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Research Article

Fatty Acids and Amino Acids Content of *Heterotis niloticus* (Cuvier, 1829) and *Protopterus annectens* (Owen, 1883) from Lake Alau, Northeast Nigeria

Olanrewaju, Adewale Nurudeen

Federal College of Freshwater Fisheries Technology, Baga, P.M.B 1060, Maiduguri, Nigeria

Abstract

Background and Objective: The amino and fatty acids composition of edible fishes occupies a unique position in nutrition but information on major nutritive compositions of most freshwater fishes is scanty. Thus, this study was carried out to determine the fatty and amino acids compositions of *Heterotis niloticus* and *Protopterus annectens* from Lake Alau. **Materials and Methods:** Ten live samples (weight: 500.00-2800.00 g) of each species were analyzed for fatty and amino acids using standard procedures. Data were analyzed using descriptive and chi-square statistics. **Results:** Total acid value (105.19 ± 117.62 g 100 g⁻¹) was higher ($p < 0.05$) in *P. annectens*, than *H. niloticus* (71.55 ± 52.96 g 100 g⁻¹). Oleic acid (183.00 ± 71.00 mg g⁻¹) and palmitic acid (18.00 ± 1.50 mg g⁻¹) were higher in *H. niloticus* than those of *P. annectens*, but no significant variations were observed ($p > 0.05$). However, the lauric acid content of *H. niloticus* was slightly higher than that of the *P. annectens* ($p > 0.05$). Eighteen amino acids with varying levels were profiled from the two fish species. Methionine (1.39 ± 0.09 g 100 g⁻¹), lysine (4.73 ± 0.35 g 100 g⁻¹), threonine (1.77 ± 0.08 g 100 g⁻¹), isoleucine (4.03 ± 0.04 g 100 g⁻¹) and leucine (8.85 ± 0.06 g 100 g⁻¹) were significantly higher ($p < 0.05$) in *H. niloticus* than in *P. annectens*. However, *P. annectens* showed markedly high valine (3.98 ± 0.14 g 100 g⁻¹), arginine (4.52 ± 0.32 g 100 g⁻¹) and proline (3.78 ± 0.59 g 100 g⁻¹) than values obtained in *H. niloticus*. **Conclusion:** The study revealed that *H. niloticus* and *P. annectens* were a good sources of monosaturated omega-9 fatty acids with rich essential and non-essential amino acids.

Key words: *Heterotis niloticus*, *Protopterus annectens*, nutritive composition, Omega-9, Lake Alau

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Corresponding Author: Olanrewaju, Adewale Nurudeen, Federal College of Freshwater Fisheries Technology, Baga, P.M.B 1060, Maiduguri, Nigeria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish is one of the valuable and safe foods highly recommended for man's healthy living. It greatly complements the food supply made available largely by land-based agriculture as it provides a crucial source of animal protein, fatty acids, minerals and essential micronutrients¹. The proteins in fish are easily digestible and rich in essential amino acids which advance the healthy development of infants, growing children and expectant mothers². This justifies the ever-increasing preference for fish food consumption among rural and urban dwellers especially those in riverine and coastal areas. Nutrient composition of fish, however, varies from one species to another and also within the same species due to season, geographical regions, age, maturity stage and food availability³. Similarly, fresh and marine water fish species do not have the same composition and even within the same environment, the chemical composition varies⁴. These variations in composition constitute the basis for the classification of fish into fat or lean fish.

A fish is considered "lean" when its flesh contains less than 2% fat, while fat fish possess high-quality lipids (about 28%). Skåre *et al.*⁵ reports that fatty fish contain more fat in their tissues and have a larger variety of fatty acids than lean fish, while lean fish contain more iodine and less energy than fatty fish. Fish have been linked to many health benefits, including a lower risk of heart disease, improved mental ability, protection from cancer, alcohol-related dementia and rheumatoid arthritis⁶.

In Nigeria, fish from the artisanal fishery constitute the major chain of supply, especially in the inland waters where over 316 fish species have been so far catalogued⁷. The West African Lungfish (*Protopterus annectens*) and African Bony tongue (*Heterotis niloticus*) are widely distributed in Nigeria inland waters and grow large to the relish of riparian communities. These scaly species have good quality flesh enhanced by their high muscle to bone ratio and their bones and cartilage pose less danger of choking consumers. Extensive study has been conducted on fatty and amino acids profiling of many Nigeria inland fish species⁸⁻¹¹ but scanty reports exist on *Heterotis niloticus* and *Protopterus annectens*. The present study was therefore carried out to analyze the fatty and amino acid composition of *H. niloticus* and *P. annectens* captured from Lake Alau, Nigeria.

MATERIALS AND METHODS

Study area: Lake Alau, the second largest lake in Borno State, Nigeria is located between latitude 11°39'84"-11°40'02"N and

longitude 13°39'92"-13°40'12"E. It was created by damming river Ngadda about 22 km from Maiduguri, along Bama road. It is about 354 m above sea level with a total surface area of 56 km². The annual inflow into Lake Alau was calculated to be 329,000 cubic meters of water¹². The Lake was built in the late 1980s primarily for the provision of potable water but has since sustains a thriving artisanal fisheries industry.

Sample collection and preparation: Ten live samples each of *Heterotis niloticus* and *Protopterus annectens* (weight: 500.00-2800.00 g) were collected from fishermen landing at Lake Alau. The sampling was done during morning hours and the randomly captured samples were selected and morphometrically identified. Specimens collected were sorted and immediately transported to the Biochemistry Laboratory, Yobe State University, Nigeria in ice boxes for analyses. Thereafter, the fish samples were separated, reduced to fillet and oven-dried in the laboratory. The dried fillets were then ground in an agate pestle and mortar to obtain muscle powder which was later used for fatty and amino acids.

Fatty acids analyses: The total lipids were extracted from the powder following¹³ the (1959) method and fatty acid composition were determined as their methyl esters by gas chromatography (GC) technique¹⁴ and identified by comparing their retention time with those of several commercial standard mixtures (Supelco, USA). The concentration of individual fatty acid was calculated using Heneicosanoic acid (C21:0) as an internal standard. Each sample was analyzed 3 times and its averages were calculated (Means ± Standard Deviation (SD)) as both weight percentage (fatty acid profile) and concentration (g/100 g dry tissues).

Amino acids composition analyses: Well extracted oven-dried powdered samples of the muscle were used for estimating the amino acids in High-Performance Liquid Chromatography (HPLC) (Merck-Hitachi L-7400) using Baker and Han¹⁵ method. The results were expressed as percentages of total amino acids in each species.

Statistical analysis: Data generated were analyzed descriptively using SPSS 20.0 Statistical Software (SPSS Inc., Chicago, IL, USA). Student's t-tests were used to compare parameters between the two species. All the statistical analyses were considered at the significance level of 5% (p<0.05).

RESULTS

The body weights and lengths of *Protopterus annectens* and *Heterotis niloticus* samples used are as shown in Table 1. The average body weight of *P. annectens* and *H. niloticus* were 850.00 ± 635.09 and 1200.00 ± 1070.83 g, respectively while the standard length was 44.00 ± 9.42 cm in *P. annectens* and 38.75 ± 12.87 cm in *H. niloticus*. However, the variations in fish body weight and size were not statistically significant ($p > 0.05$) between the two species.

The total acid value (TAV) of muscle tissue of *P. annectens* was comparatively higher (105.19 ± 117.62 mg g⁻¹) than 71.55 ± 52.96 mg g⁻¹ of *H. niloticus* in Table 2. Also, a total of three fatty acids i.e., lauric acid (C12:0), palmitic acid (C16:0) and oleic acid (C18:1) were analyzed for the fish samples. These fatty acids were found oscillating between 6.00 mg and 282 mg in the two species. Oleic acid (183.00 ± 71.00 mg g⁻¹) and palmitic acid (18.00 ± 1.50 mg g⁻¹) were higher in *H. niloticus* than those of *P. annectens*, but no significant

variations were observed ($p > 0.05$). However, the lauric acid content of *H. niloticus* was slightly higher than that of the *P. annectens* ($p > 0.05$).

The muscle tissue of *P. annectens* and *H. niloticus* has nine essential amino acids (EAA) in varying amounts in Table 3. The dominance of the identified EAA is in the order of Leu>Val>Phe>Trp>His>Met>Lys>Ile>Thr in West African Lungfish and Leu>Met>Lys>Ile>Trp>Phe>Val>His>Thr in African Bony tongue. Valine (3.98 ± 0.14 g) and threonine (2.59 ± 0.30 g) quantity were significantly higher in *P. annectens*, whereas, methionine (4.73 ± 0.35 g), isoleucine (4.03 ± 0.04 g), leucine (8.85 ± 0.06 g) and lysine (4.73 ± 0.35 g) were markedly superior in *H. niloticus*. Similarly, phenylalanine (3.95 ± 0.25 g) and histidine (3.70 ± 0.23 g) levels were higher in *H. niloticus* but with no significant variation ($p > 0.05$) between species. The tryptophan content (3.91 ± 0.54 g) of the African Bony tongue muscle was slightly higher than those of the West African Lungfish, but no significant differences were observed ($p > 0.05$).

Table 1: Biometry measurement of the investigated fish species

Parameters	<i>Protopterus annectens</i>		<i>Heterotis niloticus</i>		Significance value
	Range	Mean	Range	Mean	
Weight (g)	500.00-1800.00	850.00 ± 635.09	600.00-2800.00	1200.00 ± 1070.83	0.594
SL (cm)	38.00-58.00	44.00 ± 9.42	25.00-55.00	38.75 ± 12.87	0.535
TL (cm)	44.00-69.00	51.50 ± 11.79	28.00-62.00	43.50 ± 14.82	0.431

SL: Standard length, TL: Total length, * $p < 0.005$

Table 2: Total acid value and fatty acid composition (mg 100 g⁻¹) of the muscle of *Protopterus annectens* and *Heterotis niloticus* captured from Lake Alau

Parameters	<i>Protopterus annectens</i>		<i>Heterotis niloticus</i>		Significance value
	Range	Mean	Range	Mean	
TAV	28.05-280.50	105.19 ± 117.62	20.67-130.90	71.55 ± 52.96	0.621
Lauric acid	6.00-20.00	14.00 ± 2.00	6.00-18.00	13.00 ± 1.10	0.902
Palmitic acid	8.00-25.00	16.00 ± 8.00	8.00-28.00	18.00 ± 1.50	0.902
Oleic acid	85.00-282.00	176.00 ± 84.00	85.00-254.00	183.00 ± 71.00	0.902

TAV: Total acid value, * $p < 0.005$

Table 3: Essential amino-acids (EAA) profile of *Protopterus annectens* and *Heterotis niloticus* from Lake Alau, Maiduguri (g 100 g⁻¹)

Parameters	<i>Protopterus annectens</i>		<i>Heterotis niloticus</i>		FAO/WHO Pattern
	Range	Mean	Range	Mean	
Methionine (Met)	3.00-3.63	3.25 ± 0.24	4.16-4.96	$4.73 \pm 0.35^*$	2.00
Valine (Val)	3.85-4.18	$3.98 \pm 0.14^*$	3.55-3.82	3.68 ± 0.10	5.00
Tryptophan (Trp)	3.25-4.12	3.72 ± 0.33	3.25-4.66	3.91 ± 0.54	1.00
Phenylalanine (Phe)	3.75-4.35	3.95 ± 0.25	3.75-3.88	3.83 ± 0.54	3.05
Iso-Leucine (Ile)	2.00-4.05	2.97 ± 0.77	3.98-4.08	$4.03 \pm 0.04^*$	4.00
Leucine (Leu)	7.24-8.14	7.66 ± 0.40	8.77-8.93	$8.85 \pm 0.06^*$	7.00
Histidine (His)	3.45-3.95	3.70 ± 0.23	2.70-3.86	3.07 ± 0.49	2.50
Lysine (Lys)	3.00-4.12	3.25 ± 0.24	4.16-4.96	$4.73 \pm 0.35^*$	5.40
Threonine (Thr)	2.25-2.97	$2.59 \pm 0.30^*$	1.65-1.85	1.77 ± 0.08	4.00

* $p < 0.005$

Table 4: Non-essential amino-acids (NEAA) profile of *Protopterus annectens* and *Heterotis niloticus* from Lake Alau, Maiduguri (g 100 g⁻¹)

Parameters	<i>Protopterus annectens</i>		<i>Heterotis niloticus</i>		FAO/WHO Pattern
	Range	Mean	Range	Mean	
Alanine (Ala)	1.70-3.17	1.92±0.51	1.72-3.15	2.38±0.41	6.10
Aspartic Acid (Asp)	6.34-8.52	7.64±0.88	7.34-7.88	7.72±0.23	7.70
Proline (Pro)	3.15-4.66	3.78±0.59*	2.84-3.35	3.03±0.22	10.70
Glutamic Acid (Glu)	11.10-13.46	12.54±0.97	11.10-11.74	11.55±0.27	14.70
Serine (Ser)	3.45-3.89	3.74±0.18	3.40-4.31	3.91±0.36	7.70
Tyrosine (Tyr)	4.61-5.82	3.72±0.33	3.25-4.66	3.91±0.54	3.05
Cystine (Cys)	2.00-2.99	2.50±0.46	2.15-2.52	2.35±0.15	3.00
Arginine (Arg)	4.23-4.97	4.52±0.32	3.31-4.05	3.72±0.29*	5.20
Glycine (Gly)	3.04-3.42	3.25±0.18*	3.04-3.26	3.14±0.08	2.20

*p<0.005

The non-essential amino acids (NEAA) compositions of the West African Lungfish and African Bony tongue samples are shown in Table 4. The quantity of NEAA identified follow the order as Glu>Asp>Arg>Pro>Ser>Tyr>Gly>Cys>Ala in West African Lungfish and Glu>Asp>Ser>Tyr>Arg>Gly>Pro>Ala>Cys in African Bony tongue. Significantly higher (p<0.05) contents of proline (3.78±0.59 g) and glycine (3.25±0.18 g) were found in *P. annectens*. However, *H. niloticus* showed considerably high arginine content (3.72±0.29 g) as compared to *P. annectens*. Also, alanine (2.38±0.41 g), aspartic acid (7.72±0.23 g), serine (3.91±0.36 g) and tyrosine (3.91±0.54 g) were higher in *H. niloticus* but with no significant variation (p>0.05) between species.

DISCUSSION

Knowledge of the biochemical composition of fish food is important to evaluate the quality and palatability of the fillet nutrients. It is, therefore, crucial to making available the complete nutritional value of various fish species in Nigeria inland water for the benefit of consumers. The flesh obtained from the experimental fishes was characterized by a varied content of fatty acids with no statistically significant differences noted between species. The total acid value (TAV) was slightly higher in *Protopterus annectens* than *Heterotis niloticus* in the current study. This result conflicts with the previous studies on *P. annectens* from other regions¹⁶. Oleic acid (C18:1), a monosaturated omega-9 fatty acid is the most abundant fatty acid in *P. annectens* and *H. niloticus* in the current study. This is consistent with the work of Osibona *et al.*⁸, who reported that C18:1 was the dominant fatty acid in *Clarias gariepinus* and *Tilapia zilli* from Makoko and Epe Landing sites in Lagos, Nigeria. When compared with muscle oleic acid contents in *P. annectens* (0.82±0.29 g 100 g⁻¹) and *H. niloticus* (21.42±0.14 g 100 g⁻¹) reported by the previous study^{16,17} the content of fatty acid in the current study were lower. Also, the oleic level in this study

was comparatively low to the values reported in four freshwater fish species from Maga Lake, Cameroon¹⁷.

Palmitic acid (C16:0), the predominant saturated fatty acid in both freshwater and marine fish¹⁸, was very low in this study as compared to 2.3±0.05 g 100 g⁻¹ in *P. annectens*¹⁶ and 20.07±0.03 g 100 g⁻¹ in *H. niloticus*¹⁷. Also, these results differ from the findings of Osibona *et al.*⁸, who reported 22 and 32.2% for *C. gariepinus* and *T. zilli*, respectively. Lauric acid has many properties of medium-chain fatty acids that help to prevent infection upon consumption¹⁹. Lauric contents found in this study was higher than the findings of Paul *et al.*²⁰, who reported 2.73% for *Channa striatus* and 4.55% for *Channa marulius*. However, the authors did not find Lauric acid in *H. niloticus* and *C. gariepinus*. Also, high lauric values were reportedly found in *C. gariepinus* (3.1%) and *T. zilli* (0.1%) from Makoko and Epe Landing sites, Lagos⁸. According to Vřetiřková *et al.*²¹ factors that influence fat content and composition of fish muscle include size or age, reproductive status, geographic location and season.

Amino acids, often referred to as the building blocks of proteins, are compounds that play many critical roles in the body. They are needed for vital processes like the building of proteins and the synthesis of hormones and neurotransmitters. According to Osibona *et al.*⁸, the essential amino acids cannot be synthesized in the human body, so they are obtained from fish consumption. Kaya *et al.*²² however, opined that the type and the number of amino acids are related to fishing season, locality, feeding habit and fish life cycle. In the current study, the range values obtained for essential amino acid composition compared well with those observed by ElShehawey *et al.*²³, for the most important fish species of Saudi Arabia. This is an indication that the species studied are nutrient-rich and could help in body development upon consumption. The investigated species contained a superior amount of lysine in comparison to *Gymnarchus niloticus*²⁴, *Clarias lazera*²⁵ and *Tilapia mosambis*⁹, all from freshwater bodies in Nigeria. Similar observations were made

in five different freshwater fish species investigated by Elaigwu¹¹ except for *Clarias anguillaris*. The methionine and threonine levels in this study were within the ranges reported by Elaigwu¹¹, who studied proximate composition, amino acid profile and chemical indices in five freshwater fishes from Tiga Dam Reservoir, Nigeria. Tengyang *et al.*¹⁷, however, found much higher methionine and threonine contents in catfish, mullet and red carp caught from the Wouri river coast in Douala, Cameroon. Meanwhile, the isoleucine concentration recorded in the present study compared well with findings of Elaigwu¹¹ on five different freshwater fish species. This value was, however, superior to the level found in *G. niloticus*²⁴ and *T. mosambis*⁹.

About other species^{9,11,17,26}, leucine contents which is an essential amino acid for protein synthesis in the body was superior in both species investigated in this study. Phenylalanine is described as a "building block" of protein with an anti-depressant agent²⁷. Previous findings on phenylalanine contents by Elaigwu¹¹ on five different freshwater fish species corroborated the results obtained in this study, however, reports from Mohamed *et al.*²⁶ on five commercial Nile fishes show a comparatively low value. Valine is an essential amino acid that helps stimulate muscle growth and regeneration and is involved in energy production²⁸. The valine contents obtained in the study were similar to the findings of Salma *et al.*²⁹ in *Scomber scombrus* and Elaigwu¹¹ in five freshwater fishes. Similarly, tryptophan levels observed in this study was superior to the values found by Tenyang *et al.*¹⁷ in six fish species of Wouri river coast in Douala, Cameroon. This is an indication that eating *H. niloticus* and *P. annectens* could help to provide nitrogen balance in adults and growth in infants. The histidine contents in this study range between 3.07 ± 0.9 and 3.70 ± 0.23 g 100 g⁻¹, which is higher than 48.11 ± 2.10 mg g⁻¹ in *Arius maculatus*, 37.78 ± 0.30 mg g⁻¹ in *Semotilus atromaculatus* and 27.70 ± 0.55 mg g⁻¹ in *Cyprinus carpio* from Wouri River coast, Cameroon¹⁷.

The composition of non-essential amino acids in the species reported here was comparable to the pattern reported by El-Shehawey *et al.*²³, for the most important fish species of Saudi Arabia. *Protopterus annectens* and *H. niloticus* tissues contained a higher amount of serine in comparison with *Schilbe mystus* (2.74 ± 0.10 g 100 g⁻¹), *Bagrus bayad* (2.12 ± 0.06 g 100 g⁻¹), *Oreochromis niloticus* (3.03 ± 0.06 g 100 g⁻¹) and *Petrocephalus bane* (2.28 ± 0.12 g 100 g⁻¹) as reported by Elaigwu¹¹. Also, a lower value than what was recorded in our study was reported by Adefemi⁹ and Elaigwu¹¹ for cysteine in freshwater fishes from Nigerian Reservoirs. Similarly, Ibhaddon *et al.*¹⁰ and Salma *et al.*²⁹ also obtained lesser values of cysteine and tyrosine for

Atlantic Mackerel, African catfish, *Synodontis schall* and *Brycinus nurse*, however, they obtained higher values of alanine and aspartic acid for these species. Adefemi⁹ reported much lower glutamic acid values for *T. mosambi* caught from the major dam in Ekiti, Nigeria while Ibhaddon *et al.*¹⁰ determined higher glutamic acid content as 14.30% for *C. gariepinus* caught from River Kaduna. Meanwhile, Elaigwu¹¹ recorded higher glycine levels for the different five fish species studied as compared to the current study. The values of proline (3.03 ± 0.22 - 3.78 ± 0.59 g 100 g⁻¹) obtained in the present study compared well with the results verified for *T. zilli* (3.95 ± 0.21 %) and *C. gariepinus* (3.81 ± 0.21) reported by Osibona *et al.*⁸, whereas, higher values were observed for River catfish (6.00) and Atlantic Mackerel (6.02 ± 0.59 %) by Ibhaddon *et al.*¹⁰ and Salma *et al.*²⁹, respectively.

CONCLUSION

The study discovered that *Protopterus annectens* and *Heterotis niloticus* from Lake Alau were the good sources of monosaturated omega-9 fatty acid and dodecanoic acid, though in trace amounts. The species were, however, rich in essential and non-essential amino acids and compared well with other freshwater fish species and Atlantic Mackerel. Therefore, consumption of these species is highly recommended since these fish species are nutritious.

SIGNIFICANCE STATEMENT

This study has brought forward the nutritive quality of *Protopterus annectens* and *Heterotis niloticus*, which is beneficial for human consumption. This study will help the nutritionist to create awareness of the nutritive value of the species. Thus, these species can be aquaculture candidates in the near future as many researchers in Nigeria are already working on this.

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