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Microbial Diversity in A Mixed Dairy Cow Manure and Chicken Manure Compost

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Abstract
The research on composting using livestock waste raw materials has been conducted in the Pagerwangi Lembang farmer group. Composting is done by mixing dairy cow feces with chicken feces, fermented for 50 days. The study aims to determine microbial diversity in the dairy cow and chicken feces compost. This study used a Completely Randomized Design of experimental methods and exploratory methods for descriptively analyzing qualitative data. Samples were taken from six predetermined places: fresh dairy cow feces, the initial mixture of dairy cow feces and chicken feces, and a mixture of dairy cow feces and chicken feces in tubs 1, tub 2, tub 3, and tub 4 with fermentation time in each tub for 10 days, replicated three times, totaling to 18 samples. Observations were made on days 0, 10, 20, 30, 40, and 50. Fecal coliforms dominated the results of initial observations of coliforms in feces. However, in a mixture of dairy cow feces and chicken feces obtained, fecal and non-fecal coliforms, after the fermentation process in tubs 1 to 4, coliform bacteria were not detected. The results of mold identification were Rhizopus sp, Penicillium sp, Mucor sp, Aspergillus sp, and Scapuloriopsis sp, Aspergillus sp being the dominant ones. At the same time, the types of worm eggs found in the composting process were obtained from two worms: Strongylus spp and Strongyloides.
Keywords: Faeces, dairy cows, compost, chicken manure, bacteria, coliforms, worms, endoparasites

Keragaman Mikroba pada Kompos Campuran Feses Sapi Perah dan Feses Ayam

Abstrak
Penelitian tentang pengomposan dengan bahan baku limbah peternakan telah dilakukan di kelompok peternak Pagerwangi Lembang. Pengomposan dilakukan dengan mencampurkan feses sapi perah dan feses ayam yang difermentasi selama 50 hari. Tujuan dari penelitian yaitu untuk mengetahui keragaman mikroba pada kompos campuran feses sapi perah dan feses ayam. Penelitian ini menggunakan metode eksperimental, yaitu Rancangan Acak Lengkap dan eksploratif untuk data kualitatif yang dianalisis secara deskriptif. Pengambilan sampel dilakukan pada enam tempat yang sudah ditetapkan yaitu Feses Sapi perah segar, campuran awal Feses Sapi perah dan Feses ayam, campuran feses sapi perah dan feses ayam bak 1, bak 2, bak 3 dan bak 4 dengan waktu fermentasi di setiap bak 10 hari, ulangan tiga kali jumlah sampel 18. Pengamatan dilakukan pada hari 0, 10, 20, 30, 40, dan 50. Hasil pengamatan awal terhadap Koliform dalam feses didominasi oleh koliform fekal, dalam media campuran feses sapi perah dan feses ayam diperoleh koliform fekal dan non fekal, setelah proses fermentasi dalam bak 1 sampai dengan bak 4 bakteri koliform sudah tidak terdeteksi. Hasil Identifikasi kapang diperoleh Rhizopus sp, Penicillum sp, Mucor sp, Aspergillus sp, dan Scapuloriopsis sp dan didominasi oleh Aspergillus sp, sedangkan jenis telur cacing yang ditemukan pada proses pengomposan diperoleh dari dua spesies cacing yaitu Strongylus spp, dan Strongiloides.
Kata kunci: Feses, sapi perah, pengomposan, feses ayam, bakteri, koliform, cacing endoparasit

Introduction

Lembang is known as an agricultural and dairy farming area, with the problem that the organic fertilizer used for agriculture comes from broiler farm waste, known as postal, which is imported from other areas. Farmers generally dispose of dairy farm waste directly into waterways to facilitate waste handling, which can harm the environment around the farm. Pollution is potentially caused by livestock waste due to its unpleasant odor and pollution of the waterways around the farm.

Waste from dairy farms still contains microorganisms, namely bacteria, mold, and endoparasites. Proper handling is essential to overcome the problem of dairy farm waste. This condition has received attention from various domestic and foreign parties considering that Lembang is a tourist area. Efforts to preserve the environment are made by conducting a program for managing dairy cow feces mixed with broiler feces as compost.

Composting is an environmentally friendly and practical approach to converting solid waste from animal husbandry and agriculture into organic fertilizer (Wan et al., 2021). In the composting process, biological decomposition occurs due to the activity of various microbes, which are essential factors in the depolymerization of organic matter (Xi et al., 2015). Bacteria and mold are the most abundant and actively involved microbes in composting (Rastogi et al., 2020). Mould's role is to decompose complex polymers into simple polymers, which can decompose carbon under dry, acidic, and low nutrient conditions (Wan et al., 2021), while the role of bacteria during composting is in the decomposition process, which generates heat (López et al., 2015). According to Tang et al. (2007), Based on the physicochemical properties of composting, the temperature is an essential indicator for compost maturation and reflects microbial activity during composting. Temperature also affects microbial abundance, and it has been found that mold tends to be more sensitive to temperature and moisture content fluctuations than bacteria (Zhang et al., 2011). According to (Carlos et al., 2019; Velde et al., 2018), the presence of endoparasite worms in the nematode group needs to be anticipated, considering the prevalence of endoparasite worms in cattle can cause anemia, decreased appetite, a drastic decrease in body weight, dull hair and skin, and hair loss. Mold and other mesophilic microorganisms will die when the composting process is in a thermophilic phase, namely at 40 - 60ºC, as evidenced by Nasir (2017). Suwatanti and Widiyaningrum (2017) stated that thermophilic microorganisms would be present at the beginning to the middle of the compost maturation process, and at thermophilic temperatures can kill pathogenic microbes and accelerate microbial activity and the organic matter decomposition rate.

Research on compost obtained from a mixture of cow feces and chicken feces has been done by several researchers, including Hasham (2018), regarding the composting of a mixture of cow feces and chicken feces on the nutrient content of the compost obtained results followed the 19-7030-2004 SNI Standard concerning compost specifications. Nur Alim Natsir (2018) found that mixed compost of 3 parts of cow feces and 1 part of chicken feces has a natural effect on the production and growth of soybeans. Hwang et al.'s (2020) researched compost and biogas production using a mixture of dairy cow feces and chicken feces on compost quality. The results of research by Suyanta et al. (2020) regarding compost mixed with cow feces and chicken feces obtained bacteria that could play a role in the composting process. This study aimed to determine the diversity of microbes that play a role in the composting process of a mixture of dairy cow feces and chicken feces from the beginning to the end of the composting process to obtain compost that is safe from penetration of pathogenic microbes.

Materials and Methods

Composting

The composting process used a modification of the Heap method through an aerobic fermentation process which was carried out in 4 tanks, with a length of 2.5 m x a width of 1 m x a height of 1 m in a cage of a dairy farming group in Pagerwangi Village, District, Lembang, Regency West Bandung with a span of 10 days in each tub. The compost data was: CN Ratio 27: 1; pH 6 with 50% water content. Samples were taken from 6 different places. Some of them are dairy cow feces. Dairy cow feces mixed with postal (chicken feces), tub 1 (20 days old), tub 2 (30 days old), tub 3 (40 days old), and tub 4 (50 days old). Duplo samples were taken from each place randomly with three replications. When the sample is obtained, it is placed into a plastic bag and stored in a cool box.

Statistical Analysis

The study used an experimental method with a completely randomized design for data on the number of bacteria and molds and an experimental method for qualitative coliform data, identifying types of molds and types of endoparasite worms.

Parameter

1. The number of bacteria
2. The amount of mold
3. The number of endoparasite eggs
4. Coliform test (faecal and non-faecal)
5. Identification of mold
6. Identification of endoparasite worms.

Methods

1. Calculate the number of bacteria and mold using the Total Plate Count (TPC) method (Kutsanedzie et al. 2012). To calculate the number of bacteria using Nutrient Agar (NA) media with a dilution technique up to the power of 9. Calculate the number of molds using Potatoes Dextrose Agar (PDA) media with a dilution technique up to the power of 3.
2. Macroscopic and microscopic observations using slide culture for molds were conducted at the Center for Animal Health and Veterinary Public Health Cikole Lembang, West Java. The method used was slide culture (Onion in Sanjaya et al., 2010) with the following procedure: A sterile Petri dish with sterile filter paper was prepared. The sterile filter paper was cut into circles and moistened using sterile distilled water to maintain the humidity of the culture in the Petri dish. A triangular retaining rod was stored in the Petri dish, and a sterile glass object and cover glass were placed on top of the retaining rod. Drops were dropped on the object glass using a pipette to make sterile suction. The fungus mycelium was taken using a needle pipette, stuck in the middle of the solidified agar median, and covered with a cover glass. Furthermore, culture slides were incubated at room temperature. After a number of incubation days, slide cultures were observed using a microscope at 40X magnification and then identified.
3. Observation of coliforms with the complementary stage by MPN method using Eosin Methylene Blue Agar (EMBA) media for macroscopic observations based on changes in colony color (APHA, 1989; Arslan, 2011).
4. The McMaster method calculated worm eggs using a counting chamber (Ekawasti et al., 2017). The procedure for worm egg calculation was as follows: 3 grams of feces dissolved with 17 ml of water for a few minutes to make it soft and then crushed. 40 ml of saturated salt solution was added to float the nematode eggs. While stirring, the feces solution was taken with a pipette equipped with a filter. The solution was then put in the MC Master counting chamber. The tests were conducted at the Cikole Veterinary Public Health and Animal Health Laboratory, Lembang, West Java.

Result and Discussion

1. Observation Result for Total Bacteria, Mold, and Endoparasite Worm Eggs

Livestock manure is one of the raw materials commonly used in composting, and the microbial community of cattle and poultry manure has been extensively explored during composting (Awasthi et al., 2019; Chen et al., 2019). Observations on the number of bacteria, mold, and endoparasite worm eggs obtained during the composting process of a mixture of dairy cow feces and chicken feces are shown in Table 1.

The composition of the microbial population has changed from the mesophilic stage (25.60°-40°C) to the thermophilic stage (42.50°C – 58.50°C) during the composting process. Then the last stage is the stabilization stage or the stabilization or the cooling stage. There was an increase in the number of endoparasite bacteria and eggs in the mixture of dairy cow feces and chicken feces. However, the increase in temperature had a significant effect on reducing the number of endoparasite bacteria and worm eggs. The bacteria in tubs 1, 2, 3, and 4 were suspected of coming from the Gram-positive bacteria group rather than the coliform group, as Gram-positive has spores that can live at thermophilic temperatures. In line with the opinion of Arslan et al. (2016), a range of 72.5–99.9% coliform in feces reduces after the thermophilic stage. At the end of the composting period, total coliform and feces coliform fecal were reduced by 99.9-100%. Suyanta et al. (2020) obtained Gram-positive bacteria that have the potential and play a role in the composting process, such as *Bacillus sp* and *Staphylococcus*. A very significant decrease in the number of molds occurred when the fermentation started in tubs 3 and 4 at thermophilic temperatures, in accordance with the research by Huhe et al. (2019) that molds were not resistant to thermophilic temperatures.

Table 1. The number of bacteria, mold, and endoparasite worm eggs during composting.

Average	Cow Faeces	Cow Faeces & Chicken Faeces Mixture	Tub 1	Tub 2	Tub 3	Tub 4
Total Bacteria (…X10^9 CFU/ml)	65,17^a ±13,37	105,17^a ±24,86	11,67^b ±3,52	9,17^b ±6,45	6,80^b ±3,84	6,70^b ±2,34
Total Mold (.X10^3 CFU/ml)	98,00^a ± 63,2	123,70^a ± 64,7	243,20^a ±78,52	72,30^a ±6,90	18,00^b ±7,10	1,80^c ± 0,30
Number of Endoparasitre Eggs (…EPG)	240,00	510,00	100,00	30,00	0,00	0,00
Temperature (ºC)	25,60^a	27,90^b	35,50^c	42,50^d	58,50^e	33,20^f

Note: Different lowercase letters toward the column indicate significant differences

Microbial diversity during the composting process begins with the presence of mesophilic groups, then the presence of thermophilic groups, and again the presence of mesophilic groups in line with changes in temperature. The process that occurs is the decomposition of complex compounds into simpler ones. Thermophilic microbes quickly break down organic substrates as substitutes for easily destroyed substrates, such as starch, sugar, and protein. Then the microbial population will increase when it reaches the final stabilization or cooling stage. During the thermophilic phase, the heat generated can kill pathogenic microbes (>55 °C).

The compost temperature in tub 3 (58.50°C) showed a significant reduction due to bacterial metabolism capable of killing almost all bacteria except spore-forming bacteria. Huhe et al. (2017) explained that temperature is one of the main factors controlling the rate of the composting reaction because it affects the rate of microbial metabolism and population structure in various composting phases. The heat that arises due to the release of energy is part of the exothermic reaction that occurs during the composting process. The increase in temperature that occurs in the accumulation of organic matter will produce a favorable temperature for thermophilic microorganisms. According to Subali and Ellianawati (2010), organic matter will decompose into CO_2, water vapor, and heat when the microbes in the compost react using oxygen. Then the temperature will gradually decrease after most organic matter decomposes. However, the activity of microorganisms will decrease due to the death of organisms due to high heat if the temperature exceeds 65–70ºC. These results are due to the previously high mold in tub 1 slowly dying and starting to decrease. Mold death was caused by an increase in temperature that occurred in Tub 2 and Tub 3. Mold is a mesophilic microorganism that will die if the temperature in the composting process changes to a thermophilic stage.

Table 1 shows that the results of identifying worms obtained by the parasitic worm egg infection standard show a mild infection (Novyan, 2010). Fermentation of cow dung can break the life cycle of worms in livestock because the eggs or larvae that come out through the feces will decompose due to the heating process (34°C) during the decomposition of cow dung. This condition will prevent and reduce the transmission of worm infections to other cattle (Sugama & Nyoman, 2011). Several factors led to the discovery of endoparasites in samples, including temperature and surrounding environment, cage cleanliness, feed hygiene, feed quality, and preventive and treatment measures. Farmers generally collect forage grass in the morning, so the forage given to livestock remains moist, a suitable place for endoparasite worms. In general, parasitic worms live in areas with a humidity temperature of 25-37℃. This temperature is very suitable for the growth of worm eggs into larvae (Sugama & Nyoman, 2011). A range of 72.5–99.9% coliform in feces was reduced after the thermophilic stage. At the end of the composting period, a range of 99.9-100% total coliform and fecal coliform were reduced.

2. Mold, Group of Coliform, and Endoparasite Worms Identification Results

Cattle feed contains more crude fiber, while chicken feed has a higher protein and antibiotic content (Chibisa et al., 2016; Wan et al., 2021). Dietary differences will likely impact the composition of the manure microbiota (Carmody et al., 2015). The gut microbiome of herbivores has been shown to be more diverse than that of omnivores. The results of microbial identification during the composting process are shown in Table 2.

Table 2. shows the fecal and non-feces coliform bacteria detected in dairy cow feces and a mixture of dairy cow feces and chicken feces. After the fermentation process took place in tubs 1, 2, 3, and 4, no fecal and non-fecal coliform bacteria were detected, in line with the opinion of Arslan (2016), which stated that in addition to high temperatures, a decrease in organic matter causes a decrease in coliform. Coliforms generally use highly degradable materials but cannot reproduce with complex compounds such as lignin and humic materials. Coliforms only reproduce in suitable conditions with adequate nutrition.

The results of microscopic observations in Table 2 are the results of the mold identification from the Ascomycota phylum, namely the genera *Aspergillus sp.*, *Scopuloriopsis sp.*, and *Penicillium sp.*, and from the zygomycota phylum, namely the genus *Mucor sp.* and *Rhizopus sp.*, *Penicillium sp.*, and *Rhizopus sp.* molds were detected in the mixed samples after the dairy cow feces were mixed with the chicken feces. *Penicillium sp* can also be found in organic materials. *Penicillium sp* was found in a mixture with aerobic conditions and a temperature of 27.9ºC. According to Sadhasivam (2008), *Penicillium sp.* can decompose organic matter better than other molds. Ascomycota and Basidiomycota fungi have been found as core phyla in cow and chicken dung. The relative abundance of the three phyla was initially higher in herbivores than in omnivore manure composts. Both have been shown to play an essential role in the decomposition of organic matter (Meng et al., 2019; Singh et al., 2003). Such diversity occurs during the composting process, caused by initial differences in the composition of the manure microbiota, determined by the feed and the composition of the animal gut microbiota (Chen et al., 2019). *Aspergillus niger* mold can help to break rough fiber in forage feed plants, so it will be very beneficial if found in cattle manure compost. *Aspergillus* can utilize many nitrogen sources (Krappmann & Braus, 2005), while *Bacillus* bacteria are involved in the biodegradation of organic matter during composting (Wang et al., 2020).

Strongiloides and *Strongyle spp.* from the nematode worms class were found in the cattle feces samples. This was because the feces were fresh and had not gone through the composting process. In mixing cow feces with postal, there were also *Strongiloides*. Whereas in tub 1, the compost, feces, and chicken postal which had been mixed and left for 20 days, only contained *Strongiloides*, which had started to decrease, and the next day, in tubs 2, tubs 3, and tubs 4, worms were not found in the composting process. This was due to the decomposition of organic matter by microorganisms which produce high temperatures reaching 58℃. Temperatures over 30 ℃ cause endoparasites to decrease or die. This is in accordance with the statement of Abbasi et al. (2012) that endoparasite worms are unable to survive at temperatures exceeding 37ºC. Therefore, the worms cannot survive and eventually die.

Table 2. Identification results of coliform bacteria, mold, and endoparasite worm eggs during composting.

Microorganism	Feces	Mixture	Tub 1	Tub 2	Tub 3	Tub 4
Coliform	Fecal	Fecal & Non-Fecal	-	-	-	-
Mold	*Mucor sp*	*Penicillum sp* *Scopuloriopsis sp*	*Aspergillus sp* *Rhizopus sp*	*Aspergillus sp* *Scopuloriopsis sp*	*Aspergillus sp*	*Rhizopus sp* *Penicilium sp*
Endoparasite Worm	*Strongylus sp* *Strongyloides*	*Strongyloides*	*Strongyloides*			

Conclusion

The number of microbes in the compost mixed with dairy cow feces and chicken feces is affected by changes in temperature. Thermophilic temperature can reduce the number of bacteria, molds, and endoparasite eggs. The diversity of mold in compost mixed with dairy cow and chicken feces plays an important role in the decomposition of organic matter.

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