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Growth and reproduction of *Eisenia fetida* in different livestock excreta management through vermicomposting

B Venkatesh[1] and N Ramesh[1,2]*

[1]Department of Biotechnology, JJ College of Arts and Science, Pudukkottai, Tamilnadu, INDIA

[2*] PG & Research Department of Botany, Government Arts College for Men, Krishnagiri, Tamilnadu, INDIA

Abstract

In India, millions of tones of livestock excreta are produced every year. Vermicomposting is commonly adopted for the management of livestock organic wastes. The objectives of this study were to compare the quantity and quality of vermicasts produced from four different types of livestock manure (buffalo, horse, pigeon and goat) by the worms and the effects of these manures on their growth and reproductive performance. Our study explores the potential of an epigeic earthworm *Eisenia fetida (E. Fetida)* to compost four livestock excreta into value added product (vermicompost) at the laboratory scale. Each treatment group consisted of six replicates and worm vermicasts were examined after 60 days for the concentrations of total C, P, K, C:N ratio higher N content in goat manure vermicasts was observed than those in pigeon, buffalo and horse manure vermicasts. The cocoon production per worm in goat manure was higher than in other manures. In conclusion, goat manure provided a more nutritious and friendly environment to the *E. fetida* earthworm than other manure. Thus, goat, pigeon and buffalo excreta show potential as good substrates in vermicomposting using *E. fetida*, although further research is required to explore the feasibility of use of chicken, donkey and pigeon excreta in combination with goat, pigeon and horse excreta.

Keywords: *Eisenia fetida*, Livestock excreta, goat manure, Reproduction, Vermicomposting

1. Introduction

In India, the integration of crops and livestock and use of manure as fertilizer were traditionally the basis of farming systems. Vermiculture is basically the science of breeding and raising earthworms. It defines the thrilling potential for waste reduction, fertilizer production, as well as an assortment of possible uses for the future (Entre Pinoys, 2010). Nevertheless, climate has a substantial influence on earthworms (physiology, development or activity) that is reflected in the seasonal dynamics of their life history (Orgiazzi *et al.,* 2016). In India, millions of tons of livestock excreta are produced annually (Table 1). Throughout the world, earthworms play an important role in determining the balance of greenhouse gases from soils, and their impact is expected to increase in the coming decades (Lubbers *et al.,* 2013). During vermicomposting, the important plant nutrients such as N, P, K, and Ca, present in the organic waste are released and converted into forms that are more soluble and available to plants (Kaushik, 2003). Vermicompost has also been reported to contain biologically active substances such as plant growth regulators (Tomati *et al.,* 1987). Moreover, the worms themselves provide a protein source for animal feeds (Aung, 2014). Earthworm is an important animal in decomposer system. Earthworm can be divided into 3 types, epigeic, endogeic and anecies.

Table1: Livestock population and quantity of waste generated in India

Animal species	Population Animal species (in million)	Daily average excreta animal^{-1} Wet weight(kg)
Buffalo	97.92	12.2
Goat	135.2	0.75
Horse	0.75	-
Pigeon	0.98	-

Source: Livestock Census Report, 2012, Directorate of Economics and Statistics, Ministry of Agriculture, Government of India.

Earthworms feed on organic matter and utilize only a small amount for their body synthesis and excrete a large part of the consumed materials in a partially digested form as worm casts. The process involves physical/mechanical and biochemical activities. The physical/mechanical process includes mixing and grinding, whereas biochemical process includes microbial decomposition in the intestines of the earthworms. Feeding is required every 3–5 days in vigorously growing worm beds, with an optimal daily feeding rate of 0.75 kg feed/kg worm/day. (Ndegwa *et al.*, 1999). Karmegam and Daniel (2000) have undertaken studies to find out the efficiency of *E. Fetida*, a compost worm, in the decomposition of certain leaf litters which are available in plenty in this part of South India. The healthy earthworm *E. Fetida* were selected as the experimental animal for the present study. Earthworms belonging to Phylum Annelida, Class Chaetopoda, and Order Oligochaeta occupy a unique position in animal kingdom. They are the first group of multicellular, eucoelomate invertebrates who have succeeded to inhabit terrestrial environment. The taxonomic status of *E. Fetida* was confirmed by Jaenike (1982). employing electrophoretic techniques. Most earthworm communities display an aggregated spatial distribution in response to soil environmental heterogeneity at a small scale (Jiménez *et al.*, 2012). *E. fetida* is an epigeic species associated to environments with high organic matter content and their natural populations live at high densities in patchy distributions *(*Elvira 1996*)*. The basic aspects of the life cycle of *E. Fetida* are relatively well documented due to the importance of this species in waste management. The growth and reproduction of this earthworm species is affected by several factors such as food quality, moisture, temperature and population density, as showed under laboratory conditions (Domínguez 2004). Moreover *E. Fetida* is an iteroparous earthworm, with continuous and high reproduction rates, and it should respond to adverse environmental conditions modifying those rates. The optimum temperature for *E. Fetida* was 25°C (77°F), and its temperature tolerance was between 0°C (32°F) and 35°C (95°F). The present study aims to investigate the physicochemical changes effected in the waste by *E. fetida* with time. We hypothesized that during the vermicomposting of animal dung physico-chemical characteristics of the dung may affect vermicompost quality and hatchling production.

2. Materials and Methods

The Buffalo, horse, pigeon and goat manures were obtained from animal farms of pudukkottai area and air dried before use. The epigeic earthworm *E. fetida* were selected for the present study. The *E. fetida* was bought from agricultural farm at pudukkottai. The worms were kept in the laboratory under room temperature and provided food and maintained as a stock mother culture. The cocoons collected from mother culture were reintroduced into the same bed. The vermibed was kept moist by sprinkling water once in two days. The earthworm *E. fetida* was mass multiplied as per the methods of Karmegam (2002) using cow dung as the medium. *E. fetida* was collected from the mass culture tanks; Adult clitellate worms of uniform size were selected and used for the composting experiment. Healthy adult earthworms, commonly known as red wigglers, were randomly picked for use in the experiments from several stock cultures containing 500–2000 earthworms in each, maintained in the laboratory with cow dung as culturing material. Each worm weighed between 400 and 600 mg.

2.1. Animal wastes

Fresh excreta of three mammalian animals, viz., buffalo, horse, goat and an avian species pigeon were collected from different farms located in pudukkottai,Tamilnadu, India. The excreta consisted of a mixture of faeces and urine without any bedding material. The main characteristics of animals excreta are given in Table 2.

2.2. Stoichiometry

All the organic waste quantities were used on dry weight basis that was obtained by oven drying known quantities of material at 110°C in hot air oven to a constant mass.

2.3. Experimental design

One kg (on dry weight basis) of each excreta was taken as feed in circular plastic containers (volume 10 L, diameter = 50 cm and depth = 15 cm). The moisture content of these wastes was adjusted 70 to 80% during the study period by spraying adequate quantities of distilled water. These mixtures were turned over manually everyday for 15 days in order to eliminate volatile gases which may be potentially toxic to earthworms. After 15 days, 20 adult *E. fetida* individuals of known biomass were introduced into each container. The substrate was covered with a moist nylon mesh to avoid colonization by pests. All containers were kept in dark at 25 ±1°C. The experiment was done in six replicates for each substrate.

Homogenized samples of the feed were drawn at 0, 30, 45 and 60 days from each container. Day 0 refers to the time when different animal dung were filled in different container before pre-composting. The cocoons, earthworms and hatchlings were removed manually from each sample. The samples were air dried in shade at room temperature, ground in a stainless steel blender and stored in plastic vials for further chemical analysis. At the end of the experimental period (day 60) all earthworms including hatchlings were counted and weighed after washing with water and drying by paper towels.

2.4. Chemical analyses

All the chemicals used were analytical reagent (AR) grade supplied by S. D. Fine Chemicals, Mumbai, India. Alkali resistant borosilicate glass apparatus and double glass distilled water was used throughout the study for analytical work. All the samples were used on dry weight basis for chemical analysis that was obtained by oven drying the known quantities of material at 110°C. All the samples were analyzed in six times and results were averaged. The pH and electrical conductivity (EC) were determined using a double distilled water suspension of each waste in the ratio of 1:10 (w/v) that had been agitated mechanically for 30 min and filtered through Whatman No. 1 filter paper. Total organic carbon(TOC) was measured using the method of Nelson and Sommers (1982). Total Kjeldhal nitrogen (TKN) was determined after digesting the sample with concentrated H_2SO_4 and concentrated $HClO_4$ (9: 1, v/v) according to Bremner and Mulvaney (1982) procedure.

Total available phosphorus (TAP) was analyzed using the colorimetric method with molybdenum in sulphuric acid. Total K (TK) was determined after digesting the sample in diacid mixture (concentrated HNO_3: concentrated $HClO_4$, 4: 1, v/v), by flame photometer *(*Elico, CL 22 D, Hyderabad, India*)* (Bansal and Kapoor, 2000).

2.5. Statistical analysis

The data in this study were analyzed using the SPSS package, and all the values are presented as the mean ± SE. Student t-tests were used to compare the nutritional quality of vermicompost and fecundity data between the control (cow dung) and other feed wastes. The probability levels used for statistical significance were $P < 0.05$ for the tests.

3. Results and Discussion

3.1. Characteristics of the livestock excreta

Table 2 summarizes the initial physicochemical characteristics of animal wastes before use. The moisture content of the wastes varied between 20.29% and 73.42%. The pH values of the wastes were alkaline. The highest electrical conductivity was in goat waste and minimum in pigeon waste. The TOC of different wastes were in the range of goat waste and buffalo waste. The TKN content ranged from horse waste and buffalo waste. The minimum C:N ratio was in the pigeon waste; the maximum was 109.23± 0.26 in horse waste. The potassium content ranged from 0.32± 0.02% in pigeon manure to 0.69± 0.0.03% in horse dung. TKN content in our wastes was lower than that reported for goat wastes in literature and hence, C:N ratios were higher that reported by other co-workers (Bansal and Kapoor, 2000).

Seasonality had a strong influence on the growth and reproduction of *E. fetida* and consequently on the age structure of the population. This population was characterized- by a very high density and biomass with a high proportion of mature individuals throughout the year. This resulted in an unusual population structure where the young earthworms were not the most abundant stage, as in other earthworm species (Edwards 2004). High densities are common in *E. Fetida* populations when large quantities of organic matter are available, supporting up to 14-600 individualsm−2 *(*Elvira 1996*)*. Moreover, *E. Fetida* lives in environments where the food and the living substrate usually are the same *(*Fernando Monroy 2006*)* and this resource availability should improve the conditions for earthworm growth and reproduction, and promote the occurrence of high numbers of mature individuals.

Changes in the physico-chemical quality of the livestock excreta. The vermicomposts produced from buffalo, horse, pigeon and goat wastes were much darker in colour and had been processed into a homogeneous mixture after 90 days of worm activity, where as goat pigeon and buffalo, wastes remained in compact clumps. No mortality was observed in any animal waste during the study period. Gunadi and Edwards (2003) reported the death of *E. fetida* after 2 weeks in the fresh cattle dung although all other growth parameters such as moisture content, pH, electrical conductivity, C:N ratio contents were suitable for the growth of the earthworms. They attributed the deaths of earthworms to the anaerobic conditions which developed after 2 weeks in fresh cattle solids. In our experiments, all the wastes were pre-composted

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for 2 weeks and during this period all the toxic gases produced might have been eliminated. It is established that pre-composting is very essential to avoid the mortality of worms.

Table 2: Initial physicochemical characteristics (mean ±SE, *n* = 3) of the animal dung materials

Treatments	Pigeon manure	Goat manure	Horse manure	Buffalo manure
Moisture content %	72.42 ± 047^{b}	20.29 ± 0.23^{a}	54.79 ± 0.48^{a}	73.42 ± 4.7^{b}
pH	8.05 ± 0.02^{b}	8.08 ± 0.02^{a}	8.0 ± 0.02^{a}	8.01 ± 0.02^{b}
EC (dS m−1)	0.98 ± 0.02^{b}	2.57 ± 0.03^{a}	2.09 ± 0.03^{a}	2.43 ± 0.07^{b}
TKN (%)	0.22 ± 0.01^{b}	0.54 ± 0.03a	0.54 ± 0.03a	48.2 ± 0.31^{b}
TOC (%)	31.51 ± 0.04^{b}	54.58 ± 0.45^{a}	47.54 ± 0.45^{a}	49.51 ± 0.04^{b}
C:N ratio	90.22 ± 0.39^{b}	98.28 ± 0.42^{a}	109.23 ± 0.26^{a}	91.22 ± 0.42^{b}
TK (%)	0.32 ± 0.02^{b}	0.69 ± 0.03^{a}	0.68 ± 0.03^{a}	0.49 ± 0.02^{b}
TAP (%)	0.29 ± 0.01^{b}	0.36 ± 0.02^{a}	0.36 ± 0.02^{a}	0.32 ± 0.01^{b}

Table 3: Final physicochemical characteristics (mean ±SE, *n* = 3) of the animal dung materials after 60 days

Treatments	pigeon manure	Goat manure	Horse manure	Buffalo manure
pH	6.34 ± 0.02^{b}	6.89 ± 0.02^{a}	6.60 ± 0.02^{a}	6.71 ± 0.02^{b}
EC (dS m−1)	0.8 ± 0.02^{b}	1.82 ± 0.03^{a}	1.29 ± 0.03^{a}	1.48 ± 0.03^{b}
TKN (%)	0.80 ± 0.01^{b}	0.94 ± 0.03a	0.79 ± 0.03a	0.78 ± 0.31^{b}
TOC (%)	22.8 ± 0.42^{b}	29.57 ± 0.43^{a}	20.54 ± 0.45^{a}	24.17 ± 0.42^{b}
TK (%)	0.48 ± 0.02^{b}	0.42 ± 0.03^{a}	0.38 ± 0.02^{a}	0.55 ± 0.04^{b}
TAP (%)	0.49 ± 0.03^{b}	0.53 ± 0.03^{a}	0.36 ± 0.02^{a}	0.38 ± 0.01^{b}

There were little changes in the pH of feeds (Table 2 and 3). The pH decreased from alkaline pH (8.08±0.2 – 8.0±0.02) to acidic or neutral (6.34±0.02 –6.89±0.02). Other workers (Ndegwa *et al.*, 2000; Gunadi and Edwards, 2003; Atiyeh *et al.*, 2000) have also reported similar observations. However, our results are contrast with those of Datar *et al.*, (1997) who reported an increase in pH during vermicomposting. The pH shift towards acidic conditions could be attributed to mineralization of the nitrogen and phosphorus into nitrites/nitrates and orthophosphates; bioconversion of the organic material into intermediate species of organic acids (Ndegwa *et al.*, 2000). The electrical conductivity was reduced in the range of 28.4 to 46% for different feeds after vermicomposting. Gunadi and Edwards (2003) have reported that EC and pH of feed could be the limiting factor for the survival and growth of *E. fetida*. Mitchell (1997) reported that *E. fetida* was unable to survive in the cattle solids with pH of 9.5 and electrical conductivity of 5.0 dS m−1. In our experiments, significantly ($P \leq 0.05$) higher pH (9.0) of camel waste and higher EC (3.91 dS m−1) of donkey waste may have made these unpalatable for *E. fetida.*

Table 4. The concentrations of C and N and the pH in the goat manure were significantly higher ($P <$ 0:05) than in the other manure. However, the goat manure had higher ($P <$ 0:05) P and K concentrations than the other manure. The amounts of C, P and K in goat vermicasts were significantly higher ($P <$ 0:05) than in other vermicasts. The amounts of minerals differed in treatment groups, this could be attributed to variations in growth and multiplication rate of the earthworms in the different animal manures, which resulted in a differential pattern of uptake of the nutrient for their body synthesis and subsequent release of the remaining minerals in a mineralized form. The carbon content of cattle manure decreased during vermicomposting indicating a higher mineralization of organic matter. However, the N content of goat manure increased in the process of vermicomposting. This shows that the

increased microbial activity continues outside the gut in the casts and results in an increased mineralization rate of organic N and consequent further increase in concentration of NH_4 (Lavelle *et al.*, 1992).

Table 4: Nutrient contents and pH of Buffalo, Goat, Pigeon and Horse manures used in vermiculture before and after composting

Treatments	pigeon manure	Goat manure	Horse manure	Buffalo manure
Nutrient contents of manure				
C (%)[a]	50.92 ± 047[b]	58.95 ± 0.48[a]	54.28 ± 047[b]	50.83 ± 0.48[a]
N (%)	1.01 ± 0.02[b]	1. 19 ± 0.02[a]	1.02 ± 0.02[b]	1.06 ± 0.02[a]
P (%)	0.39 ± 0.02[b]	0.57 ± 0.03[a]	0.38 ± 0.02[b]	0.48 ± 0.03[a]
K (%)	0.32 ± 0.01[b]	0.62 ± 0.03a	0.46 ± 0.01[b]	0.50 ± 0.03a
C:N ratio	51.02 ± 2.41[a]	62.67 ± 3.12[a]	51.02 ± 2.51[a]	52.67 ± 3.42[a]
pH	8.05 ± 0.02[b]	8.08 ± 0.02[a]	8.0 ± 0.02[a]	8.01 ± 0.02[b]
Nutrient contents of vermicasts				
C (%)a	49.51 ± 0.34[b]	53.58 ± 0.35[a]	51.51 ± 0.34[b]	43.78 ± 0.35[a]
N (%)	0.92 ± 0.02[b]	1.08 ± 0.02[a]	0.92 ± 0.02[b]	0.93 ± 0.02[a]
P (%)	0.32 ± 0.02[b]	0.48 ± 0.03[a]	0.32 ± 0.02[b]	0.38 ± 0.03[a]
K (%)	0.29 ± 0.01[b]	0.52 ± 0.02[a]	0.29 ± 0.01[b]	0.36 ± 0.02[a]
C:N ratio	46.06 ± 2.1[b]	55.48 ± 3.21[a]	40.06 ± 2.1[b]	45.48 ± 3.21[a]
pH	6.34 ± 0.02[b]	6.89 ± 0.02[a]	6.60 ± 0.02[a]	6.71 ± 0.02[b]

Values in the same row with different superscripts are significantly different (P < 0:05).
Values are presented as mean ± SD. a % on dry weight.

In the present study, C:N ratio measurement provided an indication of degree of decomposition. The C:N ratios of the manures were higher before vermicomposting. Enhanced organic matter decomposition in the presence of earthworms has been reported, which results in lowering of the C:N ratio (Fosgate and Babb, 1972; Kale *et al.*, 1982; Edwards, 1988; Talashilkar *et al.*, 1999). During composting, the organic C is lost as CO_2 and total N increases as a result of carbon loss. The final N content of compost is dependent on the initial N present in the waste and the extent of decomposition (Crawford, 1983; Gaur and Singh, 1995). In the present study, the C:N ratio was higher in the goat manure than in other manures

4. Conclusions

This work reports the possibility of use of vermicomposting technology in live stock excreta management. Our trials demonstrated vermicomposting as an alternate technology for the recycling of different animal dung materials using an epigeic earthworm *E. fetida* under laboratory conditions. The vermicompost produced from buffalo, horse, pigeon and goat manure had more fertilizer value than camel and donkey dung materials. The dehydrogenase activity and C:N ratio indicated that cow, horse, pigeon and goat wastes are converted to vermicompost in 60. Finally, cow, horse, pigeon and goat excreta can be converted into a value added product by *E. fetida*, helping to recycle the nutrients present in these wastes and avoiding their disposal in open dumps, along the water bodies or roadsides. Further studies are required to explore the potential of utilization of buffalo, donkey and camel excreta in combination with cow or pigeon or goat dung materials while employing *E. fetida* as vermicomposting worm

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