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Formulation of Organic Wastes as Growth Media for Cultivation of Earthworm Nutrient-Rich *Eisenia foetida*

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Abstract: Inadequate management of solid organic waste can lead to the spread of diseases and negatively affects the environment. Fermentation and vermicomposting of organic waste could have dual benefits by generating earthworm biomass for a source of animal feed protein, and, at the same time, turning the organic waste into readily used compost. This study investigated the effect of an organic waste source (as a sole source or blended with others) totaling 24 media for the cultivation of the earthworm *Eisenia foetida*. Eight media sources were applied, namely cow manure, horse manure, goat manure, broiler chicken manure, market organic waste, household organic waste, rice straw, and beef rumen content. *E. foetida* was cultivated for 40 days, then the number of cocoons, earthworms, and the total biomass weight were measured at the end of the cultivation. Results demonstrated that the media source affected *E. foetida* earthworm cultivation. The most effective media were those containing horse manure that led to the production of the highest earthworms and the highest biomass. The produced cocoons and earthworms were poorly correlated with an r-value of 0.26 and *p*-value of 0.21. Meanwhile, the number and weight of the earthworms correlated well with an r-value of 0.784 and *p*-value of <0.01. However, the average numbers and weights of the produced earthworms in the media containing horse manure, cow manure, goat manure, and non-blended organic waste were insignificant. Overall results suggest that blended organic wastes can undergo composting to produce nutrient-rich earthworm biomass while turning the solid organic waste into readily used compost.

Keywords: biomass; protein source; *Eisenia foetida*; vermicomposting; organic waste; cultivation media

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1. Introduction

Humans, livestock, and crops produce approximately 38 billion metric tons of organic waste worldwide annually [1]. Such a vast amount of solid waste can have significant impacts on the disposal and methane emission from the anaerobic fermentation process. The management and safe disposal of these wastes has become a global priority. Moreover, the open dumping of organic waste also facilitates the breeding of disease vectors and creates environmental pollution issues. Fortunately, adequately processed organic waste can be used for agriculture and industries. Composting is a simple, sustainable option and is most economical for handling organic waste. Although composting has been adopted as a primary tool for on-site waste decomposition, it has a few shortcomings, and long retention time requires frequent aeration, etc. [2]. Organic wastes are naturally transformed into plant nutrients by a variety of soil decomposers involving bacteria, fungi, and earthworms [3].

Vermicomposting is a process for the stabilization of organic material through the joint actions of earthworms and microorganisms [4]. In this process, microorganisms (bacteria and fungi) are responsible for the biodegradation of organic matter, while earthworms are drivers of the process. Earthworms act as mechanical blenders. The biological activities of the earthworms lead to modification of growth media in terms of biological, physical, and chemical condition, reducing its C:N ratio gradually, increasing the exposed surface area to microorganisms and eventually making it more favorable for microbial activities and further decomposition [5]. Earthworms maintain aerobic conditions, ingest organic solid, partially convert organics into earthworm biomass and metabolite products, and expel the remaining partially stabilized product. The vermicomposting yields product with higher nutrient availability than the traditional composting systems. The nutrients in vermicompost are also readily taken up by the plants [6]. Apart from producing high-quality compost, the earthworm biomass can also be used to supply nutrition in livestock and aquaculture industries.

Eisenia foetida is an earthworm that has a high advantage in reproduction and overhauling organic matter into nutrient-rich biomass [7]. Gunya et al. (2016) [8] reported that the dried *E. fetida* contains about 45.8% saturated, 22.2% monounsaturated, 31% polyunsaturated, 23.5% of n−6 and 8.3% of n−3 fatty acids. Furthermore, it has tolerable crude fibre levels (10.9%) suitable for fish digestion, and as such promotes high-protein assimilation efficiency when used as a component of fish feed [9]. In other reports, the nutrient content of earthworm *E. foetida* and the *Lumbricus rubellus* mixture are dry matter 12.9–25%, crude protein 58.2–71%, crude fat 2.3–10%, crude fiber 0.73–3.3%, carbohydrates 21%, ash 5.2–10%, calcium 0.33–0.8%, phosphorus 0.7–1.0% and total energy 17 MJ/kg [10–14]. Meanwhile, the essential amino acid content (in g/100 g protein) are phenylalanine 3.5–5.1, valine 4.4–5.2, methionine 1.5–3.6, isoleucine 4.2–5.3, threonine 4.8–6.0, histidine 2.2–3.8, arginine 6.1–7.3, lysine 6.6–7.5, leucine 6.2–8.2 and tryptophan 2.1. The content of non-essential amino acids (in g/100 g protein) are cysteine 1.8–3.8, tyrosine 2.2–4.6, aspartic acid 10.5–11.0, glutamic acid 13.2–15.4, serine 4.2–5.8, glycine 4.3–4.8, alanine 5.4–6.0 and proline 5.1 [8,15]. *E. foetida* biomass is considered among the promising non-conventional protein sources for animal and fish feed ingredients thanks to its high protein levels, proper amino acid profile, high reproduction rate, low mortalities, fast growth and ease of production [16].

Earthworm cultivation for biomass production is highly attractive given the high nutritional value of its flour as a source of animal protein and amino acids for animal and fish feed. High earthworm biomass is generally obtained from the production of a high number of cocoons (earthworm eggs). The earthworm life cycle is divided into four stages, namely cocoon production, incubation, hatching, and growth [17,18]. The cocoons produced by earthworms are influenced by population density, temperature, humidity, and the energy content available in the feed/media. Some earthworm species naturally produce cocoons throughout the year when the soil is moist, feed reserves are sufficient and other environmental factors are favorable. The earthworm *E. foetida* can produce 14 cocoons in 70 days or one every five days [19]. The number of earthworms that hatched ranged from 1–7, with an average of 3.9 per cocoon. *E. foetida* reaches sexual maturity at the age of four weeks; this is marked by the formation of a clitelum. At that age, earthworms can mate and produce cocoons at the age of 35 days, so the entire time required for one life cycle is 40–60 days. The life span of the earthworm *E. foetida* is estimated at 4.5 years [20].

One component of *E. foetida* earthworm cultivation technology that can increase cocoon and biomass production is the use of earthworm cultivation media that is suitable for earthworm life. A good earthworm media comes from organic waste. Organic waste is the material left over from the activities of human, animal, and plant life that are wasted and have no economic value. Materials used as earthworm cultivation media must be able to retain moisture, porosity and contain sufficient food substances, including protein, carbohydrates, minerals and vitamins, fat, and crude fiber [4,21,22].

The potential of *E. foetida* biomass as a nutrient-rich feed source has long been recognized. Therefore, many reports are available on the cultivation performances of *E. foetida* in various media (i.e., cattle and goat manure [23], various organic wastes [24], etc.). The cultivation performances seemed to differ slightly and were affected by the nutritional content of the media. However, most of those reports focus on the vermi composting aspect of organic waste management. Recently, vermicomposting of different types of waste using *E. foetida* has been reported. The use of those waste for cultivation of E. foetida increased the organic nitrogen, organic carbon and phosphorous content significantly [7]. More recently, vermi composting using *E. foetida* was reported for conversion of vegetable solid waste amended with wheat straw, cow dung, and biogas slurry [25]. It resulted in agronomic potentials of nutrient-rich vermicompost with acceptable C:N ratio ranges (≥1:20), demonstrating that *E. foetida* facilitated conversion of organic wastes into nutrient-rich biofertilizer if mixed with bulking materials in appropriate ratios. This study focused more on the exploration of organic waste directed to nutrient-rich biomass production yields. Eight main sources of the cultivation media (and 16 other combinations) were evaluated to assess their suitability as cultivation media for *E. foetida* biomass production.

This study investigated the effect of cultivation media on the produced number and the biomass weight of the *E. foetida* earthworm. Eight sources of media were applied, namely cow manure, horse manure, goat manure, broiler chicken manure, market organic waste, household organic waste, rice straw, and beef rumen content. They were also blended to form a total of 24 cultivation media. After media preparation, *E. foetida* was cultivated for 40 days. At the end of cultivation, the number of cocoons, earthworms, and the total weight were measured. The relationships between media source and composition with the production of cocoon, earthworm, and biomass were later analyzed and also linked with the nutritional composition of the media.

2. Materials and Methods

2.1. Preparation of the Cultivation Media

The compositions of all growth media evaluated in this study are summarized in Table 1. The base media for the cultivation were cow manure, horse manure, goat manure, broiler chicken manure, cow rumen contents, wet market organic waste, household organic waste, rice straw, and some combinations thereof. Few criteria were used as the basis for selecting the organic waste base media, namely: its local availability in the field (potential), not competing for its use for basic human/animal needs, and a good source of carbon (C) and nitrogen (N) for the earthworm growth building block and nutrition.

The preparation of the growth media was carried out in stages, as follows below. The market and the household organic waste were separated from glass, metal, and plastic materials, leaving only the organic fraction. Most of the organic fraction contained spoiled vegetables, fruit, and food waste. The organic waste was washed with clean water to remove the adhering dirt and to release odors. The organic material was then finely chopped or blended to sizes of 2–3 cm, then further grounded and screened with a mesh number of 18, resulting in a maximum particle size of 1 mm to ease the composting. Ten kg of each type of waste was then fermented. After collection from the farm, fresh rice straw waste was chopped or blended to sizes of 2–3 cm, then finely grounded. Later, 10 kg of the fine rice straw waste was composted. Ten kg of cow manure, horse manure, goat manure, broiler chicken manure, and the cow's rumen contents were then placed in a barrel for composting.

After composting of the organic waste media, 2.5 kg of each medium was separated for cultivation. Each homogeneous medium was used for worm cultivation without mixing with others. These were cow manure (CM), horse manure (HM), goat manure (GM), broiler chicken manure (BM), market organic waste (MW), household organic waste (HW), rice straw (RS), and beef rumen content (RC), totaling eight growth media. The blended media were formed by mixing two media with equal weight composition (wt.%). They were CM + MW (CW-MW), CM + HW (CM-HW), CM + RS (CM-RS), CM + RC (CM-RC), HM + MW (HM-MW), HM + HW (HM-HW), HM + RS (HM-RS), HM + RC (HM-RC), GM + MW (GM-MW), GM + HW (GM-HW), GM + RS (GM-RS), GM + RC (GM-RC),

BM + MW (BM-MW), BM + HW (BM-HW), BM + RS (BM-RS) and lastly BM + RC (BM-RC), totaling 16 media. Each growth medium was put into a nest box, dosed with 0.3 wt.% of lime (to maintain the pH close to normal value) followed by blending, after which it underwent aerobic composting for 21 days. During the composting, the media was stirred once a week. After media preparation, their nutrition contents were analyzed approximately according to a method developed by Henneberg and Stohmann [26] to determine the content of crude protein, fat, crude fiber, and ash as detailed elsewhere [27]. The analytical method was developed to provide a top level, very broad classification of food components [27].

Table 1. Details of the growth media evaluated in the present work.

No.	Code	Media	Source of Collection
1	CM	Cow manure	Local farmer
2	HM	Horse manure	Local farmer
3	GM	Goat manure	Local farmer
4	BM	Broiler chicken manure	Local farmer
5	MW	Market organic waste	Wet market
6	HW	Household organic waste	Household waste
7	RS	Rice straw	Local rice farm
8	RC	Beef rumen content	Local slaughterhouse
9	CM-MW	CM + MW	The media were from from equal wt.%
10	CM-HW	CM + HW	
11	CM-RS	CM + RS	
12	CM-RC	CM + RC	
13	HM-MW	HM + MW	
14	HM-HW	HM + HW	
15	HM-RS	HM + RS	
16	HM-RC	HM + RC	
17	GM-MW	GM + MW	
18	GM-HW	GM + HW	
19	GM-RS	GM + RS	
20	GM-RC	GM + RC	
21	BM-MW	BM + MW	
22	BM-HW	BM + HW	
23	BM-RS	BM + RS	
24	BM-RC	BM + RC	

2.2. Cultivation Process

The study was conducted by cultivating earthworm species of *E. foetida* collected from the Zoology Laboratory of IPB University. The cultivation was done during September–December 2019 by using cultivation media detailed in Table 1. The cultivations were carried out in a cage (nest box) made from thatched roofs and bamboo. All nest boxes were placed on a plastered floor and were assigned a pre-randomized code. They were covered tightly to avoid predators, to reduce water evaporation, and to maintain moist conditions. After completion of the media preparation each nest box was filled with 2.5 kg of growth media.

The earthworms were introduced into each nest box with a stocking density of 10 g per kg of growth media. The earthworm broodstocks were placed into the middle of the piled media through a top hole and were cultivated for 40 days. The medium was mixed on days 8, 15, 22, 29, and 36 of the cultivation. The harvesting of earthworms was carried out on day 40, whereby the data on cocoon and biomass (number and weight) of the earthworms was collected.

During the research, measurements of pH, humidity, and temperature of the media were carried out every two days at 1:00 p.m. during the media composting and over the entire duration of the earthworm cultivation. In addition, the daily temperature of the environment inside the nest box was measured three times a day: at 6:00 a.m., 1:00 p.m., and 8:00 p.m. If the temperature of the media or the environment in the cage increased, it was sprayed with water. The temperature was maintained in the range of 18–27 °C.

2.3. Experimental Design and Statistical Analysis

The formulation of the media was done based on a completely randomized design, resulting in 24 combinations (as listed in Table 1). For each media, the cultivation was done in triplicate, resulting in a total of 72 nests. The data on mortality rate, cocoon production, earthworm production, and mass of the produced earthworms were analyzed statistically. The significance of each parameter was evaluated using the one-way analysis of variance (ANOVA) at 95% confidence intervals ($p < 0.05$). Then, the Tukey HSD post hoc test was performed to identify which pairs of mean were significantly different. The Pearson coefficient of correlation was applied to identify the relation between the cocoon and earthworm number, as well as the total weight of the produced biomass.

3. Results and Discussion

3.1. Mortality Rate

Figure 1 shows the mortality rate of the inoculated *E. foetida* earthworm. Four media were found not suitable because no earthworms survived at the end of the cultivation. These media were MW, BM, BM-MW and BM-HW. They were excluded from the results presented in subsequent figures. Figure 1 also shows that media containing the broiler chicken manure led to a significantly high earthworm mortality of 81.9 ± 36.3%. No earthworm survived in BM, BM-MW, and BM-HW media; the mortality rates in BM-RS and BM-RC media were 27.4 ± 2.5% and 68.1 ± 5.0%, respectively. Media containing the horse manure showed an average mortality rate of 16.6 ± 3.9%, followed by the ones constituted of the cow manure and the goat manures with average mortality rates of 3.2 ± 3.4% and 1.3 ± 2.6%. Interestingly, despite showing 100% mortality when used as the sole component of the growth media, the market organic waste can still be used in combination with other media to lower the mortality rate, suggesting that blending of organic waste from different sources can be optimized to support the growth of the earthworm *E. foetida*.

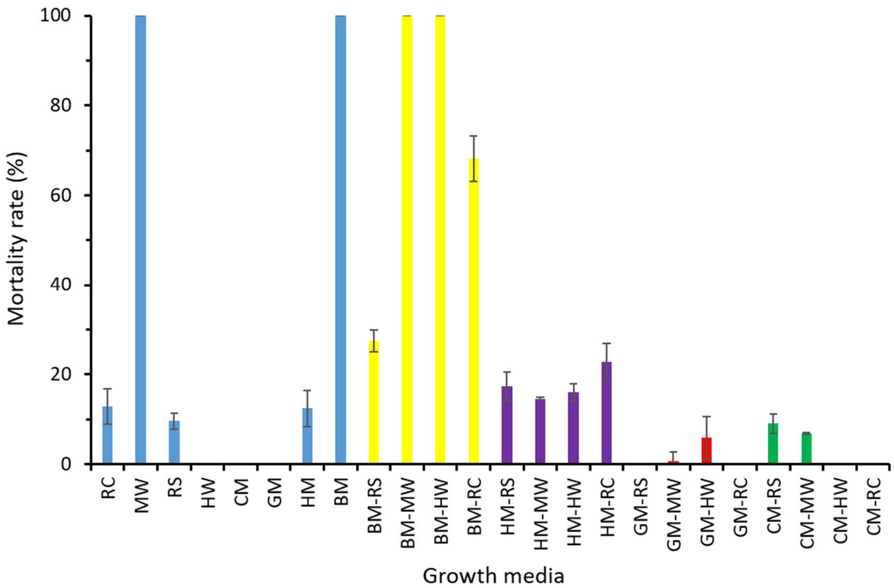

Figure 1. Earthworm mortality rate under different cultivation media.

The summary of the compositions of all media tested in this study is detailed in Tables 2 and 3. The four media with high mortality rates had relatively high phosphorous content (of 0.91–1.13%) which were much higher than the rest, with an average of 0.49%.

Exposure to a high concentrations of phosphate (i.e., iron phosphate) increased earthworm mortality, and surviving individual worms gained less mass [28].

Table 2. Composition of the cultivation media.

Cultivation Media	Water (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)
RC	16.96	14.99	17.6	0.04	33.76
MW	21.41	17.16	18.66	2.69	33.86
RS	17.61	26.88	8.33	0.07	20.83
HW	28.38	16.71	21.12	2.73	23.72
CM	15.37	51.97	10.62	0.54	16.21
GM	19.69	21.15	17.84	0.92	32.9
HM	17.74	31.54	13.2	0.14	25.73
BM	23.87	24.35	24.93	1.25	16.53
BM-RS	18.76	33	15.49	0.15	17.27
BM-MW	21.82	26.3	22.29	2.42	9.47
BM-HW	25.94	23.15	24.29	1.56	21.17
BM-RC	18.49	21.33	14.79	0.52	25.95
HM-RS	16.07	30.28	9.39	0.62	26.24
HM-MW	15.73	30.21	9.86	0.34	27.44
HM-HW	16.89	29.06	9.27	0.55	27.4
HM-RC	15.62	26.24	10.44	0.13	31.73
GM-RS	17.16	24.34	15.9	0.46	31.58
GM-MW	18.78	19.37	19.71	0.89	35.24
GM-HW	20.51	18.45	18.71	2.05	35.65
GM-RC	18.66	20.44	17.89	0.63	36.99
CM-RS	14.85	38.1	10.86	0.35	15.9
CW-MW	15.52	41.74	11.73	0.05	21.54
CM-HW	18.67	40.3	13.73	0.17	16.2
CM-RC	16.08	33.74	11.5	0.18	18.44

Table 3. Macronutrient composition of the cultivation media.

Cultivation Media	N (%)	P (%)	K (%)	C (%)	C/N (-)	Organic (%)
RC	2.82	0.78	0.7	42.23	14.98	70.63
MW	2.99	0.4	1.66	46.02	15.39	79.16
RS	1.33	0.09	0.95	40.62	30.54	69.87
HW	3.38	0.48	1.97	46.27	13.69	79.59
CM	1.7	0.49	1.11	26.68	15.69	45.89
GM	2.85	0.41	1.39	43.81	15.37	75.35
HM	2.11	0.74	1.03	38.11	18.06	65.55
BM	3.99	1.13	1.5	42.03	10.53	72.29
BM-RS	2.48	0.91	1.46	37.22	15.01	64.02
BM-MW	3.57	0.99	1.6	40.94	11.48	70.42
BM-HW	3.89	1.01	1.54	42.69	10.97	73.43
BM-RC	2.37	1.09	1.2	43.71	18.44	75.17
HM-RS	1.5	0.43	0.86	38.76	25.84	66.67
HM-MW	1.58	0.68	1.2	38.77	24.54	66.69
HM-HW	1.48	0.74	1.2	39.41	26.63	67.79
HM-RC	1.67	0.79	0.93	40.93	24.54	70.48
GM-RS	2.54	0.26	1.26	42.03	16.55	72.3
GM-MW	3.15	0.37	1.62	44.79	14.22	77.05
GM-HW	2.99	0.37	1.68	45.31	15.15	77.93
GM-RC	2.86	0.52	1.32	44.2	15.45	76.02
CM-RS	1.74	0.3	1.1	34.59	19.88	59.15
CM-MW	1.88	0.33	1.26	32.37	17.22	55.67
CM-HW	2.2	0.53	1.32	33.13	15.06	57.05
CM-RC	1.84	0.67	1	36.81	20.21	63.31

3.2. Cocoon Production

Figure 2 shows the number of produced cocoons after 40 days of cultivation. The top three highest cocoon productions were achieved by media from blended sources. There were CM-HW, GM-RC, and SW-MW, with the average number of cocoon production of 318.3 ± 0.6, 296.0 ± 1.0, and 217.7 ± 0.6, respectively. The respective average cocoon production for cultivation in media containing the goat manure, the cow manure, the horse manure, the non-blended and the broiler chicken manure were 180.8 ± 68.4, 160.1 ± 103.7, 90.3 ± 17.0, 83.7 ± 46.1, and 7.0 ± 4.2, which was in the range of earlier report [29]. Media containing broiler chicken manure were less attractive for cultivating *E. foetida* judging by both the mortality and the cocoon production rates. The low numbers of produced cocoons can be justified by the low number of surviving earthworm broodstocks cultivated in the chicken manure containing media (see Figure 1).

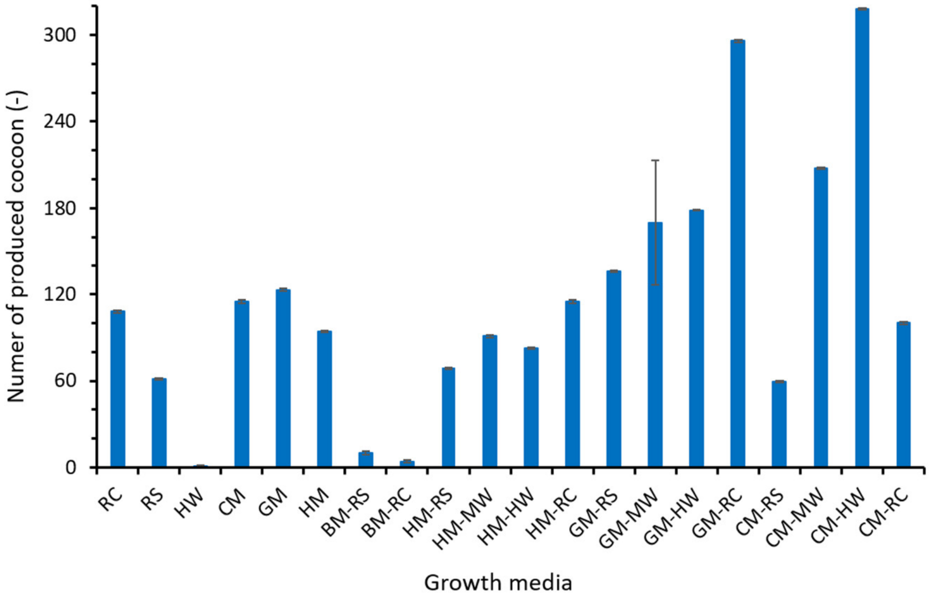

Figure 2. The rate of cocoon production under different cultivation media.

Results in Figure 2 suggest that the media source for *E. foetida* cultivation had a significant effect on the number of the produced cocoons, as also reported recently [29]. The cow and goat manures showed a higher amount of cocoon production because *E. foetida* has a natural habitat similar to the one suitable for decomposition of organic matter by soil microorganisms [21,30,31]. The presence of these microorganisms can increase the ability of earthworms to digest organic matters in their digestive tract [20].

The cocoons produced varied in terms of shape, size, weight, and color. The cocoons of *E. foetida* were generally oval to round, with an average length of 4.103 mm and a width of 2.6 mm. The weight of the cocoons also varied depending on size, with an average weight of 1.3 mg/unit, which is significantly lower than the one reported earlier (12–23 mg/unit) [32]. The color of the cocoons varied from beige (light yellow) to dark yellow or light brown, and some were even dark brown. The color of the cocoons generally depends on the age of cocoons. Newly produced cocoons were cream or light yellow and even very clear (close to white). However, with increasing age the cocoons, the color turns yellow or brown, and even before hatching the color of the cocoons approaches dark brown.

In terms of nutrition, based on the results of the proximate analysis in Tables 2 and 3, the crude protein content of the CM-HW medium was 13.73%, fat 0.17%, crude fiber 16.20%, N 2.20%, P 0.53%, K 1.32%, C 33.13%, C/N ratio of 15.06 and organic matter of 57.05%. The GM-RC contained nutrients of 17.89%, crude protein, 0.63% fat, 36.99% crude fiber, 2.86% N, 0.52% P, 1.32% K, 44.20% C, C/N ratio of 15.45 and organic matter of 76.02%. Meanwhile, the nutrient content of SW-MW was 11.73% of crude protein, 0.05% of fat, 21.54% of crude fiber, 1.88% of N, 0.33% of P, 1.26% of K, 32.37% of organic C; C/N ratio of 17.22% and organic matter of 55.67%. When compared with HW, which produced the lowest cocoons, it contained 21.12% of crude protein, 2.73% of fat, 23.72% of crude fiber, 3.38% of N, 0.48% of P, 1.97% of K, 46.27% organic C, C/N ratio of 13.69 and organic matter of 79.59%.

Analysis of the nutritional data for the mixture of *E. foetida* earthworm cultivation media listed in Tables 2 and 3 showed that to produce the most cocoons, *E. foetida* required crude protein, fat, crude fiber, N, P, K, organic C, C/N ratio and organic matter of 11.73–17.89%, 0.05–0.63%, 16.20–36.99%, 1.88–2.20%, 0.33–0.52%, 1.26–1.32, 32.37–44.20%, 15.06–17.22 and 55.67–76.02%, respectively. The application of a mixture of media containing too much protein (>18%) would not increase cocoon production. This is in accordance with an earlier report [14] that found that the best feed for earthworms contained 9–15% protein under a neutral pH. Furthermore, either excess protein interfered with the digestive system of the *E. foetida* earthworm, or protein poisoning occurred in the form of swelling of the cache, thus affecting the health of the earthworms and ultimately affecting their productivity, and even causing death [33,34]. The cultivation media of MW contained 21.12% protein, which made it unsuitable for *E. foetida* earthworm cultivation, as shown in Figures 1 and 2.

The rate of cocoon production is also affected by the nutrient content of the cultivation media. Among these nutrients, phosphorus (P) is positively correlated with the production of cocoons (eggs). This is in line with earlier reports [11,35] that the minerals that play a major role in the process of egg formation are calcium and phosphorus. Phosphorus is important in energy metabolism, carbohydrates, amino acids and fats, fatty acid transport, and coenzyme parts. Therefore, in the selection of organic waste as a medium or feed for earthworms, it is necessary to pay attention to the content of these two minerals, especially when aiming to produce a high number of cocoons. However, phosphorous in the form of iron phosphate increased earthworm mortality, and surviving individuals gained less mass [28]. As shown in Section 3.1, phosphorous content of less than 1% is recommended to avoid the poor survival rate of the earthworm broodstock.

In addition to media nutrients content, cocoon production during cultivation of *E. foetida* was also influenced by environmental factors such as temperature, pH, and humidity [3,30,36]. For the CM-HW that resulted in the highest cocoon production, the average temperature of the media was 28.92 °C, pH 6.46, and with a relative humidity of 56.55%. In addition, earthworm communities are generally very sensitive to physicochemical properties of the media, which directly or indirectly influence the earthworm's survival. The difference in physicochemical properties of media at different sites contributed to the formation of population patches for the earthworm species [37].

3.3. Cocoon Hatching

Data in Figure 3 show that the use of various types of organic waste as media material for the cultivation of *E. foetida* had a significant effect on biomass production, both in number and weight. When viewed as the media content, the average earthworm production from the highest to lowest were 1168.7 ± 383.3, 620.9 ± 489.4, 578.7 ± 328.6, 508.0 ± 291.1, and 47.5 ± 13.9 for the horse manure, the non-blended, the goat manure, the cow manure, and the broiler chicken manure, respectively. Analysis using the Tukey HSD test confirmed that the numbers of produced earthworms for the first four media were insignificant, with a Q-critical of 4.11 higher than the Q-statistic range of 0.55–3.65 for all possible pairs.

Figure 3 shows the ratio of the produced cocoon to the produced earthworm under different cultivation media. It shows a very large variability with an average value of 11.0 ± 18.7 earthworm/cocoon. The large variability was due to the extremely high earthworm/cocoon ratio for the HW medium. The produced cocoons were 0.7 ± 0.6, and the produced earthworms were 58.0 ± 1.0. When it was excluded, the average value of the earthworm/cocoon became 7.0 ± 5.7. It is much higher than the one reported elsewhere [19], in which the average number of earthworms/cocoons was 3.9.

Results of the Pearson coefficient of correlation suggest a positive but poor correlation between the number of the cocoons and the number of the earthworms, with an r-value of 0.26 and *p*-value of 0.21. Apart from showing a clear difference in the number of produced earthworms, the size and weight of the produced earthworms are also more important in determining the yield of biomass production during cultivation. The results of each type of organic waste as a medium for 24 types of organic waste indicate that the earthworm *E. foetida* has different abilities in producing biomass depending on the type of organic waste used as cultivation media. The findings are in line with the variability shown for the mortality rate and the cocoon production.

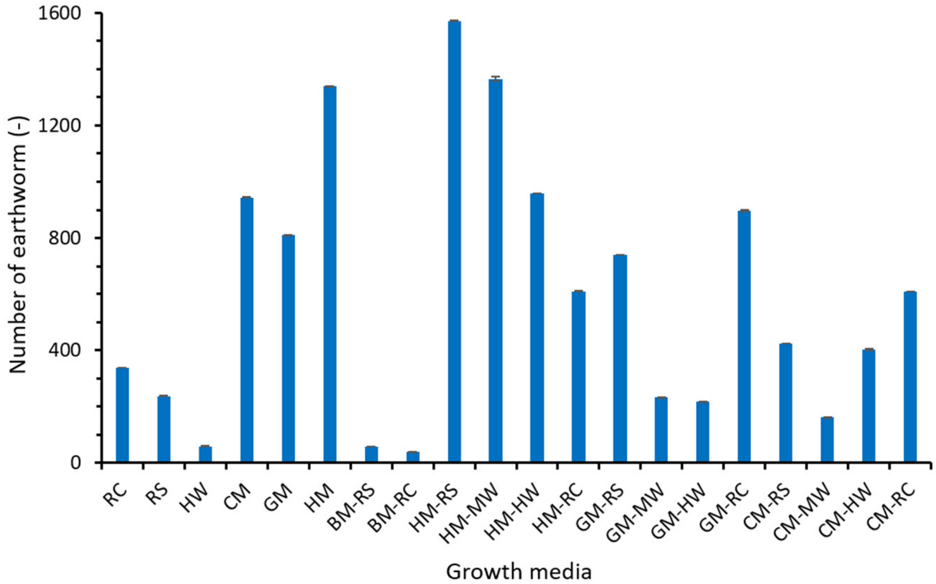

Figure 3. The transformation of the cocoon into an earthworm under different cultivation media.

3.4. Biomass Production

Figure 4 shows that the total mass of biomass produced is affected by both the number and the weight of the earthworms. Analysis using the Pearson coefficient of correlation between the number of earthworms and the total weight resulted in an r-value of 0.78 and p-value of <0.01. Indeed, more earthworms produced would lead to higher total biomass. The trend on the total weight of produced biomass is also generally in line with the number of the produced earthworms, as shown from the highest to lowest group of 1062 ± 327, 368 ± 256, 335 ± 197, and 111 ± 110 earthworms. In general, a higher number of earthworms lead to a higher yield of biomass. However, a few exceptions can be observed. For example, CM-RC, HM-RS, and HM-HW showed the three highest total biomass weights, with an insignificant difference based on the Tukay HSD test. However, there was substantial difference in the number of earthworms of 896, 1571, and 958 with an average biomass weight of 60, 34 and 54 mg/earthworm, respectively. HW media had among the lowest number of earthworms at 58.0, but still produced a relatively high biomass amount of 39.7 ± 6.4 g, corresponding to the specific weight of earthworms of 684 ± 109 mg/earthworm.

Figure 4 shows the total weight and the number of produced biomass. Based on the total weight of the earthworms produced, they can be classified into four groups that had significant differences from the Tukey HSD analysis. The first group constituted of eight media, namely GM-RC, HM-RS, HM-HW, CM, HM, CM-RC, HM-MW, and GM with an average total biomass weight of 50.8 ± 2.3 g/nest box. The second group consisted of five media of GM-RS, CM-HW, CM-RS, HW, and GM-HW with an average total biomass weight of 41.8 ± 2.3 g/nest box. The third group constituted four media of GM-MW, RC, HM-RC, and SW-MW with an average total biomass weight of 36.3 ± 0.5 g/nest box. Lastly, the media with the significantly lowest average total biomass weight of 16.6 ± 2.6 g/nest box constituted RS, BM-RS, and BM-RC.

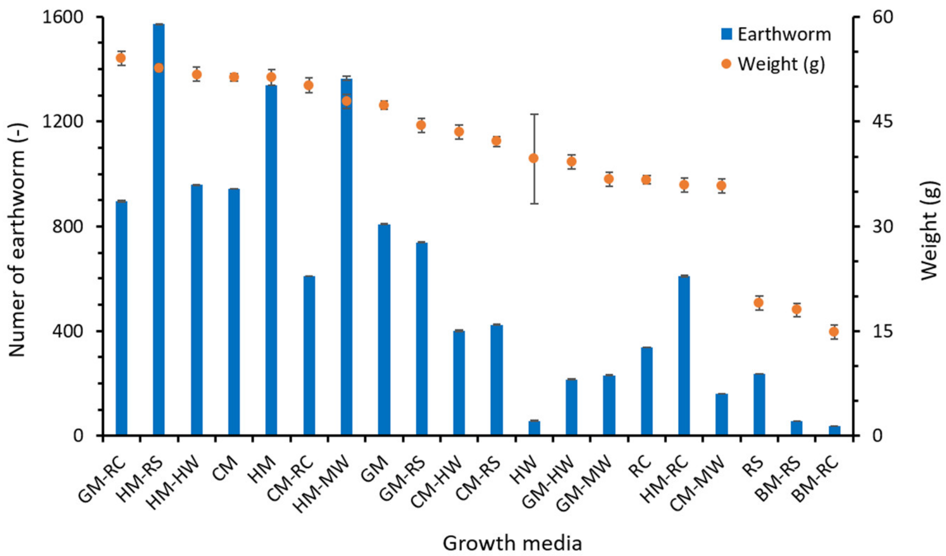

Figure 4. Number of the grown earthworm and their total weight for cultivation in different cultivation media.

In terms of the content of the media, the highest biomass production was obtained for cultivation in media containing horse manure, goat manure, cow manure, non-blended, and, lastly, chicken manure, with an average total biomass weight of 47 ± 8, 44 ± 8, 43 ± 6, 41 ± 12 and 16 ± 2 g/nest. Statistical analysis on the effect of the media source revealed that the biomass production rates of all media were insignificant except for the cultivation in media containing broiler chicken manure. The media contained horse manure, cow manure, goat manure, and non-blended sources yielded about similar total biomass, as ascribed from the Tukey HSD test. The Q-statistic of 4.11 was much higher than Q-critical ranged at 0.15–1.46 for all possible pairs. The similarities in the efficacy of the media can be attributed to their similarities in nutrient contents listed in Tables 2 and 3.

The yields of the biomass production were positive (greater than the weight of the inoculum) for all media in groups 1, 2, and 3. The biomass yields for the media in group 4 were negative, suggesting that the total mass of the harvested biomass was less than the one inoculated. By looking into the yield it seems that only group 1, which consisted of eight media, could be considered attractive for *E. foetida.* This resulted in a biomass yield of 103% higher than the one inoculated.

The highest amount of biomass production in group 1 was also influenced by environmental factors (pH, temperature, and humidity) and adequate media nutrition or feed so that earthworms could produce optimally. The average pH of the eight media was 6.44; the average media temperature was 27.65 °C and the media humidity was 57.5%. This is in line with an earlier report [38] that the earthworm *E. foetida* can reproduce at a temperature of 32 °C with an optimum temperature of 28 °C. The preferred humidity of the media needed for earthworms ranged from 50 to 80% [39].

The nutrient content of the eight media with the highest biomass-producing media was protein, with a content of 9.27–17.83%, a fat content of 0.14–0.63%, and crude fiber of 16.21–36.99%. Based on the results of this study, it appears that the environmental conditions (pH, temperature, and humidity) of the media are the best conditions for optimum production. Likewise, the nutrient content contained in the media or feed also meets the needs both in quantity and quality, so that earthworms can produce the most biomass. This is in line with the earlier results [14] that showed that the best feed for earthworms is one containing 9–15% protein with a neutral pH.

Among the four sources of media that resulted in the highest weight of the produced earthworms, the media containing horse manure showed the highest number and weight of the produced earthworms. These particular media had a characteristic of the highest C/N ratio of 24–54–26.63, which is significantly higher than the media containing goat and cow manure with C/N ratios of 14.22–16.55 and 15.06–20.21, respectively. *E. foetida* has advantages when compared to other species, especially its high ability to reproduce and convert organic matter as food. Earthworms can break down organic matter up to twice their body weight per day [40]. With the high ability of earthworms to break down organic matter and reduce pungent odours, earthworms can also be used as an alternative to prevent environmental pollution, especially that caused by livestock waste, market, and household wastes [41].

The overall results show that different sources of organic waste can be used as an effective medium to cultivate the earthworm *E. foetida*. The highest biomass production group consisted of unblended media (CM, HM, and GM) and blended media (CM-RC, HM-MW, GM-RC, HM-RS, HM-HW). The management of the unblended media can be done proximate to the farm in order to minimize the transportation costs in a composting and cultivation zone. The produced earthworm biomass can be used directly or sold for protein source in animal feed. For the blended media, additional transportation and man-power is required to blend the media according to the desired composition before they can be fermented and used for earthworm cultivation. The additional income from the earthworm biomass sales can enhance the economic sustainability of cow, horse and goat farming.

Data from Figure 4 also show that apart from yielding lower biomass, several media (CTRT50, CM-RS, HW, GM-HW, GM-MW and RC) show a higher specific weight (g/unit earthworm). It means that cultivation in those media lead to production of less but heavier earthworms. To maximize the biomass production, future research can be focused on a two stage cultivation consisting of breeding followed by growth. Apart from that, nutritional analysis of the produced earthworms are required in order to project their potential application. It would also be interesting to explore whether delicate control of cultivation conditions (i.e., temperature, moisture content, mixing, etc.) would lead to an increase in biomass yield and alter the process to be economically attractive. Finally, technoeconomic analysis on the production of compost from domestic waste and/or an animal farm is required. It can be treated as a by product of the composting site or of an animal farm that enhances economic competitiveness, or it can also be treated as a by product of vermicompost, as reported earlier [42].

4. Conclusions

This study demonstrated that most organic waste can be used as a medium for *E. foetida* cultivation. Some organic wastes tested in this study, namely MW, BM, BM-MW, and BM-HW lead to full mortality of the broodstocs. The other media resulted in variable cocoon production, earthworm and earthworm biomass. The findings suggest that common organic waste contains sufficient nutrients for *E. foetida* earthworm growth. However, none of the tested media yielded distinctively higher final biomass production. Yet, cultivation in certain media lead to substantially high numbers of cocoons and earthworms. This finding opens the possibility to engineer the media (i.e., cultivation in stages in different media) to maximize the final biomass yield. Moreover, technoeconomical analysis needs to be conducted to assess the feasibility of biomass production from common organic compost and the potential of co-production of the biomass from animal farms.

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