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Differences in fermentation time and various rumen liquids on the nutrient content and tannins of Calliandra leaves (*Calliandra calothyrsus*)

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ABSTRACT

Fermentation is one way to lower tannin levels and improve nutritional quality. The purpose of this study is to determine the effect of fermentation time and various rumen liquids on the nutrient content and tannins of Calliandra leaves. In this study, two factors were used, namely Factor 1 of fermentation time (L1:0 day, L2: 7 days, L3: 14 days) and Factor 2 of various types of rumen liquids (C1: sheep, C2: cattle), which were compiled in a Factorial Complete Random Design. The factors observed were crude protein, crude fiber, crude fat, and tannins. Based on the results, the interaction of Calliandra leaves fermentation using different fermentation time and rumen liquid had a very real influence ($P<0.01$) on the average value of crude protein (20.03-25.13%), crude fiber (9.27-12.84%), crude fat (0.97-1.80%), and tannins (23.22–36.65 TAE mg/g). Based on the results of this study, C1L3 and C2L3 which have crude protein content of 22.18% and 25.13%, respectively, crude fiber content of 9.84% and 11.44%, and crude fat content of 1.80% and 1.25% are the most ideal treatment combinations. However, with tannin levels of 2.32% and 3.19%, sheep rumen liquid has been proven to be more successful in reducing tannin levels than bovine rumen liquid.

Keyword: calliandra, fermentation, nutrient, rumen, tannins

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1. Introduction

One strategy to improve the low quality of forage is the use of legume. The nutrient content of legumes is higher in protein than grass. Leguminosae are the best choice for animal feed. Grass usually contains less than 10% protein, while Leguminosae contains more than 20%. Besides being rich in protein, Leguminosae also contain minerals such as calcium, phosphorus, magnesium, copper and cobalt. [1]. One of the legumes used as a source of forage for animal livestock is Calliandra.

Calliandra plants are known by the Indonesian people because they can grow on various types of land, including marginal land. This plant, which is included in the legume family, is widely used by farmers as animal feed because it has a protein content of 20-25%. Calliandra is very good as a forage that has a high source of protein for livestock, however, besides having good nutritional content, Calliandra also contains tannins, which can have a negative effect on livestock. Tannins are anti-nutritional substances that can interfere with livestock health, affect feed digestibility, reduce productivity and can cause poisoning in livestock. The tannin content in Calliandra is 11% [2]. Therefore, the use of Calliandra leaves in the ration of ruminants is only 30-40% in the ration [3]. To reduce the tannin content, one of the methods that can be done is through feed processing technology, one of which is fermentation technology.

Fermentation is one method of feed processing that can be used to reduce the content of antinutrients in Calliandra leaves. The purpose of this fermentation is to reduce antinutrient levels and increase the nutritional value of feed by utilizing microbes to simplify complex molecules into simpler molecules. Thus, it can increase the absorption of feed nutrients in the body of livestock. the fermentation process is influenced by several factors, one of which is the fermenter used during the fermentation process.

Rumen liquid is one of the fermenters that can be used to fermentation of feed. The rumen liquid of cattle and sheep contains enzymes produced by bacteria in the rumen and other microorganisms, so it can be used as one of the inoculums that can be used to reduce antinutrients. In addition, rumen liquid also contains vitamins, minerals and other nutrients that are the result of degradation by bacteria and rumen enzymes. According to [4], rumen liquid contains about 12.5% dry matter, 8.1% crude protein, 38.02% crude fiber, 0.37% calcium, 0.26% phosphorus, and 2.361 kcal/kg metabolic energy. The addition of 2-6% Bali cattle rumen liquid to agricultural waste feed can increase body weight gain by 59.09-100% compared to unfermented feed [5].

In this study researchers will conduct tests on the use of two different rumen, namely sheep and cattle rumen. The use of two different types of rumen was carried out to determine the type of microbes that are good to be used as fermenters in the fermentation process of forage, especially Calliandra. Based on the description above, the author is interested in conducting research on the effect of different fermentation times and various rumen liquids on the nutrient and tannin content of Calliandra leaves [6].

2. Methods

2.1. Research design

The research method used is an experimental method using a Factorial Complete Randomised Design consisting of two factors. Factor 1 consists of two locations of rumen liquid, namely C1 = rumen liquid comes from sheep and C2 = rumen liquid comes from cows and factor 2 consists of the length of days L1 (0 days), L2 (7 days) and L3 (14 days), with three replicates each for each treatment factor, so the total sample for this study was 18 samples.

2.2. Research procedure

This research was conducted in several stages. The first stage was the manufacture of fermenters using rumen liquid (cattle and sheep), the second stage was the fermentation process of Calliandra leaves, and the last stage was the observation of each research parameter.

2.2.1. Manufacture of fermenters using rumen liquid (cattle and sheep)

The rumen liquid used in this study was the rumen liquid of beef cattle and sheep. The rumen liquid was taken from the Medan city slaughterhouse. Rumen liquid is taken from freshly slaughtered livestock. Rumen liquid taken is a greenish colored liquid, which indicates that livestock consume the green grass. After that, the rumen was filtered to separate solids and liquids, after which it was put into a thermos that had been filled with warm water beforehand to regulate the rumen temperature to 40°C, after which the rumen liquids are ready to be used as fermenters for fermentation.

2.2.2. Fermentation of Calliandra leaves

Fermentation of Calliandra leaves was carried out using cattle and sheep rumen liquid with a dose of 175 ml/kg of the total Calliandra leaves used for each treatment, after which the Calliandra leaves were fermented for 0 days, 7 days and 14 days for each rumen liquid (beef cattle and sheep).

2.3. Research Parameters

The parameters observed in this study were the levels of crude protein, crude fiber, crude fat and tannins. The analysis of crude protein, crude fiber and crude fat refers to the method of AOAC [7], while the analysis of tannin content refers to the method of Chanwitheesuk *et al* [8], which is analysis the total tannin content in animal feed.

2.3.1. Crude protein

Crude protein analysis was carried out based on the AOAC method with the principle of the Kjeldahl Method with three stages, namely destruction, distillation and titration. The stages carried out are the process of weighing the sample (Calliandra leaves that have been fermented according to treatment and have been dried), after which H_2SO_4 solution is added for the destruction process, after that the addition of NaOH solution in the distillation process and the final stage of the titration process using HCl or H_2SO_4 solution.

2.3.2. Crude fiber

Crude fibre analysis was conducted based on the AOAC method. The stages carried out are the process of weighing the sample (Calliandra leaves that have been fermented according to the treatment and have been dried), after which the addition of H_2SO_4 solution and heated, after that NaOH was added. Then the liquid is filtered using filter paper. The remaining solution is called the crude fiber fraction. The remaining solution was then washed using hot water, K_2SO_4 and acetone. The final step was dried and weighed the sample.

2.3.3. Crude fat

Crude fat analysis was carried out based on the AOAC method with the Soxhlet method. The steps taken were to prepare a fat flask. Then the sample was weighed (fermented Calliandra leaves according to the treatment and had been dried), wrapped in filter paper and put into the fat flask. Next, it is doused with fat solvent (hexane), and attached to a soxhlet distillation device to extract fat. After the solvent has evaporated, the fat can be weighed and the percentage calculated.

2.3.4. Tannins

Testing of tannin content was carried out based on the method of Chanwitheesuk *et al* [8], which is the measurement of total tannin content. the steps taken were 10 mL of diethyl ether was used to extract 0.5 g of Calliandra leaves for 20 hours. The leaves were then filtered, and the pulp was cooked in 100 mL of distilled water for two hours before being cooled and filtered. Distilled water was added to the extract volume until 100 mL was obtained. After adding 0.1 mL of Folin Ciocalteu reagent to the extract, it was vortexed. 2 mL of Na_2CO_3 was then added and vortexed again. The absorbance was measured at 760 nm after 30 minutes of room temperature incubation. The total tannin concentration was measured in milligrams of tannic acid per kilogram of extract, and the results were plotted against a standard curve of tannic acid prepared using the same method.

3. Results and Discussion

3.1. Crude Protein

The results of the study of the difference in fermentation time and various rumen liquids on the crude protein content of Calliandra leaves can be seen in Table 1.

Tabel 1. Crude protein of Calliandra leaves fermented with various rumen liquids and fermentation time length

Factor 1 (Rumen liquid)	Factor 2 (Fermentation time)			Average ± Standard Deviation
	L1 (0 Days)	L2 (7 Days)	L3 (14 Days)	
C1 (Sheep)	20.37^{Aa}	22.48^{Ba}	22.18^{Ba}	21.68±1.03
C2 (Cattle)	20.03^{Aa}	24.06^{Bb}	25.13^{Cb}	23.08±2.35
Average ± Standard Deviation	20.20±0.412	23.27±0.911	23.66±1,646	

Note: Superscripts that are different with capital letters in the direction of the line and lowercase letters in the direction of the column indicate a real difference ($P<0.05$).

The results of ANOVA analysis showed that the effect of the use of various rumen liquids and the length of fermentation time and their interactions had a significant effect ($P<0.05$) in increasing the crude protein content of Calliandra leaves. The results of Table 1 analysis in the row between sheep and cattle rumen liquid treatments showed that the longer the fermentation time, the higher the crude protein. In sheep rumen liquid as a catalyst, the length of time showed a significant difference with 0 days and no significant difference between 7 and 14 days. Whereas in cattle rumen liquid, the increase in crude protein content was more consistent with significant differences seen on days 7 and 14.

If we compare sheep and cattle rumen fluid on day 0, there was not much change in each treatment. However, on days 7 and 14, there was a significant difference between the sheep and cattle rumen liquid treatments, where the cattle rumen liquid showed a higher crude protein concentration. The high crude protein value of Calliandra leaves fermented using cattle rumen liquid compared to sheep rumen liquid is due to differences in the type of feed consumed. Cattle rumen liquid has more proteolytic bacteria because the feed given is complete feed while the feed given to sheep is dominant forage. The existence of the percentage of microbial species that develop in the rumen is strongly influenced by the type of feed, because this type of feed will

affect the production of metabolites. If livestock are given forage feed, cellulolytic bacteria will develop. Based on Duncan's further test analysis, it shows that the highest crude protein content is found in the interaction of C1L2 and C2L3 treatments, which is 22.48% and 25.13%. Things that affect the increase in crude protein include the number of bacteria, the length of fermentation, and the decrease in pH during ensilage and the length of fermentation [9]. In this case, the length of fermentation time has an important role in increasing the protein content of Calliandra leaves. The fermentation process that takes place for 14 days stimulates microbial growth, causing an increase in microbial mass that is rich in protein. According to [10], the increase in crude protein content occurs because microbes produce crude protein as a product of their metabolism.

In addition, enzymes produced by rumen microbes hydrolyze the crude protein content to make peptides that are then converted into amino acids. In order for various organic components to decompose, rumen microorganisms require certain amino acids to be converted to ammonia (NH3) during the ammonia process and several other amino acids are used as building blocks for body proteins [11]. This is in line with the results [10] that the length of fermentation increases the amount of crude protein produced by microorganisms.

In addition, rumen microbes make enzymes that hydrolyze crude proteins into peptides, which are then converted into amino acids. The ammonia process converts certain amino acids into ammonia (NH3), which rumen microorganisms use to make body proteins and break down various organic components [11]. This is according to the results of [10] that the crude protein content produced by microorganisms increases with the duration of fermentation.

This happens because proteins get their contribution from the biomass of the microbes themselves which are rich in proteins, making up between 40 and 65% of their composition. According to different studies, by crude protein content, fermented bran can be improved by using bovine rumen liquid at various intervals [12]. However, research conducted by [13] also found that Putak fermented using sheep rumen liquid had higher crude protein values. The presence of proteolytic bacteria in the rumen liquid of fully fed cows, compared to sheep fed only forage feed, is believed to be the cause of the increased protein content in the rumen liquid of cows. Feed type has a significant influence on the percentage of microbial types that grow in the rumen because feed type affects metabolite production. The development of cellulolytic bacteria occurs when livestock are fed animal feed.

Based on the analysis of the Duncan further test (DMRT), it was shown that the interaction of C1L2 and C2L3 treatment had the highest crude protein concentration, respectively at 22.48% and 25.13%. The abundance of cellulolytic bacteria, the duration of fermentation, and the decrease in pH during ensiling and fermentation are all factors that affect the rise of crude protein [14]. Microbial fermentation activity is the cause of the increase in crude protein values in Calliandra leaves fermented with different rumen liquids and at different periods. The activity of fermentation microbes and the presence of proteolytic bacteria capable of breaking down proteins during fermentation are closely related to the increase in crude protein content in the treatment of C1L2 (7-day fermentation with sheep rumen liquid) and C2L3 (14-day fermentation with bovine rumen liquid). More microbial activity, particularly proteolytic bacteria, is produced by prolonged fermentation periods.

The protein content of Calliandra leaves flour 0 days was 24.50% [15], slightly higher compared to our study. This difference may be due to the condition of the soil containing high nitrogen, which has an impact on the quality of the Calliandra leaves used in this study. Nitrogen is essential for the development of plant parts such as leaves, stems, and roots. More protein will be produced if nitrogen is more readily available than other components.

3.2. Crude Fiber

The results of the study of the difference in fermentation time and various rumen liquids on the crude fiber content of Calliandra leaves can be seen in Table 2. ANOVA analysis showed that the effect of the use of various rumen liquids and the length of fermentation time with their interaction had a real effect ($P<0.05$) in reducing the crude fiber content in Calliandra leaves. Based on the data presented in Table 4, it can be seen that the crude fiber content in Calliandra leaves decreases with the increase in fermentation time with both sheep rumen liquid and cow rumen liquid. In sheep rumen liquid with a catalyst, the time period showed a noticeable difference with a time duration of 0 days and no real difference between 7 and 14 days. In addition, in bovine rumen liquid, the decrease in coarse fiber

showed a significant difference in the duration of 0 and 7 days while with a duration of 14 days it showed no significant difference.

Table 2. Crude fiber content of Calliandra leaves fermented with various rumen liquids and fermentation time

Factor 1 (Rumen liquid)	Factor 2 (Fermentation time)			Average ± Standard Deviation
	L1 (0 Days)	L2 (7 Days)	L3 (14 Days)	
C1 (Sheep)	12.84^{Aa}	9.27^{Ba}	9.84^{Ba}	10.65±1,701
C2 (Cattle)	12.02^{Ab}	10.86^{Bb}	11.44^{ABb}	11.44±0.551
Average ± Standard Deviation	12.43±0.56	10.065±0.94	10.64±0.89	

Note: Superscripts that are different with capital letters in the direction of the line and lowercase letters in the direction of the column indicate a real difference (P<0.05).

The fermentation time is significantly different when the treatment is applied to sheep and cattle rumen liquid with the same fermentation duration. The longer the fermentation time, the more food ingredients are broken down by rumen microbes. An increase in crude protein concentrations is often accompanied by a decrease in crude fiber, indicating a persistent negative relationship between the two variables.

The crude fiber content of Calliandra leaves fermented using sheep rumen fluid was lower than that using cattle rumen liquid. This is because the sheep consume more forage/fiber, so that the sheep rumen liquid contains more dominant cellulolytic bacteria, while in cattle, the type of feed given contains feed complete (a lot of protein), so the bacteria produced in cattle rumen liquid are more dominant proteolytic bacteria. So that the sheep rumen liquid fermenter is more effective in reducing crude fibre than the fermenter from cattle rumen liquid. Feed type has a significant influence on the percentage of microbial types that grow in the rumen because feed type affects metabolite production [10]. The development of cellulolytic bacteria occurs when livestock are fed animal feed. Hydrolyses Lamtoro leaf flour using enzymes from sheep rumen liquid. They found that adding 100 ml/kg of enzyme and letting it sit for 24 hours reduced the amount of crude fiber by 53.46% [16]. According to another study conducted by the crude fiber content of rice straw, it can be lowered from 28.6% to 26.49% by fermenting it with cow rumen liquid at a dose of 10-15% and urea 1% [14].

Based on the results of the Duncan further test (DMRT) Table 4, the C1L2 and C2L2 interaction treatments had the lowest crude fiber content values, 9.27% and 10.86%. The presence of cellulolytic bacteria in the sheep's rumen liquid that multiplies rapidly can be used to explain the decrease in the lowest crude fiber concentration over a seven-day period. The cellulase enzyme produced by cellulolytic bacteria is useful in the breakdown of crude fibers. The balance between the number of bacteria and the availability of nutrient supply is what causes the decrease in crude fiber content in fermented feed [14]. This prevents germs from competing with each other resulting in optimal microbial growth. In these circumstances, cellulolytic bacteria can make cellulase enzymes, which break down cellulose, allowing for more effective cellulose degradation activities in feed ingredients. However, on the fourteenth day, the microbes reach equilibrium due to the gradual interaction of the various rumen liquid, making the destruction of coarse fibers stable.

3.3. Crude Fat

The results of the study of the difference in fermentation time and various rumen liquids on the content of Crude Fat from Calliandra leaves can be seen in Table 3. ANOVA analysis showed that the effect of the use of various rumen liquids and the length of fermentation time with their interaction had a real effect (P<0.05) in increasing the crude fat content in Calliandra leaves. Based on Table 5 and the data presented, it can be seen that the crude fat content in Calliandra leaves has increased with the increase in fermentation time using both sheep rumen liquid and cow rumen liquid. In sheep rumen liquid with a catalyst for a long time shows a significant difference in each treatment, but in a cow rumen liquid shows no significant difference in a catalyst for fermentation time. This is suspected to be due to the lack of microbial activity in the overhaul of crude fat content.

Table 3. Crude fat content of Calliandra leaves fermented with various Rumen liquid and fermentation time length

Factor 1 (Rumen liquid)	Factor 2 (Fermentation time)			Average ± Standard Deviation
	L1 (0 Days)	L2 (7 Days)	L3 (14 Days)	
C1 (Sheep)	0.97^{Aa}	1.43^{Bb}	1.80^{Cb}	1.4±0.369
C2 (Cattle)	1.17^{Ab}	1.21^{Aa}	1.25^{Aa}	1.21±0.094
Average ± Standard Deviation	1.07±0.18	1.32±0.137	1.53±0.306	

Note: Superscripts that are different with capital letters in the direction of the line and lowercase letters in the direction of the column indicate a real difference (P<0.05).

There is a noticeable variation between treatments if you check the columns for each treatment with the same amount of fermentation time. This is because fermentation time is one of the crucial factors in animal feed processing that can affect the amount of crude fat. This study shows that changes in fermentation duration can result in important changes in the nutritional makeup of feed, particularly those related to crude fat. Variations in fermentation duration have an impact on the crude fat content of banana peel silage [17]. The crude fat content increased when tapioca was added and fermented for 28 days. During the fermentation process, carbohydrates are broken down into simple sugars, which are further converted into lactic acid by lactic acid bacteria. This technique increases the nutritional value of fats in addition to making them more soluble. To get the best feed results, it is important to understand the dynamics of fermentation. This study shows that the crude fat content of fermented Calliandra leaves increases with the presence of sheep rumen liquid. It is caused by microbial activity that converts complex carbohydrates into fatty acids such as butyric acid, propionate, and acetate [1]. The microorganisms in the ensiling process contribute to increased crude fat levels [18]

The C1L3 interaction (14-day fermentation with sheep rumen liquid) had the highest average crude fat value, with an average value of 1.80%, according to the research findings in Table 3 based on the analysis of the Duncan follow-up test (DMRT). Analysis of the variance of the data showed that the value of crude fat was significantly affected (P<0.05) by the interaction of rumen liquid (sheep) and fermentation time (0, 7, and 14 days).

The bacteria in the rumen liquid also contains 6–11% fat, which may be a factor in the increase in crude fat in fermented Calliandra leaves [18]. The crude fat content is also affected by the time of fermentation; The longer the fermentation, the higher the crude fat content. The crude fat yield of putak fermented with rumen liquid for 0 days ranged from 2.30% to 3.56% for 21 days. The crude fat content of fermented ingredients increases with the length of the incubation period [15].

3.4. Tannins

The results of the study of the difference in fermentation time and various rumen liquids on the Tannin content of the Calliandra leaves can be seen in Table 4.

Table 4. The tannins content of Calliandra leaf tannins fermented with various liquids Rumen and fermentation time length

Factor 1 (Rumen liquid)	Factor 2 (Fermentation time)			Average ± Standard Deviation
	L1 (0 Days)	L2 (7 Days)	L3 (14 Days)	
C1 (Sheep)	3.58^{Aa}	3.28^{Aa}	2.32^{Ba}	3.06±0.65
C2 (Cattle)	3.53^{Aa}	3.66^{Aa}	3.19^{Ab}	3.46±0.81
Average ± Standard Deviation	3.55±1.02	3.47±0.22	2.76±0.48	

Note: Superscripts that are different with capital letters in the direction of the line and lowercase letters in the direction of the column indicate a real difference (P<0.05).

ANOVA analysis showed that the influence of the use of various rumen liquids and the length of fermentation time with the interaction was significantly affected (P<0.05) in reducing the tannin content. The results of the analysis of Table 4 in the row section showed that there was only a significant difference in the sheep rumen liquid with a long fermentation time, while in the cattle rumen liquid there was no significant difference in the

treatment. When compared between the treatment of sheep rumen liquid and bovine rumen liquid in the same column, it shows no real difference, and only shows a significant difference in the treatment of sheep rumen liquid with a duration of 14 days. This is because the length of fermentation time is an important factor in the processing process that can reduce the tannin content. Some studies show that the fermentation process can lower tannins. Research by [19] showing that fermentation of liquid feed made from tamarind seeds can significantly reduce tannin levels. This process indicates that fermentation can break down complex tannin compounds making them less biologically active, thereby improving the digestibility of the feed.

Fermentation with various types of rumen liquid can hydrolyze carbohydrates and sugars. Since tannins have carbohydrate groups in their molecular structure, this carbohydrate hydrolysis mechanism also hydrolyzes tannins. Feed fermentation is an efficient way to lower tannin levels. The results of this study are in line with the study of [17], that the tannin content of unfermented tamarind seeds can be lowered by 5.7% through fermentation with Rhizopus oligosporus and then lowered to 0.43-0.34% after fermentation.

With an average value of 2.32%, the findings of the Duncan test in Table 4 showed a very significant influence on tannin content on the interaction of C1L3 treatment ($P<0.05$). There was a significantly difference in C1L3 when compared to unfermented Calliandra, which contained 3.58% sheep rumen liquid. This is likely because the sheep used in the rumen liquid are used to tannins. It's because the tannin-tolerant bacteria are present in Ethiopian sheep fed acacia feed [20].

Because the microbial enzymes involved in the process can hydrolyse complex tannins into simpler molecules that are easily soluble in water, it was found that fermentation utilizing rumen liquid can also lower tannin levels [21]. The tannin content can be lowered through the fermentation process. The reduction in the concentration of tannins into simpler molecules is also affected by the duration of fermentation. The longer the fermentation process continues, the more oxidation will change the tannins and reduce the tannin content in the leaves [22].

The feeding legumes containing more than 0.5% tannins can inhibit the growth of cows and goats, especially if the tannin content is more than 4%, it can even be fatal. The proteins can be bound by tannin molecules [17], thus creating complex bonds that make it difficult for protease enzymes to digest proteins [23]. However, tannins are not always bad, this is evident from in vitro results [24] showing that the addition of tannins can significantly reduce methane production. Tannins are polyphenols that interact with extracellular enzymes and bacterial cell walls. In addition to decreasing the digestibility of feed ingredients and the total synthesis of volatile fatty acids (VFAs), these interactions can inhibit the entry of nutrients into the cells. Because tannins tend to bind to proteins strongly, rumen bacteria may not be able to break down certain proteins.

4. Conclusion

Fermentation time of 14 days can increase the nutrient content and decrease the tannin content of Calliandra leaves. Cattle rumen fluid fermenter can increase the protein content of Calliandra leaf flour, while sheep rumen fluid fermenter can reduce the content of crude fiber and tannins in Calliandra leaves, so it can be concluded that the best treatment combination is obtained when using sheep rumen liquid with a fermentation time of 14 days (C1L3).

References

[1] A. S. Sudarmono and Y. B. Sugeng, *Panduan Beternak Sapi Potong*. Jakarta, ID: Penebar Swadaya, 2008.

[2] S. Nurjanah, B. Ayuningsih, I. Hernaman, and I. Susilawati "The Use of Calliandra calothyrsus, Indigofera sp. and Its Mix in Diet to Substitute Concentrate on Productivity of Male Garut Sheep," *Jurnal Ilmiah Peternakan Terpadu,* vol. 7, no. 3, pp. 293-298, 2019.

[3] R. T. Paterson, E. Kiruiro, and H. K. Arimi, "Calliandra calothyrsus as a Supplement for Milk Production in the Kenya Highlands," *Tropical Animal Health and Production*, vol. 33, pp. 115-126, 1999.

[4] S. A. Moningkey, R. A. V. Tuturoong, and I. D. R. Lumenta, "Pemanfaatan Isi Rumen Terfermentasi *Cellulomonas* Sp Sebagai Campuran Pakan Komplit Ternak Kelinci," *Zootec*, vol. 40, no. 1, pp. 352-362, 2020.

[5] T. I. Putri, T. G. B. Yadnya, I M. Mudita, and B. R. T. Putri, "Biofermentasi Ransum Berbasis Bahan Lokal Asal Limbah Inkonvensional dalam Pengembangan Peternakan Sapi Bali Kompetitif dan Sustainable," Denpasar, ID, *Laporan Penelitian Hibah Kompetitif Penelitian Sesuai Prioritas Nasional*, 2009.

[6] N. Y. Amalia, S. Surono, and S. Sutrisno, "Pengaruh Penambahan Isi Rumen dalam Ransum terhadap Konsumsi Nutrien pada Domba Pasca Sapih Dini," *Jurnal Sain Peternakan Indonesia*, vol. 14, no. 3, pp. 265–271, Sep. 2019, doi: 10.31186/jspi.id.14.3.265-271.

[7] L. George Jr. *Official Methods of Analysis of AOAC INTERNATIONAL, 22nd Edition,* New York, US, online edn, AOAC Publications, 2023.

[8] A. Chanwitheesuk, A. Teerawutgulrag, and N. Rakariyatham, "Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand," *Food Chem*, vol. 92, no. 3, pp. 491–497, 2005, doi: 10.1016/j.foodchem.2004.07.035.

[9] N. Widaningsih, S. Dharmawati, and N. Puspitasari "Kandungan Protein Kasar dan Serat Kasar Tongkol Jagung yang Difermentasi dengan Menggunakan Tingkat Cairan Rumen Kerbau yang Berbeda," *Ziraa'ah Majalah Ilmiah Pertanian*, vol. 43, no. 3, pp. 255-265, 2018.

[10] N. Nurhayati, B. Berliana, and N. Nelwida, "Kandungan nutrisi ampas tahu yang difermentasi dengan Trichoderma viride, Saccaromyces cerevisiae dan kombinasinya,". *Jurnal Ilmiah Ilmu-Ilmu Peternakan*, vol. 23, no. 2, pp. 104-113, 2020.

[11] M. D. H. Putra, R. M. S. Putri, Y. Oktavia, and A. F. Ilhamdy, "Karakteristik Asam Amino dan Asam Lemak Bekasam Kerang Bulu (Anadara antiquate) di Desa Benan Kabupaten Lingga," *Jurnal Marinade,* vol. 3, no. 2 pp 160-167, 2020.

[12] H. P. Nalar, B. Irawan, S. N. Rahmatullah, N. Muhammad, and A. K. Kurniawan, "Pemanfaatan cairan rumen dalam proses fermentasi sebagai upaya peningkatan kualitas nutrisi dedak padi untuk pakan ternak" *Seminar Nasional "Inovasi Teknologi Pertanian Spesifik Lokasi*, pp. 563-568, 2014.

[13] C. Umbu, J. Tagumara, A. Hilakore, and D. Amalo, "Pengaruh Lama Fermentasi Dengan Cairan Rumen Kambing Terhadap Perubahan Kualitas putak (Effect of fermentantion time using goat rumen liquid on changes of putak quality)," *Jurnal Peternakan Lahan Kering*, vol. 2, no. 3, pp. 1022–1028, 2020.

[14] L. Mirni "Inokulasi Bakteri Bacillus pimilus ML 08 Untuk Peningkatan Kualitas Nutrisi Jerami Padi Untuk Pakan Ternak Sapi Potong," *JurnalAgroveteriner* vol. 2, no. 2, pp. 152-153, 2014.

[15] R. Ginting, I Surahman, and Adelina, "Pengaruh pemanfaatan fermentasi tepung daun kaliandra (*Calliandra calothyrsus*) menggunakan cairan rumen sapi dalam pakan terhadap pertumbuhan ikan bawal air tawar (*Colossoma macropomum*)," Undergraduate Thesis, Fakultas Perikanan dan Kelautan, Universitas Riau, Riau 2018.

[16] I. Fitriliyani, E. Harris, and N. Nahrowi "Peningkatan Kualitas Nutrisi Tepung Daun Lamtoro Sebagai Pakan Ikan dengan Penambahan Ekstrak Enzim Cairan Rumen Domba" *Berita Biologi*, vol. 10, no. 2, pp. 135-142, 2010.

[17] T. N. I. Koni, A. Paga, and A. Jehemat, "Kandungan Protein Kasar dan Tanin Biji Asam yang Difermentasi dengan Rhyzopus Oligosporus," *Partner*, vol. 20, no. 2, pp. 127-132, 2013.

[18] I. M. Suadnyana, I. G. L. O. Cakra, and I. W. Wirawan, "Physical and Chemical Quality of Rice Straw Silage Made by The Addition of Bali Cattle Rumen Fluid," *Jurnal Peternakan Tropika,* vol. 7, no. 2, pp. 661-675, 2019.

[19] R. Wea, A. Y. Ninu, and B. B. Koten, "Kualitas Nutrisi dan Anti Nutrisi Pakan Cair Fermentasi Berbahan Biji Asam," *Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science)*, vol. 22, no. 2, p. 133, Jun. 2020, doi: 10.25077/jpi.22.2.133-140.2020.

[20] A. Odenyo, R. Bishop, G. Asefa, R. Jamnadass, D. Odongo, and P. Osuji, "Characterization of Tannin-tolerant Bacterial Isolates from East African Ruminants," *Anaerobe*, vol. 7, pp. 5–15, Feb. 2001, doi: 10.1006/anae.2000.0367.

[21] S. S. Sarnaik, G. B. Shinde, and V. V. Mahajani "Enzymatic hydrolysis of tannin from Terminalia chebula in a solid–liquid system," *Journal of Food Science and Technology*, vol. 54, no. 4, pp. 1175-1182, 2017.

[22] A. Eviza, "Pengaruh Lama Fermentasi Terhadap Mutu Teh Daun Gambir (Uncaria Gambir Roxb.)," *Agroplantae: Jurnal Ilmiah Terapan Budidaya dan Pengelolaan Tanaman Pertanian dan Perkebunan*, vol. 10, pp. 50–58, Apr. 2021, doi: 10.51978/agro.v10i1.249.

[23] A. Jayanegara, and A. Sofyan. "Penentuan aktivitas biologis tanin beberapa hijauan secara in vitro dengan polietilen glikol sebagai determinan," *Media Peternakan,* vol. 31, no. 1, pp. 44-52, 2008.

[24] C. S. Mcsweeney, B. Palmer, D. M. Mcneill, and D. O. Krause, "Microbial interactions with tannins: nutritional consequences for ruminants," *Animal Feed Science and Technology,* vol. 91, pp. 83-93, 2001.