The effect of sludge dairy cattle and expired milk powder waste as growth media for white oyster mushroom (*Pleurotus florida*)

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Abstract. White oyster mushroom media must add additional nutrients as stimulants for mushroom growth like bran, but bran is cattle feed so its availability is limited. Sludge biogas of cattle and expired milk powder waste is the result of livestock waste which is still rich in nutrients which are important substances. This study aims to determine the effect of differences in the composition of the biogas sludge media and expired milk powder waste on mushroom productivity. This study was designed with four treatments and three repetitions which difference in the ratio of the concentration of cattle biogas sludge and expired milk powder with a ratio of P0 (0g: 0g), P1 (100g: 100g), P2 (50g: 150g) P3: (150g: 50g). Data taken include mushroom growth measurement The data was processed using analysis of Variance-Completely Randomized Design Unidirectional and the average difference was tested using Duncan's Multiple Range Test (DMRT). The best result is P2 treatment, fresh weight, a diameter of caps, stem length, and harvest time obtained the results are 124.15 gram, 10.57 cm, 5.98 cm, and 46.00 days. In this study, the greater the proportion of expired milk powder waste that was given increasingly increased the growth of white oyster mushrooms.

1. Introduction

Human food needs are substantially fulfilled because they are daily needs, some of the food needs, especially animal protein, are obtained from beef cattle that are raised by humans, but as a biological industry, livestock produces waste that must be degraded so that it is safe to release into the environment. Researchers in the field of animal husbandry themselves are also required to be active in presenting breakthroughs in waste management that can provide benefits not only to tackle pollution but the waste can still increase farmers' income. Processing the waste as biofertilizer for mushroom media is one alternative way to manage waste and able to save the environment. Besides, mushrooms contain high protein and other nutrient contents that give healthy effects in the human body [1]. Moreover, mushrooms have the potential in giving an umami taste where chemical components contributing to the umami taste are supposed to be from non-volatile compounds [2]. That organic wastes could be processed to enhance the value-added by using appropriate technologies in agricultural wastes and increase the economic value [3]. Therefore, using the waste as the biofertilizer on mushroom media can give benefits to mushroom farmers.

Livestock waste is usually in the form of solid, liquid, and gas waste, this solid waste can be in the form of housing waste and solid waste from the residual products of the factory. Abundant housing
waste such as feces waste, fecal waste is usually processed into compost or biogas, but waste processing with the biogas system still produces new waste which, if not treated, will cause contamination which is bad for health and human health. The sludge waste itself, when examined in terms of its nutrient content, still has a lot of nutrient content which is still high and can be used as enrichment material for fertilizers and plants. The nutrient content contained in the sludge waste includes Mg, Ca, P, K, Zn, and Cu [4].

Other solid waste besides housing waste can also be in the form of livestock product waste, which is a consequence of the limited production period. One of the potential livestock waste products due to its abundance is milk powder waste. The waste milk powder is usually handled by burning, even though the powdered milk waste itself still has very high nutrient content in the form of P2O5, N-total, C-Organic, and K2O, and in previous studies when applied to pakchoi vegetables it can produce high yields. high, increasing the humus content in the soil and soil fertility [5]. Refined milk powder waste contains high carbohydrates, phosphate, and high potassium and other elements that can be complementary [5].

The mushroom growing media that is commonly used saw waste, but oyster mushrooms do not only grow with sawdust media because nutrition plays a very important role in the process of growth and development of the fruit bodies of oyster mushrooms [6]. Livestock waste as described above has The nutritional content is still very high, the high nutritional content is a loss if it is not used to its full potential. The nutritional content in livestock waste can be used as a medium for white oyster mushrooms, conventional white oyster mushroom media usually uses bran as a source of nutrition for planting media, but the bran is also animal feed so that its availability competes with the needs of animal feed, the bran itself is rich in protein content, and carbohydrates which are essential nutrients needed by white oyster mushrooms. The carbohydrate component is a nutrient for fungi, the hyphae threads can be used to break down carbohydrates into sugar, which will be used as energy by metabolized oyster mushrooms so that they can grow quickly or appear in baglog [7].

The nature of oyster mushrooms itself is very supportive of conducting deeper research related to oyster mushrooms, oyster mushrooms themselves have heterotrophic abilities, namely, they cannot cook their food, so this ability makes oyster mushrooms must obtain nutrients from other compounds or organisms. In this research, we want to use a potential source of nutrition from livestock waste so that it can be used as a medium for white oyster mushrooms. This study has many benefits, namely related to the function of waste milk powder and cattle dairy sludge waste, especially as an alternative nutrient source for white oyster mushrooms. It is hoped that this research will not only treat the existing waste but also increase productivity, and also become a pioneer in supporting the integrated agricultural campaign movement.

2. Materials and methods
This research was conducted at the leather science and technology, byproducts and livestock waste Laboratory, Faculty of Animal Science, Universitas Gadjah Mada, and growing chamber (kumbung) Faculty of Animal Science, Universitas Gadjah Mada.

2.1. Materials
The tools used in this study were bottles, filters, stoves, analytical scales, plastic logs, shovels, sterilization drums, pralon rings, cotton swabs, sacks, spatulas, test tubes, measuring cups, Erlenmeyer tubes, glove masks, Thermo hygrometers, spatulas, porcelain dish mixer, stirrer, bunsen lamp, laminar airflow, pH meter, petri dish, filter paper, measuring tape, spatula, micropipette, sprayer dropper, a set of destructive tools, vortex mixer, furnace oven, and spectrophotometer. The materials used in the research were milk powder, sengon sawdust, fresh cattle dairy sludge, oyster mushroom F3 seeds, water, methylated spirits, eggshells, distilled water, NaOH, H2SO4, selenium mixture, Na phenate, tartrate buffer, NaOCl 5 %, HClO4, HNO3, concentrated reagent, and ascorbic acid.
2.2. Methods

2.2.1. Preparation of oyster mushroom media. This research was carried out in the oyster mushroom kumbung belonging to the Laboratory of Leather Technology and Upholstery and Animal Husbandry Waste. This research was started by preparing oyster mushroom growing media materials, namely fresh cattle dairy sludge, water, milk powder, and eggshells. Cattle dairy biogas sludge is made from cattle feces at the UGM Faculty of Animal Science which the waste is processed into biogas, after the wet biogas sludge is prepared from the biogas digester then dried in the sun to dry, it is estimated that the water content will be below 60%, it is estimated that by dripping the sludge into a fist, if not dripping and not breaking then the water content is obtained the right moisture content. Then the expired milk waste is obtained from expired milk obtained from retailers, then the eggshell waste is obtained from households or food stalls. All research materials were then prepared and differentiated into 4 treatments. The treatments were distinguished by a combination of the proportion of the sludge media for cattle dairy biogas and milk powder waste, namely P0 (0g: 0g), P1 (100g: 100g), P2 (50g: 150g) P3: (150g: 50g) mixed with the basic media commonly used, namely sawdust and eggshells. So if it is realized in the composition of the media that is given, each consisting of different proportions presented in table 1 below.

| Materials for the media | P0 (g) | P1 (g) | P2 (g) | P3 (g) |
|-------------------------|--------|--------|--------|--------|
| Sawdust                 | 750    | 750    | 750    | 750    |
| Bran                    | 200    | -      | -      | -      |
| Biogas sludge           | -      | 100    | 50     | 150    |
| Expired milk powder     | -      | 100    | 150    | 50     |
| Eggshell                | 50     | 50     | 50     | 50     |
| Total                   | 1,000  | 1,000  | 1,000  | 1,000  |

The four types of media were left to stand overnight and then put into polypropylene plastic media with the weight of each log being 1,000 grams, each log was then compressed by pressing, because the density of the media affected the density of the mycelium, then closed using a log and cotton ring, then the media put into the sterilization tube or drum, for approximately 8 hours, the sterilized media is left to stand overnight after which the white oyster mushroom F3 seeds are inoculated, the inoculation is carried out aseptically and as quickly as possible to reduce contamination, the seeds are taken from the bottle with using a sterile spatula by heating it using a Bunsen lamp, and spraying it on the surface of the media using methylated spirits. Then the inoculated media is allowed to stand in the incubation room for 2–4 weeks with a temperature between 22–28°C, observed until the mycelium evenly covers the surface of the baglog, then after the mycelium covers the surface of the baglog, the fungus is ready to fog in the media to maintain as much moisture as possible 2 times a day.

2.2.2. Oyster mushroom growth measurement. Measurement of oyster mushrooms includes measurements of fresh weight, stalk length, caps diameter, first time to grow mycelium, and harvest time of oyster mushrooms, fresh weight is measured using a digital scale, the weight is calculated in grams, then the length of the stalk and the width of the caps are measured using a measuring tape in cm. Then for the parameters of the first time to grow and the length of harvest seen from the time since the inoculation was set in days, all data were then recorded and statistical analysis.

2.2.3. Data analysis. The data processing of this research involved the calculation of the Completely Random Design (CRD) pattern. The average difference was tested with Duncan's Multiple Range Test (DMRT).
3. Results and discussion

The growth of white oyster mushrooms produced by various media was seen with various observation parameters, namely fresh weight, the diameter of the cap, stem length, number of caps, first time to grow pinhead, and harvest time. The result of the Oyster mushroom growth measurement is presented in Table 2.

Table 2. Oyster mushroom growth measurement

| Variable                        | P₀     | P₁     | P₂     | P₃     |
|---------------------------------|--------|--------|--------|--------|
| Fresh weight (gram) ns          | 115.56±32.31 | 113.23±31.08 | 124.15±17.22 | 93.04±7.21 |
| Diameter of cap (cm)            | 7.03±1.79 b | 10.15±0.73 a | 10.57±0.80 a | 9.34±0.13 a |
| Stem length (cm) ns             | 5.38±0.67 | 5.50±0.86 | 5.98±0.32 | 4.92±1.13 |
| Number of caps (pieces)         | 13.00±1.00 a | 10.33±2.08 ba | 11.66±1.52 ba | 9.33±1.52 b |
| Time to grow pinhead (days) ns  | 44.33±0.57 | 45.33±3.21 | 43.66±0.57 | 42.66±0.57 |
| Harvest time (days)             | 48.00±1.73 a | 46.66±0.57 ba | 46.00±0.00 b | 46.66±1.11 ba |

a,b,c different letter on the same line shows the difference (P<0.05)
ns nonsignificant
P₀: Mushroom media with a composition of biogas Sludge 0 g and expired milk powder 0 g.
P₁: Mushroom media with a composition of biogas Sludge 100 g and expired milk powder 100g.
P₂: Mushroom media with a composition of biogas Sludge 50 g and expired milk powder 150g.
P₃: Mushroom media with a composition of biogas Sludge 150 g and expired milk powder 50g.

3.1. Fresh weight (gram)

Based on the research conducted, it was found that the weight of fresh mushrooms, when harvested, did not give a significant difference (p> 0.05) to the given control media. Based on the results of the weighing carried out, the results obtained in treatment P₀, P₁, P₂, P₃ are 115.56, 113.23, 124.15, and 93.04 grams, respectively. The results showed that the highest fresh weight treatment was treatment (P₂) of biogas sludge and 50g: 150g expired milk powder waste, namely 124.15 grams, while the lowest fresh weight was treatment (P₃), namely 93.04 grams. The highest fresh weight was obtained in the media with the addition of oyster mushroom media with the addition of expired milk powder waste, expired milk powder had a high sugar content compared to biogas sludge, and P₂ media with low lignin content, the addition of medium sugar with high media could provide a significant difference to weight. Fresh from oyster mushrooms, hyphae threads release enzymes that can hydrolyze sugar into simple compounds and are used as energy for metabolism used for the growth of oyster mushrooms [8].

3.2. The diameter of the cap (centimeter)

Based on the research conducted, the results of the diameter of the cap (cm) showed a significant difference (p<0.05) to the control media (P₀). Based on the measurement results, the results obtained are P₀, P₁, P₂, P₃ respectively 7.03, 10.15, 10.57, and 9.34 grams. The results showed that the best treatment was obtained in treatment (P₂), the ratio of biogas sludge and expired milk powder waste was 50 g: 150 g which gave the best results on a diameter of 10.57 cm, while for the lowest caps diameter was found in treatment (P₀), namely 7.03 cm. Based on this research, the addition of high powdered expired milk waste gives a greater effect on the diameter of the caps compared to other media. Based on previous research, the higher the addition of sugar affects the number of fruit caps, which produces a smaller diameter [8].

3.3. Stem length (centimeter)

Based on the results of statistical analysis showed that the results of stalk length (cm) were not significantly different (p> 0.05) from the control planting medium (P₀). Based on these results, the length of the growing media stalk P₀, P₁, P₂ and P₃ were obtained respectively, namely 5.38, 5.50, 5.98, and 4.92 cm, and the highest results were obtained on the length of the stalk, namely treatment [8].
(P2), namely treatment the ratio of biogas sludge and expired milk powder waste is was 50 g: 150 g which is 5.98 cm. Whereas for the lowest stalk length was P3 treatment which was 4.92 cm. The addition of media with expired milk powder waste which is rich in carbohydrates shows the best results at stem length, carbohydrates are the main substrate for carbon metabolism in mushroom [9].

3.4. Number of caps (pieces)
Based on the results of the study, the average results obtained were the number of caps in each treatment P0, P1, P2, P3, the results were as follows: 13.00, 10.33, 11.66, and 9.33. The best results were found in the control treatment (P0), namely 13.00. Based on the statistical analysis, the results of the number of caps (fruit) showed significantly different results (p <0.05) to the control media (P0), while the least number of caps was in treatment (P3), namely 9.33 pieces. Based on the results of this study, the number of caps is much influenced by small diameter, this is supported by the result of the previous study which states that fungi that have a small number of fruit bodies, then the diameter of the mushrooms will grow larger [8]

3.5. Time to grow pinhead (days)
Based on the results of the research, the average results obtained were the first time the pinhead (days) of each treatment P0, P1, P2, P3 were obtained sequentially, namely 44.33, 45.33, 43.66, and 42.66 days. The fastest result on pinhead growth rate was treatment (P3), namely the treatment of media with 150 g: 50 g a ratio of biogas sludge and expired milk powder waste, namely 42.66 days, while late pinhead growth was found in treatment (P1), namely 45.33. Based on statistical analysis, the treatment showed results that were not significantly different (p> 0.05) from the control media (P0). The high carbohydrate content in the biogas sludge-based media supports the metabolism of fungi for mycelium growth [9]

3.6. Harvest time (days)
Based on the results of the study, the average yields of harvest time (days) for each treatment P0, P1, P2, P3 were obtained sequentially, namely 48.00, 46.66, 46.00, and 46.66. The best results with the fastest harvest were found in the P2 treatment, namely the comparison of biogas sludge and 50 g: 150 g expired milk powder waste, namely 46.00 days, while for late results there was treatment (P0), namely 48.00 days. Based on the statistical analysis carried out on the treatment given showed significantly different results (p <0.05) to the control media (P0). The high content of nitrogen, carbon, and carbohydrates is thought to supply the nutritional needs of white oyster mushrooms so that the harvest time can be faster. The nutritional content needed for mushroom growth includes carbon, nitrogen, minerals, and vitamins. [10].

4. Conclusion
The best growth of white oyster mushrooms was obtained in the treatment using P2 media which used a composition of 50 g biogas dairy cattle sludge waste and 150 g of expired milk powder waste showed the best results on fresh weight, caps diameter, and stalk length. In this study, it can be concluded that the addition of expired milk powder waste can affect, namely an increase in the growth of white oyster mushrooms.

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