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RESEARCH ARTICLE

Adaptability comparison of *E. fetida* in vermicomposting against sludge from livestock wastewater treatment plant based on their several growth stages

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Abstract Vermicomposting is a low-cost, eco-efficient process to deal with organic wastes. Mixtures of swine manure (SM), cow dung (CD), and animal wastewater treatment plant sludge (S) were applied as feeds, and *Eisenia fetida* was employed in this study to investigate the vermicomposting efficiency based on their several growth stages. The hatching test resulted in a 100 % hatching rate in $S_{60}SM_{40}$ (60 % S + 40 % SM) mixture, while 4.40 hatchlings per cocoon were observed. The growth of infancy performed best in 0–20 % CD mixtures (0.05 ± 0.002 g), followed by in SM + CD (0.04 ± 0.003 g). The highest growth rate of young and adult *E. fetida* was noticed in CD + S mixtures (11.14 ± 0.01 and 6.00 ± 0.02 mg/d/worm, respectively), while the higher cocoon production of adults was noticed in S + SM mixtures especially in $S_{40}SM_{60}$ (537 ± 5 worms). Moreover, the conversion of solids; the modified pH value; the reduction in total organic carbon (TOC); total Kjeldahl nitrogen (TKN), NH_4-N, NO_3-N, and C:N ratio; and the rich in total available phosphorus (TAP) and total potassium (TK) content by young and adult *E. fetida* were related to the growth of worms. Such work would benefit understanding and to increase the efficiency of vermicompost processing of different wastes.

Keywords Vermicomposting · Animal manure · Livestock wastewater treatment plant sludge · Mixtures · *E. fetida* · Growth stages

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Introduction

Vermicomposting had been proven to be an eco-efficient, low-cost method (Yadav and Garg 2011). During the vermicomposting process, earthworms convert organic material into worm biomass and, physically, nutrition and biochemically allied stabilized product (Aira and Domínguez 2009). Furthermore, the vermicompost has been called organic gold (Adhikary 2012) while the earthworm biomass can be restored as protein feed. The life cycle of *Eisenia fetida* has been studied over periods ranging from 28 to 120 days (Manyuchi and Phiri 2013). Studies on organic materials like swine manure, cattle dung, goat dropping, poultry excrete, rabbit manure, and plant litter have been conducted (Elvira et al. 1997; Manna et al. 2005; Molina et al. 2013; Song et al. 2014). And the sludge from sewage, textile mill, tannery, and other factories amended with animal or plant wastes was used to examine the growth and reproduction of earthworms (Manyuchi and Phiri 2013; Xing et al. 2015). Little research reported on the sludge from livestock wastewater treatment plant which is still an issue in China.

Generally, the life cycle of earthworms was studied in the same substrate in the whole experimental period. However, the nutrient requirement of worms in different growth stages may be different. Loh et al. (2005) demonstrated that the cocoon hatchability of *E. fetida* in goat manure was higher while significantly ($P < 0.05$) more cocoons were produced in cattle manure. And in a period of 21 weeks of vermicomposting, the maximum mean individual biomass was observed in pig manure during the 4–6 weeks, and it was most in chicken manure during the 7–21 weeks (Coulibaly and Zoro Bi 2010). Besides, it has been found that the adults always are trying to escape from the substrates in the breeding practice when small worms exist, and the production process can be greatly affected. Therefore, it is meaningful to investigate the technology of breeding earthworms separately during their several growth stages to improve the efficiency of vermicomposting.

The aim of this study was to investigate the growth and reproduction performance of *E. fetida* in mixtures of swine manure (SM), cow dung (CD), and sludge (S) from animal wastewater treatment plant; measure their vermicomposting efficiency on substrates; and figure out the proper proportions of the wastes. Besides, the feasibility of vermicomposting in terms of *E. fetida*'s growth stages was examined, including the hatching of *E. fetida* cocoons in various feed mixtures.

Materials and methods

Animal wastes and *E. fetida*

The sludge (S), cow dung (CD), and swine manure (SM) were all obtained from the farms of Sichuan Agricultural University (SAU, Ya'an, Sichuan, China). The sludge (S) was procured using anaerobic baffled reactor-cyclic activated sludge system-artificial wetland (ABR-CASS-artificial wetland) wastewater treatment plant where the wastewater mainly came from the pig, dairy, and poultry farm. Fresh S, CD, and SM were spread for 4–7 days for air-drying before use, so that unwanted gases and heat which may cause harm to earthworms were removed. The earthworms (*E. fetida*) in different growth stages were also provided by the SAU in China.

Mixture preparation, earthworm feeding, and vermicomposting

The swine manure (SM), cow dung (CD), and sludge (S) were mixed with two of them. Fifteen mixtures were prepared (dry weight basis). All the treatments were signed T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, and T15 in sequence for short (Table 1). A homogenization process of the mixing materials and the mixtures were carefully carried out. The initial C:N ratio in each mixture was evaluated, and the rice bran was used to adjust the final C:N ratio to 25 which is the proper level for vermicomposting process. The moisture content was regulated to 70 % around by tap water. The other initial properties are listed in Table 1.

The *E. fetida* in several growth stages were feeding in containers with different conditions. Specifically, experiments were conducted as follows:

Cocoons: 40 g (dry weight) of each mixture were placed into glass culture dishes (0.12-m diameter). Twenty healthy cocoons which were new born (24 h) in the same day and same breeding conditions were introduced into each of the dishes, with each of the cocoon owned 2.0 g of the feeds in an area of 5.65×10^{-4} m^2. The cocoons were incubated in 25 °C for 49 days until the hatching process was finished completely.

Infant *E. fetida*: dishes with mixtures were used as above. Twenty infant worms were introduced into each of the dishes. The worms were incubated in 25 °C for 30 days until their weights were 0.03 g around.

Young *E. fetida*: 250 g (dry weight) of each mixture were placed into foam boxes measuring 0.15 m × 0.15 m × 0.1 m (length × width × depth). This provided 0.023 m^2 of exposed top surface. The bottom of the box distributed many pinholes to prevent from accumulating water. Known weights 0.030 ± 0.001 g of 10 earthworms were incubated into each of the boxes. The trail was conducted in an empty barn in the farm which belongs to SAU (Ya'an, Sichuan, China) with daily temperature fluctuating from 20 to 23 °C and continued for 49 days. All the boxes were covered by gauzes to prevent the worms from escaping.

Adult *E. fetida*: same boxes with mixtures were used as above. Known weights 0.5 ± 0.1 g of 35 non-clitellated earthworms were incubated into each of the boxes. The trail was conducted in the same barn and with the same gauzes for 30 days.

Each treatment was conducted in triplicate. Overnight tap water was spayed every day to maintain the 70 % moisture content during the trial. And the substrates were turned over once a week during the young and adult stages.

The biological parameters of worms

The numbers, weights, clitellum development, and cocoon production were determined during the several growth stages. Specifically, the number of hatched cocoons, death cocoons, and newly hatched worms were recorded at 10 am every day. The biomass of the infant worms was assessed on the 30th day, while it was weighted in an interval of 7 days in 7 weeks (except for the first week) for young worms. The total cocoon production was recorded weekly, and the biomass of adult *E. fetida* was measured when the vermicomposting finished. Earthworms were separated by hand, counted, and weighed manually on a live weight basis. The adhering materials were removed by washing them in distilled water and drying on paper towels. Then, biomass of adults was determined by taking them in a water-filled weighing boat on an analytical balance (Sartorius AG, BS124S, Göttingen, Germany; accuracy: 0.1 mg) to prevent the worms from desiccating, which affects weight.

Table 1 The initial physicochemical properties of various mixtures (dry matters)

Treatments	SM (g)	CD (g)	S (g)	pH value	NH_4-N (mg/kg)	NO_3-N (mg/kg)	TK (mg/kg)	TAP (mg/kg)
T1 (SM_{100})	250	0	0	7.80 ± 0.02	437 ± 12	291 ± 5	55 ± 2	85 ± 5
T2 ($SM_{80}CD_{20}$)	200	50	0	7.93 ± 0.05	466 ± 5	267 ± 10	72 ± 1	93 ± 4
T3 ($SM_{60}CD_{40}$)	150	100	0	8.01 ± 0.01	498 ± 6	242 ± 15	88 ± 2	101 ± 3
T4 ($SM_{40}CD_{60}$)	100	150	0	8.04 ± 0.01	532 ± 10	214 ± 12	105 ± 2	109 ± 1
T5 ($SM_{20}CD_{80}$)	50	200	0	8.07 ± 0.03	571 ± 9	184 ± 6	121 ± 6	117 ± 4
T6 (CD_{100})	0	250	0	8.19 ± 0.02	612 ± 11	151 ± 5	138 ± 2	125 ± 2
T7 ($CD_{80}S_{20}$)	0	200	50	7.95 ± 0.01	473 ± 8	123 ± 12	115 ± 3	113 ± 1
T8 ($CD_{60}S_{40}$)	0	150	100	7.91 ± 0.03	378 ± 11	104 ± 10	91 ± 1	100 ± 2
T9 ($CD_{40}S_{60}$)	0	100	150	7.58 ± 0.07	308 ± 15	90 ± 12	68 ± 5	88 ± 2
T10 ($CD_{20}S_{80}$)	0	50	200	7.38 ± 0.05	255 ± 7	80 ± 7	44 ± 4	76 ± 2
T11 (S_{100})	0	0	250	7.30 ± 0.02	213 ± 3	71 ± 4	21 ± 2	63 ± 3
T12 ($S_{80}SM_{20}$)	50	0	200	7.25 ± 0.09	241 ± 6	99 ± 4	28 ± 2	68 ± 4
T13 ($S_{60}SM_{40}$)	100	0	150	7.38 ± 0.01	275 ± 5	133 ± 2	35 ± 1	72 ± 1
T14 ($S_{40}SM_{60}$)	150	0	100	7.57 ± 0.03	317 ± 6	174 ± 14	41 ± 3	77 ± 4
T15 ($S_{20}SM_{80}$)	200	0	50	7.59 ± 0.03	370 ± 14	225 ± 9	48 ± 1	81 ± 6

The initial TOC, TKN, and C:N is around 50 %, 2 %, and 25, respectively

SM swine manure, *CD* cow dung, *S* sludge from animal wastewater treatment plant

Physicochemical analysis

The pH was measured in 1:10 (*w*/*v*) aqueous solution (sample/deionized water) that had been stirred mechanically for 30 min and filtered using a digital pH meter (pHS-25, Chengdu Yiheng Technical Co. Ltd., Chengdu, Sichuan, China). According to National Standard of Environmental Protection of People's Republic of China (NSEP of PRC), the standard method coded as HJ613 was used to test the moisture and dry weight, while the HJ615-2011 was used to analyze the levels of total organic carbon (TOC) by potassium dichromate oxidation spectrophotometric (PDOS) method. The total Kjeldahl nitrogen (TKN), NH_4-N, and NO_3-N contents were measured after digesting the sample with concentrated H_2SO_4-H_2O_2 by the semi-micro Kjeldahl method (Bremner and Mulvaney 1982). The spectrophotometry and flame photometer were used to analyze the total available phosphorus (TAP) and total potassium (TK) after digesting (Olsen et al. 2002). All the determinations were carried out in triplicate.

Statistical analysis

All analyses were undertaken using SPSS software (Window Version 19.0, IBM, Chicago, USA). Values were expressed as the mean and standard deviation. One-way analysis of variance (ANOVA) was used to analyze the significant differences among mixtures. The significant difference of various parameters was evaluated by Tukey HSD test at the 5 % probability level.

Results and discussion

The hatching of cocoons

One hundred percent swine manure (SM), cow dung (CD), and sludge (S) from animal farms were taken as controls in this study to investigate the hatching process of the cocoons. It can be seen from the Table 2 that more than 70 % of the cocoons were hatched in the first 15 days in most of the feed mixtures, while the number of hatched cocoons increased significantly in the 13th–15th days of experiment. And the hatched cocoons per day decreased with increasing of the time in the following days; only 1–6 cocoons were hatched. It means that the 15th day could be a proper time spot to observe the hatching of cocoons. Similar result was reported by Reinecke and Viljoen (1988) who indicated the incubation period for 166 cocoons of *Eudrilus eugeniae* was 16.89 days, and Bakthavathsalam et al. (2010) indicated that 100 % hatching success of *E. eugeniae* cocoons was obtained in 14–15 days.

In the first 15 days, the maximum number of hatched cocoon (19.33) was found in T10 ($CD_{20}S_{80}$) followed by 18.33 in T12 ($S_{80}SM_{20}$) and T13 ($S_{60}SM_{40}$). During the whole experimental period, the maximum hatching rate of 100 % was noticed in T1, T10, and T13, while it was the minimum (93 %) in T8 ($CD_{60}S_{40}$) and T11 (S_{100}) in which the highest mortality rate 6.67 % of cocoons was shown. Besides, the number of newly hatched worms in SMCD mixtures was in the range of 57.00–60.00, while it fluctuated in 75.67–82.00 in CDS (except for T7) and 81.33–92.67 in SSM mixtures (except for

Table 2 The hatching of cocoons in various feed mixtures

Treatments	No. of hatched cocoons (13–15 days)	No. of hatched cocoons (0–15 days)	Mortality rate of cocoons (%)	Total no. of hatchlings	Mean no. of hatchlings per cocoon
T1	15.00 ± 1.73[cde]	18.00 ± 1.73[bc]	0[a]	65.00 ± 5.29[abcd]	3.25 ± 0.26[abc]
T2	14.00 ± 0.00[bcde]	13.33 ± 1.53[a]	1.67 ± 2.89[bc]	58.00 ± 5.57[ab]	2.95 ± 0.31[ab]
T3	14.00 ± 1.73[bcde]	17.00 ± 1.73[abc]	3.33 ± 2.89[bc]	57.67 ± 5.77[ab]	2.98 ± 0.27[ab]
T4	13.00 ± 2.83[abcd]	15.33 ± 2.08[abc]	1.67 ± 2.89[bc]	60.33 ± 4.04[abc]	3.07 ± 0.18[ab]
T5	12.00 ± 1.00[abc]	14.00 ± 1.00[ab]	5.00 ± 5.00[bc]	57.00 ± 7.21[ab]	3.02 ± 0.54[ab]
T6	10.00 ± 0.00[ab]	15.50 ± 2.12[abc]	3.33 ± 2.89[bc]	49.33 ± 5.86[a]	2.81 ± 0.29[a]
T7	13.00 ± 0.00[abcde]	15.00 ± 1.73[abc]	3.33 ± 5.77[bc]	54.33 ± 7.02[ab]	2.82 ± 0.41[a]
T8	12.67 ± 1.53[abcde]	17.33 ± 0.58[abc]	6.67 ± 2.89[c]	82.00 ± 6.56[de]	4.39 ± 0.23[cd]
T9	14.33 ± 1.53[bcde]	17.67 ± 1.53[abc]	1.67 ± 2.89[bc]	75.67 ± 4.51[cde]	3.85 ± 0.26[bcd]
T10	11.00 ± 1.00[abc]	19.33 ± 0.58[c]	0 [a]	76.33 ± 7.64[cde]	3.82 ± 0.38[bcd]
T11	9.00 ± 1.00[a]	15.67 ± 1.15[abc]	6.67 ± 2.89[c]	57.33 ± 6.11[ab]	3.07 ± 0.32[ab]
T12	13.33 ± 1.53[abcde]	17.00 ± 1.00[abc]	1.67 ± 2.89[bc]	92.67 ± 6.43[e]	4.71 ± 0.26[d]
T13	15.33 ± 1.53[cde]	18.33 ± 1.53[bc]	0 [a]	88.00 ± 4.36[e]	4.40 ± 0.22[cd]
T14	16.33 ± 1.53[de]	18.33 ± 1.53[bc]	1.67 ± 2.89[bc]	81.33 ± 3.06[de]	4.14 ± 0.26[cd]
T15	16.50 ± 0.71[e]	17.00 ± 2.00[abc]	1.67 ± 3.54[bc]	68.00 ± 6.08[bcd]	3.59 ± 0.23[abcd]

The experimental period is 49 days. The initial worm cocoons are 20. Values in the same column followed by different superscript lowercase letters are significantly different ($P < 0.05$) based on Tukey HSD test

T15). The maximum number of small earthworms was recorded in T12 ($S_{80}SM_{20}$) and T13 ($S_{60}SM_{40}$), which was significantly higher ($P < 0.05$) than in others. This result indicated that the sludge from animal wastewater treatment plant amended with SM or CD was good for hatching process, and SM in proportions of 20–40 % is better than CD. This may attribute to the lower levels of NH_4-N and enough nutrition to cocoons in the feed mixtures (Table 1). As shown in the table, the pure CD was the most nutrient substrate, followed by SM, and S was the poorest one, but it was found the highest level of NH_4-N in CD (612 mg/kg) which affected the hatching process of cocoons greatly.

The number of hatchlings per cocoon also was affected in the same way above. Furthermore, the biggest mean number of hatchlings per cocoon was 4.71 in the T12 ($S_{80}SM_{20}$) feed mixture which was significantly higher ($P < 0.05$) than others, and the number decreased with the SM proportion increasing in this range. In previous researches, the maximum 2.12 hatchlings per cocoon were produced after incubation in cattle manure and distilled water and on moist filter paper (Reinecke and Viljoen 1988). No report on hatchlings per cocoon of more than three was found. It suggested that the sludge from animal wastewater treatment plant amended with 20 % swine manure is a more suitable feed for *E. fetida* cocoons.

Growth of infant *E. fetida*

The individual biomass of infant earthworms in various feed mixtures is illustrated in Fig. 1. The weights of the worms were detected only at the end of the growth stage because of their vulnerability. Significant differences ($P < 0.05$) were obtained between the controls (T1, T6, and T11), and the individual biomass increased in the order of T11 (0.0156 g) < T1 (0.0303 g) < T6 (0.0467 g). It means that 100 % CD (T6) was the best feed for growth of infant *E. fetida* in three wastes, while 100 % S (T11) was the worst. Totally speaking, the individual biomass increased in the mixture of SSM < CDS < SMCD.

Specifically, the weights of infant *E. fetida* varied in a CD dose-dependent manner in mixtures of T1–T11. The highest biomass increase was obtained in 0–20 % CD mixtures. Meanwhile, the individual biomass decreased with increasing of S dose (0–100 %) in T6–T11. This result was in accordance with the report of Garg et al. (2008), who indicated that the highest growth rate of *E. fetida* was in 100 % cow dung compared to textile mill wastewater sludge, which attributed to the easily metabolizable organic matter in cow dung. The abundant nutrition in CD may also be a reason (Table 1). Different from cocoons, the NH_4-N in CD was no longer a threat to infants.

Growth of young *E. fetida*

The growth of young *E. fetida* in various waste foods during 7 weeks is summarized in Fig. 2. The individual biomass increased from 0.354 to 0.601 g in T1–T5 (except for T4) with increasing CD in SM and decreased from 0.576 to 0.473 g in T6–T10 with increasing S in CD. The individual biomass in T11–15 was ranged from 0.419 to 0.459 g. The maximum value of the individual biomass was obtained in T5

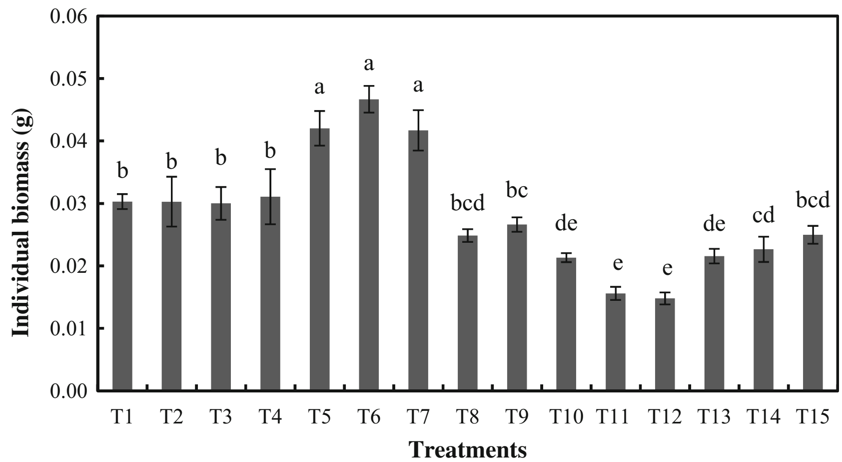

Fig. 1 Average individual biomass of *E. fetida* obtained at the end of the infancy stage. *Different letters* indicate significant differences ($P < 0.05$) between the column graphs determined by Tukey HSD test

($SM_{20}CD_{80}$) while the minimum value was in T1. The results indicated that CD can greatly improve the growth of young *E. fetida*, which may attribute to the abundant nutrient in CD. Similar finding was reported by Coulibaly and Zoro Bi (2010)), who demonstrated that the growth rate of *E. eugeniae* in cow dung was higher. Moreover, the higher growth rate and stability of young *E. fetida* were observed in T6–T10, which indicated that the mixtures of CD and S from the farms are proper medium for young *E. fetida*.

Biological parameters of adult *E. fetida*

Data on the individual biomass of adult *E. fetida* in 15 waste mixtures are given in Fig. 3. The mean weight of worms varied in waves from T1 to T15, and the highest peak was in T6 (CD_{100}; 0.680 g). Besides, the minimum mean individual biomass was shown in T10 ($CD_{20}S_{80}$; 0.434 g) and T3 ($SM_{60}CD_{40}$; 0.444 g); there was no significant differences ($P \geq 0.05$) existed between them. This illustrated that the growth of adult worms was in a CD proportion-dependent manner, and adding some sludge from animal wastewater treatment plant was good for adult *E. fetida*'s growth.

The total number of cocoon production of 35 E. *fetida* during the experimental period is shown in the same figure (Fig. 3). The cocoon production also varied in waves from T1 to T15. The highest peak was found in T14 ($S_{40}SM_{60}$; 537) followed by T8 ($CD_{60}S_{40}$; 476). However, the reproduction of adult *E. fetida* in SM, CD, and S was the least; any mixture in the treatments was a more suitable medium than single of them. The result indicated that the sludge from animal wastewater treatment plant amended with 60 % CD or SM can be served as good food for reproduction of adult *E. fetida*. And the proportion of S can significantly affect the cocoon production.

In addition, the growth and reproduction of adult *E. fetida* were connected in this experiment. Specifically, the total number of cocoons increased with decreasing individual biomass in most of the treatments. But both the weight and cocoon

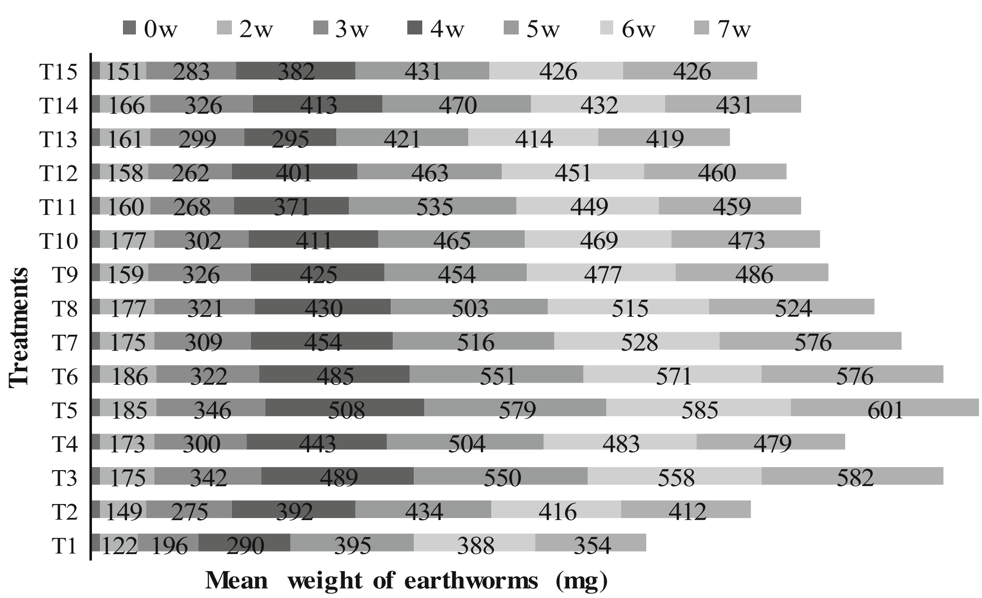

Fig. 2 The mean individual biomass of young *Eisenia fetida*

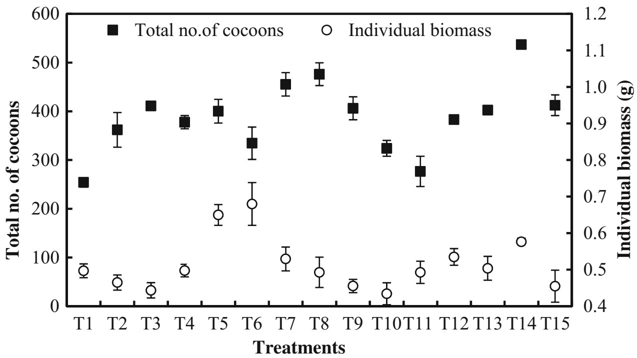

Fig. 3 Individual biomass and cocoon production of adult *E. fetida* in 30 days

numbers increased from T11 to T14. It has been revealed that the kind, palatability, and quality of food directly affected the survival, growth rate, and reproduction potential of earthworms (Ndegwa and Thompson 2000; Tripathi and Bhardwaj 2004). This may be due to S amended with 60 % SM probably provides earthworms with sufficient amount of easily metabolizable organic matter and non-assimilated carbohydrates, which favor growth and reproduction of this animal (Edwards and Fletcher 1988).

Physicochemical parameters of the mixtures

The vermicomposting by young and adult *E. fetida* significantly modified the physical and chemical properties of each feed mixture tested (Table 3). The content differences of TOC, TKN, NH_4-N, NO_3-N, TK, and TAP in the final mixtures were significantly shortened comparing with kinds of parameters in Table 1. It means that the substrates had been processed more or less into homogeneous mixtures after the activity of earthworms. Specifically, the 49-day process of vermicomposting by young *E. fetida* resulted in total weight reductions of 16.7–35.2 % in various mixtures. The average weight loss of substrates in three kinds of mixtures was SSM (30.1 %) > SMCD (24.5 %) > CDS (20.4 %). According to Bai et al. (2007), the weight loss of substrates was caused by the daily intake of earthworms and their metabolism process. Zhao et al. (2010) revealed that the presence of earthworms led to significant stabilization of the sludge by enhancing the reduction in volatile suspended solids (VSS) by 25.1 %, and digestion by earthworms and the earthworm-microorganism interactions were responsible for 54 and 46 % of this increase, respectively.

Moreover, there was no big difference on weight loss between SMCD (21.9 %), CDS (19.3 %), and SSM (23.9 %). The average individual biomass maintained in 0.51 mg in all treatment (Fig. 3). However, the total numbers of cocoon production were increased in this order: SMCD (361) < CDS (399) < SSM (402). The microbes and nutrients in S could be probably the reason of good worm reproduction in CDS and SSM, and the same levels of cocoon production could be attributed to more matters consumed in SSM (23.9 %).

Furthermore, the final pH values were increased in most of the treatments. This increase may be due to excess of organic nitrogen not required by microbes is released as ammonia, which gets dissolved in water (Rynk et al. 1992), whereas the pH values were reduced in some feeds containing sludge especially in T6 (CD) and T11 (S). The lower pH recorded in T11 has been due to the production of CO_2 and organic acids by microbial activity during the process of bioconversion (Elvira et al. 1998). In both young and adult worm breeding experiments, the minimum pH value and maximum reduction were noticed in 100 % sludge. The decrease in T6 could be caused by significant NH_4-N content reduction (468 and 540 mg/kg by young and adult worms, respectively), and the *E. fetida* played an important role in this mixture, which was in agreement with the higher growth rate in it (Figs. 2 and 3).

The young and adult earthworms remarkably decreased 22.3–29.7 and 13.0–28.8 percentage points of TOC value, respectively. Specifically, the reduction of TOC increased in SMCD mixtures and decreased in CDS and SSM feeds from T1 to T15 after young worms' breeding. The higher decline was noticed in CDS mixture. Similar data variation is observed in Fig. 2, and it is corresponding with the individual biomass of young *E. fetida*, which indicated that the transformation of organic matter in the substrate was related to the earthworms in it (Vig et al. 2011). Furthermore, it has been reported that earthworms modify the substrate conditions, which subsequently enhance the carbon losses through microbial respiration in the form of CO_2 (Hait and Tare 2011; Tognetti et al. 2007; Tripathi and Bhardwaj 2004). However, the TOC change in adult trials was not so clear; it may be due to the combined action of growth and reproduction of earthworms.

The average levels of N in the forms of TKN, NH_4-N and NO_3-N decreased in most treatments. Specifically, the content reduction of NH_4-N increased in SMCD mixtures from 191 to 372 mg/kg, decreased in CDS from 468 to 145 mg/kg, and increased in SSM feeds from 72 to 136 mg/kg, respectively, after young worms' breeding. The maximum decline was obtained in CDS, which was related to the higher growth rate of worms in it (Fig. 2). Similar NH_4-N content variation was observed in adult *E. fetida*'s feeding process, which is corresponding to the data of initial values. The results suggested the vermicompost could be affected by the initial substrates. This conclusion was supported by the initial and final NO_3-N contents. However, the TKN reduction varied from 0.07 to 0.4 and 0.22 to 0.69 percentage points after vermicomposting used young and adult *E. fetida*, respectively, whose variation was not in a pattern and may be influenced by many reasons. Zhang et al. (2012)

Table 3 The final physicochemical properties after vermicomposting by young and adult *E. fetida*

	Substrates (%)	pH value	TOC (%)	TKN (%)	NH_4-N (mg/kg)	NO_3-N (mg/kg)	C:N (%)	TK (%)	TAP (%)
Young *E. fetida*									
T1	29.2 ± 0.1	8.19 ± 0.02	36.4 ± 1.6	1.60 ± 0.02	246 ± 18	163 ± 2	17.9 ± 1.0	0.707 ± 0.021	1.111 ± 0.050
T2	31.3 ± 0.4	8.24 ± 0.02	30.8 ± 1.7	1.69 ± 0.02	246 ± 17	172 ± 17	15.1 ± 1.2	0.901 ± 0.013	1.238 ± 0.031
T3	26.9 ± 0.4	8.27 ± 0.01	24.8 ± 1.0	1.77 ± 0.11	244 ± 3	155 ± 9	12.4 ± 0.2	1.085 ± 0.027	1.266 ± 0.023
T4	17.7 ± 0.5	8.18 ± 0.04	25.2 ± 1.2	1.67 ± 0.08	197 ± 5	147 ± 13	14.9 ± 0.1	1.250 ± 0.033	1.292 ± 0.047
T5	17.6 ± 0.8	8.19 ± 0.04	21.3 ± 1.7	1.73 ± 0.05	198 ± 8	152 ± 11	11.9 ± 0.4	1.446 ± 0.054	1.340 ± 0.034
T6	18.4 ± 0.2	8.06 ± 0.07	24.1 ± 0.9	1.82 ± 0.05	144 ± 9	155 ± 1	12.4 ± 0.8	1.529 ± 0.022	1.431 ± 0.056
T7	13.8 ± 0.3	7.99 ± 0.02	31.8 ± 1.3	1.83 ± 0.08	115 ± 2	94 ± 5	17.0 ± 0.1	1.310 ± 0.021	1.316 ± 0.073
T8	16.7 ± 0.4	7.72 ± 0.02	32.0 ± 1.2	1.87 ± 0.10	114 ± 4	87 ± 3	16.8 ± 0.9	0.997 ± 0.032	1.113 ± 0.020
T9	22.9 ± 1.0	7.54 ± 0.03	36.6 ± 0.5	1.89 ± 0.03	120 ± 1	56 ± 4	19.2 ± 0.5	0.806 ± 0.034	1.033 ± 0.033
T10	30.0 ± 0.6	7.39 ± 0.10	37.0 ± 0.6	1.93 ± 0.03	110 ± 3	52 ± 3	19.1 ± 0.1	0.470 ± 0.051	0.905 ± 0.054
T11	23.6 ± 1.7	6.41 ± 0.03	31.2 ± 0.5	1.93 ± 0.06	141 ± 5	99 ± 3	16.0 ± 0.2	0.248 ± 0.030	0.749 ± 0.022
T12	29.3 ± 0.7	7.07 ± 0.02	36.2 ± 0.7	1.86 ± 0.04	166 ± 11	92 ± 7	19.3 ± 0.3	0.285 ± 0.029	0.812 ± 0.025
T13	35.2 ± 0.6	7.53 ± 0.07	34.2 ± 0.5	1.78 ± 0.01	189 ± 3	83 ± 5	18.7 ± 0.2	0.422 ± 0.011	0.858 ± 0.039
T14	33.3 ± 1.3	7.77 ± 0.03	38.6 ± 0.5	1.78 ± 0.04	216 ± 5	91 ± 3	19.6 ± 0.1	0.512 ± 0.031	0.974 ± 0.028
T15	29.1 ± 0.6	7.98 ± 0.02	39.0 ± 0.9	1.68 ± 0.01	233 ± 5	119 ± 2	20.1 ± 0.6	0.981 ± 0.012	1.067 ± 0.043
Adult *E. fetida*									
T1	24.0 ± 0.2	8.17 ± 0.03	30.7 ± 2.0	1.31 ± 0.11	76 ± 3	70 ± 2	17.4 ± 7.2	0.759 ± 0.013	1.077 ± 0.026
T2	26.3 ± 0.4	8.18 ± 0.04	27.5 ± 1.7	1.41 ± 0.10	79 ± 1	73 ± 1	15.9 ± 9.9	0.989 ± 0.011	1.172 ± 0.035
T3	21.6 ± 0.3	8.15 ± 0.03	25.4 ± 0.7	1.51 ± 0.02	77 ± 8	111 ± 7	15.0 ± 0.3	1.125 ± 0.029	1.345 ± 0.010
T4	18.8 ± 0.3	8.20 ± 0.03	21.0 ± 2.7	1.62 ± 0.08	92 ± 5	127 ± 7	12.8 ± 1.1	1.184 ± 0.020	1.310 ± 0.068
T5	18.6 ± 0.4	8.15 ± 0.04	20.3 ± 1.6	1.49 ± 0.11	65 ± 1	65 ± 3	14.0 ± 1.0	1.403 ± 0.016	1.321 ± 0.023
T6	17.0 ± 0.2	8.18 ± 0.04	25.0 ± 0.4	1.75 ± 0.10	72 ± 3	87 ± 5	13.4 ± 0.7	1.597 ± 0.011	1.363 ± 0.011
T7	18.8 ± 0.5	7.85 ± 0.07	24.8 ± 1.5	1.55 ± 0.13	70 ± 1	69 ± 3	15.8 ± 0.9	1.222 ± 0.019	1.219 ± 0.056
T8	16.4 ± 0.3	7.59 ± 0.13	24.5 ± 2.1	1.48 ± 0.09	80 ± 5	69 ± 7	16.1 ± 0.8	1.000 ± 0.022	1.090 ± 0.017
T9	21.9 ± 0.8	7.44 ± 0.05	24.8 ± 0.5	1.48 ± 0.09	67 ± 1	38 ± 1	16.4 ± 0.6	0.844 ± 0.020	0.955 ± 0.037
T10	22.3 ± 0.5	6.97 ± 0.08	27.7 ± 0.7	1.67 ± 0.03	56 ± 2	47 ± 1	16.3 ± 0.3	0.591 ± 0.019	0.900 ± 0.012
T11	19.1 ± 0.3	6.81 ± 0.12	21.2 ± 0.6	1.54 ± 0.10	33 ± 1	76 ± 3	13.5 ± 0.8	0.263 ± 0.014	0.728 ± 0.013
T12	22.8 ± 0.6	7.16 ± 0.09	21.4 ± 1.0	1.59 ± 0.05	45 ± 2	55 ± 3	13.4 ± 1.0	0.519 ± 0.011	0.790 ± 0.033
T13	24.3 ± 0.7	7.31 ± 0.01	21.6 ± 1.4	1.57 ± 0.05	93 ± 6	58 ± 9	13.5 ± 0.8	0.498 ± 0.012	0.978 ± 0.041
T14	27.4 ± 0.7	7.62 ± 0.08	25.4 ± 1.6	1.56 ± 0.04	91 ± 5	66 ± 2	14.8 ± 0.1	0.638 ± 0.014	0.926 ± 0.032
T15	26.3 ± 0.3	7.78 ± 0.09	26.3 ± 1.0	1.78 ± 0.08	94 ± 4	67 ± 2	12.4 ± 0.4	0.714 ± 0.032	1.013 ± 0.012

illustrated that the N levels in vermicompost were dependent upon the initial nutrient status in feeds. Meanwhile, a significant decrease in C:N ratio that varied in a TOC and TKN content-dependent manner was observed during experimental period. The young and adult *E. fetida* reduced the C:N ratio in the range of 8–12.6 and 4.9–13.1, respectively.

The TAP and TK content increased in various mixtures; significant differences ($P < 0.05$) were observed. The maximum increase was noticed in T6 (CD_{100}; TAP: 1.418 and 1.351 percentage points, TK: 1.515 and 1.583 percentage points) after vermicomposting by young and adult worms, respectively. Higher growth rate of earthworm in T6 was in agreement with this result. And increase in TAP during vermicomposting was probably due to mineralization and mobilization of phosphorus as a result of earthworm gut phosphatases and further release of phosphorus might be by P-solubilizing microorganisms (Le Bayon and Binet 2006). The same increased TK content result was reported by Kaushik and Garg (2004) who revealed that there was no excess water drained through mass could avoid the leaching of minerals with runoff water. Proper quantities of water were sprinkled in this study. Moreover, the increase variation in SMCD, CDS, and SSM were in accordance with the initial values in substrates, which was increased in SMCD, decreased in CDS, and increased in SSM from T1 to T15. This may due to the K and P levels in vermicompost were dependent upon the initial nutrient status in feeds.

Conclusion

The sludge from animal wastewater treatment plant amended with SM or CD was a good feed for *E. fetida*, which showed different adaptability to mixtures in their several growth stages. The hatching success of cocoons was improved in SSM mixtures especially in $S_{60}SM_{40}$. Besides, 0–20 % CD mixtures followed by SMCD and CDS mixtures were found to be the best food for growth of infant and young worms, respectively, while the SSM mixture was a better medium for cocoon production. Moreover, the weight of substrates was reduced greatly, the pH values were modified, and the variations of all physicochemical parameters were related to the growth of earthworms. All in all, the *E. fetida* can be fed separately in several growth stages with their favorite food mixtures to improve the production efficiency. Meanwhile, the earthworm vermieactor behaved as a highly effective process for converting animal manure and sludge from animal wastewater treatment plant to valuable organic fertilizer.

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