Changes in the nutritional value of lamtoro (*Leucaena leucosephala*) leaves fermented using *Trichoderma koningiopsis*

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Abstract. Lamtoro leaves can be a protein source for poultry feed, but the cells protect the protein. Cellulolytic fermentation is needed to degrade the cell walls and liberate proteins in cells. The study aimed to determine the effect of using *Trichoderma Koningiopsis* in fermentation on changes in nutrient content and weight of lamtoro leaf biomass. The study used a completely randomized design with a one-way pattern. The treatments consisted of fermentation duration: 0, 2, 4, and 6 days. Each treatment used three replications. The variables observed included temperature, pH, soluble protein content, cellulose, and biomass weight. The results showed that the fermentation of lamtoro leaves using *Trichoderma koningiopsis* significantly affected all observed variables. Fermentation temperature, soluble protein, and cellulose content initially increased (2 days: temperature; 4 days: dissolved protein and cellulose) and then decreased as fermentation continued.Meanwhile, pH decreased in 2 days of fermentation, and the value remained constant for 4 and 6 days. Biomass weight decreased by 18% in 6 days of fermentation. *Trichoderma koningiopsis* significantly affects temperature, pH, soluble protein content, crude fiber, cellulose, and Lamtoro leaf biomass weight. Six-day fermentation is the best fermentation.

1 Introduction

Protein-source feed ingredients are essential in determining the nutritional needs of livestock in feed, mainly feed ingredients for poultry, where protein-source feed ingredients have a minimum protein content of 20%. The animal protein source feed ingredient currently widely used is fishmeal, in addition to its high protein content, because it is easily digested by non-

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ruminant livestock such as poultry/chicken [1, 2]. However, the availability of fishmeal feed ingredients is still challenging, and the price is relatively high, depending on imports [3]. Therefore, alternative feed sources of protein that are more economical and abundantly available are the hope for cheap feed.

Lamtoro leaves *(Leucaena leucocephala)* are legumes with more than 20% crude protein content and are rich in carotene, minerals, and vitamins [4, 5]. The potential of Leucaena leaves as ruminant feed has been tested by various researchers [6, 7]. However, the potential as poultry feed is currently a different challenge considering the antinutrient content of mimosine in lamtoro leaves, which can inhibit the process of digesting feed in poultry because the digestive tract is unable to secrete the cellulase enzyme to digest crude fiber [8, 9].

Applying fermentation biotechnology to plant raw materials can increase digestibility and nutritional quality, including lamtoro leaf feed greens. Fermentation biotechnology involves biological agents such as *Trichoderma*, which is thought to be able to change complex compounds in the cell walls of green feed to be more easily digested. *Trichoderma* is known for deleting crude fiber, which consists of cellulose, hemicellulose, and lignin. *Trichoderma* converts cellulose into glucose by producing cellulase enzymes, which can significantly increase feed ingredients' nutritional value and digestibility [10 - 12].

Although lamtoro leaves are already known as an alternative source of protein for plant-based feed, in-depth research on the potential of lamtoro leaves as an alternative poultry feed needs to be done. This study explores the possibility of lamtoro leaf fermentation with *Trichoderma koningiopsis* AA1 to increase digestibility and be used more optimally as poultry feed. Through fermentation biotechnology with the newly discovered species *Trichoderma koningiopsis* AA1, it is hoped that it will increase feed digestibility in poultry digestion.

2 Materials and Methods

Microbial preparation. Four ml of molasses was dissolved in 120 ml of distilled water in a 300 ml Erlenmeyer flask, then sterilized using an autoclave at 15 psi for 15 minutes. After that, the solution was cooled to room temperature. Four g of *Trichoderma koningiopsis* AA1 inoculum in flour (in a carrier mixture of dry mud and glutinous rice) were added to the molasses solution and then incubated for 24 hours.

Medium Preparation. Four hundred grams of lamtoro leaf flour were mixed with 400 ml of distilled water in a plastic bag. The bag was then sterilized using an autoclave at 15 psi for 15 minutes. The medium was then cooled to room temperature.

Fermentation experiment. A solid medium of lamtoro leaves and *Trichoderma koningiopsis* AA1 solution were mixed evenly and then spread on a sterile stainless steel tray. The tray was covered with cotton cloth and put into an incubator chamber. The culture was incubated aerobically for six days. On days 0, 2, 4, and 6 of fermentation, fermented lamtoro leaf biomass sampling was carried out. The harvested fermented lamtoro leaf biomass was dried to constant weight and then weighed.

The experiment was designed using a Completely Randomized Design with one treatment factor, fermentation time. The types of fermentation time treatments include 0 (control), 2, 4, and 6 days. Each type of treatment was repeated using three fermentation experiment units. The variables observed included Fermentation temperature, PH, Satpathy *et al.* (2020), Crude fiber AOAC (2019), Cellulose Chesson (1981), and Biomass weight. The data were analyzed using a One-way Analysis of Variance, and further testing was performed using Duncan's multiple-range test [16].

3 Results and Discussion

Lamtoro leaves are included in the legume plant group with high protein content Lamtoro leaves are included in the legume plant group with high protein content Palupi *et al.*, (2020), but the protein is protected in the cells (Rinduwati *et al.*, 2023). Meanwhile, plant cell walls are classified as compounds that cannot be digested by chicken digestive enzymes (Mulyono *et al.*, 2021). In this study, lamtoro leaf fermentation was carried out using *Trichoderma koningiopsis* AA1 to see how much the nutrient and biomass content changed. The results showed that lamtoro leaf fermentation using *Trichoderma koningiopsis* AA1 had a significant effect ($P < 0.05$) on all observed variables (Table 1).

Table 1. The effect of fermentation of lamtoro leaves (Leucaena leucocephala) on temperature, pH, soluble protein content, cellulose, and dry matter of biomass.

Variable	Fermentation duration (days)			
	0	2	4	6
Temperature (ºC)	26,47 ^{a}	32,57 ^{c}	30,13 ^{bc}	28,80 ^{ab}
pH	5,00 ^{b}	3,67 ^{a}	3,67 ^{a}	3,67 ^{a}
Soluble protein content (% DM)	19,90 ^{a}	19,93 ^{a}	22,73 ^{b}	23,30 ^{b}
Cellulose content (% DM)	16,20 ^{a}	18,75 ^{b}	19,74 ^{c}	18,63 ^{b}
Biomass weight (g DM)	400,0 ^{a}			327,3 ^{b}

$^{(a,b,c)}$ Different superscripts in the same row indicate significant differences ($P<0.05$); DM: Dry Matter.

In this study, lamtoro leaves were used as a variable substrate. Therefore, significant changes in all research variables indicate that *Trichoderma konginiopsis* AA1 undergoes a metabolic process by utilizing lamtoro leaves as its substrate.

3.1 Temperature

Significant changes and increases in temperature occurred on day 2, reaching 32.57ºC and decreased on day 4 (30.13ºC) and 6 (28.80ºC). This substantial increase is suspected of intensive microbial activity, especially from day 0 to day 2 in the early stages of fermentation. Microbial metabolic activity causes an increase in temperature in response to the activity of substrate decomposition by microbes [17, 18]. Fermentation after day 2 shows that the fermentation process has reached a stable point, and feedback from the substrate decomposed by microbes begins to decrease.

Fermentation after the second day shows that the fermentation process has reached a stable point, and feedback from the substrate decomposed by microbes begins to decrease. The availability of easily digestible substrates in this phase begins to decline; of course, this has an impact on reducing heat production from microbial metabolism. In addition, a decrease in temperature can be caused by the accumulation of organic acids from fermentation, causing a reduction in microbial activity and having an impact on the fermentation temperature [19]. The phenomenon of temperature changes that occur indicates a transition from the active phase to fermentation stabilization [20, 21].

3.2 Fermentation pH

The results show the production of organic acids as a by-product of fermentation, as seen in Table 1. There was a decrease in pH from 5 to 3.67 on the second day, and so on. The ligninolytic *Trichoderma* species used in the fermentation process can degrade the lignocellulose components in green fodder through the microbial biochemical cycle [22, 23].

The decrease in pH impacts microbial fermentation by-products in the form of organic acids, such as lactic acid and acetic acid. Legodi *et al*, (2023) found that the ligninolytic microbe *Trichoderma* is responsible for decreased pH because it produces acid during fermentation [25].

pH stabilization occurs after the second day of fermentation with the same pH value consistency. The pH stability indicates that fermentation has reached a stable condition and achieved optimal efficiency, as seen from the production of acid and degradation by *Trichoderma koningiopsis* AA1, which has reached equilibrium.

3.3 Soluble protein content

The soluble protein content significantly increased from 19.90% to 23.30% on day 6. That shows that fermentation with *Trichoderma koningiopsis* AA1 on lamtoro leaves effectively increases the levels of protein available in feed ingredients. *Trichoderma koningiopsis* AA1 is known to have the ability to break down plant cell walls consisting of cellulose, hemicellulose, and lignin, which are thought to inhibit digestive enzymes against essential nutrients such as protein [26, 27]. Iram *et al*. (2020) show that fermentation by ligninolytic microbes such as Trichoderma can break down the structure of cell walls so that previously trapped proteins are released and easily absorbed by poultry digestion.

In addition, the increased levels of dissolved protein are due to the proteolytic activity of the microbe *Trichoderma koningiopsis* AA1, where the enzyme secretion by the microbe can break down proteins into simpler forms such as peptides and amino acids Cruz-Casas *et al*, (2021) and has the potential to be used as poultry feed [30, 31].

The soluble protein content in lamtoro leaf flour feed is high, has positive feedback in the poultry feed industry, and feed efficiency and production costs can be reduced. Feed with high soluble protein content makes it easier for poultry to digest the feed, has implications for FCR, and ultimately increases livestock productivity.

3.4 Cellulose content

Cellulose levels fluctuate during fermentation, with the highest increase on day 4 of 19.73%. The rise in cellulose levels during the fermentation process is due to microbes' degradation of the lignocellulose structure [28]. *Trichoderma koningiopsis* AA1 can secrete cellulase and ligninase enzymes, which effectively break down the bonds between lignocellulose in plant cell walls so that lignin and cellulose are separated [32, 33]. Lignin in the cell wall has a function of strength and protection from degradation. Cellulose not bound to lignin will be more easily degraded by cellulolytic microbes because lignin is released from the lignocellulose bond [34].

Cellulose levels on the fourth day showed the highest increase because microbial activity in breaking down cellulose peaked before cellulose levels decreased along with the decomposition into simple sugars (glucose) [35]. After the 4th day, there was a decrease in cellulose because most of the cellulose had been released from the lignin bond, so further degradation into simple sugars (glucose) by *Trichoderma koningiopsis AA1* occurred. *Trichoderma* can secrete cellulase enzymes, effectively breaking cellulose down into glucose [36].

3.5 Biomass weight

The decrease in biomass weight from 400 g to 327.3 g indicates that Trichoderma koningiopsis AA1 is able to utilize lamtoro leaves as a source of microbial nutrition. Gandra

et al. (2017) stated that microbial fermentation decreases biomass weight due to the conversion of organic matter into simple gases.

During fermentation, *Trichoderma koningiopsis* AA1 breaks down plant cell walls and utilizes polysaccharides and organic compounds as energy. The decrease in biomass weight is caused by most organic matter being converted into secondary metabolites and gases. Flodman and Noureddini (2013) and Xu *et al.* (2022) stated that biomass decomposition by microbes not only reduces biomass weight but also increases the availability of microbial nutrients such as *Trichoderma koningiopsis* AA1.

Thus, the decrease in biomass not only shows the success of fermentation by Trichoderma koningiopsis AA1 but also shows the potential of lamtoro leaves as an alternative feed. This process shows the possibility of improving the quality and availability of nutrients in alternative feed [34, 40].

4 Conclusions

Leucaena leucosephala leaf fermentation using *Trichoderma koningiopsis* has a significant effect on temperature, pH, soluble protein content, crude fiber, cellulose, and biomass weight. Six days fermentation is the best fermentation with the highest soluble protein content, and crude fiber and cellulose begin to decrease.

5 Recommendation

Referring to the best fermentation time (6 days), further research is needed to test fermented *Leucaena leucocephala* leaves regarding their nutritive value as poultry feed ingredients.

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