GROWTH AND REPRODUCTION OF *EISENIA FOETIDA* IN VARIOUS ANIMAL WASTES DURING VERMICOMPOSTING

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Abstract. The effect of various animal wastes on growth and reproduction of an epigeic earthworm *Eisenia foetida* was studied under identical laboratory conditions. For each waste, viz., cow, buffalo, horse, donkey, sheep, goat and camel, five hatchlings per 100 g of waste were inoculated and monitored for biomass gain, mortality, sexual maturity, cocoons production periodically for 15 weeks. No mortality was observed in any waste. The earthworms grew rapidly in cow, sheep, and goat wastes. Maximum weight gain and highest growth rate were attained in sheep waste. Net biomass gain/earthworm in different animal wastes was in the order of: sheep > donkey > buffalo > goat ≈ cow ≈ horse > camel. The number of cocoons produced per earthworm per day in different wastes was in the order: sheep > cow ≈ horse ≈ goat > camel > donkey > buffalo. Increase in the number of earthworms was 39.5-fold in horse waste and 26-fold in cow waste.
Keywords. *Eisenia foetida, animal waste, physicochemical characteristics, biomass, cocoon*

Introduction

Animal wastes are considered as important resources that fertilize crop fields, supplement organic matters, and improve soil conditions, but are a source of environment pollution too. The US geological survey found that the increase in in-stream loads of nitrogen and phosphorus was strongly correlated with increased animal concentrations. Eutrophication from animal waste runoff has been linked to the outbreak of toxic micro-organisms such as *Pfiesteria piscicida*. The outbreak of *Pfiesteria* has been implicated in massive destruction and disease of fish and wild-life population. Animal wastes also significantly contribute to the excess bacteria and nitrates that are frequently found in groundwater. The nations which have large livestock operations experience local lagoon spills which lead to massive destruction of fish.

Currently the fertilizer values of animal wastes are not being fully utilized resulting in loss of potential nutrients. Animal wastes are also causing concern due to odour problems [29]. Organic wastes can be ingested by earthworms and egested as a peat-like material termed as vermicompost. As opposed to traditional microbial waste treatment, vermicomposting results in bioconversion of the waste streams into two useful products: the earthworm biomass and the vermicompost. The former product can further be processed into proteins (earthworm meal) or high-grade horticultural compost [30]. The latter product (vermicompost) is also considered as an excellent product since it is homogeneous, has desirable aesthetics, reduced levels of contaminants and tends to hold more nutrients over a longer period, without adversely impacting the environment. During this process, the important plant nutrients, such as nitrogen, potassium, phosphorous and calcium present in feed material are converted into forms that are much more soluble and available to plants than those in the parent compounds [25]. Vermicompost has also been reported to contain biologically active substances such as plant growth regulators [33].

The most promising earthworm species used for vermicomposting are *Eisenia foetida, Eisenia andrei, Eudrilus eugeniae* and *Perionyx excavatus. Eisenia foetida* is commonly used for cow dung vermicomposting in Northern India. Studies are not available on the use of *E. foetida* for vermicomposting of other animals' waste. In order to utilize this species successfully for outdoor vermicomposting of different animal wastes, its survival, growth and fecundity in different wastes should be known. The life cycle of *P. excavatus* for different animal wastes has been documented [11, 19]. But such data on life cycle of *E. foetida* in different animal wastes are not available.

The chemical composition of animal waste is known to be influenced by the feed of the animal, bedding material used and the way waste is collected, stored and handled before utilization [21]. This paper aims to investigate the effect of different animal wastes (cow, buffalo, horse, donkey, sheep, goat and camel) on the life cycle of *E. foetida*. It was hypothesized that waste of different animals would affect the life cycle of *E. foetida* due to differences in physico-chemical characteristics.

Review of literature

Various organic wastes tested in past as feed material for different species of earthworms include sewage sludges [4, 9, 10], paper mill industry sludge [6], pig waste [7, 28], water hyacinth [14], paper waste [15], brewery yeast [6], crop residues [3], cow slurry [17], cattle manure [24], vine fruit industry sludge [2], rice stubbles, mango leaves [32] and activated sludge [18], textile mill sludge [20] etc.

Loh *et al.* [22] reported that biomass gain and cocoon production by *Eisenia foetida* was more in cattle waste than goat waste. Kale *et al.* [19] reported the potential of *Perionyx excavatus* to vermicompost different wastes (sheep dung, cow dung, biogas sludge and poultry manure and sand as control). The worms readily accepted cow and horse waste. Sheep waste was consumed 3 or 4 days after it was added. The growth, fecundity and mortality of *E. foetida* was studied by Gunadi & Edwards [16] in a range of different wastes (cattle manure solids, pig manure solids and super market waste) for more than one year. Worms could not survive in fresh cattle solids, pig solids, fruit wastes and vegetable wastes. The growth of *E. foetida* in pig wastes was faster than in cattle solid. The multiple additions of substrates prolonged the fecundity of worms, but there was a tendency of decreasing of the weight by worms after 60 weeks of the experiment. Singh *et al.* [31] studied the optimum moisture requirement during vermicomposting by *P. excavatus*. The study showed that a moisture content of 80% was optimum for stabilization of waste in minimum processing time.

Aquino *et al.* [1] studied earthworm fecundity in cattle dung and sugarcane bagasse mixed in different ratios. Maximum reproduction was observed in 1:1 and 3:1 ratios. The peels of bitter cassava root, a major source of food carbohydrate in tropics, form toxic waste which is lethal to the soil invertebrates and inhibit the root growth of the plants. Investigations by Mba [23] highlighted the ability of *Eudrilus eugeniae* to partially detoxify the wastes and convert the toxic cassava peels into valuable vermicompost. Vermicomposting of neem (*Azadirachta indica*) was studied by Gajalakshmi & Abbasi [13] in 'high-rate' reactors operated at the earthworm (*E. eugeniae*) densities of 62.5 and 75 animals per litre of reactor volume. Contrary to the fears that neem – a powerful nematicide – might not be palatable to the annelids, the earthworms fed voraciously on

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the neem compost, converting up to 7% of the feed into vermicompost per day. Indeed the worms grew faster and reproduced more rapidly in the neem-fed vermireactors than in the reactors fed with mango leaf litter earlier studied by the same authors.

Materials and methods

Young non-clitellated specimens of *Eisenia foetida*, weighing 200–250 mg live weight were randomly picked from several stock cultures containing 500–2000 earthworms in each, maintained in the laboratory with cow dung as culturing material.

Fresh waste of seven different mammalian animals, viz., cow, buffalo, horse, donkey, sheep, goat and camel were collected from different animal farms located in Hisar city, India. The dung consisted of a mixture of faeces and urine without any bedding material. The main characteristics of animal wastes are given in *Table 1*.

All the samples were used on dry weight basis for biological studies and chemical analysis that was obtained by oven drying the known quantities of material at 110 °C. All the samples were analyzed in triplicate and results were averaged.

Experimental design

Seven circular 1 l plastic containers (diameter 14 cm, depth 12 cm) were filled with 100 g (DW) of each dung material. The moisture content of wastes was adjusted to 70–80% during the study period by spraying adequate quantities of distilled water. The wastes were turned over manually everyday for 15 days in order to eliminate volatile toxic gases. After 15 days, 5 non-clitellated hatchlings, each weighing 200–250 mg (live weight), were introduced in each container. Three replicates for each waste were maintained. All containers were kept in dark at temperature 25±1 °C.

Biomass gain, clitellum development and cocoon production were recorded weekly for 15 weeks. The feed in the container was turned out, and earthworms and cocoons were separated from the feed by hand sorting, after which they were counted, examined for clitellum development and weighed after washing with water and drying them by paper towels. The worms were weighed without voiding their gut content. Corrections for gut content were not applied to any data in this study. Then all earthworms and feed (but no cocoons) were returned to the respective container. No additional feed was added at any stage during the study period. All experiments were carried out in triplicate 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 & Sommers [26]. Total

Table 1. *Initial physico-chemical characteristics of various animal wastes*

animal waste	moisture content (%)	pH (1 : 10)	EC (dS/m)	TOC (%)	TKN (%)	C : N ratio	TK (%)	TAP (%)
cow	56.0	8.2	2.10	47.3	0.53	89.4	0.48	0.33
buffalo	72.3	8.4	2.60	51.9	0.56	93.0	1.07	0.50
horse	54.0	8.0	2.03	48.4	0.35	137.1	0.78	0.70
donkey	54.4	8.1	3.91	48.5	0.50	97.1	1.31	0.50
sheep	73.4	8.2	0.90	32.3	0.37	88.9	0.70	0.31
goat	21.8	7.6	2.56	43.8	0.47	93.5	0.72	0.37
camel	58.0	9.0	1.56	46.3	0.40	116.1	0.50	0.30

Kjeldahl nitrogen (TKN) was determined after digesting the sample with concentrated H_2SO_4 and concentrated $HClO_4$ (9:1, v/v) according to Bremner & Mulvaney [5] 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 (cc HNO_3 : cc $HClO_4$ = 4 : 1, v/v), by flame photometer (Elico, CL 22 D, Hyderabad, India) [3].

Results and discussion

Physico- chemical characteristics of the animal wastes

Table 1 summarizes the initial physico-chemical characteristics of animal wastes before use. The moisture content of the wastes varied between 21.8% and 73.4%. The pH values of the wastes were in alkaline range (9.0–7.6). The highest electrical conductivity 3.91 dS/m was in the donkey waste and minimum in sheep waste (0.90 dS/m). The TOC of different wastes were in the range of 32.3% in sheep waste to 51.9% in buffalo waste. The TKN content ranged from 0.35% in horse waste to 0.56% in buffalo waste. The minimum C:N ratio was 88.9 in the sheep waste; the maximum was 137.1 in horse waste. The potassium content ranged from 0.48% in cow dung to 1.31% in horse dung. Phosphorus content ranged from 0.30% in camel waste to 0.70% in horse waste. TKN content in our wastes was lower than that reported for cattle wastes in literature and hence, C:N ratios were higher that reported by other co-workers [3]

Growth of Eisenia foetida in various animal wastes

No mortality was observed in any animal waste during the study period. Gunadi and Edwards [16] reported the death of *Eisenia foetida* after 2 weeks in the fresh cattle solids although all other growth parameters such as moisture content, pH, electrical conductivity, C:N ratio, NH_4^+ and NO_3^- 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 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.

The growth curves of *E. foetida* in studied animal wastes over the observation period are given in *Fig 1*. Maximum worm biomass was attained in sheep waste (1294±245 mg/earthworm) and minimum in horse waste (800±137 mg/earthworm). The maximum

Table 2: *Growth of Eisenia foetida in different animal wastes*

animal waste	mean initial weight / earthworm (mg)	maximum weight achieved / worm (mg)	maximum weight achieved on	net weight gain / worm (mg)	growth rate / worm /day (mg)	worm weight gained per unit dry animal waste (mg/g)
cow	196±69	889±90	6th week	686±22	16.3±0.52	34.0±1.9
buffalo	190±30	1026±210	13th week	836±186	9.2±2.04	41.9±2.6
horse	138±48	800±137	10th week	662±90	9.5±1.28	33.4±1.2
donkey	182±40	1116±208	9th week	930±169	14.±2.71	46.6±1.1
sheep	192±67	1294±245	6th week	1102±197	26.2±4.70	55.3±1.9
goat	210±29	904±174	6th week	694±148	16.5±3.54	34.5±1.7
camel	212±46	806±47	6th week	574±34	13.7±0.82	32.5±1.4

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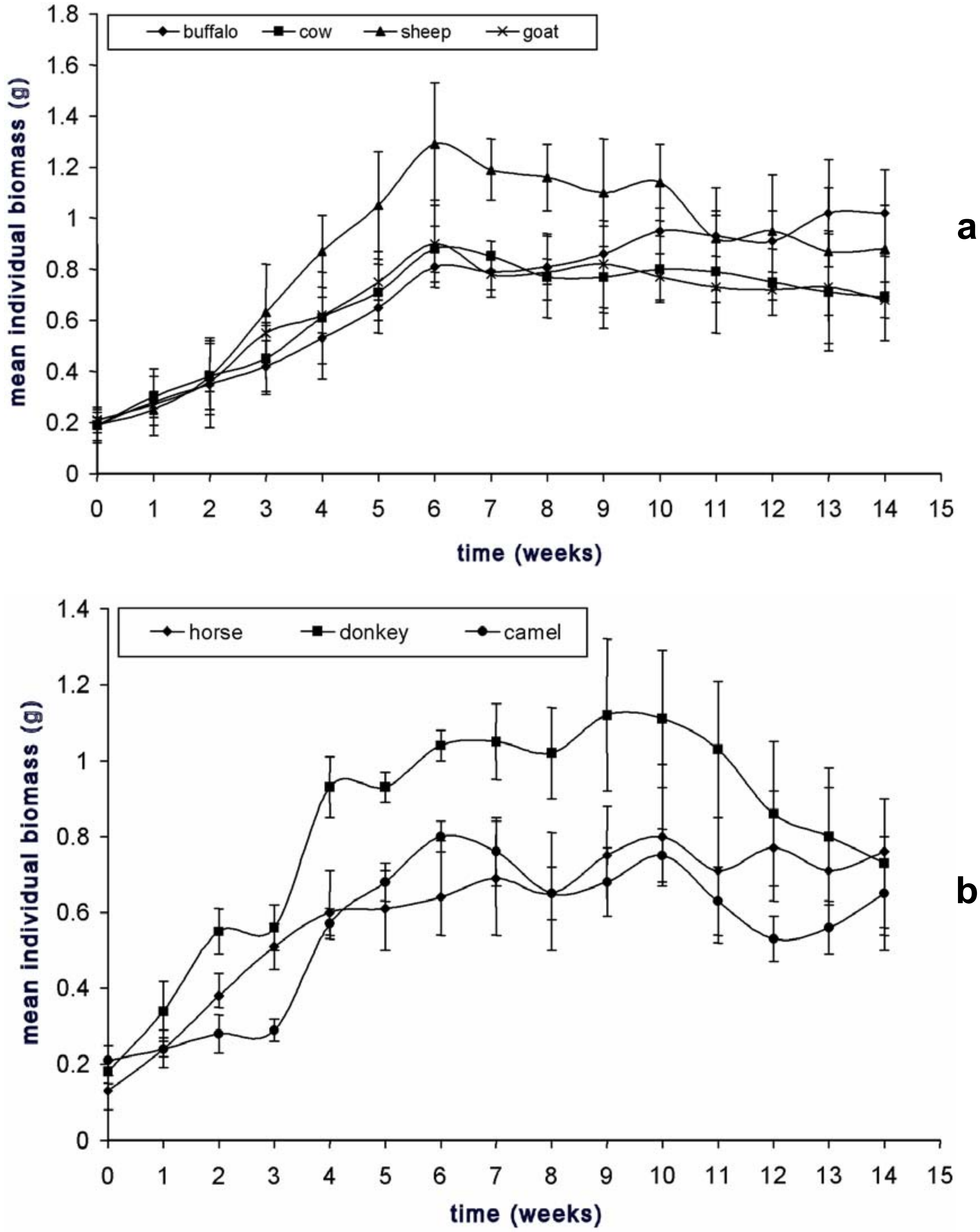

Figure 1. *Growth pattern of Eisenia foetida on different animal wastes*

weight by earthworms was attained in the 6th week in cow, sheep, goat and camel wastes, where as it took 9, 10 and 13 weeks in donkey, horse and buffalo wastes respectively. Initially worms gained biomass but later after few weeks, weight loss by earthworms was observed in all the tested animal wastes. The loss in worm biomass can be attributed to the exhaustion of food. When *E. foetida* received food below a maintenance level, it lost weight at a rate which depended upon the quantity and nature of its ingestible substrates [27].

The biomass gain for *E. foetida* per g dry weight of feed (DW) was highest in sheep waste (55.3±1.9 mg/g) and smallest in camel waste (32.5±1.40 mg/g). Edwards *et al.* [11] have reported a biomass gain of 292 mg/g cattle waste by *P. excavatus* at 25 °C. However, in our experiments, the biomass gain was only 34.0±1.90 mg/g by *E. foetida* species in cow dung at 27 °C. In horse waste, biomass gain by *P. excavatus* species was about 2 times higher than *E. foetida*. This difference could be due to the difference in species morphology and initial characteristics of the feed waste. Nauhauser *et al.* [27] reported that rate of biomass gain by *E. foetida* was dependent on population density and food type. Net biomass gain/earthworm per unit feed material in different feeds followed the order: sheep > donkey > buffalo > goat ≈ cow ≈ horse > camel. Net biomass gain by earthworms in sheep waste was 1.92 times higher than in camel waste (*Table 2*).

The growth rate (mg weight gained/day/earthworm) has been considered a good comparative index to compare the growth of earthworms in different wastes [11]. The buffalo (9.2± 2.04 mg/day/earthworm) and horse wastes (9.5± 1.28 mg/day/earthworm) supported the least growth of *Eisenia foetida*; camel and donkey wastes were marginally better than buffalo and horse wastes (Table 2). Lower growth rate in buffalo waste, in spite of attainment of more body weight than cow waste, was due to the fact that the time taken to achieve the maximum biomass was longer for buffalo waste than cow waste. Similar observations have been reported by Chaudhuri & Bhattacharjee [8] for vermicomposting of cow dung and kitchen waste by *Perionyx excavatus*. The worm growth rate was highest in sheep waste which was about twice than in camel waste. Earthworms grew at relatively similar rates in cow and goat wastes (Table 2).

Sexual development and cocoon production

Table 3 summarizes the sexual development and cocoon production by *E. foetida* in different feeds. All individuals in all the feeds developed clitellum before day 21 except camel waste (day 28) after the start of the experiment. Cocoon production by earthworms was started by day 28 in horse, donkey, sheep and goat wastes; and by day 35 in cow, buffalo and camel wastes. *Fig. 2* shows the cumulative cocoon production by earthworm in different feeds. After 15 weeks maximum cocoons (155±18.36) were counted in sheep waste and minimum (62±23.57) in buffalo waste. The mean number of cocoons produced per worm per day of 0.44±0.052 in sheep waste was 231% greater than 0.19±0.072 cocoons produced per day in buffalo waste. The number of cocoons produced per earthworm per day in different wastes was in the order: sheep > cow ≈ horse ≈ goat > camel > donkey > buffalo. The difference between rates of cocoon

Table 3. *Cocoon production by Eisenia foetida in different animal wastes*

animal waste	clitellum development started in	cocoon production started in	total no. of cocoons produced after 15 weeks	no. of cocoons produced / worm	no. of cocoons produced / worm / day	cocoon production ceased after
cow	3rd week	5th week	109±14.9	21.8±3.0	0.39±0.05	12th week
buffalo	3rd week	5th week	62±23.6	12.3±4.6	0.19±0.07	13th week
horse	3rd week	4th week	143±29.5	28.6±5.9	0.37±0.08	14th week
donkey	3rd week	4th week	97±11.3	19.4±2.3	0.28±0.03	13th week
sheep	3rd week	4th week	155±18.4	31.0±3.7	0.44±0.05	13th week
goat	2nd week	4th week	124±28.4	25.4±5.7	0.36±0.08	13th week
camel	4th week	5th week	89±15.7	17.8±3.1	0.32±0.06	12th week

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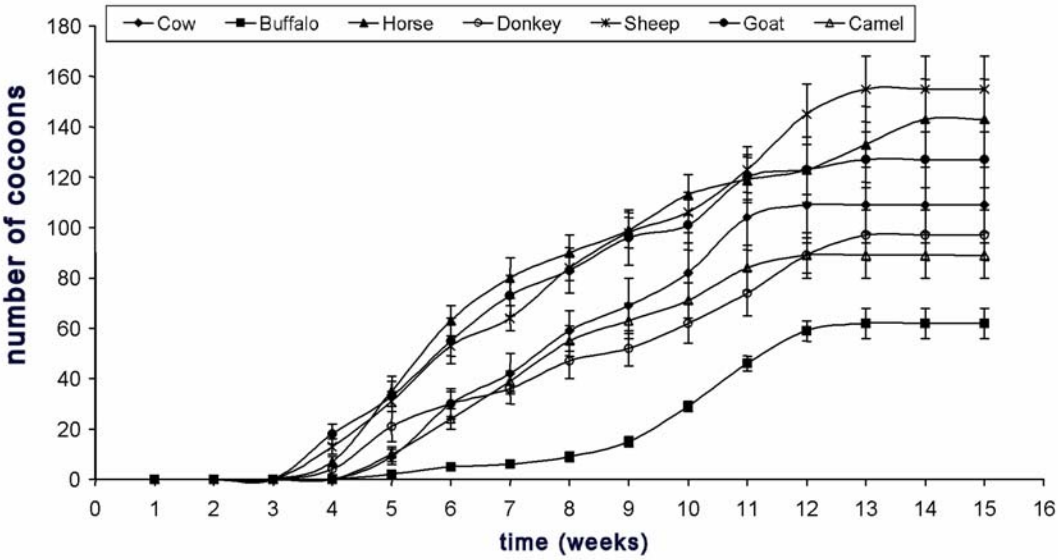

Figure 2. *Cumulative cocoon production by Eisenia foetida on different animal wastes*

production could be related to the biochemical quality of the feeds, which is an important factor in determining the time taken to reach sexual maturity and onset of reproduction [11, 12]. Feeds which provide earthworms with sufficient amount of easily metabolizable organic matter and non-assimilated carbohydrates, favour growth and reproduction of earthworms [12]. But in our experiments, buffalo and donkey wastes were in contrast to this observation. The weight gain by earthworms was more in these feeds but cocoon production was lower than other feeds tested. It indicates that buffalo and donkey wastes are a good biomass supporting medium but not good for reproduction. A large proportion of the energy of mature worms is used in cocoon production. When cocoons are not produced the energy is utilized for tissue growth [8, 19]. The cocoon production was ceased by day 84 in cow and camel feed wastes; by day 91 in buffalo, donkey, sheep and goat wastes and by day 98 in horse waste.

Conclusions

Disposal of animal dung materials is a serious problem. Currently the fertilizer values of animal dung are not being fully utilized in India resulting in loss of potential nutrients. Our trials demonstrated vermicomposting as an alternate technology for the recycling of different animal dung materials using an epigeic earthworm *Eisenia foetida* under laboratory conditions. The dung materials strongly influenced the biology of *E. foetida.* Net biomass gain/earthworm in different feeds was in the order: sheep > donkey > buffalo > goat ≈ cow ≈ horse > camel. The biomass gain for *E. foetida* (live weight) per g dry weight of feed source (DW) was highest in sheep waste (55.3±1.9 mg/g) and smallest in camel waste (32.5±1.40 mg/g). The mean number of cocoons produced per worm per day of 0.44±0.052 in sheep waste was 231% greater than 0.19±0.072 cocoons produced per day in buffalo waste. The number of cocoons produced per earthworm per day in different dung materials was in the order: sheep > cow ≈ horse ≈ goat > camel > donkey > buffalo. Finally, cow, horse, sheep and goat wastes supported the growth and reproduction of *E. foetida,* hence can be used as feed materials in large scale vermicomposting facilities. Further studies are required to explore the potential of utilization of buffalo, donkey and camel wastes in mixture with cow or sheep or goat wastes.

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