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Increasing the potential of Cajuput leaf waste as cattle feed through fermentation pretreatment

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ABSTRACT. Waste of Cajuput leaves (*Melaleuca cajuputi* Powell) has the potential to be used as an alternative cattle feed because it has a high nutritional content. The nutritional content of Cajuput leaf waste needs to be improved before being used as an alternative feed. The fermentation process can increase nutrient content in Cajuput leaf waste and increase the organic matter digestibility (OMD), dry matter digestibility (DMD), ammonia (NH_3) and volatile fatty acid (VFA). This study aimed to determine the improvement of Cajuput leaf waste nutrition with the help of yeast as well as the effect of fermented Cajuput leaf waste along with a mixture of concentrates and field grass on digestibility, NH_3 and VFA which were carried out by in vitro test. The results showed the nutritional content of Cajuput leaf waste fermented by yeast of *Aspergillus chevalieri* had the best value of protein 16.03%, fiber 16.92% and fat 5.93%. The treatment R4 (50% Cajuput leaf waste + 25% concentrate + 25% field grass) had the best digestibility (DMD= 46.12%; OMD=32.08%), NH3 (8.37 mM) and VFA (168.5 mM) production.

Keywords: *Aspergillus chevalieri;* dry matter digestibility; *Melaleuca cajuputi*; nutritional content; organic matter digestibility

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INTRODUCTION

Organic waste is the alternative feed commonly used in the farms. Organic waste is used because it is cheap, easy to obtain, abundant viability, and has good quality. Feed ingredients consumed by livestock must have good nutritional value for animal health. One alternative feed that can be used as a substitute for conventional forage which is usually reduced its availability, especially in the dry season is Cajuput leaf waste (Widiana *et al.*, 2014). Waste of Cajuput leaves consists of 50% organic compounds of cellulose, lignin and other organic compounds (Rattanaburi *et al*., 2013; Dewi, 2016). The main obstacle for the use of organic waste for animal feed is the low nutritional value and digestibility.

The protein content of Cajuput leaf waste is still quite low, which was around 7% and high fiber content of 21.47% which could reduce digestibility in animal feed (Widiana *et al*., 2014). This data shows that Cajuput leaf waste protein has not met the optimum standard for animal feed. Animal potential feed ingredients can be seen through the parameters of organic matter digestibility (OMD), dry matter digestibility (DMD), production of ammonia (NH_3) and volatile fatty acids (VFA) (Noziere *et al*., 2011; Al-Arif *et al*., 2017).

VFA concentration in rumen fluid can be used as an indicator of feed fermentability and is closely related to rumen microbial activity (Zebeli *et al.*, 2012; Maulfair & Heinrichs, 2013; Hall *et al*., 2015). Ammonia content in the rumen is positively correlated with microbial protein synthesis, if the concentration of ammonia (NH_3) and VFA in the rumen increases, microbial protein synthesis will also increase (Syapura *et al*., 2013; Uddin *et al*., 2015). As the previous studies, efforts to increase the nutritional content of Cajuput leaf waste can be carried out by several treatments such as physical (enumeration), chemical, and biological (fermentation) (Sitindaon, 2013; Widiana *et al*., 2019; Noor *et al*., 2020). Fermentation with the help of microbes (mold) is widely used to help increase the nutritional content/potential of feed ingredients. Some species of mold are known could help in increasing the nutritional content of feed ingredients. The protein content increased in the Cajuput leaf waste fermented by mold of *Trichoderma viride* and *Aspergillus niger*, but the crude fiber content was still high (Yudiar *et al.*, 2014). Decreasing the fiber content can be provided by adding the other molds. Other research showed that fermentation using the mold of *Aspergillus niger* and *Phanerochaete chrysosporium* reduced the levels of crude fiber in rice straw (Supriyatna, 2017). For this reason, research was conducted to increase the potential of Cajuput leaf waste with fermentation pretreatment by *Aspergillus chevalieri* mold which is an endophytic mold on Cajuput leaf waste.

MATERIALS AND METHODS

Fermentation. Cajuput leaf waste was obtained from the Jatimunggul Cajuput oil factory, Indramayu. Fermentation of Cajuput leaf waste by *Aspergillus chevalieri* was carried out at 31°C for eight days. Fermentation results were measured by nutritional content with proximate analysis.

***In vitro* test.** According to research conducted (Setiyaningsih *et al.*, 2012), *in vitro* DMD and OMD analysis (Tilley & Terry, 1963) can be calculated by the formula:

$$DMD\ (\%) = \frac{DM\ sample\ (g) - (DM\ residue\ (g) - DM\ blank\ (g))}{DM\ sample\ (g)}\ x\ 100\%$$

$$OMD\ (\%) = \frac{OM\ sample\ (g) - (OM\ residue\ (g) - OM\ blank\ (g))}{OM\ sample\ (g)}\ x\ 100\%$$

Notes:
DMD= dry matter digestibility DMD; OMD= organic matter digestibility; DM= dry matter; OM= organic matter

Production of NH_3 analysis (Microdiffusion Conway). Production of NH_3 can be calculated by formula (Conway & O'malley, 1942):

$$N - NH_3 = (ml\ H_2SO_4 \times NH_2SO_4 \times 1000)\ mM$$

Production of VFA analysis. Production of VFA can be calculated by formula (General Laboratory Procedures, 1966):

$$\text{Concentration of total VFA} = (b - s) \times N\ HCl \times 1000/5$$

Notes:
b= volume of titran blank; s= volume of titran sample; N= liquid normality of HCl

Experimental design. Cajuput leaf waste was mixed with field grass and concentrate to increase its potential as raw material for animal feed. This study used a completely randomized design (CRD) with five treatments and three replications. The ration consisted of four treatment types as follows:

R1= 100% fermented Cajuput leaf waste
R2= 50% fermented Cajuput leaf waste + 50% concentrate
R3= 50% fermented Cajuput leaf waste + 50% field grass
R4= 50% fermented Cajuput leaf waste + 25% field grass + 25% concentrate
R5= 25% fermented Cajuput leaf waste + 25% field grass + 50% concentrate

Data analysis. The OMD, DMD, NH_3, and VFA data were subsequently analyzed using the SPSS software ver. 16.0.

RESULTS AND DISCUSSION

Fermentation results of Cajuput leaf waste. Fermentation using *Aspergillus chevalieri* produced the largest crude protein (16.03%), exceeding the standard for feed by 12% (Table 1). It was due to the proteolytic mold activity which breaks down proteins into amino acids to increase the dissolved nitrogen. *A. chevalieri* is a mold that produces many extracellular enzymes, such as pectinase, cellulase, exo-β-1.4glucanase, hydrolase, protease, α-amylase, glucoamylase, maltase, β-galactosidase, α-glucosidase, glucose oxidase, phosphodiesterase, ribonuclease, 4-glycohydrolase, β-xylosidase, xylanase, and lipase (Ajayi *et al*., 2014; El-said *et al*., 2017; Takenaka *et al*., 2020). Furthermore, high protein content is probably due to an increase in the number of mold cells (Nasseri *et al*., 2011; Cairns *et al*., 2019).

Table 1. The value of nutrient content in fermented Cajuput leaf waste with various single and consortium inoculum treatments.

Content	Raw Cajuput leaf waste	Fermented Cajuput leaf waste
Protein (%)	7.04	16.03
Fiber (%)	21.47	16.92
Fat (%)	9.06	5.93
Ash (%)	6.57	4.36

Crude fiber consists of cellulose, hemicellulose, lignin, and silica, where the content is influenced by age as well as the species of plants (Jamarun *et al.,* 2020). The fermentation process reduced the value of crude fiber in all treatments. This is in line with SNI standards that the good fiber content used in feed ingredients is <35%. Crude fat is a lipid contained in feed ingredients and organic compounds that are insoluble in water. However, when dissolved in organic solvents, it becomes a source of energy. Fermentation of Cajuput leaf waste by *Aspergillus chevalieri* degraded fat to 5.93% (initial fat value is 9.06%). The mold has several hydrolytic enzymes such as lipase which can degrade fat and oils (Gopinath *et al*., 2013; Chandra *et al.*, 2020).

The ash is an inorganic residual from combusted organic matter, with high content caused by increased mineral components The ash content of the leaf waste fermented by *A. chevalieri* was 4.36% from 6.57% (unfermented). This was due to a high mold spore density, hence the available minerals were used as an energy source for growth. Several fungi can utilize inorganic compounds as a source of energy.

Organic matter digestibility, dry matter digestibility, production of NH_3 dan VFA. Digestibility is a reflection of nutrients number in feed ingredients which can be utilized by livestock. It can be measured by the difference between the amount of food consumed and excreted in feces, which is considered absorbed in the digestive tract. According to Mackie *et al*. (2015), the presence of microbial activity in the digestive tract greatly influences digestibility. Meanwhile, bacteria, fungi as well as protozoa are organisms that live in the rumen and appear several weeks after birth. The digestibility of organic matter shows the number of nutrients (fats, carbohydrates, and protein) digested by livestock, while dry matter digestibility indicates levels of food digested by microbes and rumen digestive enzymes. The high percentage of dry digestibility ingredients indicates the higher quality of the feed (Suardin *et al.*, 2014; Hidayat *et al.*, 2019). The digestion value of organic matter (OMD), as well as dry matter digestibility (DMD), are presented in Fig. 1 and Fig. 2.

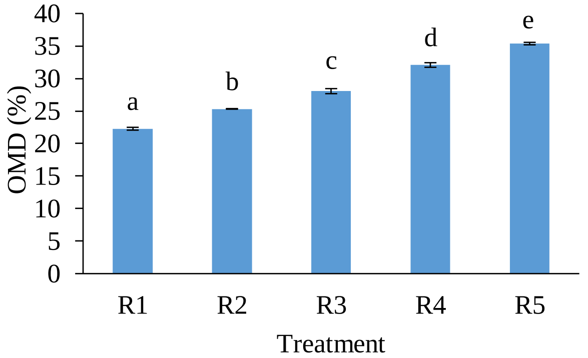

Fig. 1. The digestibility value of organic material in fermented Cajuput leaf waste and its mixture.

Organic digestibility reflects the number of digestible substances, especially nitrogen, carbohydrates, fats, and vitamins. The normal digestibility of organic matter in ruminants is affected by the rumen condition, microbial components as decomposers of organic material in rumen fluid, and certain compounds' content in the feed (Brask *et al*., 2013; Thirumalesh & Krishnamoorthy, 2013). Rumen microbes have a very important role in livestock because they can utilize feed nutrients efficiently as an energy source (Das & Qin, 2012). Furthermore, they ferment forage and fibrous material as basal feed for ruminants. Carbohydrates are broken down by *Ruminococcus albus, Ruminococcus flavefaciens, Butyrivibrio fibrisolvens, Megaspaera elsdenii,* and *Selenomonas ruminantium* microbes into three main fermentation products: acetic, propionic, and butyric acids (Flint *et al.*, 2012; Darwin *et al.*, 2018).

The treatment R5 (50% fermented Cajuput leaf waste and 50% concentrate) showed the highest digestibility value of organic matter at 35.37%. Also, concentrate affects digestibility because it has a high nutritional value. Hence, the increasing crude protein content causes a rise in microbial activity of the rumen and digestion of organic matter. Meanwhile, the percentage of treatment R4 (50% Cajuput leaf waste, 25% field grass, 25% concentrate) was 32.08%. The factors affecting digestibility include the composition of feed ingredients as well as its comparison, feed treatment, enzyme supplementation, species of livestock, and the feeding level.

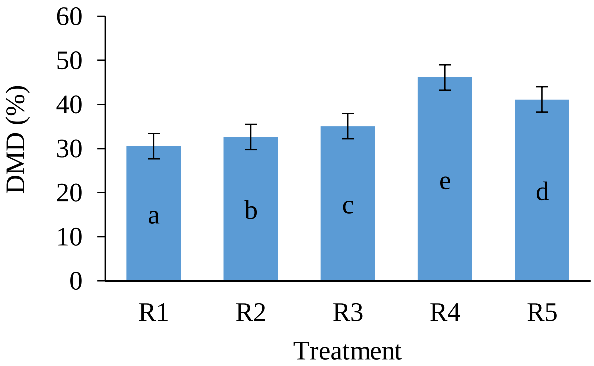

Fig. 2. The dry matter digestibility value in fermented Cajuput leaf waste and its mixture.

The fermented leaf waste in sheep rumen fluid based on in vitro tests of NH_3 and VFA production are presented in Fig. 3 and Fig 4. NH_3 as the main source of nitrogen is very important for microbial protein synthesis. This shows it is very important for ruminants because microbial protein precursors are ammonia and carbon source compounds (Dewi, 2016).

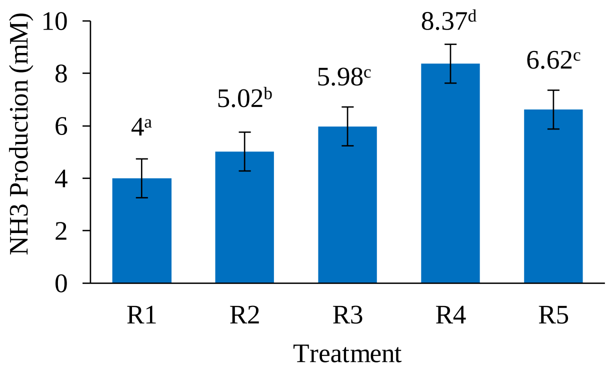

Fig. 3. The NH_3 productionin the rumen with the addition of fermented Cajuput leaf waste and its mixture.

The R4 treatment, which had the highest value of NH_3 production by 8.37 mM, met the production standard of 4-12 mM in the rumen (Sutardi, 1979). Meanwhile, NH_3 production is associated with protein degradation by microbes.

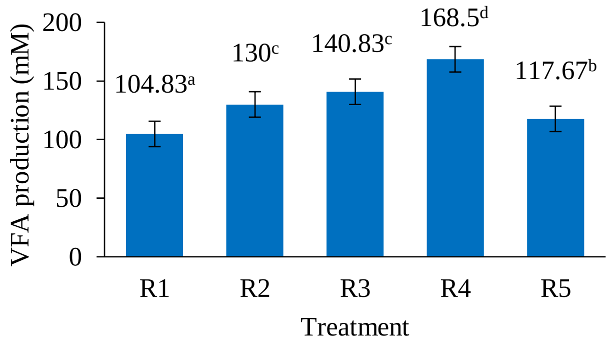

Fig. 4. The VFA production in the rumen with the addition of fermented Cajuput leaf waste and its mixture.

Low protein content in the feed results in low concentrations of rumen NH_3 (lower than 50 mg/L or 3.57 mM), which may slow microbial growth. However, when the protein degradation is faster than microbial protein synthesis, NH_3 accumulates and exceeds its optimum concentration with ranges between 85-300 mg/L or 6-12 mM. VFA concentration is influenced by the conditions of the rumen such as microbes, absorption, and fermentability of carbohydrates (Hindratiningrum *et al.*, 2011). The most important functional occupant is bacteria with 10^9 to 10^{10} cells in 1 ml of rumen gum and constitutes 5-10% dry mass of large bowel contents. The VFA concentration results (Fig. 4) are quite high for the survival of ruminant animals as the needed concentration for the livestock optimal growth is 80-160 mM. The R4 treatment produced the highest VFA concentration compared to others. This was due to the ration composition consisting of fermented Cajuput leaf waste, field grass, and the concentrate which caused high cellulose content. It has been explained that the VFA composition in the rumen changes with differences in physical form, feed composition, level or frequency of feeding, and processing. In addition, one of the factors influencing its production in the rumen is the amount of fermentability in carbohydrate source feed. High VFA production provides sufficient energy for livestock. It provides 50-70% of the energy digested by ruminants. Fermentation with *Aspergillus chevalieri* increases the crude protein content in the leaf waste by 8.19% and reduces crude fiber by 4.55%. This affected its potential as an animal feed ingredient.

This study emphasized the explanation of the QS. Al-Imran verse 191. Humans are given guidance in the form of reason to be used as well as possible in their activities. Among the tasks or activities of the mind mentioned in the verse is meditating on Allah's creation. None of Allah's creation is in vain, everything is correct and worthwhile, even when wasted. Therefore, utilizing waste through micro-organisms treatment is very useful. Further studies need to be carried out with *in-vivo* techniques to observe the effect on livestock.

CONCLUSION

The fermentation of Cajuput leaf waste using *Aspergillus chevalieri* produced animal feed ingredients with nutritional content that met the SNI requirements (protein 16.03%, fiber 16.92%, and fat 5.93%), as well as the best digestibility (DMD= 46.12%; OMD= 32.08%), NH_3 (8.37 mM), and VFA (168.5 mM) production.

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