The utilization of microbes as a fermentation agent to reduce saponin in Trembesi leaves (Sammanea saman)

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Submission date: 15-Nov-2020 05:22PM (UTC+0700)
Submission ID: 1446423474
File name: 2018_IOP_Conf._Ser.__Earth_Environ._Sci._142_012041_-revisi.docx (187.42K)
Word count: 2287
Character count: 12863

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Abstract. This objective of this research was to observe the utilization of microbes as a fermentation agent of trembesi leaves that can increase the quality of trembesi leaves as ruminants feed. Before fermentation, trembesi leaves were divided into three treatments. They were control = non-agentic in fermentation, D-An = the addition of *Aspergillus niger* as fermentation agent, and D-Lp = the addition of *Lactobacillus plantarum* as fermentation agent. Each treatment experienced five repetitions. The experimental design used a randomized direct pattern group design. The analysis included proximate analysis consisting of water content, crude protein content, crude fiber content, lipid content, mineral content (ash) and saponin content after fermentation. It could be concluded that the utilization of *Aspergillus niger and Lactobacillus plantarum* in fermentation could decrease saponin content and could increase the nutrient content of trembesi leaves by increasing crude protein content otherwise by decreasing content of crude fiber trembesi leaves.

1. Introduction

Ruminant livestock has a four plural compartments and constant of rumination process. Sustainability of rumination depends of crude fiber content. In the forage there is a high crude fiber content. This is because of the cellulose in the cell walls. Cellulose is a bond of D-glucose polymer with ß-1 and 4 glycosides.[1].

As a tropical area, Indonesia has 2 extreme seasons, namely the rainy season and the dry season. The tropical climate has high temperature also humidity.This condition less favorable for ruminants because a forage of the low availability. In rainy season the amount of forage is quite abundant but in dry season it is lacking, in certain areas there is absolutely not forage stock.

For example in the Wonogiri area, the availability of forage is very low during the dry season so that livestock will be feed from improper forage.

Trembesi from Mimosoideae family with their leaves, seeds and bark contain saponins. Moreover, Trembesi leaves and seeds contain polyphenols [2]. Trembesi leaves contain saponins whose numbers vary depending on the time of cutting.

Saponins are glycosides that will produce glycon (sugar) and aglycone (sapogenin) after hydrolysis. Saponin active compounds have soap-like activity and are detected based on

the ability to foam and have a bitter taste which results in decreased turgor so that it can result in lysis which cell membranes are damage, activates enzymes and damages proteins cell.

Saponins affect and metabolize the body's nutrients by inhibiting the work of the chymotrypsin enzyme which will inhibit livestock productivity and growth. The main effect of saponins is to cause hemolysis of red blood cells due to the interaction of saponins with erythrocyte membranes. Hemolysis is the release of hemoglobin due to damage to erythrocytes.

The saponin content of 0.20% in feed will adversely affect to growth, feed intake also feed efficiency. In alfalfa, saponin can bloating in ruminants because saponins are the active agents in producing a foamy of soap. Low levels of alfalfa usage decrease the average growth in poultry, the main effect of the saponin content is the palatability and feed intake compared to the effects of metabolism. The use of low strain increases the level of alfalfa saponins into feed for growth ruminants without degradation of performance.

Fermentation is fission of carbohydrates into alcohol, , butyric acid, carbonic acid , lactic acid also heat release. Protein reforms into ammonia, amides, amino acids, butyric acid ,water and acetic acid. In fermentation, there is a removal of anti nutritional substances that are toxic such as glucoside [3]. Furthermore, cassava leaves fermentation by *Aspergillus niger* increases digestibility protein and decreases crude fiber value [4].

2. Methods

The research was conducted in Laboratory of Biology, Chemistry and Microbiology of Agriculture Veteran Bangun Nusantara Sukoharjo University. Proximate analysis was done at Laboratory of Agricultural Technology of Sebelas Maret University and saponin analysis was done in Biology Laboratory of Pharmacy Faculty Gadjah Mada University. The study was conducted by following these procedures:

Preparation of fermentation media

The trembesi leaves were separated from tertiary stems (*dipritil*) then being collected and weighed. Each experimental unit required 0.5 kg of trembesi leaves.

Preparation of Aspergillus niger inoculum

Creating media to grow *Aspergillus niger* which is potatoes dextrose broth (PDB). In this study, 150 ml of GDP were poured into 10 ml of each reaction tube for 4 treatments of An-1 and 15 ml in 4 test tubes of An-2 treatment. Then being sterilized. Growing the *Aspergillus niger* in GDP media for each treatment. Incubating for 5 days at room temperature aerobically.

Implementation of fermentation

The collected trembesi leaves were then divided into treatments:

Control : fermentation of trembesi leaves without microbial addition as an agent
D-An : *Aspergillus niger* inoculum addited in fermentation of trembesi leaves
D-Lp : *Lactobacillus plantarum addited* in fermentation of trembesi leaves

Each treatment was repeated 5 times then stirred evenly and put into polyethylene plastic, pressed compressed and then pressed with siller. After three days of dismantling, we measured the content of fermented trembesi leaves nutrients through proximate analysis and saponin analysis.

In this study the variables observed were: nutrient content including water content, crude protein, crude fat, ash and crude carbohydrate through proximate analysis and saponin content of trembesi leaves [5].

The data obtained were analyzed by using a complete randomized design (RAL) on unidirectional pattern. If the treatment factor showed significant effect ($P < 0.05$) Duncan's Multiple Range Test (DMRT) at 5% level were performed [6].

3. Results and Discussion

The nutrient content of fermented trembesi leaves

To know the difference of quality of fermented trembesi leaves with the addition of different microbes such as *Aspergillus niger* and *Lactobacillus plantarum*, it is needed to do the proximate analysis. The result of proximate analysis in Table 1.

Table 1. The content of trembesi leaves fermented Nutrient

Treatment	Nutrient Content (%)				
	Water	Ash	CL	CP	CF
Kontrol	8.78	3.87[a]	5.85[c]	10.80	70.67[c]
D-An	8.79	4.15[b]	5.26[a]	20.74[c]	60.72[b]
D-Lp	8.57	4.40[c]	5.44[b]	9.54[b]	60.31[a]

Different superscripts on the same column showed significant differences ($P < 0.01$)

From Table 1 it was seen that with the addition of water content, the addition of *Aspergillus niger* and *Lactobacillus plantarum* provided a significant effect on the controls in conten of water, lipid, crude protein, crude fiber and ash. This showed that with the addition of microbes both *Aspergillus niger* and *Lactobacillus plantarum* in the fermentation of trembesi leaves could streamline the fermentation process. Fermentation was an effort to utilize microbes to increase the added value of a substance or substance with food or feed products [7].

The addition of *Aspergillus niger* and *Lactobacillus plantarum* in fermentation may increase the water content in the trembesi leaves although this increase is not significantly different when compared to the controls. Increased water content is due to fermentation produced molecular transformation so that the composition of nutrients contained in the leaves of trembesi experienced changes. This could be due to the activity of *Aspergillus niger* would produce acid namely citric acid. the acid state would inhibit the activity of microorganisms in the fission of carbohydrates and proteins which products are water vapor.

Table 1. showed that the ash content of the fermented leaves was relatively low but the addition of *Aspergillus niger* and *Lactobacillus plantarum* gave a marked difference compared to the subsequent controls. *Aspergillus niger* addition gives a marked difference compared to the addition of *Lactobacillus plantarum*. The ash content in the proximate analysis indicated the mineral content of the material. [8] stated that all human body tissues, animals and plants contained inorganic substances called minerals. Meanwhile, according to [9], proteins in plant leaves were existed in the cytoplasm of about half to one-third of the total protein, chloroplasts of which one-third to one-half of the total and the remainder of the nucleus were often called nucleoproteins.

Crude fiber content was found only in plant products. The presence of crude fiber in plants was due to the presence of lignin content that surrounds cellulose and hemicellulose in plant cell walls. The older the age of the plant the higher the crude fiber content of the plant [10]. Fermentation means changing the structure of feed ingredients into more easily digested, reducing allergen, anti-nutritive or indigestible components, to adding important anti-pathogenic, antioxidant and anti-carcinogenic metabolites [11]. Cellulose and hemicellulose are carbohydrates, when forage is fermented, microbes multiply so rapid and carbohydrates fermentation in organic acids [12]. The existence of *Aspergillus niger* and *Lactobacillus plantarum* in the fermentation process turned out to significantly lower the content of crude fiber of trembesi leaves. With the decline in the content of crude fiber will be more easily digested forage. *Lactobacillus plantarum addited* in the fermentation of trembesi leaves was more able to reduce the coarse fiber content compared with the addition of *Aspergillus niger*. This suggested that bacteria more rapidly multiplied to have a higher ability in break the lignin bonds in cell walls. By breaking the lignin bond it would decrease the lignin content in the cell wall which would further decrease the crude fiber content. The reduction of the content of crude fiber, the forage would be more easily digested.

Saponin content of fermented trembesi leaves

This study was to determine the effectiveness fermentation agent of microbes in trembesi leaves which could improve the trembesi leaves quality as ruminants feed. To see the quality of a material as animal feed, it was necessary to see the nutrient and anti-nutrient content of the material. Trembesi leaves contain saponin anti-nutrient substances [13]. The content of saponin of trembesi leaves which has been fermented by the addition of *Aspergillus niger* and *Lactobacillus plantarum* in Table 2.

Table 2. The content of trembesi leaves fermented Saponin

Treatment	Saponin Content (%)
Kontrol	1.468[a]
D-An	0.920[b]
D-Lp	0.888[b]

Different superscripts on the same row and column showvery significants differences (P <0.01)

Table 2. showed that *Aspergillus niger* and *Lactobacillus plantarum addited* in the fermentation of the trembesi leaves gave a significant effect on the decrease of saponin content in trembesi leaves compared with the control. Although it had a significant effect but the decrease in saponin content in this study has not yet reached the tolerable limit of saponin content in feed. [14] states that feeds containing more than 0.20% saponin will adversely affect growth, feed consumption and feed efficiency.

The addition of *Aspergillus niger* to the fermentation of trembesi leaves was not significantly different with the addition of *Lactobacillus plantarum* in fermentation as reducing the saponin content of trembesi leaves. This was in accordance with the opinions of [8] stating that fermentation is the utilization of microbes to increase the added value of a substance or material. And with the fermentation occurs elimination of anti-nutritional substances that are toxic [4].

4. Conclusion

Aspergillus niger and Lactobacillus plantarum addited in fermentation could reduce saponin content in trembesi leaves and increase the trembesi leaves' nutrient content. Increasing the crude protein content and decreasing the crude fiber content.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6