

## Amino acid profile of four earthworms species from Nigeria

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### ABSTRACT

The amino acid profile of four species of earthworms from Nigeria namely *Eudrilus eugeniae*, *Hyperiodrilus africanus*, *Alma millsoni* and *Libyodrilus violaceus* were evaluated in this study. The amino acid analysis was conducted using the High Performance Liquid Chromatography (HPLC) specifically the Technichon TSM-1 (technosequential multisample) analyser for amino acid. Nine essential namely Lysine, Histidine, Arginine, Threonine, Valine, Methionine, Isoleucine, Leucine and Phebylalanine and eight non-essential amino acids namely Aspartic acid, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine and Tyrosine were recorded in this study. Cystine with a range of 0.64-0.80 g/100kg protein had the least concentration while Glutamic acid with a range of 11.50-13.22 g/100kg protein was the highest. Lysine and methionine, which were limiting amino acids in most feedstuffs, were present in all the earthworm species and ranges from 4.95-5.70 g/100g protein for lysine and 2.08-2.30 g/100g protein for methionine

**Keywords:** Essential, Non-essential, amino acids, Earthworm species, chromatogram, dietary, *Eudrilus*

### INTRODUCTION

The potential value of earthworms as a protein source has been established by several authors. Guererro (1981) working on *Perionyx excavatus* showed that the worm is a good source of protein. Staford and Tacon (1984) showed that *Dendrodrilus sububicundus* contains 65 percent crude protein. The study of Edwards and Niederer (1988) also revealed that earthworms are an excellent source of protein. The study of Vielma-Rondon (2003) also showed that earthworms are a rich source of protein (>60%w/w). On the average earthworms have been shown to contain 60-70% crude protein (dry weight) and very little crude fibre (<5%) which result in a high concentration of highly digestible energy (16-17kJ/g) (Sabine, 1983; Lee, 1985). Furthermore studies have shown that not only could earthworms serve as a rich protein source, but also as a source of essential amino acids, especially lysine which, is limiting in many basic foodstuffs (Albarran, 1996). The content of lysine in earthworm flour is significant (5.9%w/w), representing the daily requirement for children between the age of 2 and 5 years (Segovia, 1996). Earthworm meal was shown to have an amino acid composition very similar to that of fishmeal and potentially superior to meat meal and the protein was shown to contain such essential amino acids as phenylalanine, leucine, lysine, methionine and valine (>3%w/w).

Against these backdrops and the prohibitive market cost of fishmeal due to increasing demand for high quality protein for use in intensive animal feed

industries and ethical issues of extensive fish harvesting, earthworm could fit into this mold.

Therefore if earthworm will fit this role especially in a developing nation as Nigeria, there is a need to undertake a comparative analysis of likely earthworm candidates that have a high potential for protein and amino acid supplementation in animal diet. The objective of this present study is to determine the amino acid profile of four species of earthworms namely *Eudrilus eugeniae*, *Hyperiodrilus africanus*, *Lybodrilus violaceus* and *Alma millsoni*.

### MATERIALS AND METHOD

**Study location:** The study was conducted at Olabisi Onabanjo University Mini Campus Ago-Iwoye, Ijebu-North Local Government Area, Ogun State, in south west Nigeria.

**Study animals:** Four species of earthworms were used for this study; namely *Hyperiodrilus africanus* Beddard, 1891; *Eudrilus eugeniae* Kinberg, 1866; *Libyodrilus violaceus* Beddard, 1891 all belonging to the family Eudrilidae and *Alma millsoni* Grube, Beddard, 1891 belonging to the family Almidae. The choice of the earthworms is informed by these two points (a) majority of the earthworms in this region including those described by Owa (1992) belong to the family Eudrilidae. Though *A. millsoni* is not a eudrilid earthworm, it is a limicolous earthworm (marsh dwelling), which occur in most cases side by side with *L. violaceus*. (b) relative occurrence (% frequency) of the earthworms except *Alma* as calculated by Owa (1992) who revealed that these

earthworms have a higher occurrence than other eudrilid earthworms in the rainy season.

**Collection of earthworms:** The earthworms were collected in Ago-Iwoye SW Nigeria. It lies on latitude 7°30'N and longitude 4°32'E at an altitude of 76 m above sea level with mean annual rainfall of 1,779mm and mean of 110 days, mean annual temperature of 27 °C and mean maximum temperature of 32 °C. Collections were made in the months of August to October, 2006 during which the adult stages of earthworms were available.

Collection of *A. millsoni* and *L. violaceus* were made along the banks of streams with high humus (limicolous environment). *H. africanus* and *E. eugeniae* were collected from friable soils (not water logged). Digging and hand-sorting Method according to (Owa, 1992) was employed in the collection of the earthworms. The earthworms were transferred in plastic containers filled with humus to the laboratory for Identification and further sorting into species.

**Amino acid analysis:** The amino acid analysis was determined at the Department of Zoology, University of Jos, using the High Performance Liquid Chromatography (HPLC) specifically the Technichon TSM (technosequential multisample) analyser for amino acid.

10mg of the defatted earthworm sample was weighed into glass ampoule and 7mL of 6N HC1 was added and oxygen expelled by passing nitrogen into the ampoule (This is to avoid oxidation of some amino acids during hydrolysis). The glass ampoule was then sealed with Bunsen burner flame put into the oven at 105 °C for 22 hours. The ampoule was allowed to cool before broken opened at the tip and the content was filtered to remove humins. The filtrate was then evaporated to dryness at 40 °C under vacuum in a rotary evaporator. The residue was dissolved with 5mL of acetate buffer (pH 2.0) and stored in plastic specimen bottles and kept in the freezer.

5 to 10 microlitre of the hydrolyzed sample was loaded into the cartridge of the analyzer (the amount of hydrolyzed sample loaded was dependent on whether the amino acids to be eluted were basic or acidic/neutral. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 minutes.

**Calculating amino acid values from the chromatogram peaks:** The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half -height of the peak on the chart was found and the width of the peak at half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the Formula  $NE = \text{Area of Norleucine Peak} \div \text{Area of each amino acid Peak}$

The amount of each amino acid present in the sample was calculated in g/100g crude protein or g/16gN using the following formula

Concentration (g/100g protein) =  $NH \times \text{width} @ NH/2 \times S_{std} \times C$

Where  $S_{std} = NE_{std} \times \text{mol. Weight} \times V_{MAA_{std}}$  and

$C = \text{Dilution} \times 16/\text{Sample Wt (g)} \times N\% \times 10 \times \text{Vol. loaded}$

$NH \times W$  (nleu)

Where NH = Net height

W or Width @ NH/2 = width at half-height

Nleu = Norleucine.

## RESULTS

A total of seventeen (17) amino acids consisting of Nine (9) essential namely Lysine, Histidine, Arginine, Threonine, Valine, Methionine, Isoleucine, Leucine and Phebylalanine and eight (8) non-essential amino acids namely Aspartic acid, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine and Tyrosine were recorded in this study through the high performance liquid chromatography (Fig 1-4) from each species of earthworm.

**Essential amino acids** The lowest value of Lysine and Histidine was recorded from *Hyperiodrilus africanus* while *Alma millsoni* recorded the lowest value of Arginine, Threonine, Valine, Methionine, Isoleucine and Phenylalanine. On the other hand the highest value of Lysine, Arginine, Valine, Isoleucine, and Phenylalanine was recorded from *Eudrilus eugeniae*; The highest value of Histidine and Methionine was recorded from *Libyodrilus violaceus*, while *Hyperiodrilus africanus* and *Alma millsoni* recorded the highest value of Threonine and Leucine respectively (Table 1).

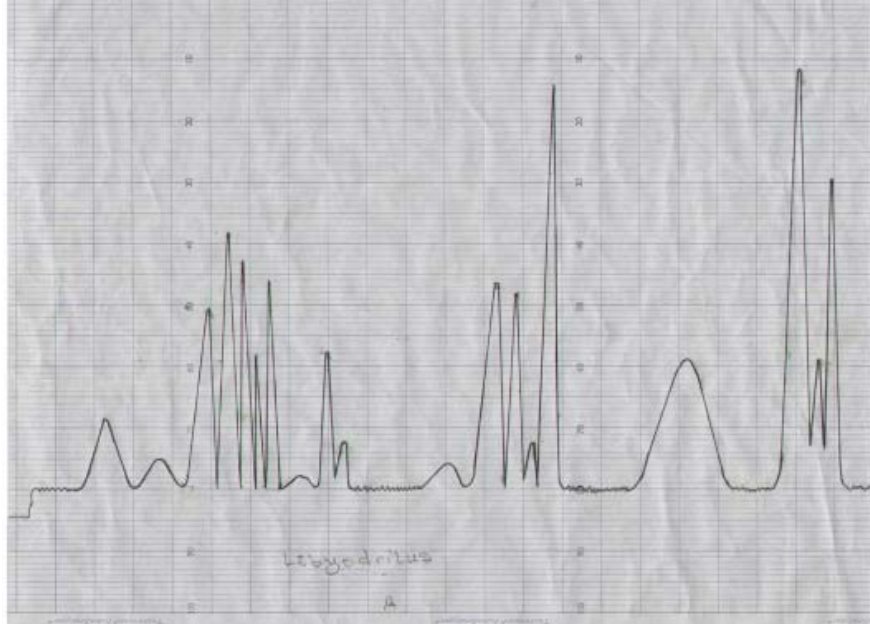
**Non-essential amino acids** The lowest value of Aspartic acid and Proline was recorded from *Hyperiodrilus africanus* while *Alma millsoni* recorded the lowest value of Cystine and Glutamic acid. The lowest value of Serine and Alanine was recorded from *Eudrilus eugeniae* while *Libyodrilus violaceus* recorded the lowest value of Glycine and tyrosine.

On the other hand the highest value of Serine, Glutamic acid, Glycine and Alanine was recorded from *H. africanus*; The highest value of Aspartic acid, Proline and Cystine was recorded from *L. violaceus*, while *A. millsoni* recorded the highest value of Alanine and Tyrosine (Table 1).

The amino acid with the least concentration is Cystine with a range of 0.64-0.80 g/100kg protein while Glutamic acid with a range of 11.50-13.22 g/100kg protein was the highest (Table 1).

The range of essential amino acids obtained from the different species of earthworm in this study was within the same range for different fishmeal brands by BOANR (1993) Lysine and methionine, which were limiting amino acids in most feedstuffs, were present

ranging from 4.95-5.70 g/100g protein for lysine and 2.08-2.30 g/100g protein for methionine and these values were within the range obtained for different fishmeal brands by BOANR (1993) (Table 2).



**Fig 1: The Chromatogram showing the amino acid profile of *Libyodrilus violaceus***

\*Nineteen (19) peaks were recorded. From the right, the peaks represent Lysine, Histidine, Ammonia, Arginine, Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Norleucine (standard), Tyrosine, Phenylalanine.



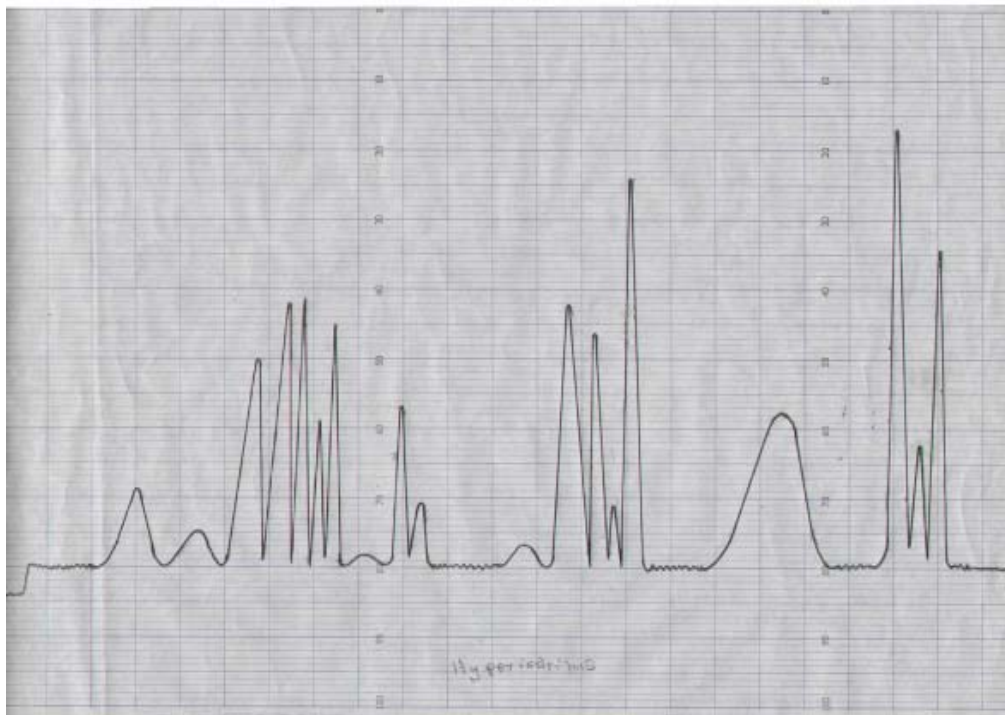
**Fig 2: The Chromatogram showing the amino acid profile of *Alma millsoni***

\*Nineteen (19) peaks were recorded. From the right, the peaks represent Lysine, Histidine, Ammonia, Arginine, Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Norleucine (standard), Tyrosine, Phenylalanine.



**Fig 3: The Chromatogram showing the amino acid profile of *Eudrilus eugeniae***

\*Nineteen (19) peaks were recorded. From the right, the peaks represent Lysine, Histidine, Ammonia, Arginine, Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Norleucine (standard), Tyrosine, Phenylalanine.



**Fig 4: The Chromatogram showing the amino acid profile of *Hyperiodrilus africanus***

\*Nineteen (19) peaks were recorded. From the right, the peaks represent Lysine, Histidine, Ammonia, Arginine, Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Norleucine (standard), Tyrosine, Phenylalanine.

Table 1: Amino acid concentration (g/100g crude protein) in earthworms (this study) compared with earthworms (other sources)

Amino acid	This study				Dynes, 2003	Sabine, 1978
	<i>Eudrilus eugeniae</i>	<i>Libyodrilus violaceus</i>	<i>Hyperiodrilus africanus</i>	<i>Alma millsoni</i>	<i>Eisinia foetida</i>	Earthworm meal (unnamed)
<b>Essential</b>						
Lysine	5.70	5.50	4.95	5.00	6.8	4.3
Histidine	3.30	3.36	2.80	3.01	2.6	1.6
Arginine	8.66	8.01	8.25	7.25	6.0	4.2
Threonine	0.95	1.02	1.12	0.80	5.2	3.0
Valine	4.22	4.00	4.09	3.96	4.7	3.0
Methionine	2.24	2.30	2.19	2.08	NG	NG
Isoleucine	5.50	4.80	5.00	4.50	4.3	2.6
Leucine	6.51	6.71	6.05	7.02	7.2	4.8
Phenylalanine	4.52	4.40	4.32	4.05	3.8	2.3
<b>Non-Essential</b>						
Aspartic acid	10.10	10.68	9.04	9.73	8.9	NG
Serine	3.60	4.03	4.23	3.91	4.7	NG
Glutamic acid	12.10	11.80	13.22	11.50	16.4	NG
Proline	2.25	2.56	1.95	2.08	4.0	NG
Glycine	0.91	0.85	1.03	0.99	5.8	NG
Alanine	3.30	3.50	3.65	4.02	6.0	NG
Cystine	0.71	0.80	0.70	0.64	NG	NG
Tyrosine	3.30	3.18	3.32	3.50	3.3	NG

NG: Not given

Table 2: The Concentration (g/100g protein) of Essential amino acid in earthworm meals (this study) compared with Fishmeal brands (other source)

Essential Amino acid	Concentration (g/100g protein) of essential amino acids				
	Fishmeal brand (72%) (BAONR, 1993)	Fishmeal brand (64.5%) (BAONR, 1993)	Fishmeal brand (59.9%) (BAONR, 1993)	Fishmeal brand (62.2%) (BAONR, 1993)	Earthworm meals (Range) (This Study)
Lysine	5.57	4.72	4.06	4.53	4.95-5.70
Histidine	1.65	1.45	1.75	1.34	2.80-3.36
Arginine	4.54	3.82	3.43	4.21	8.01-8.66
Threonine	2.90	2.50	2.31	2.57	0.80-1.12
Valine	4.30	3.22	2.77	3.02	3.96-4.22
Methionine	2.08	1.75	1.47	1.68	2.08-2.24
Isoleucine	3.13	2.66	2.45	2.67	4.50-5.50
Leucine	5.19	4.48	3.79	4.52	6.05-7.02
Phenylalanine	2.71	2.41	2.15	2.34	4.05-4.52



**DISCUSSION**

The amino acid concentration (g/100g crude protein) in *Eudrilus eugeniae*, *Libyodrilus violaceus*, *Hyperiodrilus africanus* and *Alma millsoni* were similar to those reported by other authors.

The amino acid profile obtained in this study is comparable to that of fishmeal brands in the study of BOANR (1993) and it revealed that the amino acid profile of these earthworm species is at par with that of fishmeal. The concentration of Arginine ranges from 8.01-8.66 g/100g crude protein in these earthworms whereas it ranged from 3.43-4.54 g/100g crude protein in the fishmeal brands. Similarly, Methionine, which is usually the first limiting amino acid ranges from 2.08-2.24 g/100g crude protein in these earthworms while it ranged from 1.47-2.08 g/100g crude protein in the fishmeal brands.

The value of essential amino acids concentration obtained in this study were on the higher side of the range and this agrees with the study of Edwards (1985) which showed that earthworm protein contain a higher content of essential amino acids such as lysine and methionine than either meat or fishmeal. Dynes (2003) also confirmed that the amino acid concentrations of earthworm such as *Eisenia fetida*, *Lumbricus terrestris*, *Perionyx excavatus* were similar to that of fishmeal or even better, as the case is in this study.

This suggests that earthworm species from Nigeria has comparable amino acid profile and concentration with their counterparts from the temperate region. As such these earthworm species could therefore be incorporated as dietary supplement to supply such limiting amino acids as lysine and methionine in the diets of fish or other animals.

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