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Effect of different organic substrates on reproductive biology, growth rate and offtake of the African night crawler earthworm (*Eudrilus eugeniae*)

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Abstract There is limited knowledge about how organic substrates influence reproductive characteristics and growth of African night crawler earthworm (*Eudrilus eugeniae*). It is, however, essential to understand the reproductive characteristics, fecundity, longevity and growth patterns of earthworms to promote vermiculture biotechnology as a strategy for ecological livestock intensification and sustainable production of earthworm biomass as a source of protein and vermicompost. To generate this information, reproductive characteristics, growth and offtake of *E. eugeniae* were studied using four organic substrates including abattoir waste (AW), cattle manure (CM), soya bean crop residue (SBCR) and a binary mixture of cattle manure and soya bean crop residue (CM + SBCR) aged for 2 weeks. Growth rate was 17.7, 15.8, 15.6 and 14.3 mg/worm/day when earthworms were fed AW, CM + SBCR, CM and SBCR, respectively. Irrespective of the substrate, length and biomass of earthworms increased at a decreasing rate between the 1st and 11th weeks. Clitellum appearance was initiated at 31.5 ± 2.4, 32.8 ± 3.2, 33.7 ± 3.3 and 35.5 ± 2.4 days for AW, CM, CM + SBCR and SBCR, respectively, while cocoon initiation was at 69.0 ± 1.4 (AW), 54.9 ± 2.3 (CM), 51.7 ± 1.7 (CM + SBCR) and 60.0 ± 2.4 (SBCR) days. Cocoon production rate (0.41 cocoons/worm/day) was highest ($P < 0.05$) in earthworms fed CM + SBCR but abnormally lowest for AW. Cocoon incubation period ranged between 9 and 16 days for CM but was 11–16 days for SBCR and CM + SBCR but no sufficient cocoons were available for incubation from AW. Hatching success was 88%, 82% and 68% in CM, CM + SBCR and SBCR, respectively. Similarly, the highest mean number of hatchlings per cocoon was 3.08 ± 0.73 from CM. Consequently, earthworm offtake in CM, CM + SBCR, AW and SBCR was 86%, 78%, 70% and 62%, respectively. These results demonstrate that the type of organic substrate influence reproductive biology, growth and offtake of *E. eugeniae*, but suggesting that inadequately aged substrates negatively impact cocoon production and may cause earthworm death.

Keywords Cocoon initiation · *Eudrilus eugeniae* · Growth rate and offtake · Organic substrate · Vermicomposting · Reproductive biology

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Introduction

That earthworms can reproduce parthenogenetically is not only surprising but that *Eudrilus eugeniae* is one of the fastest growing (280 mg/week) and productive tropical earthworm species when grown in animal organic waste is remarkable (Dominguez et al. 2001). *E. eugeniae* is an earthworm species indigenous to Africa although it has been bred in USA, Canada, Europe and Asia as a fish bait (Sivasankari et al. 2013). It is normally referred to as the African night crawler because it originated from West Africa (Sivasankari et al. 2013).

Rapid growth, high fecundity and ubiquitousness of earthworms render them potential commercial vermicomposting agents while their high protein content renders them suitable for use under optimum conditions as an alternative source of animal feed protein (Tacon et al. 1983; Bhat et al. 2015). Increased attention is, therefore, being given to this species of earthworm as a possible waste decomposer and a prospective protein source (Dominguez et al. 2001) that can safely dispose of organic wastes of domestic, agricultural and industrial origin. Vermicomposting is envisaged as a feasible strategy for earthworm protein production in lieu of the more expensive silver fish (*Rastrineobola argentea*) for livestock. Domestication of the earthworm is therefore one of the win-win strategies to accelerate diversity of species at the farm and ecological livestock intensification among smallholder farmers incentivised by recycling the crop and livestock waste in vermibeds for sustainable crop-livestock integration. The use of earthworms in organic waste management and its conversion into richer fertilizers (casts) as well as production of high protein earthworm biomass (50–70% CP) for livestock with a rich profile of essential amino acids necessitates a detailed understanding of the influence of different organic substrates on reproductive biology, growth and offtake of such potentially useful earthworm species (Edwards and Bohlen 1996).

Although *Eisenia fetida* and *Eisenia endrei* are the most commonly used earthworm species in vermicomposting, in Uganda, *Eudrilus eugeniae* is an indigenous and readily available earthworm (Lalander et al. 2015). The earthworm is bigger but with a similarly high reproductive rate as *E. fetida* but with somehow smaller tolerable temperature range compared with other species (Lalander et al. 2015). Furthermore, a comparative analysis of the three epigeic species (*Eisenia fetida*, *Perionyx excavatus* and *E. eugeniae*) in southern Africa indicated that *E. eugeniae* has better growth and fecundity, making it a more superior candidate in terms of its potential to be utilized in large-scale vermiculture (Reinecke et al. 1991). With a sexual maturation time of 45 days, a life cycle of 60 days, a relatively high cocoon production rate (0.42–0.51), a short incubation time of 17 days, a high mean number of hatchlings per cocoon (2.7) and a mean body mass of 2100 mg (Viljoen and Reinecke 1989) make *E. eugeniae* an ideal species for vermiculture. However, there is a paucity of information about the reproductive biology, growth rate and offtake of the earthworm raised on organic substrates like cattle manure (CM), abattoir waste (AW), soya bean crop residue (SBCR) and a binary combination of cattle manure and soya bean crop residue (CM + SBCR).

Currently, *E. eugeniae* is being targeted in Uganda for exploitation, without causing harm to the environment, as one of the organisms of major biological importance in the agricultural sector where it can serve as a source of cheaper protein for livestock in lieu of the more expensive silver fish (*Rastrineobola argentea*) (Lalander et al. 2015). It is, therefore, hypothesized that reproductive characteristics (i.e. clitellum appearance, cocoon initiation, rate of cocoon production, incubation period, hatching success and hatchlings per cocoon), growth (length and biomass increase/earthworm/day) and offtake (percentage number of earthworms weighing at least 1.0 g in 77 days at 29 ± 2 °C, 60–65% moisture content) of *E. eugeniae* are influenced by organic substrates. However, there is a paucity of information about the reproductive performance, growth and offtake of *E. eugeniae* when grown using different organic substrates and yet, these parameters are crucial for mass production of earthworm. The objective of this study was, therefore, to assess the effects of different organic substrates on reproductive biology, fecundity, longevity and offtake of the *E. eugeniae* earthworms as an alternative source of livestock protein and vermicompost.

Materials and methods

Study site and experimental design

Experiments to assess the effects of different organic substrates on reproductive biology, fecundity, longevity and offtake of the *E. eugeniae* earthworms were conducted at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) from January to June 2016. The experiment consisted of four types of organic substrates conducted in three separate experiment or phases to culture *E. eugeniae* earthworms. Four types of test substrates namely CM, AW and SBCR and a binary mixture of soya bean crop residue and cattle manure (CM + SBCR) were used. In the first phase, a pair of clitellate worms was introduced into each of the digit and colour-coded buckets containing the respective test substrates. The aim of this phase was to enable estimation of cocoon production by earthworms feeding on the different substrates. Ten replicates were made for each substrate making 40 experimental units. The second phase contained the same arrangements of 40 units with similar substrate replications as in phase one maintaining similar colour and digit coding. A known number of cocoons from different substrates in phase one were accordingly incubated into the second set of buckets corresponding to the colour, digits and ultimately the substrate as in phase one. The third phase consisted of the same arrangements of substrates in a separate set of buckets with similar colour and digit coding as in phases one and two for raising hatchlings developed from cocoon obtained from substrate in second phase.

Preparation of feeding material

Soya bean crop residue was obtained from the crop field at the study site; cattle manure mixed with urine was obtained from a local cattle farm at the study site while AW was obtained from a local abattoir (Gayaza slaughter house, Wakiso district, Uganda). All the organic substrates were aged for 15 days for microbial composting and temperature stabilization. This was intended to expel toxic gases like ammonia and to increase microbial population interaction. Moisture content of the substrates was maintained at 60–70% by sprinkling with water regularly.

Source of earthworms

Sexually mature adult earthworms (clitellate stage) of *E. eugeniae* were obtained from the earthworm production facility set up at MUARIK, which was maintained by regular feeding with aged cattle manure substrate collected from the study site. Pre-composted organic feeding material weighing 250 g were mixed with 500 g DM of soil and introduced into digit and colour-coded plastic buckets of 20 cm height, 28 cm diameter and covered with a mesh net for ventilation while excluding pests at the same time. Each of the test substrates including CM, SBCR and AW were mixed with dark loam soil in a ratio of 1:2 on dry matter basis. Meanwhile, for the binary combination (SBCR + CM), a ratio of 1:1:4 for SBCR:CM:dark loam soil, on dry matter basis, was used. A pair of randomly selected earthworms, which were originally bred on cattle manure, was then inoculated into each of the experimental buckets referred to as the vermibed with different substrates. The earthworms were allowed a period of 1 week to acclimatise to their respective substrates into which they were initiated under dark and humid environment at room temperature (Garg et al. 2005).

Cocoon production data

Cocoon production by earthworms feeding on different substrates in phase one was determined by emptying the respective contents of the colour-coded buckets onto plastic trays and counting the cocoons every day after hand sorting. Cocoons were lightly washed with clean water, placed on bloating paper tissue and weighed with an electronic balance. Cocoons produced in each bucket for a particular substrate were summed up. The number of cocoons produced per earthworm per day was then calculated for each substrate after 77 days of the experiment. The formula used to calculate the daily cocoon production per worm was:

Cocoons per earthworm per day

$$= \frac{\text{Total number of cocoons for 77 days in a given substrate}}{\text{Total number of worms} \times \text{number of days}}$$

From phase one set of buckets, groups of five cocoons were then placed onto soft pieces of old moist newspaper and transferred into another set of digit and colour-coded buckets of phase two, each containing a similar substrate as that of the mother buckets of phase one from which cocoons were obtained. These were monitored for hatching for a period of 18 days. The incubation period in days per cocoon group per substrate was then recorded on a daily basis. The number of hatched and unhatched cocoons was recorded, and viability percentages of the introduced cocoons were calculated per substrate according to the formula:

$$\text{viability percentage} = \frac{\text{hatched cocoons}}{\text{number of cocoons incubated}} \times 100$$

In phase two, AW medium aged for 2 weeks neither generated nor received cocoons since earthworms in donor buckets with this substrate in phase one were either dying or not producing cocoons.

The average number of hatchlings produced per cocoon was calculated by dividing the total number of hatchlings per bucket by the number of cocoons introduced according to the formula:

$$\text{Average hatchlings per cocoon} = \frac{\text{total hatchlings per bucket}}{\text{number of cocoons}}$$

From phase two buckets, five hatchlings per substrate were randomly selected and placed into another set of digit and colour-coded container of phase three but with substrate similar to those from which they were obtained. In this phase, abattoir waste aged for four instead of 2 weeks was used to receive hatchlings from donor buckets in which pairs of earthworms were also initiated and grown on AW aged for similar time as recipient buckets. The hatchlings were observed for clitellum development. The clitellum is a thickened glandular and non-segmented section of the body wall near the head or prostomium of the earthworms and leeches that secrete a viscid sac or cocoon into which the eggs are deposited. It is present about 2 cm behind the anterior end around the 14th, 15th and 16th segment. The time in days taken for clitellum development per hatchling per substrate was recorded as duration for sexual maturity. The number of hatchlings per substrate that grew up to offtake stage (i.e. percentage number of earthworms weighing at least 1.0 g in 77 days at 29 ± 2 °C, 60–65% moisture content) was recorded and was used to calculate the survivability percentage per substrate.

$$\text{Survivability percentage} = \frac{\text{Hatchlings that grew to offtake stage}}{\text{Hatchlings introduced}} \text{x100}$$

In order to determine the onset of reproduction cycle, the substrates and worm hatchlings were examined daily to see if a well-developed and bulged clitellum had emerged as evidence of sexual maturity. Cocoon production was later observed between 50 and 73 days to estimate cocoon initiation period when the initial cocoons were produced under different substrates, which signified the lifecycle of the worm. Cocoon initiation was observed as time in days taken for day old hatchling earthworms to start producing cocoons.

Growth rate of hatchlings

The initial average length and biomass (wet weight) of hatchlings on emerging from cocoons was measured and recorded. This was repeated every after a period of 7 days. To determine the length of hatchlings, a thread was placed on the worm at full stretch and marked off the end points of the worm body length from the thread. This length was then carried by the thread and read off from a foot ruler. This was confirmed by employing an alternative method which involved allowing an earthworm to crawl straight by grazing at the surface of the ruler, and its photo taken at moments when the worm was straight and at full stretch to the scale and the reading was taken directly from the photo. The biomass (wet weight) of worms was weighed with the aid of an electronic balance, and the worm's growth rate in (mg/worm/day) for specific periods was calculated using the formula:

$$\text{Growth rate} = \frac{W_2 - W_1}{T_2 - T_1}$$

where W_1 and W_2 were the body weights of hatchlings at the beginning and end of a specific period, respectively, while T_2 and T_1 were the age of the hatchlings in days at the end and beginning of the specific growth period.

Earthworm offtake

As a measure of sustainable harvest, offtake was estimated at least after the hatchlings had attained a weight of 1.0 g each and so a worm weighing about 1.0 g and more qualified for harvest, since it had also been deemed to have attained sexual maturity.

Chemical analysis

Physico-chemical composition of the aged substrates was determined at the Soil Science Laboratory of Makerere University. Substrates were analysed for pH using a glass electrode pH meter (TS625 pH meter TM), and cation exchange capacity (CEC) was determined by leaching method using sodium as an index ion. Available phosphorus (P) was extracted using the Bray 1 method and determined by ascorbic acid-molybdate blue colour method while exchangeable bases (K^+, Ca^{2+}) were determined according to the standard methods (Okalebo et al. 2002). Vermicomposted organic carbon (C) of the substrates was determined using the wet combustion technique earlier reported (Musinguzi et al. 2015) while total nitrogen (N) was determined using the Kjeldahl distillation (AOAC 1990).

Statistical analysis

Least square means for increase in biomass/worm/day, length/worm/day, cocoon production/worm/day, cocoon length, hatchling success, days to cocoon initiation, survivability, growth and offtake were analysed using a one-way ANOVA with SAS (2003). Probability of difference option of SAS was used to separate the means at $P < 0.05$.

Results and discussions

Physico-chemical composition of substrates used

Physico-chemical characteristics differed ($P < 0.05$) for all aged substrates used as growth media for culturing the earthworms except for calcium (Table 1). Variation in some of the major minerals, CEC, C and the carbon-to-N (C:N) ratio of the different substrates suggests that their preference by earthworm for vermicompositing process may also differ. Moreover, the survival, growth rate and reproduction potential of earthworms has been reported to be affected by the type of substrate, its palatability and quality (Bhat et al. 2015). Ganesh et al. (2009) argues that substrates rich in polyphenols and lignin are not preferred by most species of earthworms and may lead to weight loss and mortality when earthworms are forced to feed such materials. Low CEC and N but high C:N ratio of SBCR are indications that this is a feed of poor quality for earthworms. While it has been established that N content of the substrate generally affects cocoon production rate, hatching success and earthworm biomass production rate, the number of hatchlings per cocoon has been shown to be correlated with substrate N and C:N content when cultured with *E. eugeniae* and *P. excavatus* (Suthar and Ganganagar 2007). This implies that while the broad categories of optimal physico-chemical requirements for organic waste composting by earthworms has been established (Kaplan et al. 1980), nutritional requirements for mass rearing of different species of earthworms will remain unclear until a clear interaction between available nutrients, biology and ecology is established (Suthar and Ganganagar 2007).

Cocoon production

Effect of the different organic substrates in phase one on mean cocoon production/worm/week is presented in Fig. 1. Ability of clitellated earthworm to start producing cocoons within 1 week of acclimatisation is proof that such epigeic earthworms are well fitted to quickly colonise organic materials as surface feeders. The highest mean cocoon production/worm/week observed with CM + SBCR was

Table 1 Physico-chemical composition of substrates

Parameters	CM	AW	SBCR	CM:SBCR (1:1)	SEM	*P* value
K (%)	1.8165^a	0.8148^b	0.4716^b	1.1066^b	0.174	0.011
Ca (%)	0.31	0.32	0.25	0.29	0.013	0.226
P (%)	0.41^a	0.24^b	0.13^c	0.25^b	0.031	< 0.0001
pH	8.27	8.03	7.4	8.4		
CEC	24.33^a	21^{ab}	3^c	13.5^b	2.710	0.002
C (%)	31.7^b	45.7^a	30.0b	34.5b	2.3	0.03
N (%)	1.8^a	0.6^b	0.3^b	1.4^a	0.2	0.001
C: N	17.5^c	82.7^b	101.4^a	27.0^c	12.7	0.001

^{abc} Least square means in the same row with different superscripts differ significantly at $P < 0.05$; CM is cattle manure; AW is abattoir waste; SBCR is soya bean crop residue; CM:SBCR (1:1) is binary combination of cattle manure with soya bean crop residue in a ratio of 1:1

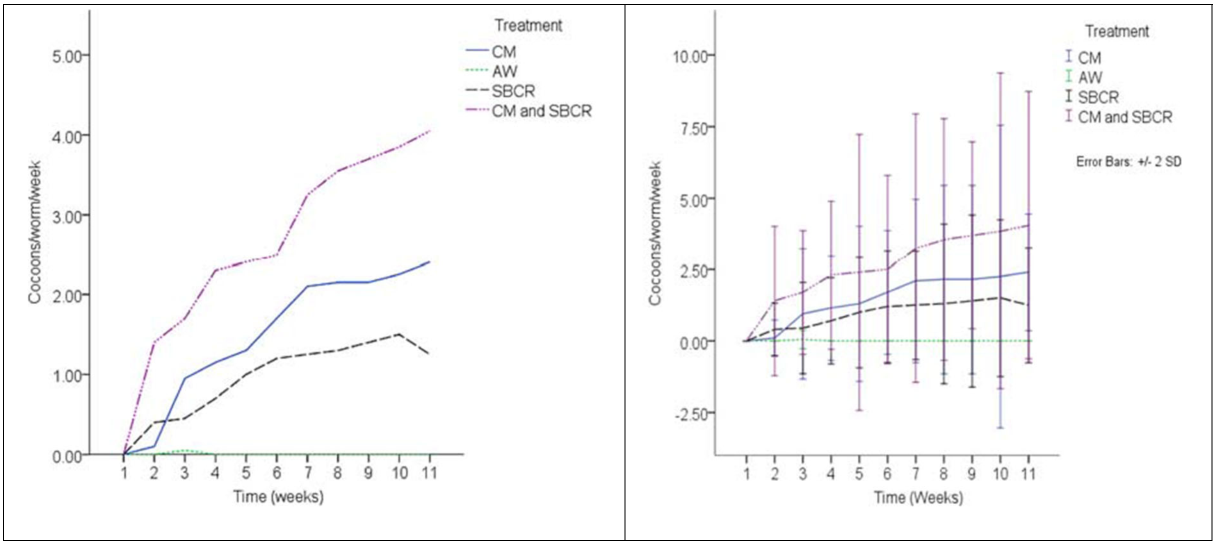

Fig. 1 Cocoon production curves showing effect of the different organic substrates (CM, cattle manure; AW, abattoir waste; SBCR, soya bean crop residue; CM and SCBR, a mixture of CM and SBCR in the ratio of 1:1 weight for weight) on cocoon production/worm/week in *E. eugeniae* earthworm

possibly because the binary combination ensured suitable aeration coupled with readily available protein and microbial load that ensured joint bio-oxidation and stabilisation of the organic materials. Perhaps a combination of CM, with high rumen microbial load, and the highly fibrous SBCR provided a diversity of bacteria, protozoa and fungi that facilitated fermentation of the substrate in the worm gut, which provided metabolites that are needed to meet energy and protein requirements for cocoon producing earthworms.

Rate of cocoon production, cocoon initiation and cocoon weight as influenced by substrate type is presented in Table 2. All these parameter are related to cocoon production efficiency of *E. eugeniae* as it interacts with the physico-chemical properties of the substrate. While the binary combination of CM + SBCR resulted into cocoon production rate of 0.41/earthworm/day similar to the earlier reported values of 0.42–0.51 cocoons/earthworm/day (Viljoen and Reinecke 1989; Garg et al. 2005), lower values of 0.15 and 0.23 cocoons/earthworm/day were observed in SBCR and CM, respectively. Suthar and Ganganagar (2007) argue that the chemical, physical and biological properties of the organic materials influence the cocoon production behaviour by the earthworms including cocoon weight. Perhaps variation in aeration and microbial populations of the substrate materials influenced microbial colonisation and enzyme production potential for degradation of the substrate that consequently affected biological

Table 2 Effect of different organic substrates on average weekly and daily cocoon production, total cocoon production, time for cocoon initiation and cocoon average weight of *Eudrilus eugeniae* earthworms

Variables	AW	CM	SBCR	CM: SBCR	SEM	*P* value
Total cocoon production*	1	325	209	574		
Average cocoon production per worm per day	0.0007^{c}	0.23^{b}	0.15^{b}	0.41^{a}	0.1696	< *0.001*
Average weekly cocoon production	0.005^{c}	1.625^{b}	1.045^{b}	2.870^{a}	0.18656	< 0.001
Time for cocoon initiation	69.0^{a}	54.9^{c}	60.8^{b}	51.7^{d}	1.1085	< 0.001
Cocoon average weight (mg)	-	15.8^{a}	11.6^{b}	14.6^{a}	0.42885	< 0.001

*Total number of cocoons produced during the experimental duration

CM, cattle manure; *AW*, abattoir waste; *SBCR*, soya bean crop residue; *CM* and *SCBR*, a binary mixture of CM and SBCR in the ratio of 1:1 weight/weight.

^{abcd} Least square means within a row followed by different superscripts are significantly different ($P < 0.001$).

Table 3 Effect of the different organic substrates on incubation days, mean hatchlings per cocoon and hatchling success of cocoons for *Eudrilus eugeniae* earthworms

Parameter	CM	AW	SBCR	CM + SBCR	*P* value
Incubation days	10.79 ± 1.13	-	12.41 ± 1.80	11.18 ± 1.45	0.441
Hatching success (%)	88.0	-	68	82	0.067
Hatchlings per cocoon	3.08 ± 0.73	-	2.84 ± 0.58	2.92 ± 0.62	0.441

CM, cattle manure; *SBCR*, soya bean crop residue; *CM + SBCR*, a mixture of CM and SBCR in the ratio of 1:1 w/w

activities of the earthworms in different substrates. Similarly, different substrates had different ($P < 0.05$) durations for cocoon initiation with CM + SBCR having the shortest. While little is known about the nutrition for mass rearing of earthworm, it is possible that differences in biochemical quality of used substrates (Garg et al. 2005) may have retarded growth of earthworms differently thus negatively impacting on the efficiency of cocoon production. This is consistent with earlier observation by Edwards and Bohlen (1996), who indicated that substrates which provide earthworms with both sufficient amount of readily fermentable organic matter and non-assimilated carbon favour growth and reproduction of earthworms. However, the drastic fall in cocoon production in phase one when earthworms were raised on AW was possibly an indication that ageing for 2 weeks was insufficient and such inadequately aged substrates negatively impacts cocoon production and may cause death of earthworms. Kaplan et al. (1980) noted that earthworms will die at pH lower than 5 and at pH greater than 9 and that optimum weight gain is achieved at pH around 7.0. The authors also argue that cattle manure contaminated with urine or abattoir wastes may contain ammonium compounds that may cause 100% earthworm mortality. This explains the death of earthworms experienced when a pair of earthworms was introduced into abattoir waste aged for only 2 weeks. Excessive ammonia, carbon dioxide gas, methane, hydrogen sulphide and volatile fatty acids of inadequately aged AW possibly affected cocoon production and survivability when aged for only 2 weeks. Ammonium acetate, which is likely a lethal component of inadequately aged abattoir waste, has been reported to cause 100% mortality of earthworms at a concentration of 0.1% just like manure contaminated with urine or cattle slurry may be lethal to earthworms (Kaplan et al. 1980).

Table 4 Effect of the different organic substrates on hatching pattern of cocoons as shown by incubation period, number of hatchlings and percentage hatchling of *E. eugeniae*

Parameter	CM		SBCR		CM + SBCR	
Incubation period (days)	Number of hatchlings	Hatchling (%)	Number of hatchling	Hatchling (%)	Number of hatchling	Hatchling (%)
8	1	0.73	4	3.28	6	4.8
9	18	13.14	0	0	4	3.2
10	13	9.49	1	0.82	3	2.4
11	17	12.41	10	8.20	17	13.6
12	14	10.22	13	10.66	12	9.6
13	17	12.41	19	15.57	16	12.8
14	17	12.41	14	11.48	27	21.6
15	14	10.22	26	21.31	19	15.2
16	16	11.68	30	24.59	15	12.0
17	6	4.38	3	2.459	4	3.2
18	4	2.92	2	1.64	2	1.6
Total hatchlings	137		122		125	

CM, cattle manure; *SBCR*, soya bean crop residue; *CM + SBCR*, a mixture of CM and SBCR in the ratio of 1:1 w/w

Table 5 Effect of the different organic substrates on clitellum development in *Eudrilus eugeniae* earthworms

Treatments	Age (days)	Number of clitellates	Clitellate worms (%)
Abattoir waste (AW)	28	6	12
	29	10	20
	30	22	44
	31	26	52
	32	36	72
	33	41	82
	34	46	92
	35	50	100
	31.5 ± 2.4		
Cattle manure (CM)	27	3	6
	30	10	20
	31	20	40
	32	28	56
	33	35	70
	34	39	78
	35	45	90
	36	48	96
	37	50	100
	32.8 ± 3.2		
Crop residue (SBCR)	32	3	6
	33	16	32
	34	24	48
	35	27	54
	36	36	72
	37	38	76
	38	41	82
	39	50	100
	35.5 ± 2.4		
Cattle manure + soya bean crop residue	29	2	4
	30	12	24
	31	16	32
	32	18	36
	33	23	46
	34	32	64
	35	39	78
	36	43	86
	38	45	90
	39	50	100
	33.7 ± 3.3		

Incubation

Incubation period, hatching success and hatchlings per cocoon in phase two did not differ with the substrate used (Table 3). However, hatching success of 88%, 68% and 82% was recorded for earthworms cultured on CM, SBCR and the binary combination of CM + SBCR, respectively. The number of hatchlings per cocoon was

3.0, 2.8 and 2.9 when *E. eugeniae* was cultured on CM, SBCR and a binary combination of CM + SBCR, respectively. The observed hatching success and number of hatchlings per cocoon were higher than the values earlier reported for *E. eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus* when cultured in different organic materials (Suthar and Ganganagar 2007). Incubation period of cocoons, irrespective of the substrate, ranged between 10.8 ± 1.1 to 12.4 ± 1.8 days. This period was shorter than the 12–26 period of incubation earlier reported for *Perionyx ceylanensis* and that of *Perionyx excavatus* (Karmegam and Daniel 2009).

While the hatching pattern for the earthworm cultured on CM showed more hatchlings emerging between the 9th and 16th days of incubation, in the SBCR and binary combinations of CM + SBCR, more hatchlings emerged later between the 11th and 16th days of incubation (Table 4). The shorter period of hatching pattern combined with a higher number of hatchlings per cocoon suggests that *E. eugeniae* has a higher fecundity compared with *P. ceylanensis* cultured either singly, in batches of four and of 8 on cow dung medium where the largest number of cocoons hatched was during 17–21 days (Karmegam and Daniel 2009).

Clitellum development

In phase three, clitellum development of *E. eugeniae* started on the 27th day for the hatchlings raised in CM and on the 28th day for the worms raised in AW aged for 4 weeks, on the 32nd day for the worms raised in SBCR and on the 29th day for the worms raised in the binary mixture of CM + SBCR (Table 5). However, the average time for clitellum development was 31.5 ± 2.5, 32.8 ± 3.2, 35.5 ± 2.4 and 33.7 ± 3.3 for AW, CM, SBCR and CW + SBCR, respectively. Since development of the clitellum is an indication of sexual maturity of earthworms, its emergency is a very important feature in the reproduction and life cycle of earthworms. The observed duration for clitellum development of 31.5 ± 2.5 to 35.5 ± 2.4 days, irrespective of the substrate into which *E. eugeniae* was cultured is in agreement with results of Karmegam and Daniel (2009) who indicated that the number of days taken for clitellum growth ranged from 15 to 35 days for another tropical *P. ceylanensis* earthworm species. These results, however, suggest that sexual development in *E. eugeniae* raised on AW, CM, SBCR and a binary combination of CW + SBCR takes a longer period than *E. foetida*, which took less than 21 days when fed aged cattle manure (Garg et al. 2005). Since clitellum is a modification of a section of the body wall of the earthworm consisting of a glandular and saddle-like thickening near the gonad pores called gonopores, during copulation, the clitellum secretes mucus-like substance that keeps the worm paired while sperms are being exchanged. After copulation, the clitellum secretes a substance for making a cocoon which surrounds the worm and into which the eggs and sperm are deposited after which the worm manipulates the sealed cocoon with eggs and sperm until it falls off its head. In the sealed cocoons

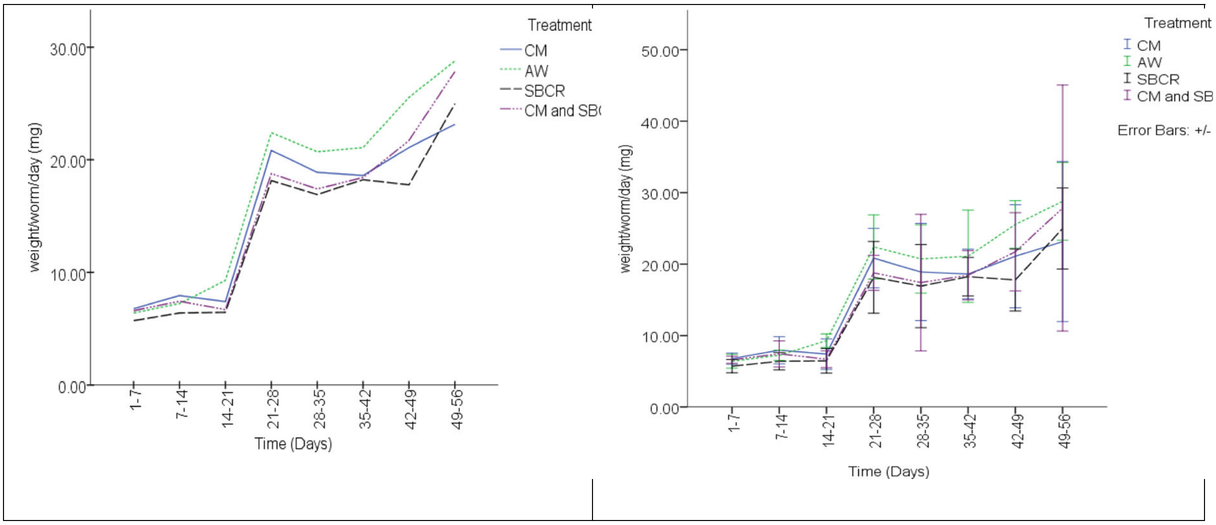

Fig. 2 Mean growth rate (wet weight) of *E. eugeniae* raised on different organic substrates (CM, cattle manure; SBCR, soya bean crop residue; CM + SCBR, a mixture of CM and SBCR in the ratio of 1:1 w/w) for a period of 8 weeks

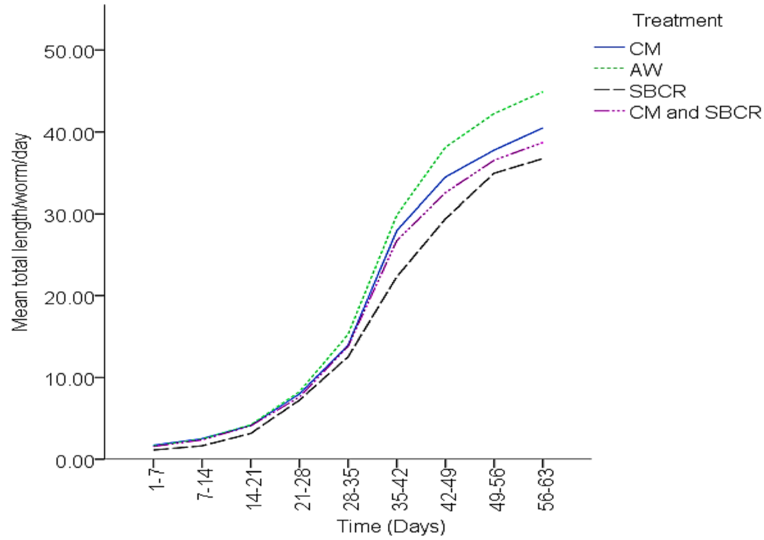

Fig. 3 Growth (length) of *E. eugeniae* cultured on different organic substrates (CM, cattle manure; SBCR, soya bean crop residue; CM and SCBR, a mixture of CM and SBCR in the ratio of 1: 1 w/w) for a period of 9 weeks

is where fertilisation and development takes place until hatching. Although the shortest time from when a hatchling emerged from a cocoon to sexual maturity was 31.5 ± 2.4 days in AW (Table 5), the lowest time taken for cocoon initiation was 51.7 days in the CM + SBCR substrate. This is in agreement with similar days of cocoon initiation of 51 days for *E. eugeniae* (Mba 1984) but longer than 46 days for the same species (Reinecke et al. 1992) and 35 days for *E. foetida* raised on cattle manure (Garg et al. 2005).

Growth rate

The growth rate of *E. eugeniae* cultured on AW, CM, SBCR and CM + SBCR in phase three is presented in Figs. 2, 3 and 4. Increase in biomass at a rate of 17.7 mg/worm/day was highest in AW aged for 4 weeks, followed by CM, binary combination of CM + SBCR and least in SBCR (Table 6). Similar trends were also observed for increase in earthworm length. Higher rate of increase in length of earthworms in AW may mean that if well aged,

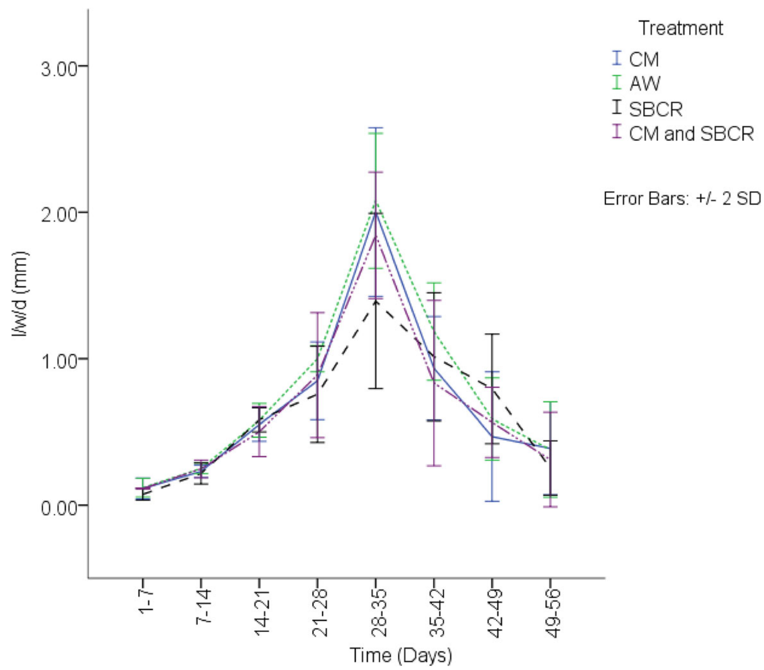

Fig. 4 Growth rate curves of *E. eugeniae* cultured on different organic substrates (CM, cattle manure; SBCR, soya bean crop residue; CM and SCBR, a mixture of CM and SBCR in the ratio of 1:1 w/w) for a period of 9 weeks (error bars indicate ± SD)

Table 6 Effect of the different organic substrates on growth rate of *E. eugeniae* earthworms

Substrate	AW	CM	SBCR	CM: SBCR	SEM	*P* value
Weight per worm per day (mg)	17.67[a]	15.58[b]	14.32[c]	15.79[b]	0.23	< 0.0001
Length per worm per day (cm)	0.08[a]	0.06[b]	0.06[b]	0.06[b]	0.001	< 0.0001

the substrate has some unidentified growth factors that favour rapid growth rate but with delayed stimulation of reproduction in *E. eugeniae*. It is, however, argued that high microbial load associated with the rumen environment promotes earthworm growth. This is in agreement with the findings of Suthar and Ganganagar (2007), who reported that along with feed quality, the microbial biomass and decomposition activities are influential on growth rate and reproduction. While the earthworm reaches its sexual maturity within a range of 31.5 ± 2.4 to 35.5 ± 2.4 days, it continues to grow indicating that the nutrient supply has to be increased possibly through both quality and frequency of supplying the feeds to the vermibeds. However, the rate of increase in the length of the earthworm increased at a decreasing rate with highest rate observed at intermediate period of 28–35 days (Figs. 3 and 4). The maximum growth rates coincided with the sexual maturity, which has a strong implication on nutrient supply, aeration and water supply.

CM, cattle manure; *SBCR*, soya bean crop residue; *CM + SCBR*, a mixture of CM and SBCR in the ratio of 1:1 w/w

Highest rate of earthworm biomass accumulation in phase 3 was attained in AW followed by CM then the binary combination of CM + SBCR and lastly SBCR (Figs. 3 and 4). A generalized growth pattern was evident which involved rapid increase in growth rate during pre-productive phase followed by a phase of steady increase in growth at a decreasing rate after attainment of sexual maturity at 28–35 days. Accelerated growth rate during the pre-reproductive phase was more or less comparable with the findings of Viljoen and Reinecke (1989) who observed a curvilinear growth pattern in *E. eugeniae* up to 50 days from hatching. Worms in all the substrates exhibited slow growth during the first 14–21 days, which is consistent with earlier findings (Parthasarathi 2007). The slow growth rate was, however, followed by an accelerated growth and attainment of a biomass of 877.2 mg for CM, 996.0 mg for AW, 807.0 mg for SBCR and 880.0 mg for the binary combination of CM + SBCR at 8 weeks. This resulted into a mean growth rate of 15.5, 17.7, 14.3 and 15.8 mg/worm/day for CM, AW, SBCR and CM + SBCR, respectively, after 56 days.

The reduced increase of worm biomass growth rate observed from 35 to 56 days (Fig. 4) for all the four substrates was possibly due to the physiological body preparations for onset of cocoon production. It was earlier reported that such a decrease in worm biomass was possibly due to the requirement of large amount of energy partitioned towards cocoon production and also for an elaborate process of copulation and continued reproduction (Mba 1983; Viljoen and Reinecke 1994; Garg et al. 2005).

Biomass harvest, offtake and survivability

The partially degraded abattoir waste aged for 4 weeks resulted into total biomass yield of about 50 g after

Table 7 Effect of the different organic substrates on offtake and survivability of *E. eugeniae* as determined at 11 weeks from hatching

Variables	AW	CM	SBCR	CM:SBCR
Total worm biomass (g)	49.6 ± 2.0	58.1 ± 1.4	43.0 ± 0.6	52.2 ± 2.1
Average weight of clitellate worms (g)	0.4 ± 0.05	0.37 ± 0.04	0.32 ± 0.04	0.34 ± 0.05
Offtake* (%)	70.2 ± 1.3	85.6 ± 1.1	61.9 ± 1.8	78.0 ± 1.9
Average weight of worm at 8 weeks (g)	1.0 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
Average weight of a worm at harvest (g)	1.2 ± 0.16	1.3 ± 0.18	1.0 ± 0.18	1.1 ± 0.18
Survivability (%)	86 ± 1.3	92 ± 2.4	76 ± 2.2	92 ± 1.9

CM, cattle manure; *AW*, abattoir waste; *SBCR*, soya bean crop residue; *CM:SCBR*, a mixture of CM and SBCR in the ratio of 1:1 w/w

*Percentage number of mature earthworms weighing at least 1.0 g in 77 days at 29 ± 2 °C, 60–65% moisture content

77 days (Table 7). At clitellate stage, AW had the highest average weight of 0.4 g. This implies that when sufficiently aged, AW contains inestimable diversity of plant materials rich in non-protein N, minerals and vitamins from various sources of plant materials eaten by cattle that favoured microbial proliferation. The diversity of forage collected by cattle possibly gave AW an advantage in favouring rapid growth. However, this never lasted up to the harvesting time since CM and the binary combination of CM + SBCR had higher offtake than AW (Table 7). Less efficiency in utilisation of SBCR is consistent with earlier studies which indicated that presence of more recalcitrant polyphenols such as lignin and related substances leads to least biological potential of earthworms to utilise such crop residues (Ganesh et al. 2009).

The highest mean survivability of worms in CM and the binary combination of CM + SBCR was 92% but lowest (76%) for SBCR. The high C:N ratio in SBCR is an indication of the high polyphenol and lignin that are not preferred by most species of earthworms (Ganesh et al. 2009). This may perhaps be the reason for the high rate of mortality and weight loss of earthworms fed of SBCR. These results are consistent with the assertion that survival, growth rate and reproduction potential of earthworms are affected by type, palatability and quality of food (Bhat et al. 2015). Therefore, survival, biomass formation and reproduction of earthworms are indicative of the extent of the vermicomposting process.

Conclusion

These results demonstrate that the type of organic substrate used has an impact on reproductive biology, growth and offtake of *E. eugeniae*. Higher survivability, total earthworm biomass accumulation and offtake when cultured on CM and a binary combination of CM + SBCR are indications that growing earthworms in conjunction with livestock and crops offer an option to ecological diversity for alternative sources of livestock protein and vermicompost for sustainable farming. However, the drastic fall in cocoon production when earthworms were raised on abattoir waste was an indication that ageing for 2 weeks was insufficient and that inadequately aged substrates negatively impacts on cocoon production and may cause earthworm death.

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Compliance with ethical standards

Disclaimer The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the supporting organizations.

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