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# NUTRIENT CONTENT OF NATIVE EARTHWORM, *EUTYPHOEUS GAMMIEI* (BEDDARD) OF TRIPURA (INDIA)

Debnath Madhusudan<sup>1</sup>, Chaudhuri Priyasankar<sup>\*2</sup>, Chakraborty Pradosh Prasad<sup>3</sup> and Sil Samir Kumar<sup>1</sup>

Departmental of Human Physiology<sup>1</sup>, Department of Zoology<sup>2</sup>, Tripura University, Suryamaninagar - 799022, Tripura, India.

Indian Institute of Chemical Technology<sup>3</sup>, (CSIR), Hyderabad - 500007, Telangana, India.

#### Keywords:

*Eutyphoeus gammiei*, Nutrients, Protein, fat, Carbohydrate, Essential amino acid, Essential fatty acid

Correspondence to Author: Priyasankar Chaudhuri

Professor, Department of Zoology, Tripura University, Suryamaninagar -799022, Tripura, India.

E-mail: priya\_1956@rediffmail.com

ABSTRACT: Eutyphoeus gammiei (Beddard) is a large native earthworm which is commonly used as fish bait and feed of chicken in Tripura. Eutyphoeus tissue comprising of both body wall and gut wall contains 63.98% protein, 15.79% carbohydrate, 7.78% fat, 1.90% crude fiber and total ash 10.55%. The result showed that essential amino acid of earthworm tissue was dominated by lysine (1.45 g /100 g), methionine (1.56 g/100 g), valine (2.78 g/100 g) and leucine (5.52 g /100 g). The non essential amino acid of earthworm was dominated by glutamic acid (1.37 g / 100 g) and ornithine (3.68 g / 100 g). Interestingly, the proportion of unsaturated fatty acids was relatively high (poly unsaturated fatty acids 26%, mono unsaturated fatty acids 27.4%). In poly unsaturated fatty acids, arachidonic acid (20: 4  $\omega$  -6) and linoleic acid (18: 2  $\omega$  -6) represented 36.39% and 29.50% respectively. Importance of essential amino acids and essential fatty acids in earthworm tissue for animal nutrition has been given in the present paper. Nutritionally important several minerals such as iron, calcium, potassium, magnesium, zinc and copper are also present in the tissue of *Eutyphoeus gammiei*. The results indicated that the native earthworm E. gammiei earthworm could be considered as food source alternative to fish meal and meat and a supplement for both human and livestock consumption in India in general and its north-eastern states in particular.

**INTRODUCTION:** Protein in the feeds of animals is essential for their growth and health. One of the major sources of protein for animal diet is fish meal which is limited in supply and increasing in cost.

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The first analysis of the constituents of the tissue of different species of earthworms was by McInroy<sup>1</sup>. Thereafter, there have been several studies on nutrient analysis of earthworm tissues <sup>2-7</sup>. The results of these studies clearly suggest that the overall composition of earthworm tissue does not differ greatly from that of many invertebrate and vertebrate. Earthworm tissues contain а preponderance of long chain fatty acids many of which cannot be synthesized by non-ruminant animals, and an adequate content of minerals and also an excellent range of Vitamins rich in niacin<sup>8</sup>.

Chicken fed on earthworm meal in their rations have shown equal to or better growth rates than those fed with conventional protein meals containing fish meal<sup>3</sup>. Research findings from several laboratories indicate that earthworm body tissue contains a higher level of protein (50% -70% of dry weight) and can be used as feed materials for fish, poultry and pigs <sup>9</sup>. A group of scientists of Royal Society (London, UK) have published the study on nutritive properties of the earthworms and their usage in the diet of Yekuana people from Venezuela 7. In their diet they used two species: Andiorrhinus moto<sup>10</sup> and Araujo which lives in sludge, and popularly named as "motto", and Andiorrhinus kuru<sup>11</sup> which lives in forest soil, and is known as "Kuru". The Yekuana people eat them raw (uncooked) or after treatment in water at the temperature 60-70 °C or after drying in the smoke. These smoked earthworms were considered as "special" and the price was very high. On the basis of nutrient analysis of "motto" and "Kuru" <sup>11</sup> it was concluded that these earthworms were the best food for fulfilling all requirements in the human body <sup>7</sup>.

Many restaurants in Mexico have in their menu variety of earthworms, and in Japan the earthworms are much liked as a food in combination with soyabean or supplementation in juices. Earthworms have been introduced in everyday diet in some parts of Asia, Africa and South America<sup>12</sup>. Studies on nutrient contents of earthworms were restricted mostly to Eisenia fetida, Perionyx excavatus and Eudrilus eugeniae which feed on organic wastes and can be easily cultured <sup>13</sup>. Our present paper aims to study the nutrient contents of giant, geophagous and native earthworm of Tripura, Eutyphoeus gammiei (Octochaetidae) which is widely distributed in Northeast India including Tripura and Bangladesh. This species is easily recognized by its large tower shaped surface cast and is collected easily using aqueous extract of plant Polygogum hydropiper<sup>14</sup>. Interestingly, Eutyphoeus gammiei is the commonly used earthworm as fish bait and feed of poultry birds in Tripura.

# **MATERIALS AND METHODS:**

**Collection of Earthworms:** Ten (10) adult earthworms, *Eutyphoeus gammiei* (average length 400mm, weight 15g each) were collected by digging and hand sorting method from east Shibsagar, Agartala, at an early morning during September 2014. The earthworms were taken to the laboratory along with their native soils and acclimated under laboratory conditions (temperature 27 - 30 °C and humidity 80-90%)

**Preparation of Earthworm Extract:** The earthworms were washed in running tap water to remove dirts if any over the body surface. They were then submerged in warm water (40 °C) for 2 hours to allow its gut-soils to be excreted out. Later on, the earthworms were washed thoroughly with distilled water and then kept in a hot air oven at 45 °C for 15 days to get it completely dried on a petridish.

**Estimation of Carbohydrate, Protein and Fat contents:** Small pieces of dried earthworm (body wall and gut wall) tissue was considered for estimation of protein (Kjeldahl method), fat (Soxlet method) and carbohydrate (nitrogen free extract) contents. For estimation of carbohydrate content 5g of tissue was considered.

Total Carbohydrate (nitrogen free extract) % = 100 - [Crude protein % + Ether extract(fat) % + (crude fiber and total ash)%]

Analysis of Amino Acid Contents: Dried earthworm tissue sample (0.5g) was dissolved in 1ml. of distilled water and vortexed according to sample size. The sample mixed with 4ml of methanol was incubated overnight at -20 °C. The sample was then centrifuged at 4 °C and the supernatent was collected. The supernatent was completely dried under nitrogen flow at 60 °C. The dried pellet was derivatized. The derivatization mixture (50µl) was taken in a vial and kept in a thermomixer for 1 hour at 45 °C. The sample was then injected to HPLC (Aglilent 1100 series)

Analysis of Fatty Acid Contents: The earthworm tissue sample (5g) was first lyophilized to powder form using scanvac coolsafe 110 freeze dryer (Labogene APS, Denmark). Lipid was extracted from the lyophilized earthworm sample using n-hexane as solvent in a Soxhlet apparatus and then filtered. The lipid was then converted to fatty acid methyl esters by reacting with 2% H<sub>2</sub>SO<sub>4</sub> in methanol for 4-5 hours under reflux condition. Then fatty acid methyl ester was extracted with

ethyl acetate, washed with water, dried over anhydrous sodium sulphate and then concentrated using rotary evaporator. This lipid sample was finally analyzed by Gas Chromatography following AOCS Method (Ce 1b-89).

Analysis of Mineral Content: Earthworm sample (0.5g) mixed with 10 ml of diacid mixture (200ml of HNO<sub>3</sub> and 80ml of perchloric acid 2.5 : 1) in 15 ml test tube was digested at 200 °C. The digested material was filtered (Whatman paper No. 42) and added with 3-5ml concentrated HCl. Again the

sample was filtered to estimate its contents of potassium and calcium by flame photometer and micronutrients (Fe, Mn, Cu, Zn) by Atomic absorption spectrophotometer.

**RESULTS AND DISCUSSION:** Analysis of earthworm (*E. gammiei*) tissue revealed occurrence of crude protein 63.98%, fat (ether extract) 7.78%, carbohydrate (nitrogen free) 15.79%, crude fibre 1.90%, total ash 10.55%, dry matter 89.98% and moisture 10.02%. **Table 1** compares our result with those of other workers.

Chemical composition	Kale <sup>15</sup>	Guerrero <i>et al.</i> , <sup>16</sup>	Istiqomah <i>et al.</i> , <sup>17</sup>	Edwards and Neiderer <sup>13</sup>	Present study
Crude protein %	56.27	68.0	63.06	78-88	63.98
Fat (ether extract) %	6.05	9.57	18.50	6-11	7.78
Carbohydrate %	-	11.05	12.41	5-21	15.79
Crude fibre %	0.56	-	0.19	-	1.90
Mineral %	-	-	-	2-3	1.58
Total ash %	10.49	9.07	5.81	-	10.55
Moisture %	7.70	-	9.03	-	10.02

 TABLE 1: CHEMICAL COMPOSITION OF EARTHWORM MEAL

Protein content of *E. gammiei* (64%) is almost similar to that of earthworm, *Lumbrius rubellus*<sup>17</sup>, edible orthopteran insect, *Oedaleus abruptus*<sup>18</sup> and marginally higher than that of fish (55-60% *crude protein*) indicating that earthworm tissues are very rich in protein source.

Recently Bhattacharjee and Ghosh <sup>19</sup> and Debnath *et al.*, <sup>20</sup> reported antibacterial properties of the coelomic fluid and body wall of *E. gammiei*. Because of its giant size, easy availability in north-east India including Tripura and also in neighbouring country Bangladesh <sup>21</sup>, *E. gammiei* is commonly used as a fish bait and also as feed of poultry birds. In contrast to *E. gammiei*, a geophagous deep burrowing earthworms, some epigeic species such as *Eisenia fetida*, *Perionyx* 

excavatus, Eudrilus eugeniae and Dendrobaena veneta feed on wide range of organic wastes, such as sewage biosolids, animal manure food wastes and organic industrial wastes and convert them to organic manure *i.e.* vermicompost.

This latter group of earthworms, according to Edwards and Niederer <sup>13</sup> can be used as very good sources of animal feed protein. Infact, difference in the composition of earthworm meal in **Table 1**, is due to the difference in the earthworm species which differs in their food habit and habitat. It is likely that earthworms feeding on organic wastes with rich content of nitrogen, such as pig manure, cow dung, poultry manure possess more protein than those inhabiting mineral soils such *E. gammiei*.

Essential amino acid	WHO*	McInroy <sup>22</sup>	Schulze <sup>2</sup> and graff	Sabine <sup>3</sup>	Graff <sup>6</sup>	Istigomah 17	Present study
Arginine		6.1	6.1	4.2	6.1	1.24	
Cysteine	2.0	1.8	1.4	2.3	1.4	0.39	
Histidine		2.2	2.3	1.6	2.3	1.91	0.21
Isoleucine	4.2	4.6	4.7	2.6	4.7	1.98	
Leucine	4.8	8.1	8.2	4.2	8.2	1.16	5.52
Lysine	4.2	6.6	7.5	4.3	7.5	1.43	1.45
Methionine	2.2	1.5	1.8	2.2	1.8	1.72	1.56
Phenylalanine	2.8	4.0	3.5	2.3	3.5	1.03	
Threonine	2.8	5.3	4.7	3.0	4.7	1.03	
Tyrosine	2.8		3.0	1.4	3.0	0.82	0.79
Valine	4.2	5.1	5.2	3.0	5.2	1.30	2.78

TABLE 2: ESSENTIAL AMINO ACID CONTENTS (g PER 100g) OF EARTHWORM TISSUE

\*Requirement of essential amino acid in animal feed

Amino acid contents (g /100g) of earthworm tissue (*E. gammiei*) were aspartic acid 0.614, glutamic acid 1.37, Serine 0.915, 1-Methyl histidine 0.210, anserine 0.304, tyrosine 0.793, valine 2.78, methionine 1.56, leucine 5.520, lysine 1.450, OH lysine 3.560, ornithine 3.687 and cystathionine 0.235. Essential amino acid contents (g per 100g) of earthworm tissue reported by different authors are compared with that of *E. gammiei*.

By comparing the essential amino acid contents of a sample protein with that of the World Health Organization (WHO) standard protein, one can calculate the protein chemical score and identify the limiting amino acid in the nutritional source '. Higher values of essential amino acid contents in earthworm tissue as obtained <sup>2, 6, 22</sup> compared to that of E. gammiei were probably due to the fact that the authors used epigeic species such as Eisenia fetida and Eudrilus eugeniae in their studies which inhabit organic wastes rich in nitrogen contents, while E. gammiei feeds on soils with very low nitrogen contents. Contents of essential amino acids *i.e.* lysine, valine, leucine and methionine in the amino acid constituents of E. gammiei also corroborates with the observation of Sabine<sup>3</sup>, Graft <sup>6</sup>, Istiqomah *et al.*, <sup>17</sup> and Reinecke *et al.*, <sup>23</sup>.

Highest level of essential amino acid in E. gammiei was leucine (5.52g / 100g). In other species of earthworms also leucine represented the highest level of amino acids in their tissue 3, 6, 17, 22, 23. Interestingly the level of leucine (g /100g) in the earthworm species studied by different workers was much higher than those of salmon fish (1.62 g/100g), soybean (2.86g /100g), chicken egg (1.44 g/100g), chicken broiler (1.48g /100g) and almond (1.48g /100g) (USDA National Nutrient Data base for standard references September 2009). Leucine works with the amino acid isoleucine and valine to repair muscle, regulate blood sugar level and provides the body with energy. Lysine is important for proper growth, converts fatty acids to energy and reduce blood cholesterol level. Methionine is a powerful antioxidant and critical for proper neurological, immunological and gastrointestinal functions.

Histidine supplements in food help to relieve symptoms of rheumatoid arthritis. It is also important to normal sexual functioning. The nonessential amino acid in earthworm (*E. gammiei*) is dominated by glutamic acid (1.37g / 100g). Highest level of glutamic acid among non-essential amino acids in earthworms was also reported in *Perionyx excavatus* (1.42g / 100g) and *Lumbricus rubellus* (1.52g / 100g)<sup>17</sup>. Glutamic acid is involved in protein synthesis and as source of energy for cells lining the intestine. It also facilitates immune function and improves anti-inflammatory effects, aids in preventing and healing of peptic ulcer and ulcerative colitis<sup>17</sup>.

EARTHWORM TISSUE (E. GAMMIEI)				
Type of Fatty	Name of	% per		
Acid	Fatty Acid	100 gm		
A. Saturated Fatty Acid				
12:0	Lauric acid	0.9		
13:0	Tridecylic acid	0.7		
14:0	Myristic acid	2.3		
15:0	Pentadecanoic acid	1.4		
16:0	Palmitic acid	10.7		
17:0	Margaric acid	5.0		
18:0	Stearic acid	14.4		
20:0	Eicosanoic acid	1.0		
21:0	Heneicosylic acid	3.1		
22:0	Behenic acid	3.1		
23:0	Tricosylic acid	2.2		
24:0	Lignoceric acid	1.6		
B. Monounsaturated Fatty Acid				
16:1	Palmitoleic acid	1.6		
17:1	Margaric acid	0.4		
18:1(w-9)	Oleic acid	16.6		
20:1(w-9)	D11-eicosenoic acid	8.3		
22:1 (w-9)	Euric acid	0.5		
C. Polyunsaturated Fatty Acid				
18:2 (w-6)	Linoleic acid	7.7		
18:3 (w-6)	<sup>y</sup> -linolenic acid	1.8		
20:2 (w-6)	D11.14-eicosadienoic acid	2.7		
20:3 (w-6)	Dihomo-g-linolenic acid	2.1		
20:4 (w-6)	Arachidonic acid	9.5		
20:5 (w-3)	Eicosapentaenoic acid	1.7		
22:6 (w-3)	Clupanodonic acid	0.6		

TABLE 3: FATTY ACID COMPOSITION OFEARTHWORM TISSUE (E. GAMMIEI)

In fact the essential amino acid content of earthworm meal would produce great result when added in animal feed stuff ratio. Due to proper balance between vitamins and amino acids present in earthworm tissue, around 98% of the protein of its body tissue are readily absorbed in the animal gut <sup>17</sup>. Guerrero *et al.*, <sup>16</sup> reported that Tilapia fish grew better on diets containing earthworm-protein supplements from *Perionyx excavatus* or *Eudrilus eugeniae* than those with fish meal supplements. No significant variations in the amino acid levels of *P. excavatus, E. eugeniae* and *E. fetida* from that of

the fish meal had been observed by Graft <sup>6</sup> and Reinecke *et al.*, <sup>23</sup>. This shows the possibility of accepting earthworm meal as a source of essential amino acids in place of fish meal. Chicken fed on earthworms had gained weight faster and had more breast muscles than when supplied with fish meal <sup>25</sup>. Fat is one of the major nutrients for any living organism. Fat content of *E. gammiei* tissue is much lower than that of earthworm, *Eudrilus, eugeniae* <sup>16</sup>, *Lumbricus rubellus* <sup>17</sup> and orthopteran insect *Oedaleus abruptus* <sup>18</sup>. Like the fat of insects, earthworm fat has a high nutritional value because of higher essential fatty acid contents. The crude fat contents of *E. gammiei* is almost similar to those of other earthworm species <sup>13, 15, 16</sup>, but quite lower than those of soybean meal (1 to 1.5%) <sup>24</sup>.

Table 3 represents reports on the fatty acid composition (% per 100g) of earthworm (E. gammiei) tissue. There are some remarkable aspects of the fatty acid composition in earthworm tissues. First, proportion of unsaturated fatty acids was relatively high (poly unsaturated fatty acids 26%, mono unsaturated fatty acids 27.4%). Second, within the poly unsaturated class of fatty acids, there was a much higher proportion of w-6 fatty acids than w-3 fatty acids. Third, arachidonic acid  $(20:4 \quad \omega-6)$  represented 36.39% of the polyunsaturated fatty acid content. Fourth, linoleic acid (18:2) $(\omega-6)$  accounted for 29.50% of the polysaturated fatty acid content. Predominance of poly unsaturated fatty acids with pentatonic acid, arachidonic acid, linoleic acid and linolenic acid as the major components in poly unsaturated fatty acids was reported in the earthworms <sup>26</sup>. Higher levels of linoleic and linolenic acids in earthworm tissue was essential for egg size in poultry birds<sup>15</sup>.

Mekada *et al.*, <sup>4</sup> reported that when older chickens were fed with earthworms, egg production was maintained. Recently Paoletti *et al.*, <sup>7</sup> also reported high contents of arachidonic acid and linoleic acid in the earthworms consumed by the native people of Alto Orinoco of Venezuela. The caterpillar, *Imbrasia ertli* consumed by human in Africa is one of the few food sources in which arachidonic acid contributes remarkably to the total fatty acid <sup>27</sup>. Arachidonic acid is an important component of phospholipids present in the membranes of eukaryotes. In mammals, it is precursor to prostaglandins. Along with the two essential fatty acids (linoleic acid and linolenic acid) arachidonic acid is especially important in the first six months of human life. It is to be noted that in the w-6 family of fatty acids, the proportion of GLA in the earthworm, *E. gammiei* was low, where as that of DGLA was higher. In fact, GLA is the immediate precursor to DGLA in the w-6 desaturate – elongase pathway <sup>28</sup>. These relative proportion of GLA and DGLA indicates that the elongase enzyme system is earthworms is responsible for catalyzing the conversion of GLA to DGLA in very active relative to the 5 – desaturase that converts DGLA into arachidonic acid <sup>7</sup>.

Preponderance of poly unsaturated fatty acids and mono unsaturated fatty acids in earthworm tissue makes it suitable for human consumption. Both these categories of essential fatty acids have several advantages. Poly unsaturated fatty acids (omega-3 and omega-6 fatty acids) in human, lower LDL, reduce irregular heartbeat, slow the buildup of plaque in arteries, lower blood pressure, reduce risk of diabetes and improves depression symptoms<sup>29</sup>. Monounsaturated fatty acids decrease risk of breast cancer, reduce cholesterol level, lower risk of heart disease and stroke, reduce body, pain and stiffness for patients of rheumatoid arthritis and reduce belly fat.

Earthworm tissue (*E*. gammiei) contains nutritionally important several minerals such iron  $1.03 \pm 0.005 \text{mg/g}$ , calcium  $0.01 \pm .008 \text{mg/g}$ , potassium 0.29  $\pm$  0.02%, magnesium 0.08  $\pm$ .001 mg/g, zinc  $0.16 \pm 0.008 \text{ mg/g}$  and copper 0.009  $\pm$  .0009mg/g. High content of iron in *Eutyphoeus* tissue is interesting. In Venezuela where the native Kuru people takes moto and earthworm (Andiorrhinus moto, A. Kuru) as food, high content of iron (1mg/g) could satisfy the daily recommended allowance of iron for a pregnant woman  $^{7}$ .

**CONCLUSION:** In north-east India, particularly in Nagaland, where variety of animals, such as caterpillars, termites, locusts *etc.* are taken as food, research on the nutritional values of these invertebrates is needed. Rich contents of essential amino acids, *viz.* methionine, leucine, lysine, valine and essential fatty acids such as arachidonic acid and linoleic acids and minerals indicate the suitability earthworm meal (*Eutyphoeus*) as an ideal source of food supplement for fishes and poultry in north east India. Efforts are needed to improve the local awareness and knowledge of earthworms and other invertebrates as source of protein, fat, minerals and Vitamins.

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