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The Effect of Molasses Dosage on The Production of Eco-Enzymes from Beef Cattle Feces and Rice Straw on Total Bacteria, Mold, and Yeast

Fitta Dheifiani Lutfiah[1], Eulis Tanti Marlina[2], Yuli Astuti Hidayati[3]

[1]*College of Animal Husbandry Study Program, Faculty of Animal Husbandry, Padjadjaran University, Sumedang 45363, West Java*

[2]*Lecturer at the Faculty of Animal Husbandry, Padjadjaran University, Sumedang 45363, West Java*

[3] *Lecturer at the Faculty of Animal Husbandry, Padjadjaran University, Sumedang 45363, West Java*

Corresponding Author: eulis.tanti@unpad.ac.id

ABSTRACT: *Livestock waste, especially beef cattle feces, can be processed into eco-enzymes. This research aims to determine the effect of the molasses dosage on the production of eco-enzymes from beef cattle feces and rice straw on the total bacteria, mold, and yeast. The raw material used in making eco-enzymes filtrates from the decomposition of beef cattle feces and rice straw with a C/N ratio of 30. Water and molasses are added to the filtrate and fermented by facultative anaerobes for 21 days. The research method used a Completely Randomized Design (CRD) with 3 treatments, P1=2.5%, P2=5.0%, and P3=7.5% molasses, with 6 replications. The parameters measured were total bacteria, total mold, and total yeast. The data was analyzed using the Analysis of Variance and Tukey advanced test. The results showed a significant effect (P<0.05) on giving different molasses doses by producing the highest total bacteria at a molasses dose of 7.5% and the highest total mold and yeast at 5.0%.*

KEY WARDS: *eco-enzymes, beef cattle feces, molasses, bacteria, mold, yeast*

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I. INTRODUCTION

The beef cattle population in Indonesia in 2022 increased by 3.52% from the previous year [5]. The increase in beef cattle population in Indonesia is in line with the increase in livestock waste produced, while the management of livestock waste in Indonesia is still not the focus of farmers. The waste can be in the form of livestock feces and urine, which, if not managed properly, can damage the environment. Processing livestock waste, especially beef cattle feces can be converted into something useful and economically valuable. One form of waste management is by making it into eco-enzyme products.

Eco-enzymes are the result of fermentation from organic waste that can come from fruits, vegetables, and other organic waste [12]. In addition to vegetables and fruits, beef cattle feces also include organic waste so that it can be made into eco-enzymes. Eco-enzyme is an organic solution from the fermentation of fruit waste with the addition of water and sugar that can use selective microorganisms such as yeast and produce mineral salts, organic acids, proteins, alcohols, and enzymes [17]. Eco-enzymes can be used as sanitizers, disinfectants, organic fertilizers or soil conditioners, and bio-detergents [10]. Eco-enzymes use facultative anaerobic fermentation, where microorganisms in the fermentation can live using oxygen or without oxygen. Microorganisms that grow in the fermentation process are bacteria, yeasts, and molds.

The process of making eco-enzymes from beef cattle feces and rice straw goes through several stages. The process of making eco-enzymes from beef cattle feces begins with the initial decomposition stage. Initial decomposition is the process of breaking down complex compounds in feces into simpler compounds. Organic matter in feces has not broken down into nutrients, so it needs a decomposition process first [17]. In the initial decomposition stage, the C/N ratio needs to be considered because this ratio is the content needed by decomposing microorganisms to grow. Beef cow feces have a low C/N ratio of 21.5 [17]. Huang et al. (2004), the ideal C/N ratio for decomposing organic matter ranges from 25 - 30. Therefore, to increase the C/N ratio, other materials are needed as an additional source. One of the materials that can be used is rice straw with a C/N ratio of dry rice straw of about 44%.

The next stage is extraction using hot water and filtration, which produces filtrate (liquid) and substrate (solid). Microorganisms that grow in the initial decomposition process will be extracted from the filtrate through the filtration process. This filtrate will be used as a raw material for eco-enzyme production. The filtrate will go through a facultative anaerobic fermentation process before becoming an eco-enzyme. During fermentation, living microorganisms require energy sources for their growth. One of the energy sources that can be used is molasses, which is a by-product of the production of sugar cane (*Saccharum officinarum*). Molasses will be a source of food and a carbon source for microorganisms during the fermentation process, this is because the main nutrients for microorganism growth are carbon, nitrogen, and some minerals [1].

The amount of molasses used affects the speed of microorganisms in decomposing and fermenting, so the higher the dose of molasses used, the faster the decomposition and fermentation processes that occur [25]. Microorganisms that grow in eco-enzymes fermentation are bacteria, molds, and yeasts. High total bacteria will cause a low pH because bacteria produce organic acids. Eco-enzymes that have high citric acid and acetic acid content have a low pH [9]. Generally, bacteria that grow on eco-enzymes are lactic acid bacteria. Meanwhile, molds produce a variety of enzymes, such as protease, amylase, and lipase enzymes. Molds produce amylase enzymes that can break down starch into simple sugars, and the sugar is converted into alcohol by yeast [4]. Eco-enzymes fermentation with the use of different doses of molasses can affect the total bacteria, molds, and yeasts. The study aims to determine the best molasses dose that produces the highest total bacteria, molds, and yeasts.

II. MATERIAL AND METHODS

2.1 Research Materials

The research materials used were beef cattle feces, rice straw, hot water, molasses, physiological NaCl 0,9%, distilled water, alcohol, Nutrient Agar (NA), Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and chloramphenicol antibiotics.

2.2 Research Methods

The production of eco-enzyme begins with the initial decomposition process for 7 days. In the initial decomposition, it is necessary to analyze the C/N ratio of beef cattle feces and rice straw, then weigh according to the calculation and mix until homogeneous in a compost bag. The results of initial decomposition that have reached a moisture content below 20% are extracted using hot water so that the decomposing microorganisms are suspended and continued with the filtration process using the multistage filtration method, obtaining filtrate, which is the liquid filtration results as raw material for making eco-enzymes. The filtrate was mixed with water in a ratio of 1:9 to produce an aqueous filtrate. Then, the filtrate was mixed with water and various doses of molasses in each jar, with molasses dosages of 2.5%, 5.0%, and 7.5% [16]. The diluted filtrate and molasses were homogenized, and the jars were tightly closed using duct tape to prevent leakage. Subsequently, it was fermented for 21 days in a facultative anaerobic.

2.3 Parameter Measurement

The media used to grow bacteria is Nutrient Agar (NA), the media for mold is Potato Dextrose Agar (PDA), and the yeast media is Malt Extract Agar (MEA). To grow bacteria, molds, and yeasts using the pour plate method and calculated by Total Plate Count (TPC) [14]. Stages of work include the sterilization of equipment and media used. Then dilution is done using a test tube filled with 9 ml of physiological NaCl 0,9%. Dilution is carried out according to the growth of the desired microorganisms. The suspension of the last dilution used was put into a Petri dish aseptically and poured with growth media. Petri dishes for bacterial growth were kept in an incubator for 24 hours, mold at room temperature for 72 hours, and yeast for 48 hours. Calculation of bacteria, molds, and yeasts using the formula [24]:

$$\mathrm{CFU}/ml = \sum \mathrm{Colony} \times \frac{1}{Dilution\ factor}$$

2.4 Statistical Analysis

The research method was carried out with a Complete Randomized Design (CRD) experimentally using 3 treatments. The treatments are the addition of molasses doses in diluted filtrate as much as 2.5% (P1), 5.0% (P2), and 7.5% (P3), and all treatments were repeated six times so that 18 experimental units were obtained. Data on total bacteria, molds, and yeasts obtained were analyzed using analysis of variance and the Tukey test with a significance level of 5% using IBM SPSS 23.

III. RESULTS AND DISCUSSIONS

3.1 Total Bacteria

The results of the study on the effect of giving different doses of molasses fermented for 21 days in the production of eco-enzymes from beef cattle feces and rice straw on total bacteria are presented in Table 1.

Table 1. Total Bacteria at Different Doses of Molasses

Treatment	Total Bacteria ($\ldots \times 10^{10}$CFU/ml)
2.5% (P1)	$1.63 \pm 0.75^{a*}$
5.0% (P2)	1.27 ± 0.78^{a}
7.5% (P3)	8.64 ± 1.63^{b}

***The different superscript letters in the same column indicate significant differences (P<0.05)**

Table 1 shows that the molasses doses of 2.5%-7.5% produced different total bacteria. The highest total bacteria were in P3 where the dose of molasses given was 7.5%, compared to the other two doses. Through the analysis of variance, it was found that there was a significant effect ($P < 0.05$) of molasses doses of 2.5%, 5.0%, and 7.5% on the total bacteria in the production of eco-enzyme from beef cattle feces and rice straw. The Tukey test results showed a significant difference between the treatment of 7.5% molasses dosing with 2.5% and 5.0% molasses dosing, but the 2.5% and 5.0% molasses dosing did not show a significant difference. This indicates that the 7.5% molasses dose has significantly higher total bacteria than the other two doses. This is in line with the research of Marlina et al. (2024), where the treatment of 7.5% molasses dosing in the fermentation process of making probiotics from a mixture of milk sludge and rice straw produced the highest total bacteria.

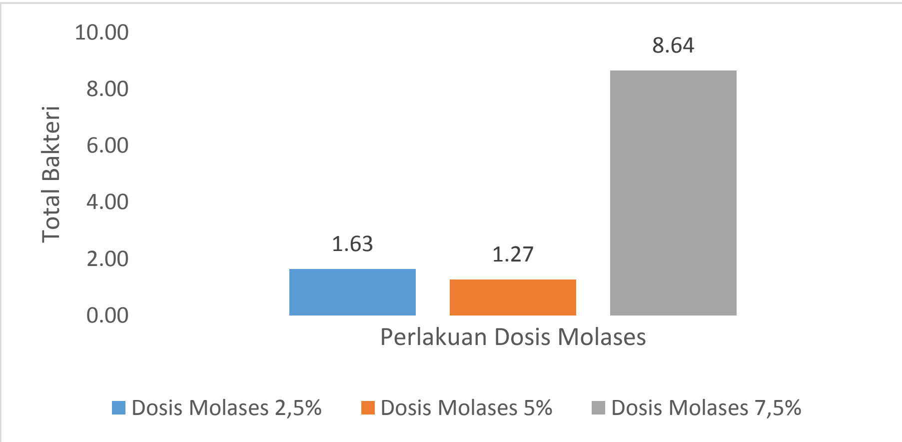

Figure 1: Total Bacteria at Different Doses of Molasses

It can be seen in Figure 1 that the highest total bacteria are in the 7.5% molasses dose where microorganisms such as bacteria need optimal nutrition for energy needs. Giving a dose of 7.5% molasses produces the highest total bacteria which can be caused by the sugar content contained in molasses optimally used by bacteria as an energy source for metabolism. Generally, bacteria that grow on eco-enzymes are lactic acid bacteria. Lactic acid bacteria can be found in environments with high carbohydrates. The more molasses added, the more carbohydrates available so that lactic acid bacteria can grow faster [6]. According to Herawati et al. (2017) [13], lactic acid bacteria can grow, and the fermentation pH becomes low with the addition of molasses which increases the availability of soluble carbohydrates. The number of lactic acid bacteria is strongly influenced by the amount of nutrients, synergy between lactic acid bacteria, fermentation time, and fermentation temperature [21].

3.2 Total Mold

The results of the study on the effect of giving different doses of molasses fermented for 21 days in the production of eco-enzymes from beef cattle feces and rice straw on total molds are presented in Table 2.

Table 2. Total Mold at Different Doses of Molasses

Treatment	Total Mold (... $\times 10^3$ CFU/ml)
2.5% (P1)	$1.62 \pm 0.58^{ab*}$
5.0% (P2)	8.35 ± 5.43^{b}
7.5% (P3)	1.12 ± 0.93^{b}

***The different superscript letters in the same column indicate significantly different (P<0.05)**

Table 2 shows the average total mold in eco-enzyme fermentation from the filtrate of beef cattle feces and rice straw in the treatment of different molasses doses. The results of the analysis of variance showed an effect (P < 0.05) on total mold in the treatment of different molasses doses during eco-enzyme fermentation. Through Tukey's test, the results were significantly different between 5.0% molasses dose and 7.5% molasses dose, but 2.5% molasses dose with 5.0% and 7.5% showed no significant difference. Total mold in eco-enzyme with 5.0% molasses dosing treatment was significantly higher than total mold in 2.5% and 7.5% molasses dosing.

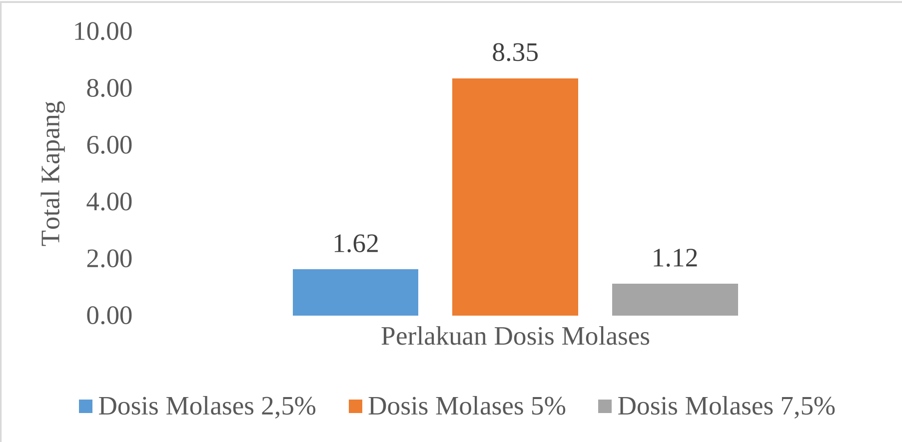

Figure 2: Total Mold at Different Doses of Molasses

Based on Figure 2, the highest total mold was at a molasses dose of 5.0%. Substrate as a provider of nutrients such as carbon sources, nitrogen sources, energy sources, and growth sources in the form of vitamin minerals greatly affect mold growth [23]. Fermentation with different dosage treatments produced different total molds. This can be caused by the fermentation rate of different molds in each dose. The highest mold growth at 5.0% molasses dose can be caused by adequate nutritional conditions for mold during fermentation. The growth of mold at a dose of 7.5% molasses was inhibited because the dose was too high. This is by the statement of Ariyanto et al. (2013) [2] that a dose of sugar that is too high results in the fermentation rate of microorganisms that play a role in running slower.

In addition, mold growth is not only influenced by the substrate, but other factors affect it, including pH, water activity, temperature, and inhibitory components. Mold can grow due to low pH conditions in eco-enzyme fermentation. Low pH conditions, low water content, low nitrogen, and certain nutrients are absent to become a place for mold growth [7]. In addition to pH, the presence of inhibitory components also affects mold growth. This is supported by the statement of Fardiaz (1992), that the component is fungistatic, which inhibits mold growth, or is fungicidal, which kills mold [11]. These components are generally produced by bacteria. Fewer molds grew at other molasses doses because these doses produced high total bacteria where molds usually lose competition with bacteria when microorganisms grow in favorable conditions. A high molasses dose will make many lactic acid bacteria grow, these bacteria dominate quickly and can defeat mold growth [15].

3.3 Total Yeast

The results of the study on the effect of giving different doses of molasses fermented for 21 days in the production of eco-enzymes from beef cattle feces and rice straw on total yeasts are presented in Table 3.

Table 3. Total Yeast at Different Doses of Molasses

Treatment	Total Yeast (... $\times 10^4$CFU/ml)
2.5% (P1)	$1.06 \pm 0.52^{a*}$
5.0% (P2)	6.78 ± 4.35^{b}
7.5% (P3)	2.78 ± 1.86^{ab}

***The different superscript letters in the same column indicate significantly different (P<0.05)**

Table 3 shows the average total yeast in the treatment of different doses of molasses on total yeast. The results of the analysis of variance showed a significant effect ($P < 0.05$) on the total yeast in the treatment of different doses of molasses in the production of eco-enzymes from beef cattle feces and rice straw. Through the Tukey test, the results were significantly different between the molasses dose of 5.0% and molasses 2.5%, but the molasses dose of 7.5% was not significantly different from the molasses dose of 2.5% and 5.0%. Total yeast in eco-enzymes with 5.0% molasses dosing treatment was significantly higher than the total yeast in 2.5% and 7.5% molasses dosing.

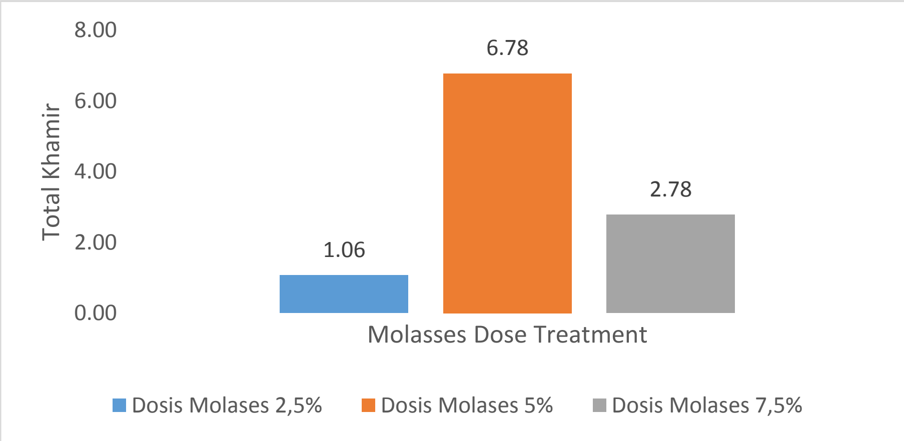

Figure 3: Total Yeast at Different Doses of Molasses

Figure 3 shows the highest total yeast was in the molasses dose of 5.0%. Eco-enzyme fermentation with a molasses dose of 5.0% resulted in the highest yeast growth compared to other doses, in contrast to research conducted by Marlina et al. [16] where the highest total yeast was in the molasses dose of 7.5%. This can be caused by differences in the length of fermentation used because one of the factors of yeast growth besides nutritional conditions is the length of fermentation [22]. Yeast during fermentation will also produce carbon dioxide, where the longer the fermentation, the more carbon dioxide will be formed [3]. This is not good for the growth of yeast, according to Datar et al. (2004), the growth of yeast during fermentation will stop when there is gas production, then it will produce alcohol again if carbon dioxide has been removed.

Giving too high a concentration of sugar in fermentation can cause cell death because the concentration outside the cell is higher than inside the cell so the osmosis process occurs [20]. Therefore, the growth of yeast at a dose of 5.0% molasses in the production of eco-enzymes is sufficient for the needs of yeast. Yeast produces ethanol and carbon dioxide in the production of eco-enzymes, the addition of sugar to the fermentation is to obtain a higher alcohol concentration, but yeast activity will be inhibited at high sugar concentrations [19]. Too high a concentration of ethanol can inhibit the growth of yeast and only alcohol-tolerant microorganisms can grow [8]. Ethanol produced by yeast in fermentation can make yeast experience protein denaturation and damage cell membranes.

IV. CONCLUSIONS AND RECOMMENDATIONS

Dosing of molasses in the preparation of eco-enzyme from mixed filtrate of beef cattle feces and rice straw affects total bacteria, mold, and yeast. The dose of molasses that produces the highest total bacteria in the preparation of eco-enzyme from mixed filtrate of beef cattle feces and rice straw is a dose of 7.5% molasses while the highest total mold and yeast is at a dose of 5.0% molasses. Based on the results of the study, a dose of 7.5% molasses is recommended to produce the highest total bacteria.

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