ISSN 2278-7763

Chemical Composition like Protein, Lipid and Glycogen of Local Three Species of Earthworms of Gulbarga city, Karnataka- India.

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Abstract:

Biochemical studies were carried out to know the content of protein, lipid and glycogen of local species of Gulbarga. Karnataka India. Local species of earthworms are *Polypheretima elongata*, *Perionyx sansibaricus* and *Dichogaster bolaui*. The studies were carried out seasonally, seasons of Gulbarga are characterized by summer (February to May) monsoon, (June to September)and winter (October to January). Our result show good seasonal in the body weight of all three worms and chemical contents like protein, lipid and glycogen has been reduced, being highest in summer, medium in monsoon and lowest in winter. Increase in their body weight, lipid and glycogen content during summer and monsoon may be attributed to the increased reproductive activities during these seasons.

Key words: Earthworm, Chemical composition, Seasons

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INTRODUCTION:

The earthworms feed on fairly large amount of protein material from decomposing plant 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 90 cm 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 (De Jorge *et al.,* 1965).

Most of the studies carried out to evaluate the chemical composition of earthworms deal with the genera, *Lumbricus, Polypheretima* and *Allolobophora,* all belonging to Lumbricidae (Laverack, 1963). These include works of Bahl (1947) on *Pheretima posthuma* and of Heidermanns (1937) on *Lumbricus terreritis*. Needham's works of 1957 and 1960 dealt, respectively, 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 Jorges *et al.* (1965) in *Glossoscolex giganteus* (Leuckart).

In recent years the work on chemical composition of earthworms are carried out by Nguekam, 1993; in *Eudrilus eugeniae*, Segbesan and Ugwumba(2008) in *Hyperiodrilus euryaulos* and Md. Hasanuzzaman *et al.* (2010) in *Perionyx excavatus*.

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Earthworms have been found to be a good source of protein (Guererro, 1983; Hilton, 1983; Tacon *et al.*, 1983 Kostecka and Paczka, 2006; Segbesan and Ugwumba, 2008) and its usage as fish bait is well known (Segun, 1978, Omorinkoba *et al.*, 1985). 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 and Tacon *et al.,* 1983; Sabine, 1986; Mason *et al.,* 1992). Among 36 earthworm species found in Bangladesh, *Perionyx excavatus* an epigeic earthworm is almost found throughout the year and is a suitably potential species for feeding roosters 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.

The aim of this study is to analyze the chemical composition of local earthworms like endogeic *Polypheretima elongata*, epigeic *Perionyx sansibaricus* and surface litter feeder *Dichogaster bolaui*. This study also provides information for formulation of fish/animal feed by incorporating these non–conventional animals by fish nutritionists and fish farmers. In the present investigation seasonal study of chemical

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composition of the earthworms has been undertaken as seasonal variations in biological activities are observed.

MATERIAL AND METHODS:

Biochemical studies were carried out seasonally to know the content of protein, Lipid and glycogen content of the earthworms. Gulbarga region is characterized by three seasons namely summer (February to May), monsoon (June to September) and winter (October to January). The estimation of the total protein was done by method described Lowry *et al.* (1951). The total lipid content was estimated by the method of Zollner and Kirsch (1962). The estimation of total glycogen was made by Glucose- Anthrone method as per Carrol *et al.* (1956).

Tissue weighing 500 mg was taken from the anterior part of the earthworms so as to cover the clitellar region for the estimation of chemical contents. The analysis was made five times for each species during each season and the average value is presented.

Estimation of Proteins:

The protein content in various earthworm tissues was estimated according to the method of Lowry *et al.* (1951). The protein containing tissue when treated with phenol reagent or Folin-Ciocalteu reagent (FCR), a deep blue colouration develops. This colour development is due to the reaction of alkaline copper sulphate solution with peptide bonds and reduction of phosphomolybdic acid and phospholungotic acid by the aromatic amino acids present in the protein. The blue colour developed is

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quantitatively proportional to the total protein in the medium which was measured spectrophotometrically.

The tissue was homogenized in 10 ml of distilled water. The homogenate was centrifuged for 15 minutes and 1 ml supernatant was collected in the test tube. To this 5 ml of alkaline copper reagent was added. Alkaline copper reagent was prepared by adding 50 ml of solution A and 0.5 ml of solution B&C (solution A was prepared by dissolving 2 g of sodium carbonate in 100 ml of 0.1N NaOH; solution B was prepared by dissolving 1 g of copper sulphate in 100 ml distilled water. Solution C was prepared by dissolving 2 g potassium sodium tartarate in solution B). After 20 minutes 0.5 ml of Folin-ciocalteau reagent (FCR) was added. After 30 minutes the optical density (OD) was read at 660 nm in a Spectrophotometer.

The calculations were made referring the standard graph. For the preparation of standard graph, standard protein solution was prepared by dissolving 20 mg of bovine serum albumin in 100 ml of 0.9% NaCl solution. This solution contains 200 µg protein per ml. From standard protein solution different concentrations were taken and as mentioned above required agents were added and optical density was measured at 600 nm in a spectrophotometer. Amount of protein in the tissues was calculated by using following formula.

$$\text{Concentration of protein} = \frac{\text{Concentration of protein in µg from standard graph}}{\text{Weight of tissue}} \text{ x } 1000$$

The concentration was expressed as µg/mg tissue.

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4.2.2 Estimation of Total Lipids:

The total lipid content of earthworm tissue was estimated by the method of Zollner and Kirsch (1962). Lipid containing sample is heated with concentrated sulphuric acid without prior deproteinization and then mixed with phosphoric acid-vanilline reagent. In this sulfo-phosphovanilline reaction, lipid develops a pink colour, which is measured photometrically. The intensity of the colour developed in samples is quantitatively proportional to the total lipid concentration in the tissue.

To the sample tubes containing 0.05 ml of tissue homogenate, 2 ml of concentrated sulphuric acid is added and was kept in boiling waterbath for 10 minutes, then cooled and centrifuged 1000 rpm for 5 minutes. From the supernatant, 0.1 ml sample was taken and 0.1 ml of standard solution (10mg/1ml) is added to standard tubes. In blank 0.1 ml of concentrated sulphuric acid is added instead of the homogenate. Then 2 ml of colouring reagent (11.9 M phosphoric acid, 0.008M/ Vanilline) was added to all the tubes. Then the tubes are allowed to stand at room temperature for 40-50 minutes. Then the absorbance of the samples and the standard was measured using a blank at 350 nm. The total lipid content calculated using the following formula.

$$\frac{As}{Ast} \times \frac{\text{Std. Conc.}}{\text{Aliquot Vol.}} \times \frac{100}{\text{Tissue wt.}} \times \text{dilution}$$

As=Absorbance of the sample.
Ast =Absorbance of the standard
Concentration was expressed as mg of total lipid content/100mg tissue wt.

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Estimation of Glycogen:

The tissue homogenate was prepared by using 10 ml of 4% TCA and centrifuged at moderate speed for ten minutes. The supernatant was decanted and the precipitate was discarded. To the 2 ml of supernatant 4 ml of Anthrone reagent was (200 mg of Anthrone was dissolved in 100 ml of concentrated sulphuric acid) added. The tubes were allowed to cool for 30 minutes. A blank is prepared using distilled water. The O.D. was measured at 620 nm wave length by using the spectrophotometer. Calculations were made using the formula.

$$= \frac{DU}{DS} \times 0.2 \times \frac{\text{Volume of extract}}{\text{Weight of the tissue}} \times 1000$$

Where,
DU → O.D of the sample,
DS → O.D of the standard,
0.2 → mg glucose in 2ml of standard volume of extract
Results were expressed in µg/mg tissue.

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RESULTS:

Seasonal changes in the biochemical parameters are observed in all three species. The details of these changes in individual species are as follow. Weight of the worms in all the species was more in summer season followed by winter season and least in the monsoon season.

1 *Polypheretima elongata:* (Table 1, Graph 1a, b and c)

The average body weight of the worm during summer is 2133.5±33.82 mg. It is slightly reduced to 1909.30±38.40 mg in monsoon and further to 1670.38±40.78 mg in winter.

Likewise, the protein, lipid and glycogen contents of the earthworm tissues show ups and downs in the various seasons. The average protein content during summer is 63.08±00.10 µg/mg tissue; it gets reduced to 59.02±00.28 µg/mg and 56.96±00.28 µg/mg tissue during monsoon and winter, respectively. Lipid content is also highest during summer and reduces during monsoon and winter seasons, as 07.25±00.06 µg/mg, 06.87±00.07 µg/mg and 06.74±00.04 µg/mg tissue is observed respectively during summer, monsoon and winter. Similarly, the average glycogen content of 15.40±00.22 µg/mg, 15.06±00.27 µg/mg and 14.22±00.28 µg/mg is observed during summer, monsoon and winter seasons respectively.

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Table –1: Seasonal gravimetric and biochemical changes in *Polypheretima elongata*

Parameters	Weight of earthworm (mg)	Protein (µg/mg)	Lipid (µg/mg)	Glycogen (µg/mg)
Summer	2133.5 ± 33.82	63.08 ± 00.10	07.25 ±00.06	15.4 ± 00.22
Monsoon	1909.30 ± 38.40	59.02 ±00.28	06.87 ± 00.07	15.06 ± 00.27
Winter	1670.38 ± 40.78	56.96 ±00.28	06.74 ± 00.04	14.22 ± 00.28

M±SE =Mean ± Standard error

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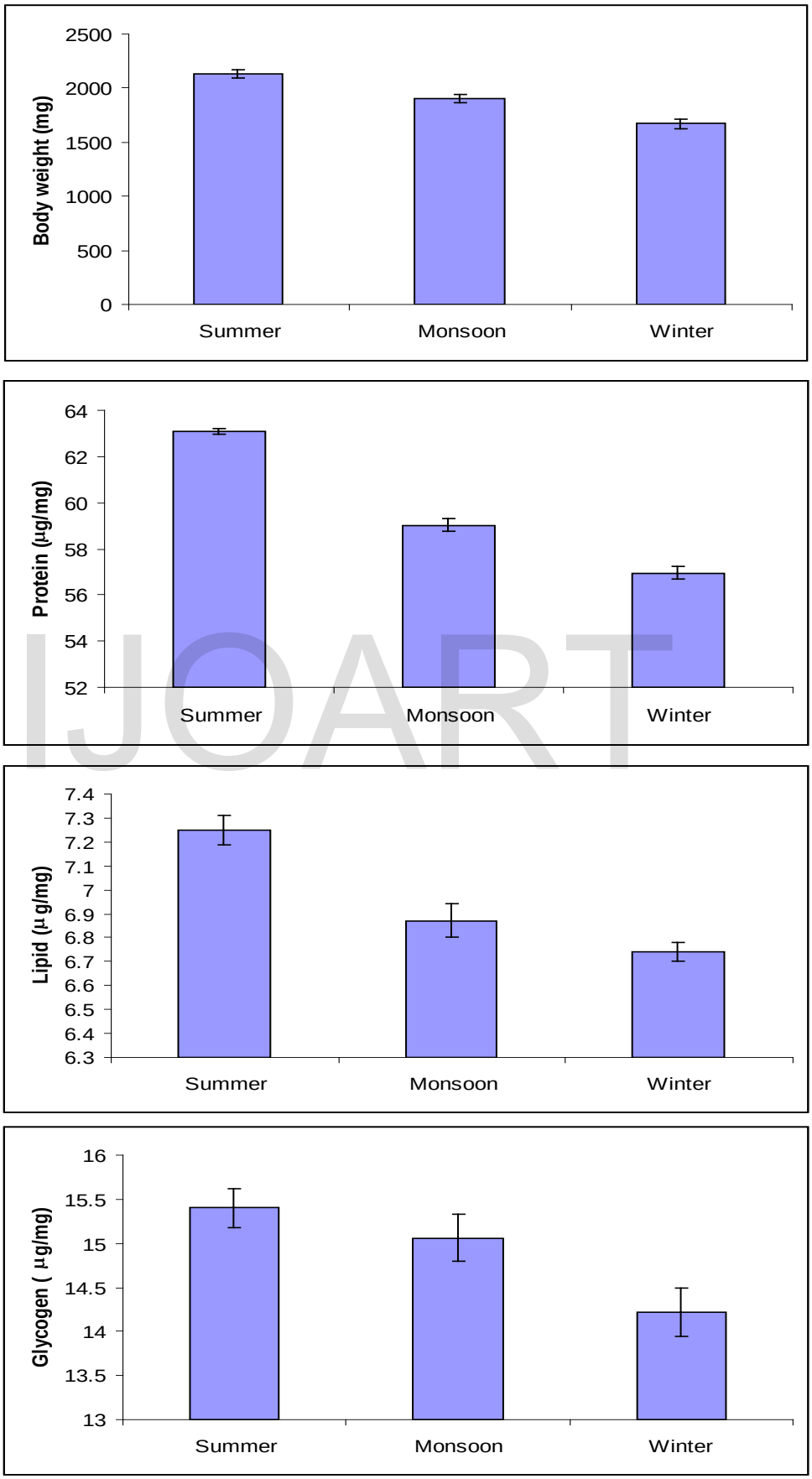
Graph –1: Seasonal changes in body weight and biochemical parameters *Polypheretima elongata*



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2 *Perionyx sansibaricus:* (Table 2, Graph 2a, b and c)

The body weight of this species is highest during summer and reduces gradually during monsoon and winter. The average body weight during summer is 543.14±01.59 mg, during monsoon is 479±01.50 mg and during winter it is 401±22.86 mg. The average protein content of the worm is observed 46.9±00.16 µg/mg, 45.8±00.26 µg/mg and 45.20±00.27 µg/mg during summer, monsoon and winter seasons, respectively. The average lipid content is 09.67±00.10 µg/mg during summer. During monsoon it reduces to 08.46±00.12 µg/mg and further reduced to 07.10±00.10 µg/mg which is observed during winter. The average glycogen content is observed to be 15.84±00.24 µg/mg, 14.44±00.11 µg/mg and 13.94±00.22 µg/mg during summer, monsoon and winter seasons, respectively.

3 *Dichogaster bolaui:* (Table 3, Graph 3a, b and c)

The average body weight of this earthworm during summer 400.50±04.20 mg, 350.30±05.62 mg during monsoon and 330.50±04.08 mg during winter.

The average protein content is 53.62±00.19 µg/mg, 53.12±00.22 µg/mg and 52.38±00.15 µg/mg during summer, monsoon and winter seasons, respectively. The average lipid content of earthworm is 07.00±00.17 µg/mg, 06.75±00.10 µg/mg and 05.39±00.09 µg/mg respectively during the summer, monsoon and winter seasons. The glycogen content of the earthworm is 13.90±00.22 µg/mg during summer, 13.34±00.18 µg/mg during monsoon and during winter it is 11.20±00.23 µg/mg.

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Table –2: Seasonal gravimetric and biochemical changes in *Perionyx sansibaricus*

Parameters	Weight of earthworm (mg)	Protein (µg/mg)	Lipid (µg/mg)	Glycogen (µg/mg)
Summer	543.14 ± 20.59	46.90 ± 00.16	09.67 ± 00.10	15.84 ±0 0.24
Monsoon	479.00 ± 21.50	45.80 ± 00.26	08.46 ± 00.12	14.44 ± 00.11
Winter	401.00 ± 22.86	45.20 ± 00.27	07.10 ± 00.10	13.94 ± 00.22

M±SE =Mean ± Standard error

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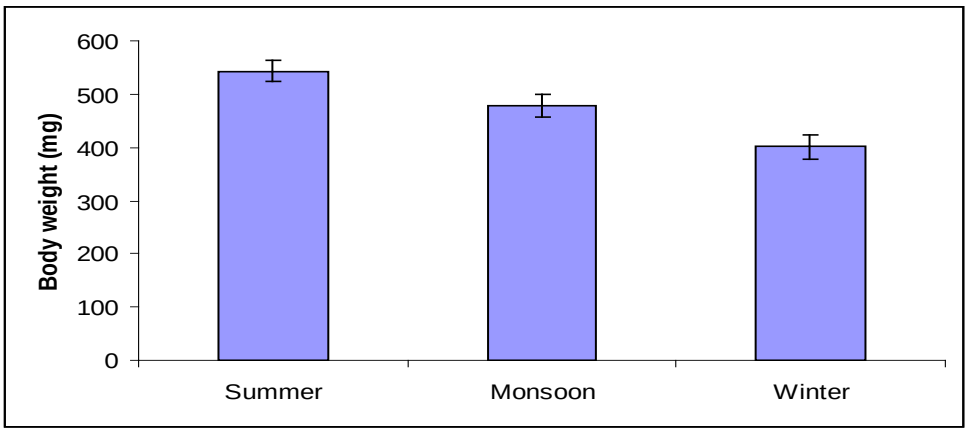
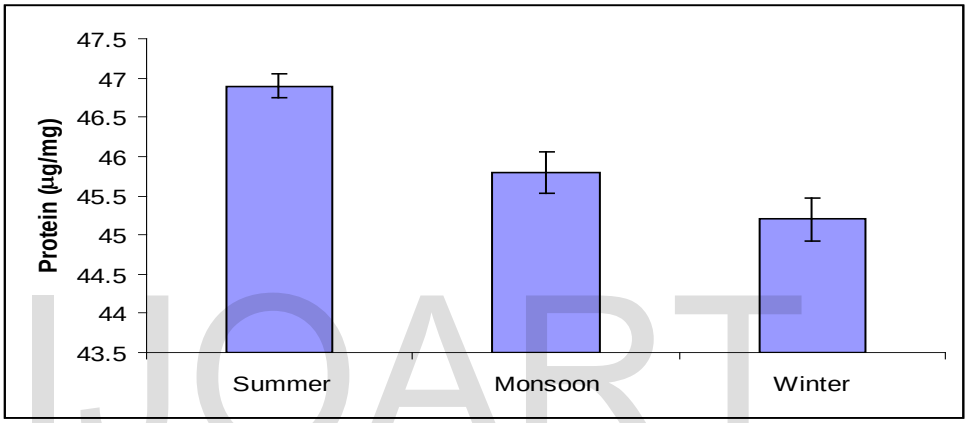
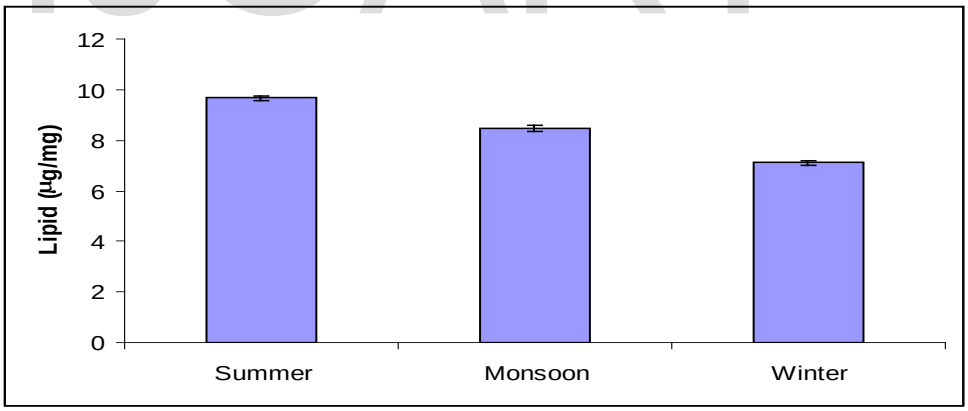
Graph –2: Seasonal changes in body weight and biochemical parameters *Perionyx sansibaricus*







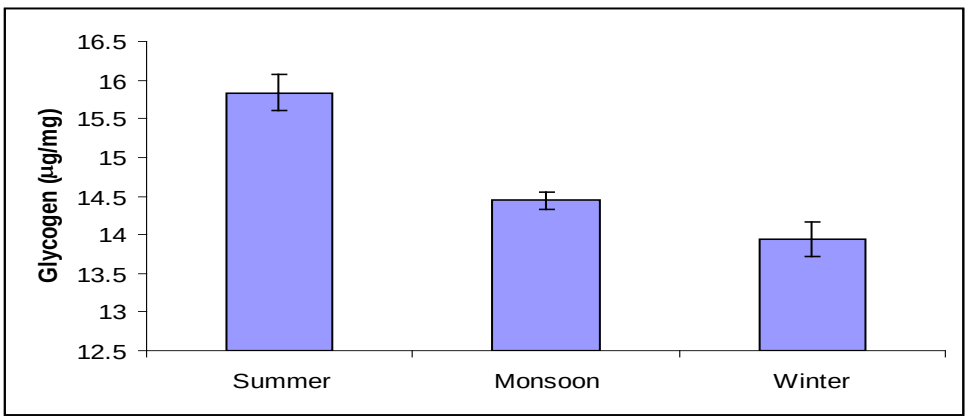


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Table –3: Seasonal gravimetric and biochemical changes in *Dichogaster bolaui*

Parameters	Weight of earthworm (mg)	Protein (µg/mg)	Lipid (µg/mg)	Glycogen (µg/mg)
Summer	400.50±04.20	53.62 ± 00.19	07.00 ± 00.17	13.90 ±0 0.22
Monsoon	350.30±05.62	53.12 ± 00.22	06.75 ± 00.10	13.34 ± 00.18
Winter	330.50±04.08	52.38 ± 00.15	05.39 ± 00.09	11.20 ± 00.23

M±SE =Mean ± Standard error

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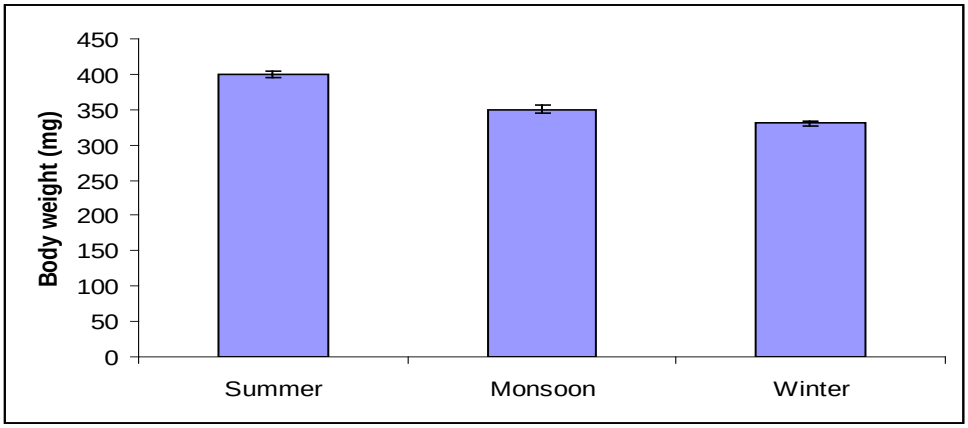
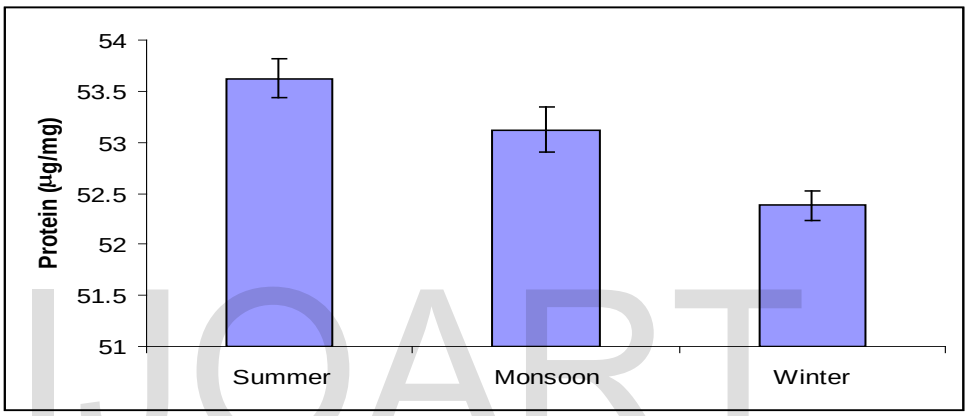
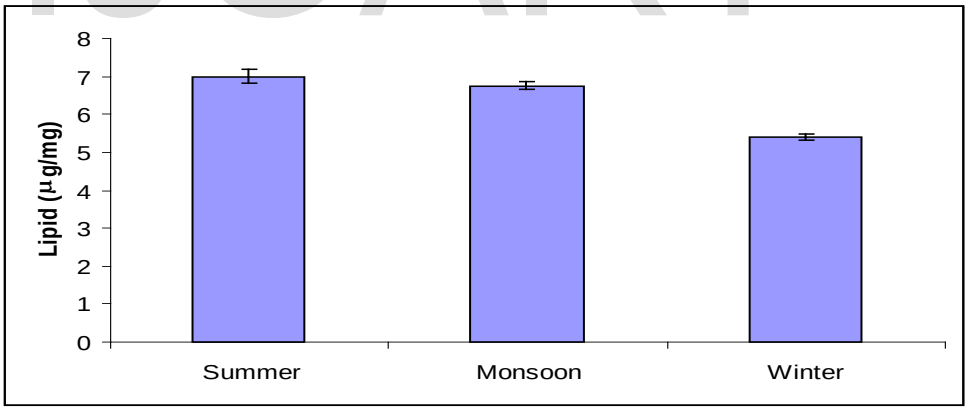
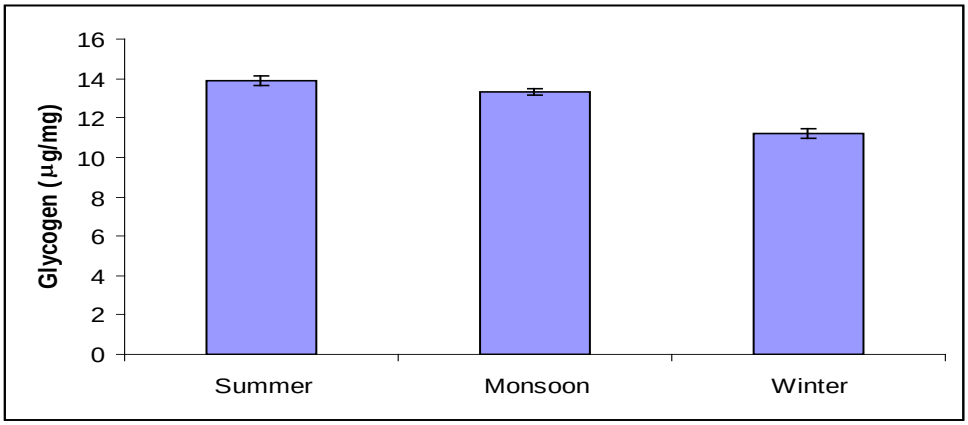
Graph –3: Seasonal changes in body weight and biochemical parameters *Dichogaster bolaui*









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In case of all the three species of earthworms, the average body weight during the summer is highest and gets reduced gradually in the monsoon and winter. The lipid and glycogen contents of the body tissue also vary along with body weight. However, no significant change in the protein content of the body is observed during different seasons of the year. The increase in the body weight, lipid and glycogen contents during summer and monsoon may be attributed to the increased reproductive activities of the worm during these seasons.

DISCUSSION:

One of the benefits of earthworm culture is the production of a valuable protein source (Lieberman, 2002). It is used as a good nutritional feed for live stock and fish production.

The growth and weight of all the three species and the tissue components (protein, lipid and carbohydrates) vary from species to species and from season to season. Mason *et al.* (1990) and Pennino *et al.* (1991) have observed that this variation is proximately associated with specific ecology, food, season, life style, reproductive state, etc. Our results show that a good seasonal variation in the weight of all three worms and chemical contents like protein, lipid and glycogen. They are highest in the summer, medium in monsoon and lowest in winter.

Protein content is considered to be the building material and involved in the alteration of almost every physiological function. It is always proportional to the growth of worm. The protein content,

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body weight and reproductive activities of the three species viz., *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui,* are highest during summer. When the growth and reproductive activities of the worms are gradually reduced during monsoon the protein content is also reduced to intermediate level. As the growth and reproduction is least during winter, the protein content too of all the three species is found to be the lowest.

The information regarding the seasonal variation on the growth and protein content of earthworm is scanty. However, a comparative account on protein among the species content among the species is available. It is highest in *Polypheretima elongata* medium in *Hyperiodrilus euryaulos* and lowest in *Perionyx excavatus* (Guerrero, 1983; Sogbesan *et al*., 2007; Md. Hasanuzzaman *et al*., 2010). Edwards (1985) and Ghatneker (1995, 2000) 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.

In the present study the seasonal variation in protein content is made it is highest during summer slightly reduced in monsoon and winter in all the species studied.

The lipid content of various earthworms is reported to vary. It is 5.9% in *Hyperiodrilus euryaulos* (Sogbesan *et al*., 2008), 5.8% in *Perionyx excavatus* (Guerrero, 1983) and 5.15% in *Eisenia fetida* (Dynes, 2003).

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The present study is also aimed to find out the seasonal variation of lipids in three species of earthworms. It is highest during summer followed by monsoon and least in winter. The lipid content is also proportionate to the reproductive activities of earthworms.

The glycogen content in the tissue indicates the energy source for the metabolic activities including reproduction. There are reports that the dry matter of earthworm body contains 8 to 20% carbohydrate (Edwards, 1985; Ghatneker, 1995, 2000).

In the present study the glycogen content of the body is found to be highest in summer and lowest in winter, which is proportionate with the rate of growth of the worms and their reproductive activities. It appears that biochemical studies on the composition of the earthworms in relation to seasonal changes are lacking.

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 (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 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

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potentially useful quantities of nutrients that are critical to the health of human beings (Paoletti *et al.,* 2003; Dedeke, 2010b).

Md. Hasanuzzaman *et al*. (2010) worked on the nutritional composition of wild earthworm *Perionyx excavatus* 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 mentioned 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).

The present study is aimed to find the seasonal variations in protein, lipid and carbohydrate contents of the three earthworm species, viz., *Polypheretima elongata, Perionyx sansibaricus* and *Dichogaster bolaui.* A good seasonal variation in the body weight of all three worms and chemical contents like protein, lipid and glycogen has been recorded, being highest in the summer, medium in monsoon and lowest in winter. Because of their high amount of protein, lipid and glycogen, the earthworms viz.,

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Polypheretima elongata, Peronyx sansibaricus and *Dichogaster bolaui* can be used as a good source of food for fisheries, poultries and piggeries and also for the consumption of human beings.

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