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EVALUATION OF IN VITRO DIGESTIBILITY OF DRIED MATTER AND ORGANIC MATTER OF SOLID WASTE OF BIOETHANOL FERMENTATION FROM CASSAVA BY *TRICHODERMA VIRIDE* AND *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

This study aimed to evaluate the digestibility of dry matter and organic matter of Solid Waste of Bioethanol Fermentation by Trichodermaviride and Saccharomyces cerevisiae. The research was conducted in the Laboratory of Animal Nutrition Ruminant Livestock and Food Chemistry, Faculty of Animal Husbandry, Padjadjaran University, using the in vitro with sheep rumen fluid. The design of the study was a completely randomized design with 4 treatments and 5 replications. The treatment consisted of four rations as followed: R1 used 100% of grass; R2 = 50% grass + 50% concentrate; R3 = 50% of grass + 25% of concentrate + 25% of fermentation products; and R4 = 50% of grass + 50% of fermentation products. Parameters measured were dry matter digestibility and organic matter digestibility. The results of this study indicate that the addition of fermentation products into the ration had real effect (P <0.01) on dry and organic matter digestibility. The R3 ration treatment had the highest level digestibility of dry matter and organic matter, which was 77.06% and 49.59% respectively.

Keywords: Fermentation, Dry matter digestibility, Organic matter digestibility, Solid waste on bioethanol, *Trichoderma viride, Saccharomyces cerevisiae.*

Contribution/ Originality

This study originates new formula which used indigenous isolated microbial from solid waste of bioethanol fermentation from cassava (*Manihot esculenta* Crantz) combined with minerals.

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1. INTRODUCTION

Food security can be defined as an access for all population every time to fulfill the need of nutrition and food safety to active and healthy life. It cannot only be guaranteed by food material product, furthermore, but also be processed for consumption security as an added value for agricultural product [1].

One attempt to protect food security and sustainability in agriculture is to keep the chain of agricultural process based models of organic farming, by utilizing agricultural and organic residue as livestock feed. Indonesian farms are generally relying on natural forage and agricultural waste, but the problem is quality of the forage in Indonesia that is low. The low quality of the forage, made farmers then use concentrates as complementary nutrient for requirements of ruminants. But in certain periods, the raw materials were often getting fluctuation prices as well as concentrate feed which results in increased production costs.

This paper discusses the digestibility of dry matter and organic matter of solid waste of bioethanol fermentation by *Trichoderma viride* and *Saccharomyces cerevisiae*.

2. LITERATURE REVIEW

Bioethanol is a product of fermentation process of carbohydrate source that uses microorganisms. One of the materials for bioethanol production is cassava (*Manihot esculenta*). 20 tons of Manihot or cassava can produce 3,332 liters of bioethanol [2].

One of the byproduct of bioethanol process is a solid waste enriched in carbohydrates but low in protein, so if these given directly to the animals, they will get deficient diet. Some efforts to improve the quality of the solid waste are processed through the fermentation process.

The fermentation process is a bioprocess technology that involves the activity of a microorganism in order to improve the nutritional quality of food and decrease the anti-nutritional substances [3]. Fermentation is the process of breakdown of organic materials (carbohydrates, fats, proteins, and others) through the action of the enzyme produced by microorganisms [4]. In this case, microorganisms are catabolic, that is breaking the components of more complexes into simpler ones so that the material is easy to digest. In addition, fermentation can also be used as a medium for microbial synthesis that can change inorganic minerals into organic minerals as well as a medium for improving the value of nutrients [5].

Fermentation is generally done by molds and yeasts, including using *Trichoderma viride* and *Saccharomyces cerevisiae*. Molds are fungi that have hyphae while the yeast does not have it. *Trichoderma viride* is one type of fungus which is cellulolytic because it can produce cellulase. *Trichoderma viride* can also be regarded as microorganisms which has capable of breaking down the high level cellulose and have the ability to synthesize some essential factors to dissolve the cellulose strongly bound with hydrogen bonds [6].

Saccharomyces cerevisiae is known by its characteristics to ferment various carbohydrates. *Saccharomyces cerevisiae* can ferment maltose quickly (lean yeast dough), improve the osmotolerance (sweet yeast dough), rapid fermentation kinetics, tolerance to extreme heating and cooling, and has the ability to metabolize a substrate. From the difference of the two types of the fungus, *Trichoderma viride* can hydrolyze cellulose more by generating many cellulolytic enzymes, while *Saccharomyces cerevisiae* produces more amylolytic enzymes to hydrolyze starch. It is expected that when a consortium made, it will result a mutually beneficial relationship in the processing of solid waste of bioethanol. However, according to Suryani, et al. [7] the increasing of protein from solid waste bioethanol fermented by *Trichoderma viride* and *Saccharomyces cerevisiae* without any addition of N (nitrogen) has only ranged between 2.8% to 3.8%, while the requirements livestock body is more than 12% [8]. Therefore, this fermentation process requires adding a source of N to increase protein content.

Most of the microbes used in the fermentation can utilize inorganic compounds and organic compounds as a source of nitrogen as well. Urea is one source of non-protein nitrogen in the form of white crystals, is easily soluble in water, and contains 45% nitrogen [9]. Urea as one of a nitrogen source has the function in forming the body proteins of microorganisms that are part of proteins, nucleic acids and enzymes [10]. The activity of protein biosynthesis by microbes on the treated substrate with urea is higher than the addition of NPK and Vitamins B1 [5]. By adding the urea in the solid waste bioethanol fermentation process, it is expected to supply the needs of the rumen microbial with food substances so as to increase the substrate degradation, which in turn increases digestibility.

3. MATERIALS AND METHODS

3.1. Material and Procedures

The material used in this study is the solid waste of bioethanol fermentation product from cassava by *Trichodermaviride* and *Saccharomyces cerevisiae*, grass, concentrates, artificial saliva, and rumen fluid. The solid waste was obtained with cassava (*Manihot esculenta* Cranzt) of bioethanol fermentation residue which treated by *Trichoderma viride* and *Saccharomyces cerevisiae* together.

Nutrients contained in the organic matter are constituent component of dry ingredients. The composition of organic matter is composed of fat, crude protein, crude fiber, and non nitrogen free extract (NNFE). This dry matter has the same chemical composition with organic ingredients, but including ash [11].

Values of dry matter and organic matter digestibility were calculated by using the Tilley and Terry [12]. The equation as follow:

a. Dry Matter Digestibility (DMD)

$$DMD\% = \frac{DMinput - (DMremaining\ undigested - DMblank)}{DMinput} \times 100\%$$

b. Organic Matter Digestibility (OMD)

$$OMD\% = \frac{OMinput - (OMremaining\ undigested - OMBlank)}{OMinput} \times 100\%$$

Note:

DMinput and OMinput = sample weight (g) before incubation

DMremaining undigested and OMremaining undigested = sample weight (g) after incubation

Blank = Rumen Liquid + artificial saliva without material tested

The procedures used the method of Tilley and Terry [12]. The process of in vitro experiment was carried out in two stages, namely:

1. **Stage 1: Fermentation Process Digestion**
   a. Rumen fluid was taken from sheep. It was filtered with muslin cloth filter and collected in a flask. The capacity of the flask was fully charged to maintain anaerobic conditions.
   b. 30 ml of solution consisting of artificial saliva (solution McDougall) and rumen fluid was mixed and inserted into the fermenter tube. The ratio of artificial saliva and rumen fluid was 4:1. Artificial saliva serves as a buffer and rumen fluid serves as a source of microbial inoculums; then add 1 gram of sample treatment (dry matter) into the solution mentioned above and covered by a nipple rubber cap.
   c. The tube was flowing by CO_2 gas to keep in a state of anaerobic environment.
   d. The solution was then stored in a water bath containing water cooler rack 39°C, the tube is covered and incubated in the fermenter tube for 48 hours.
   e. The fermentation process was stopped by adding HgCl to kill microbes.

2. **Stage 2: Enzymatic Digestion Process**
   a. Pepsin and HCl solution was added to the tube fermenter.
   b. Again, it was then incubated (anaerobic) at a temperature of 39°C for 48 hours.
   c. The rest of the samples which is not digested were separated by whatman 41 filter paper using a vacuum pump.
   d. Remaining filtering was oven dry, then weighed and proceeds with the analysis of dry matter and organic matter.

3.2. Data Analysis Techniques

This research was conducted with an experimental method and research design used was Completely Randomized Design (CRD) consisting of 4 treatments and each treatment was repeated 5 times so that there are 20 experimental units.

The treatments consist of:

a. R1 = 100%grass

b. R2 = 50% grass + 50% concentrates

c. R3 = 50% grass + 25% concentrates + 25% fermentation products

d. R4 = 50% grass + 50% fermentation products

The analysis of variance was used in determining the use effect of the fermentation products of solid waste bioethanol in this ration, by F-stat bigger than F-table's, that is132.48 for F-stat and 3.24 for F-table's ($P<0.01$) and followed by Duncan's Multiple Range Test to examine the differences among treatments.

4. RESULTS AND DISCUSSION

4.1. The Effect of Treatment on Dry Matter Digestibility

The value of the dry matter digestibility of ration with fermented solid waste of bioethanol by *Trichoderma viride* and *Saccharomyces cerevisiae* are presented in Fig.1.

Based on Fig.1, it can be seen that the average values of dry matter digestibility of rations with/ or not fermented solid waste of bioethanol by *Trichoderma viride* and *Saccharomyces cerevisiae* are between 56.25% and 77.06%. The highest average value of dry matter digestibility was obtained with ration treatment (R3) containing a mixture of 50% grass, 25% concentrate and 25% fermentation products (77.06%).

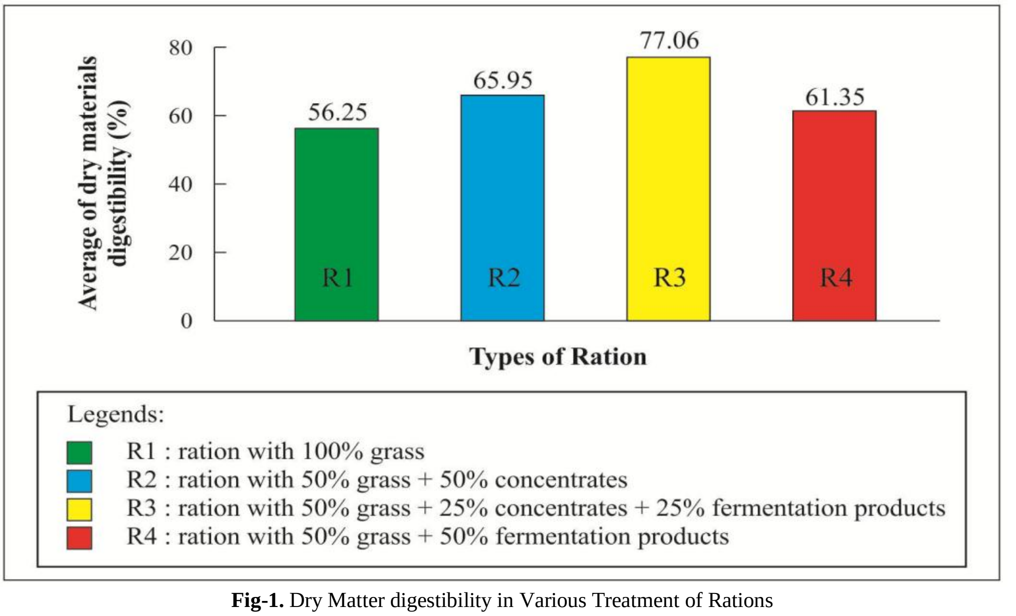

Fig-1. Dry Matter digestibility in Various Treatment of Rations

Source: Data Study

The value of the dry matter digestibility on the fermented solid waste of bioethanol, ordered from the lowest to the highest was R1, R4, R2, and R3. According to Sutardi [13] dry matter digestibility values within normal limits ranged between 50 - 60%, therefore the dry matter digestibility values are still in the normal range.

The results of statistical analysis show highly significant differences ($P<0.01$) among treatments as presented in Table 1.

Table-1. The advanced test of treatment effect on dry matter digestibility

Treatment	Average (%)	Significance (p <0,01)
R1	56,25	a
R4	61,35	b
R2	65,59	c
R3	77,06	d

Note: Different letters in the same column indicates means significantly different (P <0.01)

From Table 1 it is shown that the addition of concentrate and fermentation products to grass (R3) increase significantly the digestibility of dry matter than the ration of grass alone treatment (R1). The dry matter digestibility of ration containing 50% concentrate (R2) was higher than the ration containing 50% fermented products (R4), but still lower than mixed rations which contain 25% concentrate and 25% fermentation products (R3).

The low level of ration digestibility which contains grass alone could be explained by the content of nutrients, lower than other treatments, especially protein content. Preston and Leng [14] noted that the proteins required for ruminants are divided into two components, namely ammonia and amino acids. Amino acids are absorbed from the rumen only in small amounts. Most of the amino acids are delaminated to leave the bond chain volatile fatty acids, carbon dioxide, and methane. Therefore, the compounds containing nitrogen that can be utilized by ruminants for growth and production consist of proteins, and non-protein nitrogen. Ammonia and ATP are used to build the microbial cells, whereas CH_4 and CO_2 are discharged through eructation.

Ammonia is produced from the metabolism of proteins, peptides, amino acids, urea, nitrate, and other compounds, which are mostly used by microbes to synthesize microbial protein. The higher the protein content of the ration, the higher the production of ammonia due to the increasing activity of proteolytic bacteria [15] so that the microbial protein produced will also increase. The main determinant of the amount of amino acids, both essential and non-essential which is available for ruminants are the supply from microbial protein biosynthesis that depends on the availability of carbohydrates and N which has not necessarily in the form of protein [9]. The difference of protein content has shown among all treatments (R1, R2, R3 and R4) that were 8.2%, 11.6%, 10.91% and 10.65% respectively. Thus, that the digestibility of the ration is associated with ration protein content and rumen microbial life; and the low level of the dry matter digestibility in ration (R1) treatment could be the result of the low protein content.

Meanwhile, the treatment containing 50% fermented products (R4), the digestibility was lower than the treatment ration which contains 50% concentrate (R2). It is suspected that the fermentation products used was less sulfur-containing amino acids such as methionine, which is required for optimal growth of rumen bacteria, since the fermentation process is only supplemented by urea alone without the addition of sulfur. R3 ration which consists of grass, concentrate and fermentation products has the highest digestibility among all treatments. This could be due to the balance of the nutrients contained in this ration. Fermentation products from bioethanol waste are degraded either because of the high starch food source for microbes, and/ or the presence of N from urea.

4.2. The Effect of Treatment on Organic Matter Digestibility

The organic matter digestibilities of ration are presented in Fig.2.

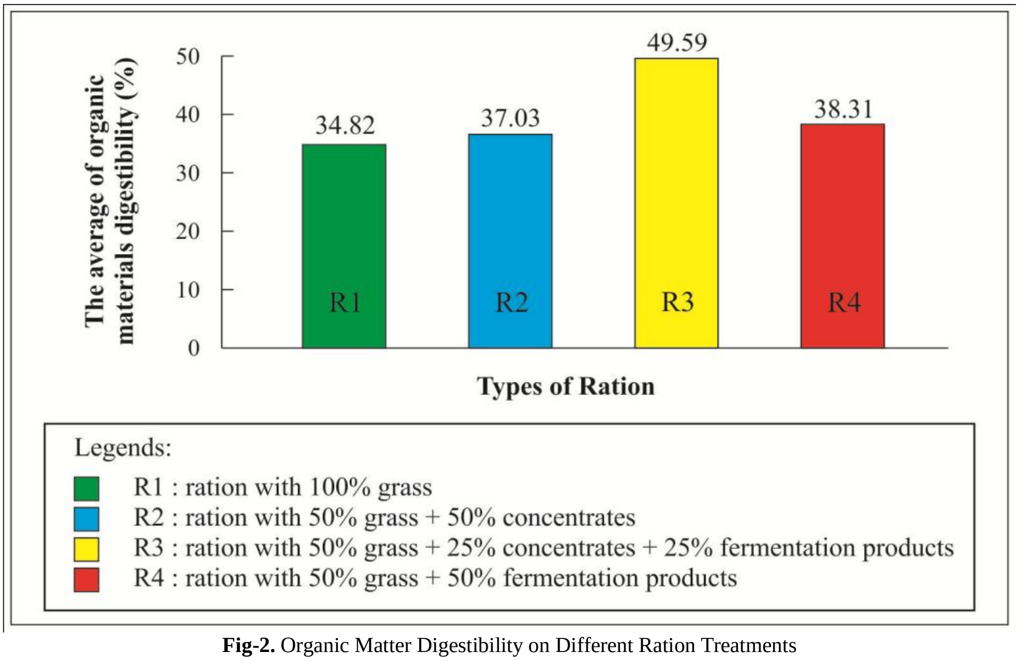

Fig-2. Organic Matter Digestibility on Different Ration Treatments

Source: Data Study

The values of the rations' organic matter digestibility ranged from 34.82% to 49.59% and these values are lower than the ones of dry matter digestibility. The highest average of the organic matter digestibility values was obtained with the ration treatment containing a mixture of 50% grass, 25% concentrates and 25% fermentation products (R3) of 49.59%. According to Firsoni, et al. [16] the complete feed of organic matter digestibility was ranged from 48.32 to 53.75%. Therefore, in general, organic matter digestibility was under normal, except R3 treatment. The statistical analysis indicates that each treatment has a significant influence on the value of the organic matter digestibility. The significance of each treatment can be seen in Table 2 Duncan below.

Table-2. The Duncan advanced test of treatment effect on organic dry matter digestibility

Treatment	Average (%)	Significance (p<0,01)
R1	34,82	a
R2	37,03	b
R4	38,31	b
R3	49,59	c

Note: Different letters in the same column indicates means significantly different (P <0.01)

The treatment R1 has lower organic matter digestibility than other treatments; while R2 and R4 treatments have the same value. The highest value of organic matter digestibility was obtained with R3 treatment. As for the dry matter, the digestibility of organic material that looks the best among all the treatment is ration with the composition of 50% grass, 25% concentrates and 25% fermented products. This is because the mixture of concentrate with fermented products achieves the required balance of nutrients, which is needed by rumen microbes so that they can grow optimally and easily degrade organic matter.

Many factors affect the digestibility of organic matter including ingredient chemical composition, feed preparation (cutting, grinding, cooking, etc.), type of animals, age of animals, the number of rations [17] and crude fiber and mineral content of feedstuffs [18]. Organic material is a material that is lost during combustion [17]. Ash, as the materials left over from combustion is a mineral that is contained in the ration.

5. CONCLUSION

There is a significant influence on the addition of solid waste fermentation products of bioethanol by *Trichoderma viride* and *Saccharomyces cerevisiae* in the ration on a dry matter digestibility and organic matter digestibility. The best results of the dry matter and organic matter digestibility is obtained with the ration composed of 50% grass, 25% concentrates and 25% fermentation products (R3).

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