Evaluation of Microorganism from Integrated Processing Mixed Sheep Feces and Rice Straw as Starter Culture

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Abstract. This study aims to determine the potential of microorganism in processing of mixed sheep feces and rice straw, used as a starter in the preservation of forage feed. The method is, in the 1st phase with 3 treatments of carbon and nitrogen ratio with 6 repetitions and the 2nd phase with 3 treatments of molasses in the base medium, which are grouped in dry matter content with 6 replications. The results of the 1st phase showed the preferred treatment of T2 (C/N ratio 30) yielded an initial number of bacteria of $2.87 \times 10^{14}$ cfu/g and fungi $9.6 \times 10^{13}$ cfu/g, and decreased into $4.2x \times 10^{10}$ cfu/g and $3.7 \times 10^{11}$ cfu/g, respectively. At the end of initial degradation, bacteria identified were Bacillus sp and fungi identified were Aspergillus niger, Aspergillus glaucus, Aspergillus terreus, Rizophus sp. The results of the 2nd phase showed that the highest bacterial colonies were achieved in T2 with DM3 of $38 \times 10^{6}$ cfu/ml and the bacterial consortium identified were Bacillus sp and Lactobacillus sp. The number and types of bacterial consortium isolated and identified in the liquid medium can be used as a starter in the preservation of forage feed.

1. Introduction

In the sheep farming, waste is produced in the form of feces, urine and the remaining feed in the cage. The amount of feces produced 5 - 10% per head per day, a considerable amount if concentrated in one location. Feces contain a number of microorganisms that can be utilized and pathogenic microorganisms, in addition feces contain organic materials that can be exploited by microorganisms to grow. Based on this, feces have the potential as a source of nutrients and sources of microorganisms that can be utilized further through integrated processing.

Integrated processing is a system of processing waste gradually and produces a wide range of products, one of which inoculum a bacterial consortium that can be used as a starter in the preservation of forage feed. This bacterial consortium can be obtained by isolating and identifying bacteria and fungi from the substrate (sheep's feces and rice straw) through an integrated treatment that begins with the initial degradation stage of the substrate. The number of bacteria and fungi growing in the substrate, in the later stages is extracted to obtain the source of inoculum and nutrients in the form of single cell proteins, and the next step adds carbon source as a source of energy for the growth and activity of bacterial consortium to be used as starter for pickling of forage feed.

Forage feed is needed in its availability, through the processing of forage feed preservation is guaranteed its availability. In the processing of preservation of forage feed is needed starter that has been obtained by buying a starter with a price that is quite expensive, so it is necessary to make starter by utilizing available resources that utilize sheep feces with integrated processing. To ensure the quality of starter produced, it is necessary to evaluate the content of bacterial consortium to be used as starter.

This research is needed to evaluate the potential of bacteria in starter, obtained from sheep feces through integrated processing. The results of this study, in addition to producing inoculants of a
bacterial consortium that can be used as a starter in preserving forage feed, is also friendly to the environment, because in the process of processing can kill pathogenic microorganisms in the feces.

2. Material and Methods

2.1. Materials

The material used in this research is sheep feces and rice straw, and materials for bacterial and fungal isolation (500 g Nutrient Agar medium, 500 g Potato Dextrose Agar, 500 ml NaCl, distilled water, materials for identification of bacteria and fungi, carbol gentiana violet, lugol, 250 ml alcohol 95%, 100 ml air fuchsins, immersion oil, alcoholic acid, H2SO4, methylene blue, carbol fuchsins).

The research method used in this research, the 1st phase is experimental method in laboratory using complete randomized design with 3 treatments (T1 = C / N ratio 25, T2 = C / N ratio 30, T3 = C / N ratio 35 with 6 repetitions and the 2nd phase, using a randomized block design with 3 treatments (T1 = addition of 2.5% molasses in the base medium T2 = addition of 5% molasses in the base medium T3 = 7.5%), which are grouped in basic media with dry matter content of each DM1 = 3.08%, DM2 = 2.49%, DM3 = 2.26%, DM4 = 1.08% with 6 replications. The variables observed in the Phase 1 were the number and types of indigenous bacteria and fungi that grown at the beginning and end of the initial degradation process of integrated treatment of sheep feces and rice straw mixture. The variables observed in the 2nd phase were the number of colonies and types of bacteria grown on liquid media resulting from the integrated processing extraction of mixture of sheep feces and rice straw.

2.2. Research procedure

This research consisted of two phases. First phase was isolation and identification bacteria and fungi on initial degradation process. Sheep feces with 60% moisture content were used as a sample. Sample of sheep feces and rice straw were analyzed C and N content through Walkley Black and Kjedahl method respectively. In the first phase, treatments were based on C/N content of sheep feces and rice straw mixture: 25, 30, and 35. After substrate for each treatment obtained, substrate incubated for 1 week (initial degradation). Substrate aerated until the moisture content reaches 16%. Fungi and bacteria grew on before and after initial degradation were isolated and identified. Second phase was addition of molasses in the base medium (T1 = 2.5%, T2 = 5%, T3 = 7.5%) into mixture of sheep feces and rice straw to produce starter for forage preservation. Substrate from initial degradation was extracted using hot water solvent with a ratio of 1: 4 (1 kg of substrate: 4 kg hot water) to DM1 (DM = 1: 8 for DM2, 1:12 for DM3 and 1: 16 for DM4; (DM1 = 3.08%, DM2 = 2.49%, DM3 = 2.26%, DM4 = 1.08%). Growing indigenous bacteria from initial research into basic media (DM1, DM2, DM3, DM4) in liquid form which added molasses according to treatment, then incubated anaerobically for 2 weeks. Then isolated calculated the amount of bacterial consortium and identified bacteria growing at each treatment. Treatments that produce the most bacterial colonies that will be used as starter in the preservation of forage feed.

3. Result and Discussion

3.1. Temperature and pH on the initial degradation process of the sheep feces and rice straw substrate with various C/N ratio

Based on observations, the temperature and pH achieved in the initial degradation process can be seen in Figure 1 and Figure 2.

Observations on the initial degradation process of the substrate of sheep and rice straw, at all treatments, on the initial substrate, the temperature reached 65-62 °C on days 1 and 2, then tended to decrease until day 7 to a temperature of 32 °C, all treatments yielded a pattern decrease the same temperature, this describes the decomposition of organic matter on the substrate. This is in line with the opinion [1, 2], which states on decomposition of aerobic organic material in composting will be followed by high temperature.
Likewise at the beginning of the process, the pH values reached 9.77 - 9.49, then tend to decrease until the 7th day pH reaches 9.33-9.44. All treatments produce the same pattern of pH reduction, this illustrates that the decomposition of organic ingredients will reduce the pH value. This is in line with the opinion [2], which states the pH value of the substrate will change according to the content of organic material present in the substrate.

3.2. Number of bacteria and fungi in the early degradation process on the substrate of sheep feces and rice straw

Based on observations, the number of bacteria and fungi that were successfully isolated can be seen in Figure 3 and Figure 4.

The results of observations on various treatments, at the beginning of the degradation process, the number of bacteria isolated 241 x 10^{10} cfu / g to 287 x 10^{10} cfu / g, then at the end of the initial degradation process the number of isolated bacteria 42 x 10^{10} cfu / g to 49 x 10^{10} cfu / g, in all treatments showed a decrease in the number of bacteria, however the highest decrease was achieved in the treatment of T2 (C / N ratio 30) which reached 85.37%, this illustrates that in the treatment of T2 degradation process of organic substrates is good, this produces high temperatures and such conditions can kill pathogenic bacteria. Presumably in the treatment of T2, the nutrient balance contained in the substrate corresponds to the growing need for indigenous bacteria and activity on the substrate, this is in line with the opinion [3, 4], which states that bacterial growth is influenced by nutrients and the amount of bacterial inoculum. However, the number of isolated bacteria at the end of the degradation
The process is still possible to be used as a starter in the subsequent process in the integrated processing of sheep and rice straw. Because in the waste treatment process, there is decomposition of organic material that causes heat and causes the temperature at the beginning of the waste treatment to be high (50 °C) and results in the death of pathogenic bacteria contained in the waste, the pathogenic bacteria live at 37 °C. This is supported by the opinion [5, 6] which states the number of bacteria that can be used as a starter from $10^6$ to $10^8$ cfu / g.

**Figure 3.** The bacteria isolated before and after the initial degradation process on various C / N ratio.

**Figure 4.** The fungi isolated before and after the initial degradation process on various C / N ratio.

Similarly, the number of fungi at the beginning of the degradation process was $48 \times 10^{10}$ cfu/g to $89 \times 10^{10}$ cfu/g, then at the end of the initial degradation process the number of bacteria isolated was $4 \times 10^{10}$ cfu/g to $9 \times 10^{10}$ cfu/g. the highest decrease was achieved in T2 (C / N ratio 30) treatment which reached 94.8%. The amount of bacteria and fungi at the end of the degradation process allows to be used as a starter in the subsequent process in the integrated processing of sheep feces and rice straw.

3.3. Identification of bacteria and fungi in the initial degradation process on the substrate of sheep and rice straw

Based on observation result, bacteria and fungi identification result on early degradation process on sheep feces substrate and rice straw can be seen in Table 1. The observations showed that the bacteria identified before the initial degradation process were *Bacillus* sp, *Escherichia coli*, *Enterobacter* sp, *Pseudomonas* and after the initial process of bacterial degradation identified only *Bacillus* sp. This shows that bacterial pathogens die during the initial degradation process, because in the process of degradation a high temperature (65 °C) is formed which
can kill pathogenic bacteria. This is in line with the opinion [7], which states that *Escherichia coli*, *Enterobacter sp*, *Pseudomonas* will live at temperature 5 – 30 °C.

Fungi that were identified before the initial degradation process were *Aspergillus* sp, *Neoprenpora sitopila*, *Rhizophus* sp, after the initial degradation process of the identified fungi were *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus terreus*, *Rizopus* sp. During the initial degradation process the fungus *Neurospora sitopila* die, because the fungus only live at 20 – 30 °C environmental temperature [8], While the fungi that remain alive until the initial degradation process ends, will serve as a single cell protein source in the next process.

### Table 1. Identification of bacteria and fungi in the initial degradation process on the substrate (sheep feces and rice straw).

| Bacteria Identified | Day 1 | Day 7 | Fungi Identified | Day 1 | Day 7 |
|---------------------|-------|-------|------------------|-------|-------|
| *Bacillus* sp       | *Bacillus* sp | *Aspergillus terreus, Aspergillus sp* | | | |
| *Escherichia coli*  | | | *Aspergillus glaucus* | | |
| *Enterobacter sp*   | | | *Aspergillus terreus* | | |
| *Pseudomonas* sp.   | | | *Neurospora sitopila, Rhizophus sp* | | |

#### 3.4. Number of Bacteria Consortium on liquid media with various treatments.

Based on the observation result of the isolation of the number of bacterial consortium growing on each treatment can be seen in Figure 5.

![Figure 5](image-url)

**Figure 5.** The number of bacterial consortiums grown in each treatment

The results showed that the highest growth of bacterial consortium was obtained at treatment of T2 (T2 = addition of 5% molasses) and at DM3 = 2.26%, i.e. 780 x 10^7 cfu/ml. This is predicted because the amount of dry matter 2.26% and 5% molases in T2 DM3 support the growth of indigenous bacterial consortium on liquid media resulting from integrated processing of sheep feces and rice straw. This is in line with the opinion [3, 4], which states that the number of growing bacteria is affected by the balance of inoculum and the availability of nutrients and environmental conditions.
Figure 6. Average pH at various treatments

Average pH achieved in T2DM (addition of 5% molasses in all percentages of dry matter) treatment is 3.26 describes the acidic media conditions and suspected bacteria that grow is a group of lactic acid bacteria. This is in line with the opinion [9] which states that lactic acid bacteria grow at acid pH conditions.

3.5. Identification of Bacterial Consortium on liquid media with various treatments

Based on observations, found some bacteria with rod-shaped, gram positive, facultative anaerobic, nonspore-forming, can ferment carbohydrate by forming lactic acid and identified are Bacillus sp, Lactobacillus sp. Almost all Lactic Acid Bacteria (LAB) only get energy from sugar metabolism, so their growth habitats are limited to environments that provide enough sugar. Nutritional requirements of LAB complex include amino acids, vitamins, purines and pyrimidines. Lactic acid produced from LAB can provide bacteriocidal effect for other bacteria because the pH of the environment can decrease to 3-4.5. At that pH LAB remains alive, while other bacteria, including bacteria, decompose food will die [9].

This study will help researchers to uncover the number and type of bacteria in the starter for the preservation of forage feed that many researchers do not know. Thus a new theory of bacterial consortium as starter for forage preservation can be obtained.

4. Conclusion

Based on the result of the initial degradation, the highest number of bacteria and fungi was obtained in T2 treatment (C / N ratio 30) which was 42 x 10^6 cfu / g and 4 x 10^10 cfu / g and identified Bacillus sp and Aspergillus niger, Aspergillus glaucus, Aspergillus terreus, Rizopus sp. While, the highest number of bacteria in the substrate from second phase was obtained at T2 treatment (DM3), which was 38 x 10^6 cfu / ml and identified Bacillus sp, Lactobacillus sp. Bacillus sp and Lactobacillus sp are lactic acid bacteria that can function as forage preservatives (silage).

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