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Original article

The influence of different moisture levels on the growth, fecundity and survival of *Eisenia fetida* (Savigny) in cattle and pig manure solids

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Abstract

The growth, fecundity and mortality of the epigeic earthworm *Eisenia fetida* in cattle manure solids and pig manure solids with different moisture contents (70%, 75%, 80%, 85%, 90%) were studied for 30 weeks in the laboratory. The maximum weight of *E. fetida* in the pig manure solids, which had a moisture content of 75%, was 1100 mg after 13 weeks with a highest growth rate of 12.8 mg d^{-1}. The total number of cocoons and hatchlings produced was lower, compared to those in all the other moisture levels. Generally, earthworms grew bigger and faster in pig manure solids than in separated cattle manure solids but the mortality at all moisture levels in pig manure solids was high, ranging from 20% to 100%. The C/N ratio, NH_4 and NO_3 contents in the cattle manure solids and pig manure solids were variable both at the beginning of the experiment and in the earthworm casts or vermicomposts.

Keywords: Eisenia fetida; Cattle manure solids; Pig manure solids; Moisture contents; Earthworm casts or vermicomposts

1. Introduction

The moisture content of organic wastes used in vermicomposting is an important parameter influencing the growth of the surface-feeding (epigeic) earthworm species *Eisenia fetida* (Savigny) (Lumbricidae) since the earthworm's body contains about 80% water. The growth of *E. fetida* in organic matter substrates with different moisture contents and temperatures has been studied by various authors in the laboratory. This species gained weight maximally and survived best at temperatures between 20 and 29 °C and moisture content between 70% and 85% in horse manure and activated sludge [9]. Loehr et al. [10] reported slightly higher optimum moisture contents for the growth of *E. fetida* in activated sludge of 85% and 90%. According to Edwards [3], the optimum growth of *E. fetida* in different animal and vegetable wastes was at temperatures of 25–30 °C and at a moisture content range of 75–90%, but these units could vary in different substrates.

The moisture content preferences of juvenile and clitellate cocoon-producing (adult) *E. fetida* in separated cow manure have been investigated [12]. This ranged from 50% to 80% for adults, but juvenile earthworms had a narrower range of suitable moisture levels from 65% to 70%. Clitellum development occurred in earthworms at a moisture content from 60% to 70% but occurred later at a moisture content from 55% to 60%. The tolerance limit for low moisture conditions on the growth of *E. fetida* was reported to be below 50% for up to 1 month [11].

Studies on the growth and survival of *E. fetida*, in relation to temperature, moisture and the presence in the substrate of *Enchytraeus albidus* (Enchytraeidae), were conducted by Haukka [8]. The earthworms thrived in bedding with 80%

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moisture content at 25 °C. It was reported that the addition of fresh pig manure solids could kill *E. fetida*. By maintaining the moisture content at 80% without the addition of a new substrate, they could survive for more than 60 weeks [7].

The experiment reported here had two main objectives. The first was a 30 week assessment of the rates of growth, fecundity and mortality of *E. fetida* in cattle manure solids and pig manure solids over a range of different moisture contents (70%, 75%, 80%, 85%, 90%) to establish the optimal moisture content, for each animal waste, which could promote the highest growth and reproduction rates. The second objective was to assess the C/N ratio, NH_4 and NO_3 contents in the cattle manure solids and pig manure solids at the beginning of the experiment and follow changes in these parameters in the final earthworm casts or vermicomposts produced at different moisture contents.

2. Materials and methods

2.1. Preparation of substrates

Separated cattle manure solids and non-separated adult sow pig manure solids were collected from the Ohio Agricultural Research and Development Center (OARDC) at Wooster. The separated cattle manure solids were obtained from the OARDC dairy cattle facility, through the use of a large-scale livestock manure separator, which utilized a large auger and screen-mesh cylinder dewatering technology. Non-separated (fresh) adult sow pig manure solids were collected directly from the OARDC swine breeding facility. These substrates were dried in an oven at 60 °C until 0% moisture content was achieved. Appropriate quantities of water were added to 10 separate 150 g sub-samples of each waste with three replicates, to achieve a range of different moisture levels 70%, 75%, 80%, 85%, and 90% moisture, based on dry weights. The taxonomic determination of the test species *Eisenia fetida* (Savigny, 1826) was done using the taxonomic keys of Schwert [13].

2.2. Culture of earthworms

The 150 g (wet weight) samples of cattle manure solids and pig manure solids with a range of different moisture levels of 70%, 75%, 80%, 85%, and 90% were placed in plastic boxes (12 × 12 × 6 cm) covered with pierced lids for aeration. Five juvenile *E. fetida* obtained from F_1 cocoons, each weighing 25–30 mg (fresh weight), were placed in each plastic culturing box and each moisture content and substrate treatment was replicated three times. The earthworms were counted and weighed weekly for 30 weeks. The moisture contents of the substrate were determined every 2 weeks by weighing and oven-drying 2–3 g homogeneous sub-samples. The substrate moisture contents were adjusted to the desired moisture content by adding distilled water as needed. The number of cocoons in each culture was counted every week and the dates of cocoon production recorded. Cocoons were removed from the containers once a week, and placed in micro-plates in a plastic container filled with distilled water for observation of their times to hatching. The number of hatchling produced weekly was counted.

2.3. Chemical analyses

At the start of the experiment, the various substrates maintained at different moisture levels and the earthworm casts or vermicomposts produced at the end of the experiment, were analyzed for C/N ratio, NH_4 and NO_3 contents. Two grams of substrate or cast sub-samples of each treatment were oven-dried at 60 °C to determine the moisture contents. The same samples were also used for analyses of C/N ratios. A second 2 g substrate or cast sub-samples were used for analyses of NH_4 and NO_3 contents. Carbon and nitrogen levels were determined using a Carlo Erba NA 1500 Series 2 Nitrogen/Carbon Analyzer. NH_4 and NO_3 levels were determined using a BIO-TEK Instruments Microplate EL311 Autoreader.

2.4. Statistical analyses

One-way analysis of variance and Duncan's new multiple-range test were used to calculate the significance of differences between growth rates, mean number of cocoons per earthworm, and mean number of hatchlings per cocoon of *E. fetida* in the substrates with different moisture contents.

3. Results

3.1. Growth patterns of Eisenia fetida

The growth of *E. fetida* in the cattle manure solids and pig manure solids with moisture contents ranging through 70%, 75%, 80%, 85%, 90% followed a logistic growth pattern over the first 5 or 6 weeks (Figs. 1 and 2). The earthworm growth rate was fastest in the separated cattle manure solids with a moisture content of 90% with a maximum mean weight of earthworms of 600 mg after 12 weeks. The slowest growth rate of *E. fetida* was in the separated cattle manure solids at a moisture content of 70% (Fig. 1).

The growth rate of *E. fetida* in the adult sow pig manure solids fluctuated. In the first 8 weeks during the period of active growth, the earthworms grew fastest in adult sow pig manure solids with a moisture content of 75%. The maximum mean weight of earthworms was 1100 mg after 13 weeks (Fig. 2). The slowest growth rate of *E. fetida* was in the adult sow pig manure solids at a moisture content of 70% and all earthworms died after 21 weeks in this substrate.

3.2. Mean growth rates of earthworms over the first 2 months

The highest mean growth rate per individual earthworm, in the separated cattle manure solids over the first 2 months,

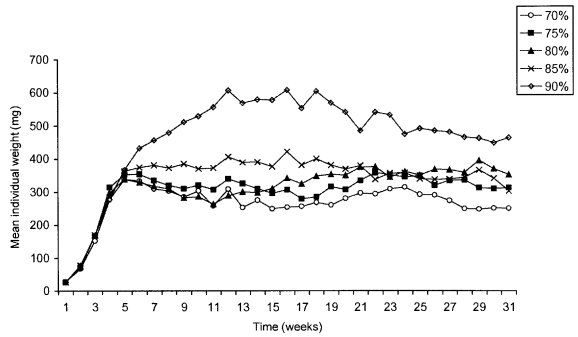

Fig. 1. The rates of growth of *Eisenia fetida* in cattle manure solids with different moisture contents over 30 weeks.

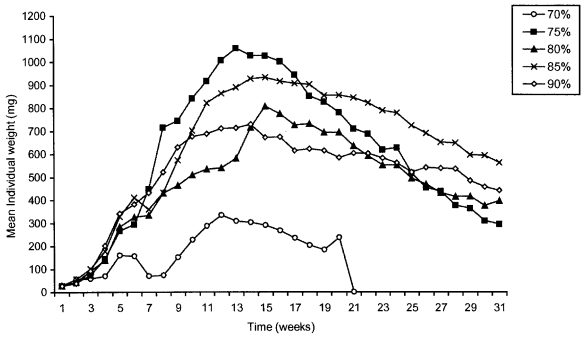

Fig. 2. The rates of growth of *Eisenia fetida* in pig manure solids with different moisture contents over 30 weeks.

was 8.7 ± 0.1 mg d^{-1} at a moisture content of 90% (Table 1). This differed significantly from the growth rates at all the other moisture contents ($P < 0.001$, $F = 740.7$). The lowest mean growth rate of earthworms in separated cattle manure solids was 4.6 ± 0.1 mg d^{-1} at moisture contents of 70% and 80%.

The highest growth rate of earthworms in the adult sow pig manure solids was 12.8 ± 0.1 mg d^{-1} at a moisture content of 75% ($P < 0.001$, $F = 2953.8$). The lowest mean growth rate in the adult sow pig manure solids was 2.2 ± 0.1 mg d^{-1} at a moisture content of 70% (Table 1).

3.3. Mortality of the earthworms

The earthworms in the separated cattle manure solids over a range of moisture contents (70%, 75%, 80%, 85%, 90%) had a low mean mortality of 7% (Table 1). The highest mortality of *E. fetida* occurred in the adult sow pig manure solids where all died at a moisture content of 70% after 20 weeks (Fig. 2). The lowest mortality of 20% was in the pig manure solids at a moisture content of 90% after 30 weeks.

3.4. Fecundity of the earthworms

The largest mean number of cocoons produced per earthworm and the largest mean number of hatchlings produced per cocoon in the separated cattle manure solids was at a moisture content of 75%: 9.8 ± 1.0 cocoons produced per earthworm ($P < 0.001$, $F = 105.9$) and 1.7 ± 0.1 hatchlings produced per cocoon ($P < 0.001$, $F = 25.1$), respectively (Table 2). At 75% moisture content, the earthworms started to produce cocoons after 4 weeks and stopped producing cocoons after 28 weeks. At this moisture content, the incubation time of cocoons was 12–67 d.

The highest mean number of cocoons produced per earthworm and the mean number of hatchlings produced per cocoon in the adult sow pig manure solids was at a moisture content of 80% with 2.9 ± 0.2 cocoons produced per earthworm ($P < 0.001$, $F = 310.5$) and 2.4 ± 0.1 hatchlings produced per cocoon ($P < 0.001$, $F = 173.2$), respectively. The earthworms started to produce cocoons after 11 weeks and stopped producing cocoons after 18 weeks. At this moisture content, the incubation time of cocoons was 12–84 d.

Table 1
Growth and mortality of *Eisenia fetida* over 30 weeks in cattle manure solids and pig manure solids with different moisture contents (five specimens, $n = 3 \pm$ standard deviation). The numbers followed by different letters are significantly different according to Duncan's multiple-range test ($P < 0.05$). In pig manure solids with 70% moisture content, all earthworms died after 19 weeks

Sources of different moistures (%)	Mean individual biomass (mg)		Mean growth of individual in the first 2 months (mg d^{-1})	Mortality after 30 weeks (%)
	At start	At end		
Cattle manure solids				
70	27.3 ± 0.2	249.7 ± 5.6	4.6 ± 0.1 a	7
75	27.4 ± 0.9	312.6 ± 7.5	5.0 ± 0.2 b	7
80	27.3 ± 0.6	352.5 ± 24.7	4.6 ± 0.1 a	7
85	26.6 ± 0.8	303.0 ± 54.2	6.4 ± 0.1 c	7
90	26.3 ± 1.3	463.3 ± 29.1	8.7 ± 0.1 d	7
Pig manure solids				
70	28.5 ± 0.5	–	2.2 ± 0.1 A	100
75	26.8 ± 1.1	295.4 ± 4.9	12.8 ± 0.1 E	53
80	27.8 ± 0.9	396.8 ± 13.8	7.8 ± 0.1 B	40
85	27.7 ± 1.0	562.5 ± 55.5	9.9 ± 0.2 C	47
90	26.6 ± 0.4	440.8 ±117.2	10.8 ± 0.1 D	20

Table 2
Fecundity of *E. fetida* over 30 weeks at different moisture levels of cattle manure solids and pig manure solids (five specimens, $n = 3 \pm$ standard deviation). The numbers followed by different letters are significantly different according to Duncan's multiple-range test ($P < 0.05$)

Treatments	70%	75%	80%	85%	90 %
Cattle manure solids					
Mean cocoons earthworm^{-1}	15.0 ± 0.6 b	19.8 ± 1.0 c	18.5 ± 1.2 c	14.8 ± 0.1 b	8.6 ± 0.1 a
Mean hatchlings cocoons^{-1}	1.6 ± 0.1 BC	1.7 ± 0.1 C	1.5 ± 0.1 BC	1.4 ± 0.1 B	0.9 ± 0.2 A
Total number of cocoons	219	291	241	208	121
Total number of hatchlings	344	506	357	299	108
Start producing cocoons after	4 weeks	4 weeks	4 weeks	4 weeks	4 weeks
Stop producing cocoons after	29 weeks	28 weeks	30 weeks	30 weeks	29 weeks
Incubation time (d)	12–55	12–67	12–91	12–76	20–63
Pig manure solids					
Mean cocoons earthworm^{-1}	–	1.9 ± 0.1 *a*	2.9 ± 0.2 *b*	2.0 ± 0.1 *a*	1.9 ± 0.1 *a*
Mean hatchlings cocoons^{-1}	–	1.5 ± 0.2 *B*	2.4 ± 0.1*C*	2.4 ± 0.1 *C*	1.5 ± 0.2 *B*
Total number of cocoons	–	13	24	16	23
Total number of hatchlings	–	15	57	38	38
Start producing cocoons after	–	10 weeks	11 weeks	5 weeks	16 weeks
Stop producing cocoons after	–	17 weeks	18 weeks	29 weeks	30 weeks
Incubation time (d)	–	25–56	12–84	19–69	14–56

3.5. C/N ratios, NH_4 and NO_3 contents of the initial feeding and earthworm casts

There was a trend for the C/N ratio in the separated cattle manure solids (C/N ratio: 40) at the start of the experiment to be more than twice that in the adult sow pig manure solids (C/N ratio: 15). The C/N ratios of the cattle solid vermicomposts had the same trend (C/N ratio about 22 for cattle vs. 8 for pig). There was no special pattern in C/N ratios in vermicomposts produced from either waste source after 30 weeks (Table 3) in response to different moisture contents of cattle manure solids and pig manure solid substrates.

There was a tendency for the greatest NH_4 content to be in the separated cattle manure solids at a moisture content of 90% (51.9 ± 20.9 mg kg^{-1}). All the moisture contents of the adult sow pig manure solids resulted in high NH_4 contents ranging from 300–1100 mg kg^{-1}. All moisture contents of the adult sow pig manure solids tested produced more NO_3 (65.4 ± 27.1 to 114.8 ± 14.6 mg kg^{-1}) than the separated cattle manure solids (10.3 ± 2.9 to 22.4 ± 5.2 mg kg^{-1}).

There was a tendency for the NH_4 contents of the earthworm casts or vermicomposts, produced from adult sow pig manure solids at different moisture contents, to be lower (11.9 ± 3.2 to 19.5 ± 4.8 mg kg^{-1}) than those of earthworm casts produced from separated cattle manure solids (18.5 ± 0.4 to 63.2 ± 4.9 mg kg^{-1}). The NO_3 contents of the earthworm casts from adult sow pig manure solids were higher (123.1 ± 26.0 to 177.7 ± 6.4 mg kg^{-1}) than those of earthworm casts from separated cattle manure solids (14.4 ± 1.2 to 27.6 ± 2.6 mg kg^{-1}). There was no specific pattern in the NH_4

Table 3
The C/N ratios, NH_4 and NO_3 content ($n = 3 \pm$ standard deviation) of cattle manure solids and pig manure solids with different moisture levels at the start and in the earthworm casts or vermicomposts at the end of the experiment after 30 weeks

Sources in different moistures (%)	C/N ratio		NH_4 content (mg kg^{-1})		NO_3 content (mg kg^{-1})	
	At start	At end	At start	At end	At start	At end
Cattle manure solids						
70	38.5 ± 2.4	24.3 ± 1.5	23.6 ± 14.2	18.5 ± 0.4	10.3 ± 2.9	14.4 ± 1.2
75	39.6 ± 4.2	22.3 ± 0.9	27.3 ± 20.2	29.4 ± 6.7	22.4 ± 5.2	20.9 ± 1.8
80	44.7 ± 3.5	20.7 ± 1.3	32.6 ± 20.2	44.1 ± 5.6	12.4 ± 3.7	22.0 ± 4.0
85	42.9 ± 3.3	18.1 ± 1.7	40.0 ± 23.8	35.9 ± 5.6	15.5 ± 7.1	21.7 ± 2.8
90	41.2 ± 3.1	20.2 ± 2.2	51.9 ± 20.9	63.2 ± 5.6	22.3 ± 3.0	27.6 ± 2.6
Pig manure solids						
70	14.7 ± 4.3	7.2 ± 0.2	1094.3 ± 115.9	18.3 ± 5.6	114.8 ± 14.6	157.4 ± 3.9
75	16.5 ± 3.1	7.7 ± 0.3	337.5 ± 122.8	19.5 ± 5.6	74.3 ± 16.1	176.0 ± 14.7
80	14.5 ± 4.6	8.2 ± 0.1	326.1 ± 137.7	11.9 ± 5.6	45.1 ± 6.8	177.7 ± 6.4
85	15.8 ± 4.7	8.2 ± 0.2	939.4 ± 224.6	18.6 ± 5.6	84.0 ± 23.1	141.8 ± 11.2
90	15.1 ± 4.2	8.2 ± 0.1	592.4 ± 242.5	16.0 ± 5.6	65.4 ± 27.1	123.1 ± 26.0

and NO_3 contents of earthworm casts produced from either cattle manure solids or pig manure solids at any moisture content.

4. Discussion

The earthworms attained their highest growth rates in the first 2 months in cattle manure solids at a moisture content of 90% (8.7 ± 0.1 mg d^{-1}), and in pig manure solids at a moisture content of 75% (12.8 ± 0.1 mg d^{-1}). Afterwards, they lost weight. The mean individual earthworm growth rate was higher than that reported by Gunadi et al. [6], where the growth rate of *E. fetida* after different pre-composting times of cattle manure solids (0, 1, 2, 3, 4, 5 weeks) ranged from 5.1 to 7.9 mg d^{-1}. It was reported that the related species *Eisenia andrei* gained weight maximally (11.8 mg d^{-1}), at a moisture content of 85% in a mixture of pig manure solids and maple leaves (85:15) [2].

The moisture conditions that resulted in most cocoons per earthworm and most hatchlings per cocoon (9.8 ± 0.1 cocoons produced per earthworm over 30 weeks and 1.7 ± 0.1 hatchlings produced per cocoon) was at 75% moisture content in the cattle manure solids which was similar to the results of Reinecke and Venter [12]. These authors reported that clitellate earthworms were found frequently at a moisture content above 70%. It seems that cocoon-producing earthworms and juveniles prefer drier substrate conditions than other adult earthworms. The clitellate earthworms started to produce cocoons after 4 weeks in separated cattle manure solids. In adult sow pig manure solids, most cocoons produced per earthworm and most hatchlings produced per cocoon (2.9 ± 0.1 cocoons produced per earthworm and 2.4 ± 0.1 hatchlings produced per cocoon, respectively) were at moisture content of 80% (Table 2). This may have been because the drier pig solid at moisture contents of 70% and 75% contained more NH_4 and NO_3. The adult earthworms started to produce cocoons after 5–16 weeks in pig manure solids. The 2.9 ± 0.1 cocoons produced per earthworm over 30 weeks in adult sow pig manure solids was similar to the numbers reported by Loehr et al. [10]. These authors reported that *E. fetida*, in aerobically digested sludge, produced 3.1 cocoons per earthworm over 20 weeks at a moisture content of 75% and a temperature of 25 °C.

The mortality of the earthworms at all moisture contents in pig manure solids was higher (from 20% to 100%) than that in the cattle manure solids (mortality 7%) (Table 1). These correspond with the results reported by Gunadi and Edwards [7], where *E. fetida* grew faster, produced less cocoons, and had a higher mortality in pig manure solids than in cattle manure solids. The high mortality of the earthworms in pig manure solids may probably be because pig manure solids had higher NH_4 and NO_3 content than cattle manure solids (Table 3). After some of the earthworms survived in the pig manure solids, they grew faster than in the cattle manure solids. This is because pig manure solids contain higher nutrition for the earthworms (e.g. protein), which is usually mixed with the left over food of the pig. The reason why *E. fetida* produced less cocoons in pig manure solids than in cattle manure solids was not clear, but it probably has a close relationship with high NH_4 and NO_3 content and low C/N ratio in pig manure solids (Table 3).

The quality of the organic waste seemed to be important for the growth and survival of *E. fetida*. The large amounts of NH_4 (1094.3 ± 115.9 mg kg^{-1}) in pig manure solids at a moisture content of 70% could have been toxic to *E. fetida* (Table 3). At 70% moisture content, the growth rate of *E. fetida* was the slowest (2.2 ± 0.1 mg d^{-1}) and all the earthworms died after 20 weeks. Kaplan et al. [9] also studied the rates of growth of *E. fetida* in different concentrations of NH_4 acetate (0, 1000, 5000, 10000 mg kg^{-1}) and reported that all earthworms died at a concentration of 1000 mg kg^{-1} of NH_4 acetate.

There was no special pattern in changes in C/N ratios, NH_4 and NO_3 content during vermicomposting of separated cattle manure solids or adult sow pig manure solids at different moisture contents. But at the end, the earthworm casts or vermicomposts from pig manure solids had NH_4 and NO_3 content at the same level at different moisture treatments. It can be stated that vermicomposts made from pig manure solids have more stabile and lower NH_4 and NO_3 content rather than pig manure solids itself (Table 3). The vermicomposts produced from separated cattle manure solids usually have a higher C/N ratio, greater NH_4 contents, and lower NO_3 contents than those produced from adult sow pig manure solids. These results agree with those of Gunadi and Edwards [7], except that in their experiments, the NO_3 content was higher in vermicomposts produced from cattle manure solids than those produced from adult sow pig manure solids (550 vs. 500 mg kg^{-1}) after 23 weeks. The different moisture contents of each type of vermicompost were less important than the types of substrate, in relation to changes in C/N ratios, NH_4 and NO_3 contents in the vermicomposts in accordance with these authors [1,4–6].

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