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# Open Access Research Article

Experimental Studies for Growth and Bioenergetics in *Eudrilus eugeniae* under Three Agro-climatic Conditions of Rainy, Winter and Summer

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### **Abstract:**

Energy transformations and energetic of oligochaete species is available in population/ecological energetic of the soil inhabitants of the temperate and tropical regions. The study of bioenergetics is relevant for both basic sciences as well as applied fields to assess the environmental impact on the economically important organisms like compost earthworms. In the present research an experiment has been implemented to study the utilization of food budget in the tropical compost earthworm *Eudrilus eugeniae* under regular seasonal patterns of rainy, winter and summer by providing ad libitum partially-aerobically decomposed cattle dung. This study was done to know the effect of season on the feeding, defecation and cocoon production of the earthworms for its best utilization from the point of commercialization under three agro-climatic conditions. The fate of eaten food from hatchlings to post reproductive periods were documented for the study of growth period, growth, food consumption, daily food intake as a function of age group, total food intake as function of age group, feeding rate, feces defecation, food assimilation, assimilation rate (ASR), assimilation efficiency (ASE), conversion of assimilated food into body substance, conversion rate, gross conversion efficiency (K1), net conversion efficiency (K2), food oxidized as an expression of metabolism and metabolic rate. The impact of season on the feeding, defecation, absorption, assimilation and cocoon production has been discussed in detail.

**Keywords:** Absorption, Assimilation Rate, Conversion Efficiency, Feeding Rate, Metabolic Rate, Oxidation.

# 1.0 Introduction:

The bioenergetics subject involves thermodynamic laws (Haynie, 2001), energy can neither be created nor destroyed although inter converted between different forms (Frayn, 1996) unless there is degradation of energy from a nonrandom to a random form (Phillipson, 1966). Study of growth and bioenergetics in the compost earthworms is a necessity from the angle of bulky conversion of biological wastes for Indian circumstances under three agro-climatic conditions of rainy, winter and summer. Previous workers have collected bioenergetics' data to assess the ecological energetic subjecting the abundantly found soil feeding earthworms and litter feeders during their active periods i.e., rainy season. Study of earthworms' role in decomposer subsystem (Coleman and Sasson, 1978), their density, biomass, burrowing, feeding, egestion and energy budgets (Boltson and Phillipson, 1976; Dash and Patra, 1977) mainly under natural ecosystems and of endogeic/anecic nature are appreciable. Earthworm Eudrilus eugeniae an experimental animal of the present study is an epigeic, humus forming compost earthworm that has become a celebrity in terms of

its research and importance under laboratory as well large-scale commercial activities. A series of work using cattle dung as their natural diet has been proved by several workers (Hand et al, 1988, 1988b; Reinecke and Viljoen, 1990; Viljoen and Reinecke, 1990; Hendriksen, 1991; Kale et al, 1994; Sunitha et al, 1994; Edwards and Bohlen, 1995, 1996; Edward et al, 1995; Sunitha and Kale, 1995a; 1995b; 1997; Sunitha, 2011a, 2011b).

Kale, (1995), observed that wooden boxes, cemented tanks, earthern pots, eathern pits lined with either stones/plastics are best suited for multiplication of *E. eugeniae* under humid and slightly dark places with a substrate moisture of av.45% and an av. temp of 25°C and of neutral pH 7. Moisture of the body surface of an earthworm is of prime physiological factor that facilitate locomotion, skin respiration, excretion of nephredial function and for lubricating act of coelomic fluid (Ghabbour, 1975; Shanthi et al, 1993; Muyima et al, 1994; Edward and Bohlen, 1996). In the present study a detailed aspect of metabolism, metabolic rate has been studied. Reinecke and Viljoen, 1990; Muyima et al, 1994 have shown a wider tolerance of

temperature by E. eugeniae as high as 42°C and a low soil temperature below 50° C. In the present study, the worms appeared to be feeding and defecating throughout the period of study irrespective of season; however, wider differences were observed when subjected to food budget and bioenergetics of the worm that affected the production of vermicompost under seasons of rainy, winter and summer. The quality and amount of food material influence not only size of earthworm population but also their rate of growth and fecundity (Dominguez et al., 2000; Chaudhari and Battacharjee 2002). The present study, a record from hatchling to extended post reproductive periods on exposure to natural diet of cattle dung under three climatic factors depicted the seasonal role-play on growth and reproduction based on rates and efficiencies of assimilation, conversion metabolism.

## 2.0 Materials and Method:

Hatchlings of Eudrilus eugeniae were collected into aerated plastic boxes containing wet filter paper to estimate food utilization budget in different seasons of rainy, winter and summer. Three replicates were maintained consisting of known weight of ten hatchlings/ box. Worms were grouped as shown in Fig: 1. The experiments were terminated at the end of every 20<sup>th</sup> day and study parameters were recorded accordingly. Worms were allowed in submerged water for a period of 18 - 20hrs for gut clearance to start the next set of 20-day period studies. When the worms were bulged due to immersion in water during gut clearance, were then put on layers of dry filter paper for 15 - 20mts to lose excess water until the original body consistency were obtained. The experiments were conducted by feeding the worms with partially decomposed cattle dung with the onset of respective seasons. Av.60% moisture were maintained in the feed substrate throughout the study period, irrespective of the season and outside temperature. A sample of the feed was kept in triplicate as control without earthworms to record the percent moisture of the feed prior to the start of the experiment and on completion of the every 20<sup>th</sup> day of the experiment. Three seasons were considered: Rainy season (28 degree C + 2 degree); Winter season (at 26 degree C + 2 degree); and summer season (32 degree C + 2 degree). The worms, their feces, given feed as well as left over feed were weighted in a single pan balance to an accuracy of 0.1mg. Ad libitum feed maintenance was ensured; feces were collected and

oven dried. The uneaten feed was collected at the termination of each set of experiments (at the end of 20<sup>th</sup> day) and oven dried. The worms' weights on fresh and dry weight basis were recorded at the beginning and at the end of the experiment. To record the growth attainment, few worms from each replicate were freeze killed and then placed in hot air oven at av.85 degree C. until the constant weights were obtained. Details of the method applied for calculations of the obtained data prior to statistical analyses are shown in **Table 1**.

### 3.0 Results and Discussion:

Type and amount of material available influence the size of earthworms, population, species diversity, growth rate and cocoons production (Edwards et al, 1998). In the present study ad libitum feed substrate cattle dung was provided for the study of growth and fate of consumed feed under three seasons as factors. Seasonal conditions under tropical climate affect the food utilization to influence growth, development of sexual stages and one must consider the possible fates of food that was consumed with a quality of organic waste as one of the factors determining the onset and rate of reproduction (Dominguez, 2000). In the present study the season has played a role in determining the age of the worm and was well known from the development of clitellum and cocoon production. As a fact cocoon production and rate of hatchling are important for multiplications of continual compost earthworms (Bhatacharjee and Chaudhuri, 2002). As per the previous record cocoon production starts at the age of 6 weeks and continues till the end of 6 months. Under favorable conditions one pair of earthworms can produce 100 cocoons in 6 weeks to 6 months (Ismail, 1997). In the present study during rainy season, the worm produced cocoons during the 40<sup>th</sup>, 60<sup>th</sup> and 80<sup>th</sup> day period with average cocoons of 6.40, 17.00 and 20.33 respectively totaling to 43.73 cocoons per worm; under winter season, the worm produced cocoons during the 60<sup>th</sup> ,80<sup>th</sup> and 100<sup>th</sup> day study period with an average cocoons of 2.40, 3.71 and 7.21 respectively totaling to 13.38 cocoons per worm; under summer season, the cocoons were produced only in the adult(2) stage between 61<sup>st</sup> to 80<sup>th</sup> day with 17.44 cocoons. Based on the morphological appearances and on the beginning and ending stage of cocoon production it can be assumed that during rainy season the worms reproductive period lasted for 60 days (2months) with an early maturity with more number of cocoons; in winter season although lasted for 60days but the reproductive days were extended with less number of cocoons compared to other seasons; however in summer only 20 days were seen as reproductive period but higher number cocoons in a short period than in winter.

Fig 1: Earthworms grouped as under for ease of the experimental study

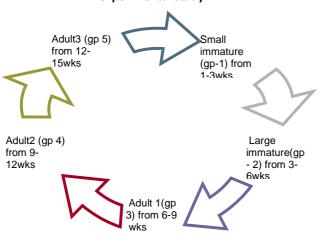


Table 1: Details of the method applied for calculations for the data prior to statistical analyses

Conversion of feed into body	Estimated by subtracting the dry weight of the experimental individuals
substance	at the beginning of the experiment and from that at the termination of
	the experiment
Food intake	was determined by subtracting the dry weight of uneaten food from the
	dry weight of the feed provided.
Food untilization budgets	were studied using IBP formula and terminology(Petrusewiez and
	Macfadyen, 1970) $I = B + M + F$ (where, $I = food consumed(ingested); F$
	= undigested food; <b>B</b> = food consumed – undigested food; <b>I</b> – <b>F</b> =
	assimilated food; <b>M</b> = assimilated food used for growth (gain in biomass,
	growth and conversion)
values	expressed in mg dry weight per age group
rates	expressed in mg dry food per mg live individual per day
Efficiencies	expressed in percentage
Growth	considered in mg live weight as function of age group
	Applied calculations
Rates of :	Each value (of feeding / assimilation / conversion / metabolic) of each
(a) Feeding	individual age group (of small immature / large immature / adult(1) /
(b) Assimilation	adult(2) / adult(3) multiplied by worm period of activity (20 days for each
(c) Conversion	age group) divided by total number of worm period from small
(d) Metabolic	immature to adult(2) / adult(3).
Assimilation efficiency (ASE):	Based on insect physiologists, Muthukrishnan and Pandian, 1987.
Conversion Efficiency:	Based on insect physiologists, Pandian, 1967 and Delvi, and Pandian,
(a) Gross conversion efficiency – K1	1972
(b) Net conversion efficiency – K2	
Metabolism	Difference between assimilation (AS) and Production (P), calculations
	based on insect physiologists, Muthukrishnan and Pandian, 1987

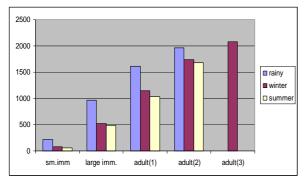


Chart 1a: Growth (values expressed in mg wet wt/worm)

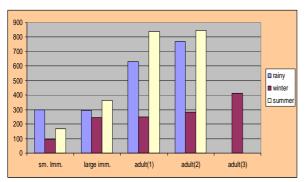
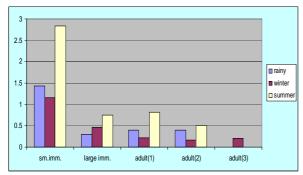
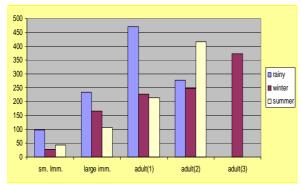


Chart 1b: Food intake (values expressed in mg dry wt/day/worm)



**Chart 1c: Feeding Rate** (values expressed in mg dry food/mg live worm/day)



**Chart 1d: Feces defecation** (values expressed in mg dry wt/day/worm)

**Chart 1a to 1d:** Showing Growth, food intake, feeding rate and feces defecation fed on cattle dung (ad libitum) in different seasons from hatchlings to post reproductive stages. Each age group is a record of 20days interval.

Earthworm biomass apart from specific species plays a pivotal role for the transformation of bulky organic wastes under mesophilic composting activities (Haimi and Huhta, 1986; Edward, 1998; Chaudhuri and Bhattacharjee, 2002; Sunitha, 2011b). Growth period, growth, food consumption, daily food intake as a function of age group, total food intake as function of age group, feeding rate, feces defecation, assimilation, assimilation (ASR), rate assimilation efficiency (ASE), conversion assimilated food into body substance, conversion rate, gross conversion efficiency (K1), net conversion efficiency (K2), food oxidized as an expression of metabolism, metabolic rate were the interconnected parameters that were calculated from the obtained experiment as shown in Tables: 2a, 2b and **2c.** The obtained data were applied to statistical analyses shown in Charts: 1a - 1d. The quantity of food taken by a worm varies from 100 to 300 mg/g body weight/ day (Edwards, 1972). In the present study assimilation was reflected in large proportion of ingested energy that was lost in feces, was the wasteful energy for compost earthworm but was a value-added product from the agricultural point of view.

Assimilation was higher in summer season than in rainy and winter. The assimilation trend slowed as the age of the worm advanced; in summer season this phenomenon was not seen wherein at all the age group the percentage of assimilation either increased and subsequently decreased or vice versa seen. It meant to say with an interval of 20day period, the assimilation trend increased at one time and decreased at another and again increased in the next showing increase-decrease pattern. Earthworm species, type of food, climatic conditions play a pivotal role in the doubling time, with reference to density and biomass (Ismail, 1997). Food intake increased in comparison to growth/age of the earthworm *E. eugeniae* in the present study. Small immature worms consumed cattle dung on dry weight basis 22 times and 55 times more food than their body weight in wet condition of av.60%. The rate of food consumption suddenly decreased with increase in body weight thus in the succeeding age

groups of large immature, adult(1), adult(2) or adult(3) the food consumption accounted to between 7.8-6.0 in rainy season; 3.9-9.3 in winter season and 10-16 in summer season.

Table: 2a – Summe	rization of fate of food utilization during rainy season
Fate of food utilization	Inference drawn
Food consumption and assimilation	Increased food consumption, increased growth Daily food intake as a function of age group was maximum for small immature compared to other groups. The feeding rate was high at small immature period (1.431mg of dry food /mg of live body wt/day) and there after reduced > 75% in other periods. Food assimilation in small immature was higher. Assimilation rate (ASR) was directly proportional to assimilation which in turn was proportional to the food intake. Assimilation efficiency (ASE) = food assimilated as a percentage of food consumed was more or less a fluctuation trend.
Feces defecation	Feces defecation was maximum for small immature group(75mg of dry wt/mg of dry body wt/day).
Conversion of assimilated food into body substance	Was more in large immature. The conversion rate was highest for small immature than other age groups. Conversion rate decreased as the growth increased. Gross conversion efficiency (K1), from small immature to large immature (i.e., the converted food material) showed efficiency of 12 to 3.2%. The conversion efficiency (K1) decreased as a function of age group and reached minimum of 0.3% to 0.6% in the older age groups. Net conversion efficiency (K2), was seen in large immature with 13.61% and least record of 1.89% for small immature.
Food oxidised (an expression of metabolism) Cocoon production	Was highest in adult(2) with 486.86mg dry wt/mg dry period; least record of 53.19mg in large immature. Metabolic rate was maximum for small immature (0.935mg) and drastically decreased to 0.055mg for large immature and 0.098mg for adult(1) and increase again an increase trend of 0.249mg in adult(2).  Cocoon production during the 40 <sup>th</sup> , 60 <sup>th</sup> and 80 <sup>th</sup> day recorded av. 6.40, 17.00 and 20.33 respectively totaling to 43.73 cocoons/worm. The period from 21 <sup>st</sup> day to 60 <sup>th</sup> day was considered as active reproductive period and 61 <sup>st</sup> day to 80 <sup>th</sup> day as post reproductive/extended reproductive period.
Growth completion	Required 80 days and later showed diminished activity, wt. loss and reduction in cocoon production. The pre-reproductive period/ small immature period lasted for 20days from hatchling. 21" day to 40" day period were extended as pre-reproductive period/large immatuse period, but cannot be called immature, because worms at this period produced cocoons; hence could be depicted as initiated reproductive period.

Fate of food utilization	Inference drawn
Food consumption and assimilation	Incressed food consumption showed increased growth. The daily food intake as function of age group was highest in small immature stage. Total food intake as function of age group for 100days accounted for 1282.67mg, the feeding rate was high at small immature stage and showed several times decreasing trend in the subsequent age groups. Food assimilation in small immature was higher than for other stages. Assimilation rate (ASR) was directly proportional to assimilation which in turn was proportional to the food intake. Assimilation efficiency (ASE) = food assimilated as a percentage of food consumed was more or less a fluctuation trend. The conversion of assimilated food into
Feces defecation	body substance was more in the adult(1).  Feces defecation was highest for small immature stage with 26.67mg of dry wt/ mg of dry body wt/day.
Conversion of assimilated food into body substance	Was highest for small immature. Conversion rate decreased with age. Gross conversion efficiency (K1) was 1.7 to 3.5% from small immature to adult(2) stage. Adult(3) showed only 0.3%. net conversion efficiency (K2) showed was 2.73% in small immature and increased to 39.43% in adult(1) and decreased from 10.6% in
ood oxidised (an expression of metabolic rate)	stage. Metabolic rate was maximum in small immature and drastically decreased to 0.018mg in adult(3) stage. Cocoon production during the 60th, 80th and 100th day
Cocoon production	stages was an av. cocoons of 2.40, 3.71 and 7.21 respectively totaling to 13.38 cocoons/worm. Later worms entered to no cocoon production stage with diminished activity.
Growth completion	Required 100 days later showed weight loss, reduced cocoon production and diminished feeding activity. The pre-reproductive period/ small immature period lasted for 20days from the date of hatchling stage. The large immature period lasted for 21 <sup>st</sup> day to 40 <sup>th</sup> day, which could be noted as extended pre-reproductive period.

Study of earthworms' role in decomposer subsystem (Coleman and Sasson, 1978), their density, biomass, burrowing, feeding, egestion and energy budgets (Boltson and Phillipson, 1976; Dash and Patra, 1977) mainly under natural ecosystems and of endogeic/anecic nature are appreciable. 'T' diagram: 1 – depicts energy allocation of consumed food in E. eugeniae. The total feces defecation accounted to 54.3% in rainy season; 81% in winter season and only 35.2% in summer season. The total amount of food converted into body substance increased as a function of age with minimum in small immature and maximum at large immature and /or at adult stages; later showed minimized conversion at the extended growth period. The converted food in percentage accounted for 2.1%, 7.4% and 1.5% in rainy, winter and summer respectively. Winter season recorded lowest oxidation or metabolism ranging between 92.8%; with an increase of 2 degree C. during rainy season, the metabolic rate slightly increased to 97.7%. In summer at higher temperatures the metabolic rate increased to 98.5%. Among the age groups of a particular season and between age groups of three seasons, summer season showed uniform levels of metabolic activity. This study reveals that seasons of rainy, winter and summer as a factor of temperature played a vital role in the metabolism/ food oxidation of the earthworm E. eugeniae that affect the defecation variation a factor to be considered under vermicompost production in terms of organic waste utilization as a value added product.

**Flow Chart 1:** 'T' Diagram showing energy allocation of consumed food cattle dung in *Eudrilus eugeniae* fed ad libitum in different seasons.

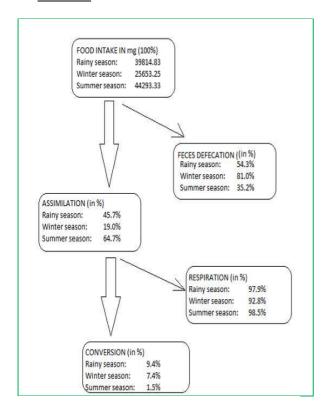


Table: 2c – Summerization of fate of food utilization during summer season				
Fate of food utilization	Inference drawn			
Food consumption and assimilation	Increased food consumption showed increased growth trend. Daily food intake as function of age group was maximum for small immature. Total food intake as function of age group for a period of 80days accounted for 2214.67mg. The feeding rate was high in small immature and showed several fold decrease trend in the later stages. Food assimilation in small immature was higher than for other stages. Assimilation rate (ASR) was directly proportional to assimilation which in turn was proportional to the food intake. Assimilation efficiency (ASE) = food assimilated as a percentage of food consumed was more or less a fluctuation trend. The conversion of assimilated food into body substance was more in the adult(2).  Feces defecation was maximum for small immature group			
Feces defecation	was 43.33mg of dry wt/mg of dry body wt/day.			
Conversion of assimilated food into body substance	Was highest for small immature and decreased as the worm period increased. Gross conversion efficiency (K1) was lower in small immature stage and increased in the subsequent stages. Net conversion efficiency (K2) was 13.6% in large immature stage and was least record of 1.89% in small immature stage.			
Food oxidised (an expression of metabolic rate)	Was highest in adult(1) 617.17mg and decreased to 42 lmg in adult(2) stage was an indication of diminished body activity as a function of dry season. Metabolic rate was highest in adult(1) 0.608mg and decressed to 0.252mg in adult(2).			
Cocoon production	Cocoon production period was $61^a$ to $80^b$ day with av. $17.44$ cocoon/worm.			
Growth completion	Required 80days, later showed weight loss, entered to no cocoon period with diminished feeding activity. The growth trend was maximum of $1678.50$ mg for adult2. The period $41$ n to $60$ h day were considered reproductive period for cocoon production.			

**Table: 3** - showing food assimilation, Rate of Food Assimilation, Food Assimilation Efficiency and Conversion of Assimilated Food when fed on cattle dung (ad libitum) in different seasons from hatchlings to post reproductive stages. Each age group is a record of 20days interval.

Season	Age group	Food assimilation	Rate of food	Food assimilation	Conversion of
		(mg dry wt/ day/	assimilation	$\textbf{efficiency} \ (\% \ \ \text{of} \ \ \text{food}$	assimilated food
		wom)	(mg dry wt/ day/	intake)	(mg dry wt/mg dry
			wom)		wt of worm/day)
Rainy season	Small immature	199.17 +/- 24.76	0.953 +/- 0.094	66.809 +/- 3.018	3.80 +/- 0.72
(28 degree C	L arge immature	60.53 +/- 27.07	0.063 +/- 0.027	20.321 +/ - 7.979	7.35 +/- 1.15
+/-2 degree C)	Adult(1)	159.38 +/- 55.53	0.100 +/- 0.040	24.935 +/- 6.433	3.79 +/- 1.65
	Adult(2)	491.39 +/- 162.29	0.251 +/- 0.085	63.845 +/- 16.791	4.53 +/- 0.60
Winter season	Small immature	65.45 +/- 26.81	0.836 +/- 0.385	69.121 +/- 15.162	1.52 +/- 0.23
(26 degree C	L arge immature	80.76 +/- 51.55	0.154 +/- 0.097	32.927 +/- 20.932	2.79 +/- 0.11
+/-2 degree C)	Adult(1)	23.33 +/- 5.66	1.020 +/- 0.004	9.238 +/- 1.508	8.83 +/- 0.33
	Adult(2)	35.38 +/- 18.32	0.020 +/- 0.011	12.129 +/- 5.434	2.81 +/- 0.16
	Adult(3)	39.33 +/- 29.72	0.019 +/- 0.014	9.468 +/- 7.211	1.47 +/- 0.72
Summer	Small immature	123.33 +/- 25.17	2.098 +/- 0.671	73.889 +/- 6.736	0.93 +/- 0.21
season	L arge immature	256.73 +/- 54.65	0.530 +/- 0.120	70.098 +/- 8.314	5.43 +/- 0.33
(32 degree C	Adult(1)	623.27 +/- 80.92	0.614 +/- 0.149	74.421 +/- 6.477	6.09 +/- 1.31
+/-2 degree C)	Adult(2)	430.83 +/- 79.09	0.258 +/- 0.053	50.576 +/- 3.489	9.10 +/- 0.31
		1			

**Table 4:** Effect of seasons in *E. eugeniae* (from hatchlings to post reproductive periods) on Conversion Rate, Gross Conversion Efficiency (K1), Net Conversion Efficiency (K2), Food Oxidized and Metabolic Rate (mean readings)

H/- 0.091
H-0.027
H/- 0.040
H/- 0.085
H-0.385
H-0.097
H/- 0.004
H-0.011
H-0.015
H-0.671
H-0.121
+/- 0.150
7-0.130

## 4.0 Conclusion:

- 1) Food intake increased in comparison to growth. Small immature worms consumed 22 times on dry weight basis and 55 times more than their body weight in wet condition of av.60%. The rate of food consumption decreased with increase in body weight and that resulted in early maturity with initiation of cocoon production.
- 2) Total feces defecation accounted to 54.3% in rainy season; 81% in winter season and only 35.2% in summer season. From the point of vermicompost production the best season for worm activity and vermicompost production would be winter season under tropical climates.
- 3) The assimilation trend slowed as the age of the worm advanced during rainy and winter season, where as in summer season at all age group the percentage of assimilation either increased/decreased from one group to the next group. From the point of bulky conversion of organic residues under commercialization, the post reproductive adults are to be sorted out, as their feeding and defecation are not commercially viable and may be used as protein meal.
- 4) Under rainy season due to prevailing rains and in summer season due to high temperatures worms' assimilated energy was utilized for oxidation as metabolic rates were higher showing the sum of energy spent for the activities of either respiration, production of body fluid (coelomic fluid). Care and planning for the procurement of vermicompost is needed during these two seasons under large scale production as compared to winter season wherein the feces defecation is negligible; however from the point transformation of bulky biological wastes as a criteria rather than commercialization these are the best seasons for feeding.

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