

## Research Article

# Organic Manure as Rearing Substrates for Red Worms (*Eisenia fetida*): Effects on Chemical Composition and Growth Performance

Zephania Maziku\* , Renalda Nanziga Munubi , Augustine Warioba Mwandya 

Department of Animal, Aquaculture and Range Sciences, Collage of Agriculture, Sokoine University of Agriculture, Morogoro, Tanzania

## Abstract

Red worms (*Eisenia fetida*) can convert biowaste and by-products into body mass and become high in protein and lipid content. However, the type of growth media used affects both larval body composition and growth performance. Using recycled organic materials from chicken manure (CM), cow dung manure (CDM) and rabbit manure (RM), the present study evaluated the production of red worms that could be used as a substitute protein source for fish meals. Two experiments were conducted, the first experiment tested the compatibility of each organic manure when mixed with soil separately, whereas the second experiment combined the three organic manures with a fixed amount of soil. The study was conducted for 60 days. The findings showed that red worms reared on 100 % CM had a significantly higher body weight ( $19.27 \pm 0.9$  g) followed by those reared on 100 % CDM and 75% RM, whereas red worms reared on 100 % RM had the lowest body weight ( $4.9 \pm 0.1$  g). A combination of 20% CM + 40% CDM + 20% RM + 20% Soil supported significantly higher body weight of red worms ( $24.9 \pm 1.1$  g), while the lowest value of body weight ( $5.1 \pm 0.2$  g) was in a combination of 20% CM + 0% CDM + 60% RM + 20% Soil). Furthermore, the results revealed that red worms reared on 100 % CDM without soil as waste substrate had the highest crude protein (73.28% DM) compared to red worms reared on other types of substrates. The study suggests the potential of reusing organic manure such as chicken and cow dung manure at different inclusion levels in the production of red worms.

## Keywords

Organic Manure, Red Worm, Growth, Proximate Composition, *Eisenia fetida*

## 1. Introduction

In many of the developing and developed regions of the world human population is still increasing which creates a relatively high demand for fish consumption (a protein source). Relatively low capture fisheries production over the past decades resulted in the intensification of aquaculture production [1], which mainly depends on quality feed (high

protein content) to ensure growth. However, these increased demands put a larger strain on limited resources such as water and energy, and have a greater negative influence on the environment [2, 3]. A study by Khoshnevisan et al. [4] demonstrated that improper management of animal manure is one of the reasons for the high quantity of untreated animal waste

\*Corresponding author: zephaniamaziku91@gmail.com (Zephania Maziku)

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from industrial farms. One way of utilizing animal manure/waste and other bio-waste, whilst obtaining high-quality nutrients to meet over-increasing demand, is through bio-conversion using invertebrates such as earthworms to convert waste into red worm biomass and vermicompost.

The red worms (*Eisenia fetida*) are one of the oligochaetes worm species that have been identified as being particularly promising food sources due to their brief lifespan, capacity to survive in a wide range of low oxygen levels and ability to ingest a wide range of substrates throughout its life cycle [5]. Earthworms can be utilized either as a direct feed or as a feed ingredient in animal feeds [6-8]. Red worms have been successfully included as feed ingredients because of their nutritional characteristics such as high crude protein that ranges from 64.4–72.9% [6], amino acid profile contains 20 out of 24 major amino acids as well as comprises suitable levels of beneficial fatty acids and fat content that range from 5–20% of dry matter [9].

To substitute fish meal, the red worm has been proposed as an alternative source of protein. However, the production of earthworms varies depending on different factors such as substrate/rearing media [10, 11], culture period [12], and physical-chemical parameters such as pH, temperature and humidity [13-15]. Among the factors, research reveals that the composition and substrate type are some of the most important aspects of red worm rearing and can significantly affect the nutritional makeup of the organism [2]. The utilization of various biowaste and byproducts such as crop wastes, household leftovers, and animal manure for raising red worms has shown that substrate with a high level of cellulose materials was the best for earthworm culture and produced the highest total biomass compared to soil substrate alone [16-19]. A study by Mahboub et al [20] showed that cow manure plus sugarcane bagasse had the highest yield than cow manure alone and cow manure plus sawdust waste.

In Tanzania, the availability of earthworms in their natural habitat varies by season and location, and their tissue is highly predisposed to the bioaccumulation of heavy metals and toxic organic residues [21]. These toxins can bioaccumulate in fish and cattle through feed. Since earthworms are used as live fish food, bait, and fish meal supplements; [22, 23]. Therefore, it is necessary to improve their culture to prevent the bioaccu-

mulation of these pollutants and guarantee the worms' availability throughout the year. Hence, the present study aimed to determine whether and how much red worms' nutritional makeup (DM, ash, protein and fat) might be altered by organic wastes/manure used as sources of substrates.

## 2. Materials and Methods

### 2.1. Experimental Setup

The study was carried out at the Training Model Farm Unit, SUA, Morogoro Tanzania. Adult red worms (*Eisenia fetida*) were bought from a reliable dependable farmer in Arusha and kept at constant condition in their original culture substrate during transportation and stayed in that media for one week. Red worms were then handpicked from the substrate and washed by using continuously flowing water ready for being stocked. For each treatment, 5 g with approximately 8-12 red worms were inoculated in each culture substrate (4 kg) and the experiment was conducted for 60 days. Three different organic manures namely, Chicken manure (CM), Cow-dung manure (CDM) and Rabbit manure (RM) were used as substrates for red worms (*Eisenia fetida*) culture. Chicken manure used as substrate contained the mixture of chicken droppings with rice hulls that are used as litter. All organic manure used was dried and collected from SUA training model farm.

### 2.2. Experimental Design and Data Collection

The productivity of red worms on different substrates was achieved by carrying out two experiments as follows: The first experiment tested the suitability of each organic manure (i.e., CM, CDM and RM) mixed with soil separately (Table 1), whereas the second experiment was a combination of the three-organic manure in different proportion with a fixed amount of soil (Table 2). Each experiment was conducted for 60 days and each organic material was studied in triplicates. The experimental containers' bottoms, measuring 13 cm in height by 41 cm in diameter were modified to include tiny drainage holes at the bottom before being lined with dry grasses [24].

**Table 1.** Percentages (%) of organic substrate material and soil used for experiment 1.

| Treatments                       | Percentages (%) |             |             |             |
|----------------------------------|-----------------|-------------|-------------|-------------|
|                                  | Level 1         | Level 2     | Level 3     | Level 4     |
| Chicken Manure (CM) + Soil (S)   | 100.0 + 0.0     | 75.0 + 25.0 | 50.0 + 50.0 | 25.0 + 75.0 |
| Cow Dung Manure (CDM) + Soil (S) | 100.0 + 0.0     | 75.0 + 25.0 | 50.0 + 50.0 | 25.0 + 75.0 |
| Rabbit manure (RM) + Soil (S)    | 100.0 + 0.0     | 75.0 + 25.0 | 50.0 + 50.0 | 25.0 + 75.0 |
| Soil (S)                         | 100             | 100         | 100         | 100         |

**Table 2.** Combination of organic materials and soil in Percentages (%) used for experiment 2.

| Substrates            | Percentages (%) |    |    |    |    |    |
|-----------------------|-----------------|----|----|----|----|----|
|                       | T1              | T2 | T3 | T4 | T5 | T6 |
| Chicken Manure (CM)   | 20              | 20 | 40 | 60 | 20 | -  |
| Cow-Dung Manure (CDM) | 20              | 40 | 20 | 20 | -  | 60 |
| Rabbit manure (RM)    | 40              | 20 | 20 | -  | 60 | 20 |
| Soil                  | 20              | 20 | 20 | 20 | 20 | 20 |

Four (4) kg of the dry substrate was measured using a weighing balance and then added to each experimental container and thoroughly mixed with water, then experimental containers were covered with enough dry grasses at the top to block direct sunlight and maintain moisture. The plastic containers used to culture red worms had a gutter at the bottom to collect the vermiliquid /vermiwash. The mixture was kept in this form for three weeks to allow for decomposition before inoculation of the red worms into the respective substrates. To keep the desired range of moisture content of the culture substrates during the experimental period, wetting was done twice per week using one litre of water from the fish pond per each experimental container [25] and there was no addition of chicken manure, cow dung manure or rabbit manure during the production cycle.

**Figure 1.** Experimental Setup for Red Worm Culture.

The weight of red worms from each treatment was then recorded at 15, 30, 45 and 60 days to determine the biomass. For this purpose, each experimental container was divided into four quadrants then one quadrant was randomly selected and the number of cocoons, hatchling juvenile and adult red worms were handpicked and then washed with water to clean the red worm's biomass. After data collection, the sampled area was then mixed with the same substrate and the red

worms were released back into the respective treatment after recording the weight gain and subsequently, the average weight in each treatment was calculated.

### 2.2.1. Determination of Growth and Reproductive Performance

Growth performance was expressed in terms of mean weight gain and specific growth rates using the following formula

$$\text{Specific Growth Rate (SGR)} = \frac{\log w_f - \log w_i}{t} * 100$$

Where;  $\log w_f$  and  $\log w_i$  are the logarithm of final weight and initial weight respectively and  $t$ , the experimental period in days.

### 2.2.2. Determination of the Chemical Composition of Red Worms

Subsamples of red worms were harvested, separated from the growth media and then washed. Clean samples were wiped with tissue paper and their wet weight was determined by using a digital weighing balance. The chemical composition of red worms was analyzed in the laboratory of the Department of Animal, Aquaculture and Range Sciences (DAARS), SUA according to the procedures of AOAC [26].

### 2.3. Data Analysis

Data are presented as mean  $\pm$  standard error (SE), unless otherwise mentioned, considering each column an experimental unit. All data were tested for normality of residuals and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. In instances where data were not normally distributed, log transformation and square root transformation were performed. The growth performance of the worms when analyzed separately (each substrate) differed significantly among sampling days with the highest mean values on day 60 compared to day 15, 30 and 45 thus analyses were based on data collected on day 60. Data on proximate composition were subject to a one-way ANOVA to compare

means among treatment groups. When significant differences were obtained ( $p < 0.05$ ), a Tukey honest significant difference post hoc test was performed. The correlation was computed between different developmental stages of red worms. Data on red worm's growth over time were analyzed using a two-way Analysis of Variance (ANOVA). All statistical tests were performed using the R statistical program (version 4.3.1).

### 3. Results

#### 3.1. Growth Performance of Red Worms

The effect of growth media (substrate) on the biomass of red worms over time is depicted in Figure 2 and Figure 3 for Experiment One and Experiment Two respectively. Experiment one aims to assess the suitability of each organic manure mixed with different levels of soil on the growth performance of red

worms. In the first experiment, the overall results revealed that after 60 days, the highest biomass of red worms was ( $19.27 \pm 0.9$  g) on 100% CM followed by ( $14.27 \pm 0.9$ g) on 100 % CDM, and the biomass of red worms was lowest ( $4.9 \pm 0.1$  g) on 100% RM (Figure 2). There were significant differences in the mean values of the biomass of red worms among treatments ( $P < 0.05$ ). The differences were recorded between the control treatment containing soil only (TC) and 100% of CM, CDM, 75% RM and CM. The biomass of red worms also differed significantly between 100% CM with all treatments of CDM and RM, while 75% of CM showed a significant difference with 100% CDM, 100% RM and 75% RM. 50% and 25% of CM differed significantly with 100%, and 75% of CDM and RM respectively. Additionally, the biomass of red worms also differed significantly between 100% RM and 100% CDM, while 75% RM showed a significant difference with 75% RM. It was also found that 50% and 25% of RM differed significantly from 100% of CDM (Table 3).

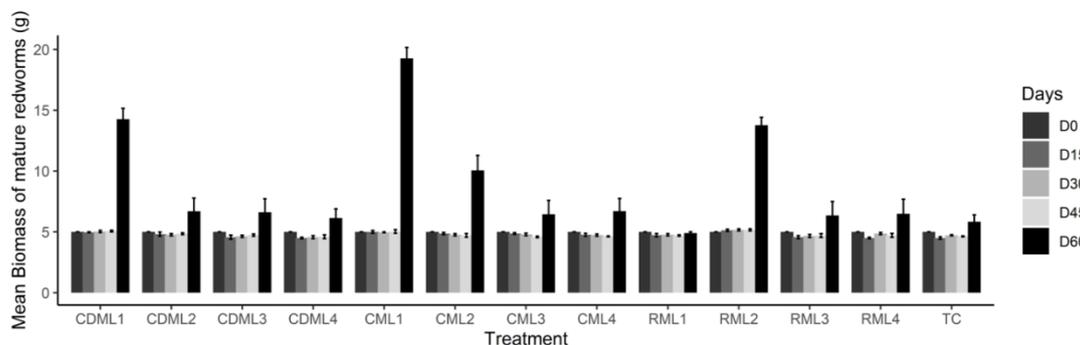


Figure 2. Mean biomass ( $\pm SE$ ) of red worms over time reared in different inclusion levels of Chicken manure (CM), Cow dung manure (CDM), Rabbit manure (RM) and Soil only as a control treatment (TC).

Table 3. Comparison of different treatments in Experiment One.

| Treatments | %   | TC  |     |    |    |    | CDM |    |    |    |     | CM |    |    |  |
|------------|-----|-----|-----|----|----|----|-----|----|----|----|-----|----|----|----|--|
|            |     | 100 | 100 | 75 | 50 | 25 | 100 | 75 | 50 | 25 | 100 | 75 | 50 | 25 |  |
| TC         | 100 | ns  | **  | ns | ns | ns | **  | *  | ns | ns | ns  | ** | ns | ns |  |
|            | 100 | **  | ns  | ** | ** | ** | *   | *  | ** | ** | **  | ns | ** | ** |  |
| CDM        | 75  | ns  | **  | ns | ns | ns | **  | ns | ns | ns | ns  | ** | ns | ns |  |
|            | 50  | ns  | **  | ns | ns | ns | **  | ns | ns | ns | ns  | ** | ns | ns |  |
|            | 25  | ns  | **  | ns | ns | ns | **  | ns | ns | ns | ns  | ** | ns |    |  |
| CM         | 100 | **  | *   | ** | ** | ** | ns  | ** | ** | ** | **  | *  | ** | ** |  |
|            | 75  | *   | *   | ns | ns | ns | **  | ns | ns | ns | *   | *  | ns | ns |  |
|            | 50  | ns  | **  | ns | ns | ns | **  | ns | ns | ns | ns  | ** | ns | ns |  |
|            | 25  | ns  | **  | ns | ns | ns | **  | ns | ns | ns | ns  | ** | ns | ns |  |

| Treatments |     | TC  |     | CDM |    |    | CM  |    |    |    | RM  |    |    |    |
|------------|-----|-----|-----|-----|----|----|-----|----|----|----|-----|----|----|----|
|            | %   | 100 | 100 | 75  | 50 | 25 | 100 | 75 | 50 | 25 | 100 | 75 | 50 | 25 |
| RM         | 100 | ns  | **  | ns  | ns | ns | **  | *  | ns | ns | ns  | ** | ns | ns |
|            | 75  | **  | ns  | **  | ** | ** | *   | *  | ** | ** | **  | ns | ** | ** |
|            | 50  | ns  | **  | ns  | ns | ns | **  | ns | ns | ns | ns  | ** | ns | ns |
|            | 25  | ns  | **  | ns  | ns | ns | **  | ns | ns | ns | ns  | ** | ns | ns |

Note: ns and \*\*: are non-significant and 1% probability levels, respectively. TC: Control treatment

In the second experiment, the suitability of the combination of different organic manures at different inclusion levels on the red worm growth performance was assessed. The overall results revealed that the highest biomass of red worms was (24.9±1.1 g) on T2 (20% CM + 40% CDM + 20% RM + 20% Soil), followed by (18.9±0.6 g) on T6 (0% CM + 60% CDM + 20% RM + 20% Soil). The biomass of red worms was lowest (5.1 ±0.2 g) on T5 (20% CM + 0% CDM + 60% RM + 20%

Soil) (Figure 3). There were significant differences in the mean values of the biomass of red worms among treatments (p < 0.05). The differences were recorded between T1 with T2 and T1 with T6. The biomass of red worms also differed significantly between T2 with all treatments, while T3 showed significant differences with T4, T5, T6 and TC. The results also showed that T4, T5 and TC differed significantly with T6 (Table 4).

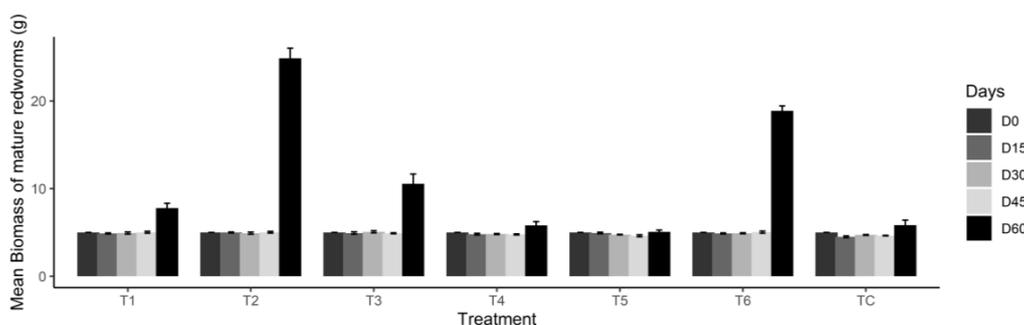


Figure 3. Mean biomass (±SE) of red worms in seven different treatments during 60 days.

Table 4. Comparison of different treatments in Experiment Two.

| Treatments | TC | T1 | T2 | T3 | T4 | T5 | T6 |
|------------|----|----|----|----|----|----|----|
| TC         | ns | ns | ** | ** | ns | ns | ** |
| T1         | ns | ns | ** | ns | ns | ns | ** |
| T2         | ** | ** | ns | ** | ** | ** | ** |
| T3         | ** | ns | ** | ns | ** | ** | ** |
| T4         | ns | ns | ** | ** | ns | ns | ** |
| T5         | ns | ns | ** | ** | ns | ns | ** |
| T6         | ** | ** | ** | ** | ** | ** | ns |

Note: ns and \*\*: are non-significant and 1% probability levels, respectively. TC: Control treatment.

The specific growth rate (SGR) was used to evaluate the overall red worm's growth performance during the 60-day experimental period. In the first experiment the SGR was significantly higher (0.97%) for red worms reared in 100% CM (100% Chicken manure +0% Soil), followed by a red worm (0.76%) those reared on 100% CDM (100% Cow dung manure +0% Soil and (0.73%) on 75% RM (75% Rabbit manure +25% Soil) and lowest (0.4%) for those reared on 100% RM (100%

Rabbit manure + 0% Soil) as indicated in Table 5). In the second experiment, SGR was significantly higher (1.16% and 0.96%) for red worms reared on T2 (20% CM + 40% CDM + 20% RM + 20% Soil) and T6 (0% CM + 60% CDM + 20% RM + 20% Soil) whereas the lowest SGR (0.01%) was observed for red worms reared on T5 (20% CM + 0% CDM + 60% RM + 20% Soil) as indicated in Table 6.

**Table 5.** Growth performance and productivity indices of earthworms cultured in different inclusion levels of organic manure with soil for 60 days in experiment one. Note: CDM = Cow dung manure, CM = Chicken manure, RM= Rabbit manure and TC= treatment control.

| Treatment | Parameters         |                     |                    |                        |
|-----------|--------------------|---------------------|--------------------|------------------------|
|           | Initial weight (g) | Final weight (g)    | Weight gain (g)    | Specific growth rate % |
| CDM100    | 5                  | 14.27 <sup>b</sup>  | 9.27 <sup>b</sup>  | 0.76 <sup>a</sup>      |
| CDM25     | 5                  | 6.14 <sup>cd</sup>  | 1.14 <sup>cd</sup> | 0.14 <sup>bc</sup>     |
| CDM50     | 5                  | 6.61 <sup>cd</sup>  | 1.61 <sup>cd</sup> | 0.18 <sup>bc</sup>     |
| CDM75     | 5                  | 6.7 <sup>cd</sup>   | 1.7 <sup>cd</sup>  | 0.19 <sup>bc</sup>     |
| CM100     | 5                  | 19.27 <sup>a</sup>  | 14.27 <sup>a</sup> | 0.97 <sup>a</sup>      |
| CM25      | 5                  | 6.71 <sup>cd</sup>  | 1.71 <sup>cd</sup> | 0.20 <sup>bc</sup>     |
| CM50      | 5                  | 6.45 <sup>cd</sup>  | 1.45 <sup>cd</sup> | 0.16 <sup>bc</sup>     |
| CM75      | 5                  | 10.06 <sup>bc</sup> | 5.06 <sup>bc</sup> | 0.50 <sup>ab</sup>     |
| RM100     | 5                  | 5.2 <sup>d</sup>    | 0.2 <sup>d</sup>   | 0.04 <sup>c</sup>      |
| RM25      | 5                  | 6.49 <sup>cd</sup>  | 1.49 <sup>cd</sup> | 0.17 <sup>bc</sup>     |
| RM50      | 5                  | 6.35 <sup>cd</sup>  | 1.35 <sup>cd</sup> | 0.15 <sup>bc</sup>     |
| RM75      | 5                  | 13.77 <sup>b</sup>  | 8.77 <sup>b</sup>  | 0.73 <sup>a</sup>      |
| TC        | 5                  | 5.83 <sup>cd</sup>  | 0.83 <sup>cd</sup> | 0.10 <sup>bc</sup>     |

Note: Dissimilar lowercase superscript letters represent significant differences between means within the same column ( $p < 0.05$ )

**Table 6.** Growth performance and productivity indices of earthworms in different treatments for 60 days in experiment two. Note: CDM = Cow dung manure, CM = Chicken manure, RM= Rabbit manure and TC= treatment control.

| Treatment | Parameters         |                    |                    |                     |                    |
|-----------|--------------------|--------------------|--------------------|---------------------|--------------------|
|           | Initial weight (g) | Final weight (g)   | Weight gain (g)    | RGR (%)             | SGR %              |
| T1        | 5                  | 7.77 <sup>cd</sup> | 2.77 <sup>cd</sup> | 55.4 <sup>cd</sup>  | 0.32 <sup>bc</sup> |
| T2        | 5                  | 24.87 <sup>a</sup> | 19.87 <sup>a</sup> | 397.33 <sup>a</sup> | 1.16 <sup>a</sup>  |
| T3        | 5                  | 10.55 <sup>c</sup> | 5.55 <sup>c</sup>  | 111.07 <sup>c</sup> | 0.53 <sup>b</sup>  |
| T4        | 5                  | 5.81 <sup>d</sup>  | 0.81 <sup>d</sup>  | 16.2 <sup>d</sup>   | 0.11 <sup>cd</sup> |
| T5        | 5                  | 5.07 <sup>d</sup>  | 0.07 <sup>d</sup>  | 1.33 <sup>d</sup>   | 0.01 <sup>d</sup>  |
| T6        | 5                  | 18.87 <sup>b</sup> | 13.87 <sup>b</sup> | 277.4 <sup>b</sup>  | 0.96 <sup>a</sup>  |

| Treatment | Parameters         |                   |                   |                    |                   |
|-----------|--------------------|-------------------|-------------------|--------------------|-------------------|
|           | Initial weight (g) | Final weight (g)  | Weight gain (g)   | RGR (%)            | SGR %             |
| TC        | 5                  | 5.83 <sup>d</sup> | 0.83 <sup>d</sup> | 16.67 <sup>d</sup> | 0.1 <sup>cd</sup> |

Note: Dissimilar lowercase superscript letters represent significant differences between means within the same column ( $p < 0.05$ ); RGR Relative Growth Rate (RGR), Specific Growth Rate (SGR)

There were positive correlations between the Biomass of adult worms and the number of cocoons ( $r = 0.68$ ,  $p$ -value  $< 0.05$ ;  $r = 0.74$ ,  $p$ -value  $< 0.05$ ), hatchlings ( $r = 0.71$ ,  $p$ -value  $< 0.05$ ;  $r = 0.73$ ,  $p$ -value  $< 0.05$ ) and juvenile worms ( $r = 0.67$ ,  $p$ -value  $< 0.05$ ;  $r = 0.86$ ,  $p$ -value  $< 0.05$ ) for experiments one and two respectively.

### 3.2. Chemical Composition of Red Worms

The results of the chemical composition of red worms are shown in [Tables 7 and 8](#) for experiment one and experiment two respectively. The chemical composition of red worms harvested at the end of the experiment (after 60 days) was significantly influenced by the nature of the rearing substrates. The chemical composition value of red worms differed significantly among

treatments ( $p < 0.05$ ). The crude protein content was highest (73.28% DM) in red worms reared on 100% CDM (100% Cow dung manure +0% Soil) and lowest (49.95% DM and 50.3%DM) in those reared on 25% CM (25% Chicken manure +75% Soil) and TC (100% Soil). Crude lipid was highest (7.63% DM) in red worms reared on 50% CM (50% Chicken manure +50% Soil) and lowest (3.68% DM) reared on 50% CDM (50% Cow dung manure + 50% Soil). Dry matter was highest (88.75% DM) in red worms reared on 75% CDM (75% Cow dung manure +25% Soil) and lowest (82.59% DM) in red worms reared on 100% RM (100% Rabbit manure +0% Soil). Ash content was highest (26.52% DM) in red worms reared on 25% CM (25% Chicken manure +75% Soil) and lowest (5.16% DM) in red worms reared on 100% CDM (100% Cow dung manure +0% Soil) as indicated in [Table 7](#).

**Table 7.** Proximate composition of red worms reared on different levels of manure (chicken manure (CM), cow dung manure (CDM), and rabbit manure (RM) mixed with soil.

| Treatments | Dry matter (%WW)      | Ash (%DM)             | Crude protein (%DM)    | Crude lipid (%DM)    | NFE (%DM)              |
|------------|-----------------------|-----------------------|------------------------|----------------------|------------------------|
| CDM100     | 85.3±0.0 <sup>b</sup> | 5.2±0.0 <sup>j</sup>  | 73.2±0.0 <sup>a</sup>  | 3.9±0.0 <sup>i</sup> | 17.6±0.0 <sup>e</sup>  |
| CDM25      | 83.4±0.0 <sup>b</sup> | 16.5±0.1 <sup>d</sup> | 55.3±0.0 <sup>b</sup>  | 4.3±0 <sup>h</sup>   | 23.9±0.1 <sup>bc</sup> |
| CDM50      | 83.6±0.5 <sup>b</sup> | 14.4±0.0 <sup>f</sup> | 57.3±0.1 <sup>fg</sup> | 3.7±0.0 <sup>j</sup> | 24.6±0.1 <sup>b</sup>  |
| CDM75      | 88.7±0.0 <sup>a</sup> | 13.5±0.0 <sup>g</sup> | 64.7±0.0 <sup>b</sup>  | 4±0.0 <sup>i</sup>   | 17.8±0.0 <sup>e</sup>  |
| CM100      | 84.7±0.6 <sup>b</sup> | 13.0±0.0 <sup>h</sup> | 58.1±0.0 <sup>f</sup>  | 5.7±0 <sup>d</sup>   | 23.2±0 <sup>c</sup>    |
| CM25       | 84.9±0.1 <sup>b</sup> | 26.5±0.1 <sup>a</sup> | 49.9±0.0 <sup>j</sup>  | 6.6±0 <sup>b</sup>   | 16.9±0.1 <sup>e</sup>  |
| CM50       | 84.6±0.1 <sup>b</sup> | 22.0±0.0 <sup>c</sup> | 53.4±0.0 <sup>i</sup>  | 7.6±0.0 <sup>a</sup> | 17.0±0.0 <sup>e</sup>  |
| CM75       | 84.2±0.1 <sup>b</sup> | 15.8±0.0 <sup>e</sup> | 57.1±0.0 <sup>g</sup>  | 5.8±0.0 <sup>c</sup> | 21.3±0.1 <sup>d</sup>  |
| RM100      | 82.6±0 <sup>b</sup>   | 10.2±0.1 <sup>i</sup> | 61.0±0.0 <sup>d</sup>  | 5.0±0.0 <sup>e</sup> | 23.8±0.0 <sup>bc</sup> |
| RM25       | 83.8±0.0 <sup>b</sup> | 16.4±0.0 <sup>d</sup> | 62.9±0.0 <sup>c</sup>  | 6.6±0 <sup>b</sup>   | 14.1±0.0 <sup>f</sup>  |
| RM50       | 84.8±1.6 <sup>b</sup> | 24.5±0.0 <sup>b</sup> | 59.9±0.1 <sup>e</sup>  | 4.7±0.0 <sup>g</sup> | 11.0±0.1 <sup>g</sup>  |
| RM75       | 82.9±0.0 <sup>b</sup> | 14.4±0.0 <sup>f</sup> | 59.4±0.5 <sup>e</sup>  | 4.8±0.1 <sup>f</sup> | 21.5±0.6 <sup>d</sup>  |
| TC         | 88.6±0.0 <sup>a</sup> | 14.3±0.0 <sup>f</sup> | 50.3±0 <sup>j</sup>    | 6.7±0.0 <sup>b</sup> | 28.8±0.0 <sup>a</sup>  |

Note: All values are expressed as mean ± standard error (SE;  $n = 3$ ) for percentage of wet weight (%WW), and percentage of dry matter (%DM). Dissimilar lowercase superscript letters represent significant differences between means within the same column ( $p < 0.05$ ).

In the second experiment ([Table 8](#)), the chemical composition of red worms differed significantly among treatments ( $p < 0.05$ ).

The crude protein content was highest (70.83% DM) in red worms reared in T4 (60% CM + 20% CDM + 0% RM + 20% Soil) and lowest (50.28% DM) in red worms reared in TC (0% CM + 0% CDM + 0% RM + 100% Soil). Crude lipid was highest (12.68% DM) in red worms reared in T2 (20% CM + 40% CDM + 20% RM + 20% Soil) and lowest (4.07% DM) reared in T5. Dry matter was highest (88.6% WW) in red worms

reared in TC (0% CM + 0% CDM + 0% RM + 100% Soil) and lowest (74.41% WW) in red worms reared in T1 (20% CM + 20% CDM + 40% RM + 20% Soil). Ash content was highest (16.45% DM) in red worms reared in T5 (20% CM + 0% CDM + 60% RM + 20% Soil) and lowest (8.47% DM) in red worms reared in T2 (20% CM + 40% CDM + 20% RM + 20% Soil).

**Table 8.** Proximate composition of red worms reared on different organic manure with a fixed amount of soil).

| Treatments | Dry Matter (%WW)       | Ash (%DM)              | Crude protein (%DM)     | Crude lipid (%DM)      | NFE (%DM)             |
|------------|------------------------|------------------------|-------------------------|------------------------|-----------------------|
| T1         | 74.4±0.0 <sup>d</sup>  | 11.9±0.1 <sup>c</sup>  | 64.12±0.1 <sup>c</sup>  | 11.9±0.0 <sup>b</sup>  | 12.1±0.1 <sup>d</sup> |
| T2         | 83.9±0.0 <sup>b</sup>  | 8.5±0.0 <sup>e</sup>   | 68.685±0.3 <sup>b</sup> | 12.7±0.15 <sup>a</sup> | 10.2±0.0 <sup>e</sup> |
| T3         | 81.8±0 <sup>c</sup>    | 11.5±0.3 <sup>cd</sup> | 52.915±0.0 <sup>e</sup> | 7.6±0.0 <sup>c</sup>   | 28.0±0.3 <sup>a</sup> |
| T4         | 83.7±0.1 <sup>bc</sup> | 11.2±0.0 <sup>d</sup>  | 70.83±0.0 <sup>a</sup>  | 7.1±0 <sup>d</sup>     | 10.9±0.1 <sup>e</sup> |
| T5         | 86.5±1.5 <sup>ab</sup> | 16.5±0.0 <sup>a</sup>  | 63.91±0 <sup>c</sup>    | 4.1±0.0 <sup>g</sup>   | 15.6±0.0 <sup>e</sup> |
| T6         | 84.0±0 <sup>bc</sup>   | 15.9±0.0 <sup>a</sup>  | 60.455±0.2 <sup>d</sup> | 4.6±0.0 <sup>f</sup>   | 19.0±0.2 <sup>b</sup> |
| TC         | 88.6±0.0 <sup>a</sup>  | 14.3±0.0 <sup>b</sup>  | 50.28±0 <sup>f</sup>    | 6.7±0.0 <sup>e</sup>   | 28.8±0.0 <sup>a</sup> |

Note: All values are shown as mean ± standard error (SE; n = 3) for percentage of wet weight (%WW), and percentage of dry matter (%DM). Dissimilar lowercase superscript letters represent significant differences between means within the same column (p < 0.05).

## 4. Discussion

The present study appreciable growth performance of red worms suggesting that organic manures are excellent oligochaetes worm culture substrates. Chicken manure was found to contain more nutrients than other organic manures due to the reported high biomass of red worms (*E. fetida*) at a 100% inclusion level. [27] revealed that earthworms develop and reproduce favourably in the substrate containing 70% poultry droppings as feed mixture. In addition, the high biomass of earthworms under chicken manure might be due to the ideal conditions of this manure which favoured more cocoon development, hatching rates and the presence of cellulose which speeds up the production of bacteria necessary for the breakdown of manure [28, 29]. A study by Monebi and Ugwumba [30] also reported that cellulose substrate is suitable for weight gain and growth rate of the earthworms. On the other hand, it was revealed that the substrate containing 20% chicken manure, 40% cow dung manure, 20% rabbit manure and 20% soil produced red worms with higher biomass than other substrates. This was probably due to the presence of easily metabolizable organic matter and low concentration of growth-retarding substances [31].

The red worms raised on cow dung manure-based substrate had relatively higher values of crude protein than those raised on other treatments. These findings are within the range reported in studies by Paoletti et al. [32], however, a study by

Medina et al. [33] reported higher protein contents (75.8%) than the reported protein contents in our study. High nutrient contents of the worms might be due to the type of culture media used, the nutritional quality of the substrate, the worm's gut contents and processing techniques [22, 34].

In the present study, the red worm *E. fetida* had higher mean values of ash content (26.5%) compared to the average values of 22.16% reported by García-Solís [35]. Differences in the results might be due to the harvesting and processing steps that exposed the ingredients to the soil. The lipid content in the present study ranged between 4-13% which is within the range proposed by Kostecka et al. [36] which was 12.21%. However, the studies by Kavle et al. [37] and Medina et al. [33] reported slightly higher lipid content (19.3%) than that found in the present study. A study by Gunya and Masika [38] observed that the lipid content of earthworms ranged from 5–20% of dry matter. The quality of the culture substrate and the way the earthworms are raised can have an impact on differences in the lipid content. In addition, the metabolism of lipids can change as an earthworm develops through its various developmental stages [39]. Moreover, habitat location, diet, and worm-raising practices might all influence the difference in fat content found in various organisms [39].

## 5. Conclusions and Recommendations

From the results, it can be concluded that the rearing substrate has a significant effect on both growth performance and body chemical composition. The findings of this study sug-

gest that organic manure especially chicken manure and cow dung manure can be used effectively to culture red worms which later may serve as an alternative source of protein in fish feeds. Reusing organic manure in the production of red worms encourages waste reduction and the creation of organic fertilizers (vermicompost and vermiliquid) that may be used in agricultural activities.

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## Author Contributions

**Maziku Zephania:** Formal Analysis, Methodology, Validation, Visualization, Writing – original draft editing

**Renalda Nanziga Munubi:** Formal Analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing

**Augustine Warrioba Mwandya:** Data curation, Formal Analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing

## Data Availability Statement

The data is available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare no conflicts of interest.

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