GEROLD RAHMANN, VICTOR OLOWE, TIMOTHY OLABIYI, KHALID AZIM, OLUGBENGA ADEOLUWA (Eds.) (2018) Scientific Track Proceedings of the 4TH African Organic Conference. "*Ecological and Organic Agriculture Strategies for Viable Continental and National Development in the Context of the African Union's Agenda 2063".* November 5-8, 2018. Saly Portudal, Senegal

Effect of Different Organic Substrates on Reproductive Biology, Growth and Offtake of the African Night Crawler Earthworm (*Eudrilus eugeniae*)

Fred Kabi[1], Denis Kayima[1], Abasi Kigozi[1], Eric Zadok Mpingirika[2], Ronald Kayiwa and Dorothy Okello[3]

[1]Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, P. O. Box, 7062, Kampala, Uganda.

[2]The American University in Cairo, New Campus: AUC Avenue, Department of Biology, P.O Box 74, New Cairo 11835, Egypt.

[3]Resilient Africa Network (RAN), Makerere University School of Public Health P.O. Box 7072, Kampala Uganda

Corresponding author:
fred.kabi@gmail.com

Keywords:
Clitellum, Cocoon initiation, Earthworm biomass, Hatchlings, Vermibeds, vermicomposting

Abstract

Rapid growth and high fecundity of Eudrilus eugeniae makes it a commercial vermicomposting agent. The worm is also a rich protein source (50-70%CP) in livestock diets. The major question, however, is how do we promote earthworm production as a strategy for ecological livestock intensification and integration with crops through earthworm domestication as a source of protein and vermicompost. Reproduction 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 mixture of cattle manure and soya bean crop residue (CM+SBCR) aged 15 days. Irrespective of the substrate, length and biomass of earthworms increased at a decreasing rate between the 1st and 8th 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 BCR, 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. Organic substrate used affected reproductive biology,

Introduction

That earthworms can reproduce parthenogenetically is not only surprising but that *E. eugeniae* is one of the fastest growing (280 mg/week) and productive tropical earthworm species when grown in animal organic waste is remarkable. 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; Lalander et al., 2015) makes *E. eugeniae* an ideal species for vermiculture. In order to design sustainable but intensive feeding programs based on earthworm meal as a substitute for the more expensive silver fish (*Rastrineobola argentea*) in poultry and fish diets, it is important to understand the reproductive biology, growth and offtake of the *E. eugeniae*. 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 effects of different organic substrates on reproductive biology, fecundity, longevity and offtake of *E. eugeniae* as an alternative source of livestock protein and vermicompost.

Material 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). The experiment consisted of four types of organic substrates used in three phases to culture *E. eugeniae* earthworms. Four types of test substrates namely cattle manure (CM), abattoir waste (AW) and soya-bean crop residue (SBCR) and a mixture (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. 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 into which the cocoons produced by worms from their respective substrate counterparts in phase one were incubated. The third phase consisted of the same arrangements of substrates as in phases one and two for raising hatchlings hatched from buckets in the second phase but with AW waste aged for 4 weeks.

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. All the organic substrates were aged for 15 days for microbial composting and thermo-stabilization in phase 1 and 2. This was intended to expel toxic gases like ammonia and increase microbial population interaction. The moisture content of the beddings 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. Substrate to dark loam soil ratio of 1:2 for CM, SBCR, AW and a binary combination of (SBCR +CM) with the dark soil in the ratios of 1:1:4 on dry matter basis was used. A pair of randomly selected earthworms that were originally bred on cattle manure was then inoculated into each of the experimental buckets referred to as the vermibeds with different substrates. The earthworms in phase 1 were allowed a period of one week to acclimatize to their respective substrates into which they were initiated under dark and humid environment at room temperature.

Cocoon production data, hatchlings per cocoon, fecundity, growth rate of hatchlings, earthworm offtake were estimated according to Karmegam & Daniel (2000).

Chemical analysis

Physico-chemical composition of the substrates was determined at the soil science laboratory. The substrates were analysed for organic Carbon (C), total Nitrogen (N), total phosphorus (P), potassium (K), Carbon to Nitrogen ratio (C:N), pH and Cation exchange capacity (CEC).

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 (2000). Probability of difference option of SAS was used to separate the means at $P < 0.05$.

Results

Physico-chemical characteristics differed ($P<0.05$) for all aged substrates used as growth media for culturing the earthworms except for calcium (Table 1). 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 (Fig. 1). 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 (Table 2), 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 (Table 3). 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 to 16 days for SBCR and CM+SBCR. However, no sufficient cocoons were available for incubation from AW. Hatching success was 88%, 82% and 68% in CM, CM+SBCR and SBCR, respectively. Similarly, 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.

Table 1. **Physiochemical 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

Table 2. **Effect of the different organic substrates on clitellum development in *Edrilus 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		

Table 3. **Effect of different organic substrate on average weekly and daily cocoon production, time for cocoon initiation and cocoon average weight of *Eudrilus eugeniae* earthworms**

Variables	Treatments				SEM	P-Value
	AW	CM	SBCR	CM:SBCR		
Average cocoon production per worm per day	0.0007[c]	0.23[a]	0.15[a]	0.41[b]	0.1696	<0.001
Average weekly cocoon production	0.005[c]	1.625[a]	1.045[a]	2.870[b]	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

**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 w/w*)

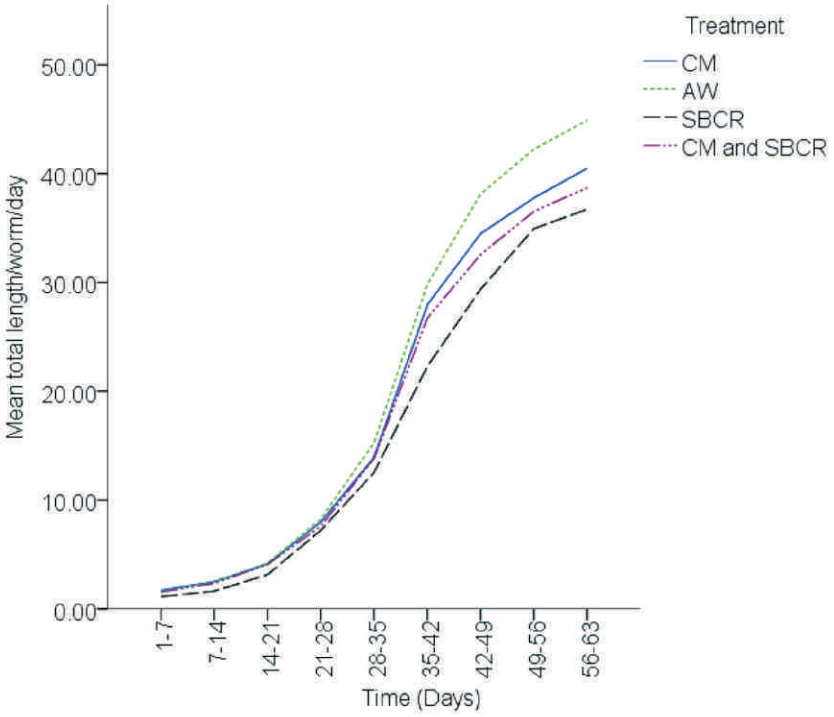

Figure 1: **Growth (length) of *E. eugeniae* cultured on different organic substrates (CM= cattle manure; SBCR= soya bean crop residue; CM and SCBR= a mixture of CM and SBCR in the ratio of 1:1 w/w) for a period of 9weeks**

Discussion

Rate of cocoon production, cocoon initiation and cocoon weight as influenced by substrate type are related to cocoon production efficiencies of *E. eugeniae* as it interacts with the physco-chemical properties of the substrate. While the binary combination of CM+SBCR resulted into cocoon production rate of 0.41/earthworm /day similar to earlier values of 0.42 -0.51 cocoons/earthworm /day (Viljoen and Reinecke 1989), lower values of 0.15 and 0.23 cocoons/earthworm/day were observed in SBCR and CM manure, respectively. Increase in biomass at a rate of 17.7 mg/worm/day was highest in AW aged for four weeks,

followed by CM, CM + SBCR and least in SBCR. 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, the substrate has some unidentified growth factors that favour rapid growth rate but with delayed stimulation of reproduction in *E. eugeniae*.

Acknowledgments

The Makerere University School of Public Health's Resilient Africa Network (RAN) and Partners supported this work. This was made possible by the generous support of the American people, through the United States Agency for International Development (USAID) - [Project ID AID-OAA-A-13-00018]. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the supporting organizations.

References

Karmegam N,Daniel T (2000) Growth, reproductive biology and life cycle of the vermicomposting earthworms, Perionyx ceylanensis Mich. (Oligochaeta: Megascolecidae). Bioresource Technology 100:4790-4796.

Lalander CH, Komakech AJ, Vinnerås, B (2015) Vermicomposting as manure management strategy for urban small-holder farms – Kampala case study. Waste Management, 39, 96 -103.

Viljoen SA, Reinecke AJ(1989). Life cycle of the African night crawler, *Eudrilus eugeniae* (Oligochaeta). *S. Afr. J. Zool*, *24*(1), 27-32.