

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

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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 crude fiber content of trembesi leaves.

1. Introduction

Ruminant livestock has a stomach with four plural compartments and is constantly in a process of rumination. Sustainability of rumination process depends on the presence of feed material which has a high content of crude fiber. Feed ingredients with high crude fiber content are found in many forage crops. Cells within the plants contain cell walls which typical nutrient composer is cellulose. Cellulose is a polymer of D-glucose by binding β -1 and 4 glycoside [1].

Indonesia is a tropical country. A special feature on this climate is that there are two seasons namely rainy season and dry season in areas, beside that the tropical climate has high temperatures and humidity. This condition is actually less favorable for livestock especially ruminants cattle because the availability of forage is not guaranteed. During the rainy season, there are abundant mount of forage but during the dry season is very lacking, even in certain areas there is absolutely no forage stock.

In drought conditions, for example Wonogiri area, there will be massive sales of existing stock. This is due to the absence of staple forage ruminants, so to maintain the existing livestock, the farmers will provide improvised forage that is usually obtained from annual crops.

Trembesi is a perennial plant that is often referred to "ever green" from the family of Mimosoideae with their leaves, seeds and bark contain saponins. Moreover, Trembesi leaves and seeds contain polyphenols [2]. Saponins exist in all parts of the plant, for example in leaves, stems, roots, flowers and seeds while the amount varies according to time of cutting.

Saponins are glycosides that after hydrolyzed will produce sugar (glycan) and sapogenin (aglycone). Surface-active compounds of saponin has the quality as soap and is detected based on the



ability to form foam on agitation and has a bitter taste that has the effect of decreasing the surface tension so can damage cell membranes and activate cell enzymes and also damage the cell proteins.

Saponins can affect the body's biological processes and nutrient metabolism by inhibiting enzymes such as productivity of chymotrypsin enzymes work that inhibit productivity and growth of livestock. The main biological effects of saponins are able to cause haemolysis of red blood cells due to interaction of saponins with membrane (proteins, phospholipids and cholesterol) of erythrocytes. Haemolysis is the release of hemoglobin into the blood plasma due to destruction of erythrocytes.

Feed containing more than 0.20% saponin would be bad for growth, feed intake and feed efficiency. Saponins in alfalfa can cause bloating in ruminants because saponins are the active agents in producing a foamy surface of soap. Low levels of alfalfa meal usage decrease the amount of 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 food for growth ruminants without performance degradation.

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

2.1. 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.

2.2. 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.

2.3. 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 : addition of *Aspergillus niger* inoculum in fermentation of trembesi leaves

D-Lp : addition of *Lactobacillus plantarum* 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

3.1. 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 can be seen in Table 1.

Table 1. The nutrient content in fermented trembesi leaves

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 water content, 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 rapidly and ferment carbohydrates into 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. The addition of *Lactobacillus plantarum* 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.

3.2. Saponin content of fermented trembesi leaves

The purpose of this study was to determine the effectiveness of microbes as a fermentation agent in trembesi leaves which could improve the quality of trembesi leaves 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* can be seen in Table 2.

Table 2. Saponin content in fermented trembesi leaves

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 show very significant differences (P < 0.01)

Table 2. showed that the addition of *Aspergillus niger* and *Lactobacillus plantarum* 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 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

The utilization of *Aspergillus niger* and *Lactobacillus plantarum* in fermentation could reduce saponin content in trembesi leaves and could increase the trembesi leaves' nutrient content by increasing the crude protein content otherwise by decreasing the crude fiber content.

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