoleucine, tryptophan, methionine, histidine, tyrosine, alanine, and serine. This observation is contrary to that of Ovie and Ovie (2007) that concentrations of amino acid did not differ significantly among different stages of growth of *Heterobranchuslongifilis*. This could be due to the fact that their study compared the amino acid composition of fry, fingerlings and broodstock, while this study focused on the grow out stages of *C.gariepinus*. This study also showed that the concentration of isoleucine, valine, threonine, histidine, cysteine, proline, serine, and aspartic acid decreased as size of fish increased from the small to the large sized fish after which a steady rise was observed as fish size further increased in group very large sized fish. This may be attributed to a gradual decrease in dietary protein requirement of fish as size increases. From a crude protein requirement of 50-55% at larval stage, would require 40-43% crude protein in their diet at adult stage (FAO, 2013). This reduction in dietary protein intake could account for the reduction in the amino acid composition observed as fish sizes increased. The observed increase in amino acid composition as size increased from 650 g to 1kg (group D) could be attributed to a decrease in the crude protein requirement in the diet of the fish as they grew older and bigger (Robinson and Li, 2007). The observations from this study agreed with the conclusions of Ovie and Ovie (2007) that amino acid composition varies with different size of fish. Amino acids act as intermediates in various metabolic pathways. They serve as precursors for synthesis of a wide range of biologically important substances including nucleotides, peptide hormones, and neurotransmitters (Mohanty *et al*., 2014). Amino acids also play important roles in cell signaling and act as regulators of gene expression and protein phosphorylation cascade (Wu, 2010), nutrient transport and metabolism in animal cells (Wang *et al*., 2013), and innate as well as cell-mediated immune responses.

In the present study, there were no significant differences ($p>0.05$) in the concentrations of leucine, isoleucine, tryptophan, methionine, histidine, tyrosine, alanine, and serine among the four studied fish sizes. In estimating the protein quality using the amino acid score, the small size fish group was found to have the lowest number for limiting amino acids – just one – followed by medium, large, and very large sizes, respectively. It can therefore be inferred, that, of the four treatments, the group with the small sized fish (150-250 g) had the highest amino acid composition and protein quality, followed by group small (251-450 g), large (651 g-1 kg) and very large size fish (451-650 g). Among the nine essential amino acids, group A (150 – 250 g) had the highest concentration of leucine, isoleucine, valine, threonine, and histidine, while group B (251 – 450 g) had the highest concentration of lysine, tryptophan and methionine. Tyrosine, methionine, histidine, lysine and tryptophan are considered to act as antioxidants (Saito *et al*., 2003) while aspartic acid, glutamine, proline, glycine and leucine have been reported by Kim *et al*., (1999) to possess strong cytotoxic activity against cancer cells. According to Ruiz-Capillas and Moral (2004), the free amino acids, such as glutamic acid, aspartic acid, alanine, and glycine are responsible for the characteristic flavour of fish.

The quality of protein supply can be partially determined by its potential to perform the constant physiological activities and meet metabolic demands (Dalibard*et al.*, 2014). The EAA composition of the small and medium sized fish groups were higher than the recommendation of (FAO/WHO/UNU, 2002) except for valine and threonine which had AAS values less than 1.0. The large sized fish had five essential amino acids – isoleucine, valine, threonine, and methionine + cysteine with AAS values less than 1.0 which is the recommended amino acid score for quality proteins; while in group D, isoleucine, methionine, and threonine with amino acid score values less than recommended (FAO/WHO/UNU, 2002). It has been reported that the essential amino acids most often acting in a limiting capacity in protein sources are methionine (and cysteine), lysine and tryptophan. However, in the present study, valine and threonine were the limiting amino acid in all size groups, while isoleucine was limiting in the large and very large sized fish.

Conclusion

Findings from this study revealed that all the four size grades of farmed adult catfish contained all eighteen amino acids, including the nine essential amino acids. The amino acid composition of *Clariasgariepinus* decreased with increasing size of the fish. This can be attributed to the fact that dietary protein requirement of catfish decreased with an increase in the size of fish. The small sized fish(150-250 g) had the highest amino acid composition and protein quality, followed by medium (251-450 g), large (451-650 g) and very large sized fish groups, respectively (651-1000 g). Therefore, the recommended size range of *C. gariepinus* to consume for optimal protein quality is 150 to 450 g.

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