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Utilization of sheep dung and rice straw with indigenous microbial agent to optimize vermicompost production and quality

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Abstract. *Hidayati YA, Nurachma S, Badruzzaman DZ, Marlina ET, Harlia E. 2021. Utilization of sheep dung and rice straw with indigenous microbial agent to optimize vermicompost production and quality. Biodiversitas 22: 5445-5451.* Sheep dung is a useful by product that can be potentially processed into more useful products with minimal pollution. Vermicomposting is a waste processing method that produces Solid Organic Fertilizer and worm biomass as the raw materials for drugs and cosmetics. This research aimed to utilize the role of indigenous fungi and bacteria to determine the influence of C/N ratio from sheep dung and rice straw mixture on vermicompost quality (N, P, K, Ca, Mg). This experimental research was conducted in a Completely Randomized Design with three treatments (T1: C/N ratio of 25; T2: C/N ratio 30; T3: C/N ratio 35) and six replicates for each treatment. Data so obtained were analyzed with ANOVA and Duncan's Multiple Range Test. The results showed that C/N ratio had a noticeable influence on vermicompost quality. C/N ratio 30 produced the highest vermicompost quality (N: 1.11 ± 023381%; P: 0.56 ± 0.010328 %; K: 0.51 ± 0.021369 %; Ca: 0.33 ± 0.0248 %; Mg: 0.14 ± 0.0228%).

Keywords: C/N ratio, indigenous microbes, rice straw, sheep dung, vermicomposting

INTRODUCTION

Sheep is one of the most common livestock in Indonesia. The 2020 statistics of livestock in Indonesia showed that West Java province is home to most sheep population in Indonesia, accounting for 12.272.435 heads of sheep or 69.06% of the total population nationwide (17.769.084 heads of sheep) (Ditjen PKH 2020). Sheep farming inevitably produces dung that weighs approximately 7-8% of cattle body weight (Penakalapati et al. 2017) like ram (90-100 kg) and ewe (50-70 kg). In other words, a farm with 100 sheep (+60 kg/head) will produce ± 420 kg of fecal waste per day which is required to be managed well to avoid harm to the environment. The uncontrolled decomposition of dung produces air polluting agents like ammonia, H_2S, CO_2, and CH_4. When it enters the water system, the sheep dung increases the Biological Oxygen Demand and Chemical Oxygen Demand but decreases the Dissolved Oxygen. In addition, sheep dung promotes the survival of pathogenic bacteria in the soil which may cause many diseases. Therefore, dung required attention to be processed properly (Zainuddin et al. 2019).

The sheep dung contained indigenous microbes, including bacteria and fungi that can be utilized as decomposing agents if used in well controlled conditions. In the right quantity, Microbes in appropriate quantity can undergo an optimal decomposition process in controlled moisture content and humidity of the organic matter. Abu-Bakar and Ibrahim (2013) stated that good levels of C and N fulfill the nutrient requirements of these indigenous and ultimately affect microbial activity. To obtain a good balance of C/N ratio, sheep dung (a nitrogen source) cannot be processed singly but be incorporated with carbon sources. The optimum C/N ratio is the main factor of composting quality (Dadi et al. 2019). The C/N ratio of sheep dung is lower than the required level for processing (C/N 25 - 35). The C/N ratio of sheep dung is 19.00 ± 0.84 (C/N 25 - 35) (Azim et al. 2014). Therefore, it is necessary to add another C source to increase C/N ratio of sheep dung.

Rice straw is one of the agricultural wastes that potentially adds nutrients to the soil and provides carbon source. However, burning straws is more popular among farmers than processing them into fertilizer because it is difficult to incorporate a large amount of straw into the soil (Jin et al. 2020). Rice straw contains 40% C, 0.6% N, 0.1% S, and 1.5% Si (Ponnamperuma 1984) and has a cell wall that builds on 39.7% cellulose in dry weight, 25.2% hemicellulose, and 4.8% lignin (Yang et al. 2020). The chemical complexity and the structure of straw make it difficult for microorganisms to decompose (Guo et al. 2018). Therefore, the straw needs shredding prior to mixture to allow a smooth composting process and to obtain quality compost (Roca-Pérez et al. 2009). Dung and rice straw can be composted with the help of anaerobic activity of indigenous microorganisms. Use of earthworms to compost organic material is another method that produces a solid organic fertilizer called vermicompost (Pathma and Sakthivel 2012).

The growth and activity of indigenous microorganisms in vermicomposting require a balanced substrate C/N ratio. Therefore, it is necessary to evaluate the effect of the C/N ratio of the substrate according to the indigenous microorganism requirement through decomposition and vermicomposting. This study aimed to determine the impact of C/N ratio of the mixture of sheep dung and rice straw on vermicompost quality (the content of N, P, K, Ca, Mg) by utilizing the role of indigenous fungi and bacteria.

MATERIALS AND METHODS

Substrate source

Two substrates namely, sheep dung (source of N) and rice straw (source of C) collected from local farms and rice farmers residing around the area of Padjadjaran University, Jatinangor, Sumedang Regency, West Java, Indonesia were used in the present. The sheep dung was first air dried and mashed to reduce ammonium levels and inhibit the decomposition or vermicompost process. The rice straws were shredded into small pieces of 5-10 cm to allow an optimum decomposition (Garczyńska et al. 2020). Organic carbon was determined using the Walkley and black methods (Gelman et al. 2012) and total nitrogen concentration by Kjeldahl method (Abrams et al. 2014).

Decomposition

After being weighed, the prepared sheep dung and rice straw were combined to achieve C/N ratios of 25, 30, and 35. The formula for calculating the C/N ratio (Azim et al. 2014) is as follows:

$$\frac{C}{N}\ (mixture) = \frac{\sum_{n=1}^{\infty}(Q_n\ [C_n\ (100 - M_n)])}{\sum_{n=1}^{\infty}(Q_n\ [N_n\ (1 - M_n)])}$$

$\frac{C}{N}\ (mixture)$ = C/N ratio of the resulting materials to compost

Q_n = Quantity of the fresh material (n)
C_n = Total carbon content of the dry material (n)
M_n = moisture content of the fresh material (n)
N_n = Total nitrogen content of the dry material (n)

To maintained moisture content of the mixture between 55-60%, the water content was determined using the gravimetric method at regular intervals. Each mixture was put into a sack, compacted, and let decomposed for 14 days while, measuring the temperature of the mixture on a daily basis (Mironov et al. 2021).

Analysis of total bacteria and fungi

Total bacteria and fungi were enumerated before and after the decomposition process to determine the activity of indigenous microbes during the decomposition. For microbiological analysis, 10 g of mixture sample was resuspended in 90 mL of sterile saline, then a 10-fold serial dilution of the suspension was performed and appropriate dilution was plated on sterile growth media (Nutrient Agar and Potato Dextrose Agar) with a subsequent incubation for 48h at 37ºC (NA) and 5 days at 25ºC (PDA) (Tamizhazhagan et al. 2016; Mironov et al. 2021).

Vermicomposting

As decomposition process was completed, the mixture was removed from the sack and air-dried for several days to release any ammonia that might have formed during decomposition (Mohammed-Nour et al. 2019). The decomposed mixture was put into a 44.5 x 35 x 1.5 cm plastic box and the water content was maintained between 60-70% (Singh and Bhadauria 2012). After that, *Eisenia fetida* (Savigny 1826) earthworms (7-10% of the total mixture) were put into the box. The vermicomposting process was carried out for at least 14 days, then the earthworms were separated from the mixture and weighed while, the vermicompost was tested further (Garczyńska et al. 2020).

Determination of P, K, Ca, and Mg

Macro elements such as Phosphate (P), Potassium (K), Calcium (Ca), and Magnesium (Mg) were determined in the vermicompost products. Total P, K, Ca, and Mg was extracted with $HClO_4$ and HNO_3 (4:1 ratio). The concentration of P was measured with Molybdovanadate reagent and scanned by UV–VIS spectrophotometry at 822 wavelength (Bhat et al. 2017; Moldes et al. 2007). The K, Ca, and Mg concentration was measured using flame photometer in the diacid digest of the samples then determined by readings obtained from flame photometer using a standard curve (Bhat et al. 2017).

Data analysis

The experiment was conducted in a Completely Randomized Design and the data were subjected to Analysis of Variance. Any differences between treatments were further subjected to Duncan's multiple range test (Tamizhazhagan et al. 2016), and any differences among means for all traits were tested for significance at 5% level (Farag et al. 2015). The statistical analysis was performed using IBM SPSS ver. 20.

RESULTS AND DISCUSSION

Total bacteria on the mixed substrate of sheep dung and rice straw at various C/N ratios

Total bacteria from the mixture of sheep dung and rice straw (substrate) was detected to ensure the ability of bacteria to generate a decomposition of the organic material. Total bacteria in the mixture (before decomposition), after decomposition, and after vermicomposting are presented in Table 1.

The results of bacteria that have been isolated and identified at the onset and the end of the decomposition process and after the vermicomposting process are shown in Table 2.

Table 1. Total bacteria on the mixed substrate of sheep dung and rice straw at various C/N ratios

Treatment	Total bacteria		
	Mixture (× 10^{11} CFU/g)	**After decomposition (× 10^9 CFU/g)**	**After vermicomposting (× 10^9 CFU/g)**
T1	197.16 ± 9.32^a	107.83 ± 7.67^b	47,5 ± 4.75^b
T2	211.16 ± 10.59^{ab}	129.16 ± 6.91^c	29 ± 5.32.32^a
T3	220.88 ± 17.75^b	99.5 ± 4.32^a	50,33 ± 4.67^b

Note: Values are mean ± SD of six replications; $^{a–b}$ Different superscripts in the same row represent significant differences (p<0.05); T1: C/N ratio 25, T2: C/N ratio 30, T3: C/N ratio 35

Table 2. Identification of bacteria on various phase

Mixture	Process	
	After decomposition	**After vermicomposting**
Bacillus sp., Gram negative, no spore	*Bacillus* sp., Gram negative, subterminal spore	*Bacillus* sp., Gram negative, no spore
Bacillus sp., Gram negative, sub terminal spore	*Bacillus* sp., Gram positive, subterminal spore	*Streptobacillus* sp., Gram negative, no spore
Streptobacillus sp., Gram negative, no spore	*Streptobacillus* sp., Gram negative, no spore	
Staphylococcus sp., Gram negative, no spore		

Total bacteria in the mixture of sheep dung and rice straw at the beginning of the decomposition (mixture) were indigenous bacteria that degraded the complex organic material into simpler organic forms. These results were higher than those found by Mironov et al. (2021) which stated the number of mesophilic microorganisms was 3 x 10^6 CFU/g and thermophilic microorganisms 4 x 10^5 CFU/g in the initial phase of decomposition.

The total number of bacteria decreased after the decomposition process because of the changed temperature during the composting due to mesophilic bacteria activities. According to Ayilara et al. (2020), high temperatures are expected in the aerobic composting process to kill disease germs, pathogenic microorganisms and weed seeds in organic waste. Thermophilic bacteria are active in a range of 40-58°C. In this study, thermophilic bacteria ceased to grow and then decreased because metabolic products had inhibited bacterial development or organic matter for bacterial nutrition had depleted. It is in line with Wu et al. (2020) which stated that the total organic matter and nitrate are the most important factors of bacterial and fungal population change during the composting process. The present study also confirms regarding interactions with indigenous microorganisms, the changes in C/N ratio and temperature affect the survival of pathogenic microbes in the composting process.

The results showed that T2 (C/N ratio 30) produced the least bacteria, namely 29 x10^9 CFU/g. In vermicomposting, microorganisms work biochemically and earthworms contribute to the direct stimulation through fermentation of organic matter. According to Ramnarain et al. (2019), in the vermicomposting process, earthworms replace the activity of microorganisms, thus increasing the rate of mineralization of organic materials rapidly. The growth of earthworms is inhibited by the availability of carbon sources in their food ingredients (Joseph 2019). As bacterial activity decreases with organic matter and ultimately the number of bacteria, *Bacillus* sp. and *Streptobacillus* sp. are isolated at the end of the composting. *Streptobacillus* sp. degrade organic matter but *Bacillus* sp. help degrade organic matter in the decomposition process.

Total fungi on the mixed substrate of sheep dung and rice straw at various C/N ratios

Fungi contribute at the beginning of decomposition. Fungi is the first microorganism that helps penetrate the enzymes of microorganisms in degrading cellulose. Total fungi in the mixture before decomposition, after decomposition, and after vermicomposting are presented in Table 3.

The results of fungi isolated and identified at the onset and the end of the decomposition process and after the vermicomposting process are shown in Table 4.

The total fungi from the decomposition results had the same effect (p>0.05) on T1 and T3 treatments but were significantly different (p<0.05) in T2. The decreased number of fungi at C/N ratio 30 was due to the role of fungi as decomposers being replaced by earthworms that consume the nutrients to reproduce and migrate. According to Medina-Sauza et al. (2019), since, earthworms and microorganisms compete for carbon sources, earthworms replace the role of fungi as the lack of carbon required for its growth. Also, fungi decreased because earthworms consumed fungi as their selective feed (Domínguez and Gómez-Brandón 2012).

Based on our observations, the fungi isolated and identified at the mixture were *Aspergillus niger* van Tieghem 1867, *Aspergillus flavus* Link (1809), *Neurospora* sp., *Trichoderma* sp., and *Mucor* sp. These fungi are active at mesophilic temperatures (27.8-38.63℃) similar to the initial temperature of composting. The fungi stop growing as the temperature hits thermophilic stage, but resume growing once it reaches mesophilic temperature.

Table 3. Total fungi on the mixed substrate of sheep dung and rice straw at various C/N ratios

Treatment	Total fungi		
	Mixture (× 10^{11} CFU/g)	After decomposition (× 10^{9} CFU/g)	After vermicomposting (× 10^{9} CFU/g)
T1	27.34 ± 4.4572^a	3.67 ± 1.21^a	4.83 ± 2.578^b
T2	28.17 ± 3.0605^a	6.17 ±1.17 ^{b}	2.50 ± 3.234^a
T3	29.83 ± 3.1885^a	4.33 ± 1.03^a	4.83 ± 2.678^b

Note: Values are mean ± SD of six replications; $^{a–b}$ Different superscripts in the same row represent significant differences (p<0.05); T1: C/N ratio 25, T2: C/N ratio 30, T3: C/N ratio 35

The fungi isolated after the decomposition process were *A. flavus*, *A. niger*, and *Trichoderma* sp. At the end of the vermicomposting process, fungi that could be isolated and identified were *A. flavus*, *A. niger*, and *Neurospora* sp. Vermicomposting process provided a suitable environment for the fungi to grow at 20-29˚C (Kaplan et al. 1980).

Changes in the temperature and C/N ratio at decomposition of dung of sheep and rice straw at various C/N ratios

The decomposition of sheep dung and rice straw mixture (substrate) by indigenous microorganisms results in temperature change and a decrease in the C/N ratio which illustrated the activity of indigenous microorganisms in degrading the substrate organic matter. The temperature and C/N ratios achieved in the decomposition process of sheep dung and rice straw mixture with different C/N ratios are presented in Figure 1 and Figure 2, respectively.

In the decomposition process, the temperature in all treatments was observed between 32ºC-34ºC. The temperature in T1 and T2 rose to 60ºC on days 2 and 4, then decreased up to 27-28ºC on day 14. Meanwhile, the temperature in T3 continued to increase until day 5, but started to decrease on day 7 (56ºC) and keep going until day 14 (28ºC). It indicated organic matter degradation in the substrate of sheep dung and rice straw mixture in all treatments. In T3, the temperature took longer to decrease than in T1 and T2, presumably because organic material in T3 contained more carbon, requiring a longer degradation process. These results are in accordance with Topal et al. (2016) and Azim et al. (2018) which stated that the biodegradation process will be followed by an increase in temperature.

The initial C/N ratio in T1, T2, and T3 was 25, 30, and 35, respectively, and decreased to 22-24 as the decomposition process, occurred. It was evident that organic matter was degraded during decomposition process as indicated by temperature change. Similarly, Nayak et al. (2013); Nunes et al. (2016) and Pourzamani and Ghavi (2016) reported a decrease in C/N ratio during the vermicomposting process from various organic waste.

Changes in temperature during the decomposition process were caused by the heat generated from the activity of mesophilic microorganisms. It was in line with Galitskaya et al. (2017) that the activity of heat-generating microorganisms would increase the temperature above 40°C, indicative of organic matter degradation. The heat generated during the thermophilic phase can inhibit pathogenic microbes (>55°C) and weed seeds (>62°C).

Effect of C/N ratio on vermicomposting of sheep dung and rice straw mixture on earthworm biomass

In vermicomposting, earthworms decompose organic material on the substrate to grow and become earthworm biomass at the end of the process. Earthworm biomass data from vermicomposting mixture of sheep dung and rice straw at various C/N ratios are presented in Table 5. The results showed that the C/N ratio had no significant effect ($p > 0.05$) on the biomass of earthworms in T1 and T2 but was significantly different in T3 treatment. The T1 and T2 treatments produced higher earthworm biomass, because T2 was assumed to have the appropriate C/N ratio for the degradation of organic matter substrate by indigenous microorganisms and earthworms. These findings are in line with Nayak et al. (2013), which stated that the C/N ratio played a vital role in the composting process. The best compost was produced from C/N ratio 30 using sewage sludge mixed with livestock manure and sawdust. According to Aira et al. (2006), the C/N ratio affects the size of the earthworm population.

Table 4. Identification of fungi on various phase

Mixture	After decomposition	After vermicomposting
Aspergillus flavus	*Aspergillus flavus*	*Aspergillus flavus*
Aspergillus niger	*Aspergillus niger*	*Aspergillus niger*
Neurospora sp.	*Trichoderma* sp.	*Neurospora* sp.
Trichoderma sp.		*Trichoderma* sp.
Mucor sp.		

Table 5. Average biomass of earthworms from vermicomposting of sheep dung and rice straw mixture at various C/N ratios

Treatment	Earthworms biomass (g)
T1	112.67 ± 2.33^b
T2	114.83 ± 2.78 ^{b}
T3	105.17 ± 1.94^a

Values are mean ± SD of 4 replications; a–e Different superscripts in the same row represent significant differences (p<0.05); T1: C/N ratio 25, T2: C/N ratio 30, T3: C/N ratio 35

Table 6. Effect of C/N ratio on vermicomposting of sheep dung and rice straw mixture to N, P, K, Ca and Mg contents in vermicompost

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
T1	0.95 ± 0.01[a]	0.36 ± 0.01[a]	0.49 ± 0.02[a]	0.34 ± 0.04[a]	0.12 ± 0.02[c]
T2	1.11 ± 0.23[c]	0.56 ± 0.01[c]	0.51 ± 0.02[ab]	0.33 ± 0.02[a]	0.14 ± 0.02[b]
T3	0.98 ± 0.01[b]	0.44 ± 0.01[b]	0.53 ± 0.03[b]	0.32 ± 0.01[a]	0.14 ± 0.02[a]

The effect of C/N ratio on vermicomposting of sheep dung and rice straw mixture on the quality of vermicompost (N, P, K, Ca, and Mg contents)

The decomposed products are used by earthworms and microorganisms for their growth and activities to convert simple organic materials into nutrients that can be absorbed by the plant roots. According to Bidabadi (2018), the nutrients as an indicator of in vermicompost include nitrate, phosphorus, potassium, calcium, and dissolved magnesium. The content of N, P, K, Ca and Mg in the vermicomposting process of sheep dung and rice straw mixture with various C/N ratios are presented in Table 6.

The results of present study showed that T2 had the highest N content (1.11%) in vermicompost because the organic matter in the substrate was parallel with the number and types of indigenous microorganisms and earthworms, thus allowing a smooth decomposition of the substrate organic matter and mineralization process of forming N. Similarly, Ayilara et al. (2020) observed that a high C/N ratio in a substrate is not suitable for the growth and activity of microorganisms in degrading organic matter. According to Kaushik and Garg (2003), the symbiotic activity between indigenous microorganisms and enzymes in the intestines of earthworms transform organic matter into macronutrients (N, P, and K) in vermicompost.

The P content in vermicompost reached the highest value (0.56%). The P content is derived from sheep dung and rice straw which contain protein that breaking down during vermicomposting process. In vermicomposting process, the P element is used by microorganisms and worms for growth and activities to degrade the substrate, then a mineralization process occurs which is left in the vermicompost. This finding supports Goswami et al. (2013) and Suthar (2009) that phosphate in the substrate is converted into P by phosphatase in the intestines of the substrate during the vermicomposting process worms and microorganisms.

The Potassium (K) content was significantly different in T3 ($p < 0.05$), Ca was not significantly different across treatments ($p > 0.05$), but Mg was significantly different across treatments ($p < 0.05$). K content in vermicompost is derived from potassium which is in the forage from sheep dung and rice straw and is influenced by the vermicomposting process. Pattnaik and Reddy (2010) reported that K content changed differently according to the types of green waste used as a substrate. While incorporating plant forage (Neem leave, Parthenium, Sialkanta) into cow dung in vermicomposting, increase in K content takes place (Mistry et al. 2015), the total K in vermicompost is dependent on the raw materials and correlated with activity of earthworms and microorganisms during vermicomposting (Yan et al. 2013).

Ca and other minerals in vermicompost are influenced by the organic matter of substrate composition and the activity of plant worms and microorganisms during vermicomposting. Similarly, Yan et al. (2013) stated that high Ca content in sago waste stimulates earthworms to produce vermicompost with high Ca content. According to Pattnaik and Reddy (2010), Ca content in vermicompost is obtained from the substrate and the activity of earthworms, and Mg is associated with earthworm activity. Furthermore, Ramnarain et al. (2019) stated that the amount of Mg in vermicompost is higher than the substrate due to the activity of earthworms.

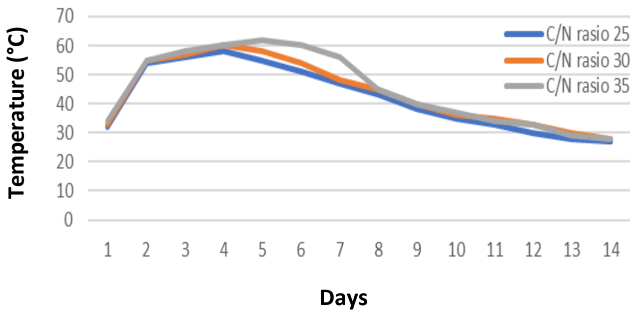

Figure 1. Temperature Changes in decomposition of Sheep dung and rice mixtures at various C/N ratios

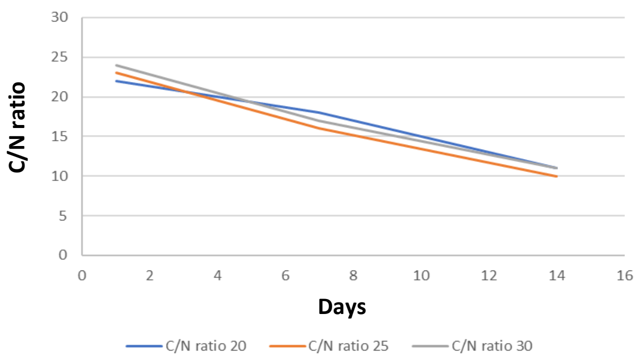

Figure 2. C/N ratio changes in the early decomposition of mixed sheep dung and rice straw

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