Effect of Various Farm Manures and Straw Containing Cattle Manure on Worm and Worm Manure Production

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ABSTRACT
The aim of this study is to determine the effect of different combinations of farm manure and farm manure on earthworm proliferation and earthworm manure production quantity and property. Accordingly, 100% horse manure (H), 100% cattle manure (C), 100% straw cattle manure (SC), 90% horse manure + 10% hen manure (H/H), 90% cattle + 10% hen manure (C/H) and 90% straw cattle manure + 10% hen manure (SC/H) six research groups were designed. The highest level of worm growth in the experimental period was in group H, followed by SC group. The lowest values were found in C/H group and C group. In the amount of worm manure produced, the highest value was found in H/H group and the lowest value was found in SC group. As a result, it was found that 100% H and 100% SC groups made a better contribution to the increase in worm growth.

Key words: Earthworm, Farm manure, Feed, Stubble, Worm feed.

INTRODUCTION
Epigeal earthworms such as eisenia fetida (red wigglers) reproduce very well as they consume organic waste (Chaoui, 2019). The habitat of earthworms should be highly absorptive thus expand in volume. Earthworm feeds should contain low level of nitrogen and have high organic manure/nitrogen rate. Likewise, there is a list of most commonly used bedding models (Munroe, 2007) which provides some information on absorptivity / volume expand potential and organic manure nitrogen (Organic manure: N) rates of each material. The list suggests that horse manure have superiority among other types of manure. That is because horse manure is high fiber and has high organic manure:N rate. These features give horse manure relatively better moisture holding capacity thus making it more expandable in volume. For these reason horse manure is considered the best habitat for E. Fetida. Humus worms also noted to be a good consumer of agricultural and animal waste. In this context these crop stubble straws are used as beddings to prevent diseases (Bai, 2011) since they provide a dry bed for cows, and when combined with animal manure they make up the best bedding model and feed for earthworms and facilitate their reproduction. On the other hand, silkworm waste was used as raw material for vermicomposting. (Venugopal et al., 2010). The manure from these reproduced earthworms can be used in plant production (Munroe, 2007; Atiyeh et al., 2000; Venugopal et al., 2010) and the earthworms themselves as hen-feed (Sözcü and Ipek, 2016) and fish-feed (Arıman Karabulut et al., 2016). In this context, comparative studies are needed to bring farm fertilizers to the economy. In order to meet this need, horse manure, cattle manure without wheat stalk, cattle manure containing wheat stalk and worm feeds prepared by combining them with 10% poultry manure were investigated. In this research, it is aimed to determine the effect of these farm fertilizers on the reproduction levels of soil worms and fertilizer production levels.

MATERIALS AND METHODS
Research groups and experiment design
The group in which worm bedding and 100% horse manure used as feed is the horse manure group (Group H). The group in which 100% cattle manure not containing wheat straw were used is the cattle manure group (Group C). The group in which 100% cattle manure containing wheat steam is used is the straw cattle manure group (SC Group). The 90 % horse manure + 10 % hen manure combination is named the horse/hen manure group (H/H group). The 90 % non-straw cattle manure + 10 % hen manure combination is named the cattle/hen manure group (C/H) group. The 90 % straw cattle manure + 10 % hen manure combination is the straw / hen manure group (SC/H). These each beddings and feed were pre-fermented. Each group (blocks) was used in experiment repeatedly 5 times. Randomized block design method is employed in the conducting of the experiment.


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Placing the bedding and the worms in cultivation compartments
For each six group with each to be tested five times, 30 cultivation compartments were built onto a concrete flat ground. The pre-fermented subject worm feeds were placed as bedding into an area with 0.4 m x 0.4 m size and 20 cm of height. 10.000 worms were placed into this bedding (Munroe, 2007).

Feeding the worms
About 1-2 kg of pre-prepared worm feed placed once in every week in the cultivation compartments. Then the compartment was springer-irritated in a way to keep humidity about 75-80%.

Harvesting the worms
On the 60th day of the experiment, worms were separated from the humus. This process was done through letting the worm compartment dry in order for worms to gather in the moist area. Then the worms in the moist area was sorted out from the manure.

Determination of the number of harvested worms
Separated worms then filled in the 10 ml measurement cups. Two of these 10 ml cups were taken to count the worm amount, then the average number was multiplied by the total cup amount, which gave the total average worm amount for each sub groups.

Calculating the reproduced worm number
Planted worm amount was subtracted from the harvest worm total.

Reproduction rate of worms
Harvest worm amount was divided by the planted worm amount, and the resulting number multiplied by 100, which makes up the reproduction rate.

Calculating the manure weight
Worm manure after separated from worms weighted for each tests. Also the manure’s dry substance was determined. With the help of the below given formula, worm manure amount obtained from dry substance in 60 days was calculated. Manure amount obtained from dry substance (kg) = weight of the post-harvest worm manure (kg) x dry material rate within manure after harvesting: 100.

Analyses on worm feed and worm manure

Measuring salinity level
Added 50 ml of water on a 20 gr. of sample from bedding and worm feed. After letting this mixture wait for one night, the mixture’s salinity level was identified using a conductivity meter.

Measuring pH
Added 50 ml of water on a 20 gr sample from bedding and manure. After letting this mixture wait for one night, pH level was measured the next day, using a pH meter.

Raw cellulose that is contained in worm feed was measured with Ankom Fiber Analyser, nitrogen was measured with Kjeldahl apparaturus, dry substance was measured in a drying chamber of 105°C, raw ash was measured in ash oven of 600°C and, organic substance was measured using the methods given in AOAC (AOAC, 1990).

Humic acid level was measured in pursuant with the Appendix-19 (analysis methods) of the Regulation on Organic, Mineral and Microbial Manures and the Regulation on Market Surveillance and Inspection of Manures, with the method stipulated by TS 5869 ISO 5073 (Anonymous, 2019a).

Magnesium level was measured with Absorption Spectrophotometer (Perkin-Elmer Instrument model 2380) in accordance with the method stipulated in AOAC (AOAC, 1990). Potassium level was measured with flame photometry (Anonymous, 2019b). The phosphor level measured with spectrophotometry (Anonymous, 2019b).

Statistical analyses
Statistical analysis of the data was performed using the SPSS 22 program and Kruskal Wallis and Bonferroni Mann Whitney-U test. Kruskal-Wallis Analysis of Variance test was performed to determine whether there was a significant difference between Bonferroni Mann-Whitney-U and subgroups. All the results were produced as average±standard deviation and p<0.05 was accepted to be the statistically significant value (SPSS, 2013).

RESULTS AND DISCUSSION
The manure with the highest level of dry substance of 100% was the straw cattle manure (p<0.05) (Table 1). This was followed by 100% horse and cattle manure. The reason behind this may be the straw which contains high level of dry substance (Çakmak and Çerçi, 1995; Çerçi and Sari, 1994). Raw ash level was highest in C/H group (p<0.05). When the non-hen manure groups are cross-examined, raw ash levels shows a downward slide respectively from the C-group to the SC and to the H-group. Hen manure contribution seemed to increase the raw ash level. This may be caused by the mineral substances which cattle and hen feeds contain (Sari et al., 2008). Organic substance (Table 1) is naturally high in group with low raw ash level and vice versa. Raw cellulose level is highest in straw cattle manure. Hen manure contribution on the other hand, seemed to decrease cellulose levels in groups which partly compose hen manure, mainly in the 90% cattle + 10% hen manure combination group. The reasons behind these differences in raw cellulose rates can be listed as follows: 1) Using roughages rich in raw cellulose in cattle and horse diets (Sari et al., 2008). High raw cellulose level in straw cattle manure group may be caused by the straw it contains (Çakmak and Çerçi, 1995; Çerçi and Sari, 1994). The decrease in low cellulose level seen in hen manure combination groups is because poultry diets contain low cellulose (Sari et al., 2008). Nitrogen level showed an increase in hen manure containing groups and...
Table 1: Nutrition composition, humic acid, salinity (%) and pH values of farm manure worm feeds.

| Feed Groups | Dry substance (mg/kg) | Raw ash (mg/kg) | Organic substance (mg/kg) | Dry cellulose (mg/kg) | Nitrogen (%) | Humic acid (%) | pH | Salt (%)
|--------------|-----------------------|----------------|--------------------------|----------------------|--------------|----------------|-----|----------|
| C group      | 27.24±1.43            | 29.57±1.59     | 50.97±1.25^*              | 2.91±0.13            | 7.92±0.05    | 30.36±1.28     | 7.92±0.05 | 2.34±0.07 |
| C/H group    | 28.8±1.01             | 33.82±1.43^*   | 24.96±0.78^*              | 7.44±2.23^*          | 8.10±0.08^*  | 31.80±1.66     | 8.13±0.05 | 3.38±0.03 |
| SC group     | 71.14±1.01            | 66.18±1.43^*   | 75.04±0.78^*              | 39.56±1.30^*         | 33.82±0.64^* | 37.58±0.56^*TK| 8.02±0.06 | 1.81±0.02 |
| SC/H group   | 37.61±1.94            | 27.44±2.23^*   | 39.56±1.30^*              | 33.82±0.64^*         | 33.82±0.64^* | 31.16±1.33^*TK| 7.92±0.05 | 1.81±0.02 |
| H group      | 2.67±0.17             | 2.91±0.13      | 2.46±0.07                 | 2.53±0.12            | 8.00±0.10    | 30.16±1.33     | 7.58±0.10 | 2.16±0.03 |
| H/H group    | 30.36±1.28            | 31.80±1.66     | 37.58±0.56^*TK            | 31.16±1.33^*         | 30.36±1.28   | 34.62±0.48^*TZ | 7.92±0.05 | 4.91±0.02 |

Data based on average ± standard deviation. Statistically regarded significant are the groups with different symbols in the same line. (Dry substance: A; those that differs from C group; B; those that differs from C/H group; C; those that differs from SC group; D; those that differs from SC/H group. Raw Ash: K; those that differs from C group; L; those that differs from C/H group; M; those that differs from SC group; N; those that differs from SC/H group; O; those that differs from H group. Organik madde: f; those that differs from C group; g; those that differs from C/H group; h; those that differs from SC group; i; those that differs from SC/H group; j; those that differs from H group. Raw cellulose: a; those that differs from C group; b; those that differs from C/H group; c; those that differs from SC group; d; those that differs from SC/H group; e; those that differs from H group. Nitrogen: k; those that differs from C group; l; those that differs from C/H group; m; those that differs from SC group; n; those that differs from SC/H group; o; those that differs from H group. Raw ash: p; those that differs from C group; q; those that differs from C/H group; r; those that differs from SC group; s; those that differs from SC/H group; t; those that differs from H group. pH: P; those that differs from C group; R; those that differs from C/H group; S; those that differs from SC group; Q; those that differs from SC/H group; X; those that differs from SC group; Y; those that differs from SC/H group; Z; those that differs from H group. p<0.01). (Table 1)

Table 2: Magnesium, phosphor and potassium levels of farm manure worm feeds.

| Feed Groups | Magnesium (mg/kg) | Potassium (mg/kg) | Phosphor (mg/kg) |
|--------------|-------------------|------------------|------------------|
| C group      | 60.165            | 301              | 284.03           |
| C/H group    | 60.525            | 395              | 252.34           |
| SC group     | 59.598            | 239              | 345.86           |
| SC/H group   | 59.751            | 308              | 344.48           |
| H group      | 59.688            | 732.5            | 282.76           |
| H/H group    | 60.228            | 715              | 317.59           |

The tests proved that the H/H group had the highest level of salinity in worm feeds. And the lowest level was the straw cattle manure group. Horse manure seemed to increase the salinity levels. Also, poultry manure increased the salinity level in worm feeds. Its reason may be the added salt in animal diets. The reason why it was low in groups may be because the straw being a vegetative feed has low salinity (Sari et al., 2008). On the other hand, a previous study (Munroe, 2007) suggests that ideal pH value for worms is between 7.5 and 8.

Magnesium levels of worm feeds (Table 2) in all groups were around 60 mg/kg. The highest potassium level was observed in H group. Potassium levels of cattle manure groups was seen to be much lower than that of horse manure group. Cattle manure groups, when combined with hen manure, slightly increased its potassium levels. The reason behind this increase may be that horse feeds consist mostly of weeds rich in potassium and potassium contribution to poultry feeds (Sari et al., 2008). The group which showed the highest phosphor level was the SC group. This was followed by the SC/H group and the H group.

C/H group showed the highest dry substance level in manures obtained from worms fed with different feeds (Table 3). The highest raw ash level in worm manures was observed in SC group. This was followed by SC/H group, C/H and C groups. The highest organic substance level was observed in H group and lowest organic substance level was observed in SC group. SC/H group showed the lowest raw cellulose level in worm manures. Highest raw cellulose level was observed in H group. While SC group had the lowest nitrogen level in worm manure with 1.68 %, highest value was observed with 2.52 % was the H group. This is possibly because the worms consume the organic substances, mainly raw celluloses, more efficiently. Indeed, as it is reported in a previous study (Chaoui, 2019), epigal worms such as eisenia fetida (red wigglers) reproduce very fast as they consume organic waste.
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Table 3: Nutrient composition and humic acid levels (%) of worm manures obtained from various farm manures (%).

| Feed groups | Dry Substance | Raw ash | Organic substance | Raw cellulose | Nitrogen | Humic acid |
|-------------|---------------|---------|------------------|--------------|---------|-----------|
| 1. C group  | 45.75±0.76    | 53.09±1.74 | 47.26±2.02       | 11.79±1.02   | 2.34±0.25 | 32.98±0.78 |
| 2. C/H group| 47.26±2.02    | 53.44±1.65 | 49.17±1.71       | 8.80±0.44    | 2.68±0.12 | 31.60±0.35 |
| 3. SC group | 44.39±1.31    | 59.12±0.75 | 57.45±1.23       | 9.22±0.72    | 2.26±0.12 | 43.67±0.21 |
| 4. SC/H group| 42.78±2.31   | 42.97±1.22 | 42.55±1.23       | 15.46±0.44   | 2.52±0.11 | 53.86±1.67 |
| 5. H group  | 46.47±0.95    | 40.88±7.05 | 45.75±1.22       | 15.46±0.70   | 2.52±0.11 | 46.56±1.65 |
| 6. H/H group| 44.75±1.23    | 42.21±1.25 | 45.75±1.22       | 15.46±0.44   | 2.52±0.11 | 46.56±1.65 |

Data based on average ± standard deviation. Statistically regarded significant are the groups with different symbols in the same line. (Dry substance: *; those that differs from C group; +; those that differs from C/H group; b; those that differs from C/H group; c; those that differs from SC group; d; those that differs from SC/H group. Organic substance: e; those that differs from C group; f; those that differs from C/H group; g; those that differs from SC group; h; those that differs from SC/H group. Raw cellulose: A; those that differs from C group; B; those that differs from C/H group; C; those that differs from SC group; D; those that differs from SC/H group; E; those that differs from H group. Nitrogen: K; those that differs from C group; L; those that differs from C/H group; M; those that differs from SC group. Humic acid: k; those that differs from C group; l; those that differs from C/H group; m; those that differs from SC group; n; those that differs from SC/H group. P value p<0.01).

Table 4: Magnesium, phosphor and potassium levels of worm manure obtained from various farm manures as feed.

| Feed groups | Magnesium mg/kg | Potassium mg/Kg | Phosphor mg/Kg |
|-------------|-----------------|-----------------|----------------|
| C group     | 60.39           | 139             | 66.18          |
| C/H group   | 60.813          | 152             | 38.91          |
| SC group    | 61.47           | 206.4           | 42.67          |
| SC/H group  | 60.444          | 224.8           | 191.41         |
| H group     | 60.858          | 256             | 217.07         |
| H/H group   | -               | 279             | 122.76         |

When looking at worm manures in terms of humic acid, the highest level is observed in SC group, which is closely followed by H/H group.

Among the obtained worm manures, the highest level of magnesium was observed in SC group with 61.47 mg/kg. Potassium levels in worm manures show a descending order from H/H (highest), H, SC/H, SC, C/H and C (lowest). In terms of phosphor level, H group is on the lead, which is followed by SC/H group (Table 4). In terms of cellulose and ingredients, H and SC groups of this study shows a resemblance to another study where 50% brewed tea and 50% cattle manure were combined, for both studies had similar mineral substance level results (Yüksek et al., 2019).

A comparison between worm feed and worm manures shows that, while raw ash level in worm manure increased by 86%; organic substance decreased by 33%, raw cellulose by 67%; nitrogen by 25%, potassium by 59% and phosphor decreased by 63% in worm manures, when compared to worm feed (Table 5). This possibly proves that worms can substantially digest the organic substances and minerals included in their diet and use it for their growth and reproduction. Because these materials directly affect the efficacy of vermicomposting (Barik et al., 2011). On the other hand, evaluating the raw ash levels which includes insoluble inorganic salt and minerals, may depend on its solubility rate.

In this 60-day period study (Table 6), worm reproduction and production rate was highest in H group, which was followed by SC group. This, again, was respectively followed by H/H and SC/H groups, while lowest rates observed in C/H and C groups. In terms of manure amount produced, highest rate was observed in H/H group while SC group had the lowest rate. Hen manure added to horse and cattle manures increased the worm manure amount, however it decreased worm reproduction rate. In a previous study (Namli et al., 2014), it is emphasized that the sludge used in incubation test which contains high level of NH4-N and ammonium, may have negatively affected worm activity and population. This emphasis partly explains the findings of this study. Furthermore, the method of combining straw with cattle manure to use as bedding, raised the worm production rate to the level that of the worms which consume horse manure. In another study where worms consumed the feed obtained from a 50% brewed tea and 50% cattle manure combination, worm amount and weight showed higher values compared to other subgroups of the same study (Yüksek et al., 2019). One other research (Yüksek, 2019) reports that fiber waste such as nutshell and tea fiber, paper and wood flour added in cattle manure showed highest worm reproduction and worm weight results. Additionally other previous researches (Chaoui, 2019; Venter and Reinecke, 1988) suggest that worms complete their growth within 40 to 60 days and can double their biomass in 60 days. However in practice, the same researches reported slightly lower values. Because many factors can be effective in this for example, winter and rainy seasons are better for conversion rate of vermicompost (%) and proliferation rate of earth worm (%) as compare to summer season (Agarwal and Arora 2011). It can be said that outcome of this study showed a correspondence with the outcomes of previous studies; especially the horse manure and straw cattle manure groups.
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### Table 5: Change (difference) rate of raw ash, organic substance, raw cellulose, nitrogen, potassium and phosphorus levels between worm feeds and worm manures and the rates of this variability.

| Nutrients and minerals (on dry substance) % | in worm feeds | in worm manures | Their increase/decrease rate within worm manure | Change rate in manure compared to worm feed, % |
|-------------------------------------------|--------------|----------------|-----------------------------------------------|---------------------------------------------|
| Raw ash                                   | 27.81        | 51.78          | +23.97                                       | +86.19                                      |
| Organic substance                         | 72.19        | 48.22          | -23.97                                       | -33.20                                      |
| Raw cellulose                             | 33.41        | 11.17          | -22.24                                       | -66.57                                      |
| nitrogen                                  | 2.97         | 2.24           | -0.73                                        | -24.58                                      |
| Magnesium mg/kg                           | 60.00        | 60.79          | +0.79                                        | +1.32                                       |
| Potassium mg/kg                           | 448.42       | 184.2          | -264.22                                      | -58.92                                      |
| Phosphor mg/kg                            | 304.51       | 113.17         | -191.34                                      | -62.84                                      |

### Table 6: Reproduction rate and produced amount of worms fed with various farm manures and manure production (Planted worm amount: 1000).

|                          | 1. C group | 2. C/H group | 3. SC group | 4. SC/H group | 5. H group | 6. H/H group | P value |
|--------------------------|------------|--------------|-------------|---------------|------------|--------------|---------|
| Harvested worm (amount)  | 15100.0±291.6 | 16060.0±315.0³ | 21070.0±781.1⁴ | 18810.0±159.7¹⁰ | 21780.0±605.8⁴₀ | 20290.0±454.7⁴₀ | 0.000   |
| Produced worm (amount)   | 5100.0±291.6 | 6060.0±315.0⁰  | 11070.0±781.5⁸ | 8810.0±159.7⁷ₓ₉ | 11780.0±605.8⁷ₓ₉ | 10290.0±454.7⁷ₓ₉ | 0.000   |
| Reproduction %           | 51.0±2.9   | 60.6±3.2⁷    | 110.7±7.8⁸   | 88.1±1.6⁸       | 117.9±6.2⁸     | 102.9±4.6⁸     | 0.000   |
| Manure production rate (kg)/KM | 17.1±0.3 | 18.7±0.5⁸ | 16.3±0.3⁸   | 21.6±0.7⁸       | 20.1±0.7⁸     | 23.0±0.3⁸      | 0.000   |

Data based on average ± standard deviation. Statistically regarded significant are the groups with different symbols in the same line. (Harvested worm amount: A; those that differs from C group; B; those that differs from C/H group; C; those that differs from SC group; D; those that differs from SC/H group. Produced worm amount: T; those that differs from C group; U; those that differs from C/H group; X; those that differs from SC group; Y; those that differs from SC/H group. Reproduction %: a; those that differs from C group; b; those that differs from C/H group; c; those that differs from SC group; d; those that differs from SC/H group. Manure production rate (kg): K; those that differs from C group; L; those that differs from C/H group; M; those that differs from SC group. p<0.01.).

### CONCLUSION
As a result, horse manure proved to be a better contributor to worm reproduction. Cattle manure mixed with wheat straw came closer to horse manure in terms of worm reproduction. Added hen manure affected worm reproduction negatively, while it affected manure production positively. Compared to worm feeds, the worm manures showed higher raw ash level, while it showed lower organic substance, raw cellulose, nitrogen, potassium and phosphorus levels.

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