Biochemical Estimation of Paste Prepared from the Earthworm Pheretima posthuma in India

Ms. Shraddha Chintalwar[1] Mr. Pratik Meshram[2]
[1,2]Department of Zoology
[1,2]Kamla Nehru Mahavidhyala Sakkardhara Nagpur, Maharashtra India

Abstract— The present study was to analyze the biochemical composition of earthworm species Pheretima posthuma. This study provides information for formulation of fish and animal feed by incorporating these non-conventional animals like earthworm which helps nutritionist and fish farmers. Biochemical studies was carried out to estimate the content of protein, glucose and cholesterol in the earthworm paste. For this analysis the earthworm, Pheretima posthuma species were collected from various places like Ambazari lake bank side, Bhandewadi dump yard Nagpur, Maharashtra, India were acclimated to the laboratory condition for few days. Pheretima posthuma is the local species of the earthworm commonly found in India. For the study of biochemical analysis of protein, glycogen and cholesterol, earthworms were collected in monsoon period between the month of August and September 2016 and artificially vermiculture in laboratory and prepare the stock culture, in Department of Zoology Kamla Nehru Mahavidyalaya Nagpur, Maharashtra, India.

Key words: Proteins, Glucose, Cholesterol, Vermiculture, Earthworm Paste

I. INTRODUCTION

The aim of this study is to analyze the chemical composition of local earthworm like P. posthuma. This study also provides information for formulation of fish/animal feed by incorporating these non-conventional animals by fish in nutrition. In the present investigation seasonal study of chemicals composition of the earthworms has been undertaken as seasonal variations in biological activities are observed. The earthworms are better known as the friend of farmers. The earthworms improve the soil fertility in various ways. Actually, the burrowing and soil feeding habits of earthworms make the soil porous which permit both aeration and quick absorption of water also permits easy and deep penetration of the plant roots. They also bring the fresh subsoil to the surface which is still finer and rich in organic matters. Charles Darwin has estimated that an acre of earth is inhabited by nearly 50,000 earthworms (a recent estimate suggests that their number may reach up to 25, 00,000 per acre) which may bring more than 18 tons of deeper subsoil to the surface in one year. This may in 20 years form a layer of 7.5 cm thick surface on the earth in the form of their castings. The castings of earthworm contain fine soil having mixed with its nitrogenous wastes and faces of nice manorial value. The faces of earthworm contain nitrate, calcium, magnesium, potassium and phosphorus which constitute an important component of the humus essential for plant growth.

They also reduce the alkalinity and acidity of the soil to provide better conditions for plant growth, worms are also known as natural ploughmen or tillers of the soil. These are used as bait and food. The earthworms were used as food by so many uncivilized people of the world and they are still used as food by Macrea people. The earthworms are eaten upon by frogs, toads, moles, hedgehogs and birds which are of many uses to mankind. Many people earn their livelihood by catching these worms and supplying to scientific laboratories. Ayurvedic and Unani system of therapy suggests that these worms were used in making medicines for the cure of diseases like bladder stones, jaundice, pyorrhea, piles, rheumatism, etc. Even today, these are used in making various medicines of vital importance in India as well as other countries.

Earthworms may damage young and tender plants by eating them bit by bit. They also damage the grass lands by making tunnels in the ground when present in huge numbers. Some earthworms act as secondary hosts for the completion of life stages of some parasites which are directly or indirectly harmful to mankind. P. posthuma are long cylindrical worms of brownish color which live in burrows in moist earth particularly that contains decaying vegetation or humus.

An adult worm measures about 15-30 cm in length and is dark brown in color due to the presence of a pigment porphyrin in its skin. The body is made of 100 to 120 segments, of which the first segment is divided into an anterior prostomium and posterior ring-like peristomium. Segments 14-16 form a girdle-like thick band of glandular tissue called clitellum that secretes mucus, albumen and cocoon inside which eggs are laid. All segments of body except the first, last and clitellum have chitinous setae embedded in each segment. The setae are used in anchoring and in locomotion. P. posthuma, also exhibits a wide variety of circadian rhythms and daily rhythms to synchronize their activities with predictable changes in the environment, availability. According to Brady, 1981 and Bennet, 1974 Endogenous circadian rhythms of locomotary behavior in earthworms are well documented.

Collection of earthworms taken place from different places like bank of Ambazari lake and Bhandewadi dumpyard in Nagpur; and accumulated them in laboratory making a bed which is made up of vermicomposting, bed of cow dung, garbage waste of vegetables, dry wooden crush etc. Many references were seen which show alteration in metabolism due to temperature. Temperature has a profound effect on the metabolism of organism. Depending on the species, earthworm possess varying temperature optima and tolerances and even adopt to temperature extreme. They may show long term responses to chronic temperature change both in laboratory and in natural habit (G. Tripati, N. Kachhawala, I. Dabi, N. Bandooni). Pomert and Zarrow (1936) show the effect of temperature on the respiration of earthworm. Saroja (1961) documented seasonal acclimatization of oxygen consumption to temperature in tropical earthworm Megascolex mauritii. Temperature in tropical may be a factor of primary importance in determining the composition and structure of earthworm communities (Lavelle, 1983; Lavelle,

et.al.1989). Hazel (1995) described thermal adaptation in biological membranes. Temperature affects the lipid composition of the earthworm Lumbricus rubellus and Eisenia nordenskioeldi (Petersen and Holmstrup, 2000).

It appears to be the most important environmental variable influencing the growth metabolism and biology of earthworms. Tripathi and Bhardwaj (2004) suggested that the earthworm population mainly decreased with the increase in soil temperature. Tripathi and Bhardwaj (2004) made a comparative assessment of biomass growth of Eisenia fetida and Lampito mauritii at different temperature. The biomass of both the species of earthworms varied significantly with the changes in temperature.

The earthworms feed on large amount of protein material from decomposing plants and animals. An adult Glossoscolex giganteus (Leuckart, 1836) a terrestrial oligochaete, found in South Brazil ranges from 30 to 35 cm in length when contracted but when narcotized can measure more than 90cm long. The worm is cylindrical, with a diameter of 1.5-1.8 cm, with a slightly larger diameter in the segment near to the clitellum. Because of its larger size and high amount of food consumption and composting ability, this is the first oligochaete subjected to the biochemical and physiological studies. Most of the studies carried out to evaluate the chemical composition of earthworm deal with the genera, Lumbricus, Polypheretima and Allolobophora, all belonging to Lumbricidae. (Laverack, 1963)

These include works of Bahl (1947) on P. posthuma and of Heidermanns (1937) on Lumbricus terreritis. Needham's works of 1957 and 1960 dealt, resp, with nitrogenous excreta and arginase activity in Lumbricus terrestris and Eisenia fetida. Osmatic relations in earthworms were studied by Ramsay (1949) in lumbricus terrestris and De Jorged et al. (1965) in Glossoscolex giganteus (Leuckart). In early years the work on chemical composition of earthworms were carried out by Nguekam, 1993 in Eudrilus eugeniae, segbesan and ugwumba in Hyperiodrilua euryaulos and MD. Hasanuzzaman et al. (2010) in perionyx excavates.

Earthworms have been found to be a good source of protein (Guerrero, 1983, Hilton 1983, tacon et all. 1983 Kostecka and Paczka, 2006, Segbesan and Ugwumba, 2008) and its usage is as fish bite is well known (Segun, 1978, Omorinkoba et al., 1992). Earthworms, because of their high protein component, are fed to chickens, pigs and rabbits and as a dietary supplement for fish species. (Akiyama et al., 1984; Stafford an Tacon et al., 1983; Sabine, 1986; Mason et al., 1992). Among 36 earthworm species found in Bangladesh, Parionyx excavatus an epigeic earthworm is almost found throughout the year and is it suitably potential species for feeding rooster and fishes(Ali, 2002). The high reproductive rate and Biomass production of this tropical earthworm species makes it ideally suited for fish meal production (Edwards and Niederer, 1988).

Incorporation of the earthworms in fish/animal feeds can be considered as non-conventional feeds. Such earthworm incorporated feeds are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production. (Devendra, 1988; Madu et al., 2003). Due to their non-competitiveness, such feeds prove cheaper.

Fig. 1:

Fig. 2:

II. MATERIALS AND METHODS

Biochemical studies were carried out to know the content of protein, lipid and glycogen content of the earthworms. Estimation of the total protein was done by method described the total liquid contain was estimated by the method of 'Protein by Biuret Method'. Estimation of total glucose was made by 'O-Toluidine method'. The total liquid contains was estimated by method 'Zak's method'.

A. Preparation of Earthworm Paste:

20 sexually mature clitellated worms were washed with running tap water and then fed with wet blotting paper for 18-20 hours to clear their gut.

The gut cleared worms were again washed with distilled water. The worms were kept in plastic troughs, covered tightly with polythene cover, and exposed to sunlight for 3 days to kill them. Mucus and coelomic fluid that oozed out digested the dead worms forming a brown colored paste earthworm paste (EP). For the estimation of chemical constituents, an Entire body crushed and sample was taken and their average value is presented. There are different sizes of earthworms which are smaller weighing 0.260-0.267 gm and of 6-7 cm in length. They are transparent and light brown in colour. After this some are larger than these earthworms weighing 0.506-0.500gm and 9-10 cm in length.

III. OBSERVATION TABLE

1	Room temperature	31°C	Protein	Glucose	Cholesterol
			(Absorbance)		
	Weight of earthworm	1.31-1.30gm	0.63	0.028	0.018
	Length of earthworm	12-14cm			
	Place where earthworm found	Bhandewadi dumpyard, Nagpur.			
2	Room temperature	22°C	0.54	0.022	0.016
	Weight of earthworm	0.506-0.500gm			
	Length of earthworm	9-10cm			
	Place where earthworm found	Ambazari lake bank side, Nagpur.			
3	Room temperature	18°c	0.46	0.017	0.0013
	Weight of earthworm	0.267-0.260gm			
	Length of earthworm	7-8cm			
	Place where earthworm found	Ambazari lake bank side, Nagpur			

Table 1:

IV. RESULT AND DISCUSSION:

One of the benefits of earthworm culture is the production of a valuable protein source. According to Lieberman, 2002 it is used as a good nutritional feed for livestock and fish production. The growth and weight of this species, P. posthuma and the tissue components (protein, lipid and glucose) vary according to length and weight. Mason et al. (1990) and Pennino et al. (1991) have observed that this variation due to ecology, food, season, life style, reproductive state etc. Results showed variation in the weight of worms and chemical contents like protein, lipid and glycogen. Protein content is always proportional to the growth of the worm. The protein content, body weight and reproductive activities of worms.

The protein level is 6.3mg in earthworm whose length is 12-14cm weighing 1.31-1.30gm. 5.4mg in earthworm whose length is 9-10cm and weighing 0.506-0.500gm, 4.6mg in earthworm whose length is 7-8cm and weighing 0.267-0.260gm in size. The protein content of worm is always proportional to the growth of worms. According to Hatti Shankerappa S. 2013, the chemical composition of the earthworm (Eisenia fetida), worm casts and worm body fluids depend on common foods and animal feeds consumed by the worms. Common nutrient analysis showed that this species meal has high protein content in the range of 54.6 -71% dry matter. Protein content and amino acids composition were close to that of fish meal and hen egg, and higher than that of cow milk powder and soybean meal according to Sun Zhenjun, Liu Xianchun, Sun Lihui, Song Chunyang, 1997.

The degree of variations in the protein contents of cytoplasm and mitochondrial fractions remained more or less constant (i.e., 26 - 41% change) with respect to the changes in surrounding temperatures in all the three earthworm species. It is interesting to note that a number of animals are used as a protein source by the human populations in some regions of the world according to DeFoliart, 1989, 1999. The earthworm species Andiorrhinus motto and Andiorrhinus kuru, commonly referred as motto and kuru, respectively are known to be widely consumed in Venezuela Right and Araujuo, 1999; Moreno and Paoletti, 2002.

The information regarding the variation on the weight and length and protein content of earthworm is scanty. However, a comparative account on protein among the species content among the species is available according to Guerrero, 1983;Sogbesan et al., 2007; Md. Hasanuzzaman et al., 2010 Edwards (1985) reported that the dry matter of an earthworm body contains 60 to 70% protein, 7 to 10 % fat, 8 to 20% carbohydrate, 2,3% minerals and variety of vitamins.

Glycogen content in the tissue indicates the energy source for the metabolic activities including reproduction. There are reports (Guerrero, 1983; Sogbesan et al., 2007; Md. Hasanuzzaman et al., 2010). Edwards 1985 and Ghatneker in 1995, 2000 reported that the dry matter of earthworm contains 8 to 20% carbohydrate. The glucose level is 0.028mg in earthworm whose length is 12-14cm weighing 1.31-1.30gm. 0.022mg in earthworm whose length is 9-10cm and weighing 0.506-0.500gm, 0.017mg in earthworm whose length is 7-8cm and weighing 0.267-0.260gm in size. There are reports that the dry matter of earthworm body contains 8 to 20% Carbohydrates according to Edward, 1985; Ghatnekar 1995, 2000, the glucose content is proportionate with the rate of growth of the worms. In the present study the glycogen content of the body is found to be highest in longer and more weighing earthworm and lowest in smaller and low body weighted earthworms.

The cholesterol level is 0.0018mg in earthworm whose length is 12-14cm weighing 1.31-1.30gm. 0.0016mg in earthworm whose length is 9-10cm and weighing 0.506-0.500gm, 0.0013mg in earthworm whose length is 7-8cm and weighing 0.267-0.260gm in size. The analysis of the whole body of the earthworms contain large amount of proteins (64.5% and 72.9% dry weight), essential amino acids, lipids, carbohydrates and minerals, indicating that these worms

contain potentially useful quantities of nutrients that are critical to the health of human beings according to Paolett et al., 2003; Dedeke, 2010b. Md. Hasanuzzaman et al. (2010) worked on the nutritional composition of wild earthworm P. exacavatus and found that it contains 46.57±0.97%, protein and 8.03±0.44% lipid. They concluded that this earthworm species has almost similar nutritional values to fish meal and thus would be an animal protein in supplementing fish meal.

Further, they opined that year round production of this earthworm species through standard mass culture system and its radical use could be a pivotal role in sustainable fisheries and aquaculture production. In all the above mention reports the biochemical composition study of earthworms has been aimed towards its food value. Therefore, it can be concluded that earthworm can be used as a replacement for fish meal and also a potential source of protein, essential amino acids, lipids, minerals and trace elements. Therefore, they are widely consumed in Venezuela (Munnoli et al., 2010).

Depending on the species, earthworm possess varying temperature optima and tolerances and even adopt to temperature extreme. They may show long term responses to chronic temperature change both in laboratory and in natural habit according to G. Tripati, N. Kachhawala, I. Dabi, N. Bandooni. Pomert and Zarrow 1936 show the effect of temperature on the respiration of earthworm. Saroja 1961 documented seasonal acclimatization of oxygen consumption to temperature in tropical earthworm Megascolex mauritii.

Temperature in tropical may be a factor of primary importance in determining the composition and structure of earthworm communities (Lavelle, 1983; Lavelle, et.al.1989). Hazel (1995) described thermal adaptation in biological membranes. Temperature affects the lipid composition of the earthworm Lumbricus rubellus and Eisenia nordenskioeldi (Petersen and Holmstrup, 2000). It appears to be the most important environmental variable influencing the growth metabolism and biology of earthworms. Tripathi and Bhardwaj (2004) suggested that the earthworm population mainly decreased with the increase in soil temperature.

Tripathi and Bhardwaj 2004 made a comparative assessment of biomass growth of Eisenia fetida and Lampito mauritii at different temperature. The biomass of both the species of earthworms varied significantly with the changes in temperature. The optimum temperature for both the species were 25-30°C, respectively. Different earthworms have their own optimum temperature Lee, .1985, Holmstrup et. al. 1999 reported that E. nordenskioeldi synthesized and accumulated glucose as an immediate response to ice formation in extracellular body fluids.

Aerobic metabolic rate of most earthworms increases two times when their body temperature increases by 10°C well, 1980; Lee, 1985; Eshky et.al, 1996. The effects of temperature on the lipid composition and oxygen consumption Petersen and Holmstrup, 2000 in earthworms has also been documented. The present study is aimed to find the variation in protein, lipid and carbohydrate contents of earthworms, viz., P. posthuma. A variation in the body weight and length of all worms and chemical contents like protein, lipid and glycogen has been recorded in Pheretima posthuma. It can be used as a good source of food for fisheries, poultries and farming.

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