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The Effect of Corn Stover and Red Calliandra in Silage on the Dry Matter, Organic Matter, in Vitro Digestibility

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ABSTRACT

This study seeks to determine the impact of corn stover and Red Calliandra on dry matter, organic matter, and in vitro digestibility. This research employed a completely randomized design (CRD) featuring four treatments and five replicates: P1 (90% corn stover + 10% Calliandra calothyrsus), P2 (80% corn stover + 20% Calliandra calothyrsus), P3 (70% corn stover + 30% Calliandra calothyrsus), and P4 (60% corn stover + 40% Calliandra calothyrsus). We used analysis of variance (ANOVA) to look at the data and then Duncan's multiple range test to look at the results. The observed variables included dry matter, organic matter, dry matter digestibility, and organic matter digestibility. The findings indicated that the combination of corn stover and Calliandra calothyrsus significantly influenced dry matter digestibility and organic matter digestibility ($p < 0.05$), although it exerted no significant impact on dry matter and organic matter content ($p > 0.05$). The combination of 80% corn stover and 20% Calliandra calothyrsus (P2) produced the optimal silage, with dry matter digestibility of 54.03% and organic matter digestibility of 59.72%. Higher inclusion levels (30-40%) significantly reduced digestibility ($p < 0.05$) due to the anti-nutritional effects of tannins, while dry matter and organic matter content were not significantly affected ($p > 0.05$).

Keywords: Corn Stover Silage, Calliandra Red, Microbial Population, Rumen pH, Methane Gas

INTRODUCTION

Indonesia is a country with significant potential for the development of the cattle farming sector. According to the Indonesian Statistics Agency, the number of beef cattle in Indonesia is projected to reach 11,749,780 head by 2024, BPS (Indonesian Statistics Agency), which requires an adequate supply of feed. However, tropical climatic conditions create challenges, especially during the dry season when the availability of forage decreases drastically, which can disrupt farmers' productivity and potentially increase the incidence of disease in livestock susceptibility to disease (Ali et al., 2024). Therefore, high-quality feed preservation technology is needed to address these issues, ensuring that feed requirements are met. One solution that can be used is silage. One technique for preserving forage through anaerobic fermentation to preserve and improve the quality of feed is silage (Pudjawati et al., 2024).

The forage preservation method that can be used is silage, which utilizes the principle of anaerobic fermentation, in which bacteria degrade complex carbohydrates that are broken into simple sugars, which form the basis for lactic acid formation (Widyastuti, 2008). The process of lactic acid formation in silage is known as ensilage (Anjalani et al., 2017). Silage is one effective method for preserving nutrient content and ensuring long-term storage during the. Success in silage production is influenced by the quality of raw materials, moisture content, carbohydrate content, and bacteria in the feed material, as well as the fermentation management practices (Borreani et al., 2018).

One potential energy source for livestock is corn stover. Corn stover is often used as a silage ingredient due to its stable availability and high soluble carbohydrates suitable for fermentation (Sahid et al., 2022). Corn cobs contain only 7.8% protein (Mustika & Hartutik, 2021). A study by Kurniawan et al. (2022) noted that adding Indigofera legumes to corn stover silage can increase the dry matter content and the quality of the silage. The results of a study by Sutaryono et al. (2023) indicate that the addition of Leucacena legumes to corn stover silage increases dry matter content, and organic matter, as well as the in vitro digestibility of the silage. Therefore, it is necessary to add high-protein feed ingredients to increase the nutrient content of the silage.

Red calliandra (*Calliandra callothyrsus)* is a tree legume used for livestock feed because it contains more than 20% protein (Susilawati & Khairani, 2017). However, calliandra contains tannins, which are anti-nutritional compounds (Sulistyawati et al., 2024). Tannins can inhibit lactic acid bacteria, disrupting the ensilage process, reduces palatability and digestibility in livestock. Therefore, an appropriate formulation is needed between corn stalks and red calliandra to produce high-quality silage.

MATERIALS AND METHODS

Materials

This study was conducted at the Laboratory of Ruminant Animal Nutrition and Food Chemistry at the Faculty of Animal Husbandry, Padjadjaran University, Sumedang, West Java, from November to December 2025. This study utilized equipment including silage-making tools, namely 10-liter plastic jar, 40×60 cm plastic bag, 8×6 m tarp, vacuum, machete, Five-point balance, for testing dry matter and organic matter as well as in vitro digestibility, electric oven, analytical balance, desiccator, forceps, furnace, fermenter tubes, and stopper with a valve, beakers, magnetic stirrer, water bath at 39–40°C, thermos, muslin cloth, pH meter, fermentor tube rack, 50-ml volumetric pipette, centrifuge, centrifuge tubes, porcelain uses. This study uses two main groups of materials. The first group consisted of corn stover (90 days after harvest), red calliandra, and molasses. The second set of materials was for in vitro digestibility testing, namely corn stover silage, McDougall's solution (pH 6.8–7.0, neutral), beef cattle rumen fluid (39°C from the Ciroyom in Bandung), distilled water, CO_2gas, 2%$HgCl_2$, 0.2% pepsin HCl solution, and Whatman filter paper 41 mm. Supporting equipment included a vacuum pump, a cleaver, and a five-pan balance.

Observation Parameters

The parameters observed were dry matter content, organic matter content, and in vitro digestibility, namely dry matter digestibility and organic matter digestibility.

Procedure

Silage Production

To produce silage, the moisture content of corn stalks was reduced by wilting them for 24 hours, and red calliandra for 24 hours. The two materials were then chopped into 2–4 cm pieces using a machete and weighed according to the treatment. Molasses was added at a rate of 4% of the total silage weight per treatment, diluted with water at a 1:1 ratio. All ingredients were then mixed homogeneously into 10-liter jars lined with plastic, compacted, vacuum-sealed to remove air, and tightly sealed to create an anaerobic environment. Fermentation was carried out for 22 days at room temperature. After fermentation, the samples were sun-dried for 2–3 days. The samples, with reduced moisture content, were then ground into flour using a hammer mill fitted with a 20-mesh screen. The resulting samples were then examined and tested according to the parameters mentioned.

Dry Matter

Dry matter analysis refers to the AOAC (2005) oven method. This method begins by weighing a 5-gram sample, which is then oven-dried at 105 °C for 24 hours; the moisture content is then determined and calculated as follows this

$$Dry\ Matter\ (\%) = 100\% \left(\frac{C - A}{B}\right) \times 100\%)$$

where A is the weight of the aluminum dish (g), B is the weight of the oven-dried sample, and C is the weight of the aluminum dish and oven-dried sample (g).

Organic Matter

The analysis of organic matter refers to the AOAC (2005) furnace method. This method begins by weighing 2–5 grams of the sample, which is then oven-dried at 105 °C for 1 hour. The sample was then placed on a hot plate until the carbonization process was complete, after which it was incinerated in a furnace at 600°C for 6 hours. The resulting content is calculated as follows

$$Organic\ Matter = DM - \left(\frac{(C - A)}{B}\right) \times 100\%)$$

where A is the weight of the porcelain dish (g), B is the weight of the oven-dried sample, and C is the weight of the porcelain dish and oven-dried sample (g).

In Vitro Digestibility

In vitro digestibility analysis refers to the method of Tilley & Terry (1963). There are two main phases in this analysis: fermentative digestion by rumen fluid and enzymatic digestion using a pepsin-HCl solution. A sample of 0.5 g per treatment was weighed, then the sample was incubated in a water bath conditioned to resemble the rumen environment at a temperature of 39◦C and pH 6.5–6.9, with each phase lasting 48 hours. After the enzymatic digestion process was complete, Whatman No. 41 filter paper was used to separate the residue, which was then placed into an aluminum dish. For the dry matter digestibility analysis, the samples were dried in an oven at 105 °C for 24 hours. Next, to determine the digestibility of the dried residue's organic matter, it was incinerated in a furnace at 600 °C for 4 hours. As a correction factor, the blank value obtained from the residue of fermentation without the sample was used. The calculation is as follows Tilley & Terry (1963)

$$DMD = \frac{Initial\ DM - (Residue\ DM - Blank\ DM)}{Initial\ DM} \times 100\%$$

$$OMD = \frac{Initial\ OM - (Residue\ OM - Blank\ OM)}{Initial\ OM} \times 100\%$$

where Initial DM and Initial OM are the sample weights before incubation, and Residual DM and Residual OM are the

Data Analysis and Method

A total of 20 experimental units with 4 treatments and 5 replicates were conducted using a Completely Randomized Design (CRD), with the following details:

T1 = Silage with a ratio of 90% Corn Stover and 10% Red Calliandra

T2 = Silage with a ratio of 80% Corn Stover and 20% Red Calliandra

T3 = Silage with a ratio of 70% Corn Stover and 30% Red Calliandra

T4 = Silage with a ratio of 60% Corn Stover and 40% Red Calliandra

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A total of 20 experimental units were used for the analysis of dry matter, organic matter, and in vitro digestibility, with three additional units serving as a blank. Statistical analysis was performed using an analysis of variance (ANOVA) via SPSS version 27. If significant differences ($P<0.05$) were found between treatments, to determine the differences in mean values between treatments, Duncan's multiple range test was performed at a 5% significance level.

RESULT AND DISCUSSION

This study evaluated the effects of different ratios of corn stover and Red Calliandra silage on dry matter, organic matter, dry matter digestibility, and organic matter digestibility under in vitro conditions. The results are presented in Table 1.

Table 1. The Effect of Corn Stover and Red Calliandra Ratio in Silage on Dry Matter, Organic Matter, Dry Matter Digestibility, and Organic Matter Digestibility (In Vitro).

Parameter	Treatment			
	T1	T2	T3	T4
Dry Matter (%)	31.77±1.47	35.04±2.98	34.74±1.99	33.22±3.13
Organic Matter (%)	30.10±1.39	33.05±2.98	32.66±1.83	31.30±2.98
Dry Matter Digestibility (%)	53.91±1.10^{c}	54.03±1.07^{c}	52.15±0.57^{b}	46.47±0.51^{a}
Organic Matter Digestibility (%)	59.07±1.16^{c}	59.72±1.23^{c}	56.74±0.48^{b}	56.52±0.44^{a}

Data are presented as mean ± SD. Based on ANOVA, only the dry matter digestibility and organic matter digestibility were significantly affected by the treatments ($P \leq 0.05$), whereas dry matter and organic matter showed no significant differences among treatments ($P > 0.05$). Different superscript letters indicate significant differences exclusively within the dry matter digestibility and organic matter digestibility row. T1 = 90% Corn Stover + 10% Red Calliandra; T2 = 80% Corn Stover + 20% Red Calliandra; T3 = 70% Corn Stover + 30% Red Calliandra; T4 = 60% Corn Stover + 40% Red Calliandra.

Dry Matter

Looking at the table of ANOVA results, the effects of the corn and red Calliandra on dry matter did not have a significant effect ($p > 0.05$). Dry matter content results range from 31.77% to 35.04%. The lowest BK content was found in P1 at 31.77%, followed by P4 at 33.22%, followed by P3 at 34.74%, and the highest average was found in P2 at 35.04%. A study by Li et al. (2022) showed higher values, ranging from 24.53% to 31.55%, in a study of corn and soybean silage. However, these values are nearly comparable to those in the study by Ayuni et al. (2017), which reported 27.28–34.16% for corn stover and gliricida leaves Although there were variations in the mean values among the silage treatments, these differences do not indicate statistically significant differences.

This result is due to the fact that treatment P1 had a higher proportion of corn stalks, whereas treatment P4 had a balance of kaliandra red. Wardana et al. (2019) increased with the addition of 20% noted in their study that the increase in red calliandra substitution only improved digestibility up to 20% inclusion of red calliandra; after a 20% addition of red calliandra, the dry matter content will decrease further. Harjono et al. (2023) in their study demonstrated that the addition of legumes to corn stover silage, with higher proportions, reduces the dry matter content. This decrease in dry matter content is consistent with the findings of Surono et al. (2006). That respiratory activity degrades nutrient content, while fermentation produces lactic acid and water, which increases the moisture content.

The increase in moisture content can act as a trigger leading to a decrease in dry matter, caused by the fermentation of simple sugars. According to Harjono et al. (2023), the addition of molasses is also thought to stimulate this fermentation process, thereby increasing water production, which causes a reduction in dry matter in silage. Thus, the fermentation of simple sugars can be a factor in the reduction of dry matter.

The increase in red calliandra substitution only increased with.

Organic Matter

Referring to the table of analysis of variance results, the effect of the corn stover and red calliandra on organic matter did not have a significant effect ($p > 0.05$). The value was highest in P3 at 33.05, followed by P2 at 32.66, P4 at 31.3, and finally P1 at 30.1. Although there were variations in results among treatments, according to statistical calculations, these results had no significant effect. The data obtained are consistent with the study by Wardana et al. (2019), which showed that the increase in red calliandra substitution only increased with 20% of red calliandra; after a 20% addition of red calliandra, the organic matter content decreased further.

Organic matter content is related to dry matter content. A high dry matter content reflects a higher organic matter content, whereas a low dry matter content implies a low organic matter content. Ash content in silage can be a factor in the decline of organic matter content.(Bagus et al., 2024) argue that the activity of bacteria that consume easily soluble carbohydrates leads to a decrease in organic matter content. This may also be due to differences in material characteristics. Red calliandra has a high protein content, whereas corn stalks have a high soluble carbohydrate, which can accelerate the decrease in pH, followed by a phase where anaerobic activity ceases more rapidly. The continued activity of aerobic bacteria during the aerobic phase can convert the substrate into CO_2 , H_2O, and heat from respiration. This decrease in organic matter content indicates that fermentation leading to degradation occurs during the ensilage process Harjono et al. (2023).

Dry Matter Digestibility

Based on the results of the analysis of variance, the effect of the corn stover and red calliandra ratio on dry matter digestibility was highly significant ($p<0.01$). Multiple range test, Duncan's test was used to examine the differences in the effects of each treatment. From the test results, it was found that P2 had a result of 54.03, followed by P1 with an average value of 53.91. This indicates that there is no significant difference between P1 and P2, but they differ significantly from P3, which had a mean of 52.15, and P4, which had a mean of 46.47. These test results are lower than those of Parwata et al. (2025), who tested elephant grass silage with various types of legumes, gliricida leaves, caliandra (Indigofera zollingeriana), with DMD values ranging from 56.22 to 69.12. However, this is consistent the study by Mudhita et al. (2024), which tested elephant grass silage with red calliandra and Lactiplantibacillus plantarum, yielding average values ranging from 45.95 to 54.00.

Dry matter digestibility is one of the crucial parameters in feed quality evaluation. Dilaga et al. (2022) state that high nutrient availability can be indicated by high digestibility. The increase in dry matter digestibility can be attributed to the high crude protein content in the silage. The results of the study by Mudhita et al. (2024) show that the addition of red calliandra increases dry matter digestibility. They explained that the increased crude protein content stimulates microbial activity to degrade silage during the dry matter digestibility test. According to Sutaryono et al. (2023), the addition of legumes can provide a source of nitrogen for rumen microbes.

An increase in dry matter digestibility occurred only at 10% to 20% substitution of red calliandra, whereas a substitution of 30% to 40% resulted in a decrease in dry matter digestibility due to lower carbohydrate content, as red calliandra contains tannins. These findings are consistent with the findings of Sulistyawati et al. (2024) that red calliandra has tannins. Tanuwiria & Hidayat (2019) note that high tannin content causes protein binding from the mastication process all the way to the rumen. This unintended protein binding significantly limits nutrients available to microbes, which results in the inhibition of growth and the activity of microorganisms in degrading feed; Zhao et al. (2025) also state that tannins can inhibit fibrolytic activity. The threshold effect observed in this study (decreased digestibility only at >20% Calliandra inclusion) suggests that tannin concentrations below a certain level may be tolerated by rumen microbes, or that the tannin-binding capacity of feed proteins is saturated only at higher inclusion levels. This is consistent with previous research of Tanuwiria & Hidayat (2019) and Zhao et al. (2025) showing that low to moderate tannin levels (2-4% of dietary dry matter) can protect dietary protein from rumen degradation without adversely affecting fiber digestion, while higher levels (>6%) suppress both protein degradation and fiber fermentation by binding to enzymes on the microbial surface or by binding to cellulose, which has the potential to suppress the growth of degrading bacteria. A study by Mudhita et al. (2024) found that adding more than 30% red Calliandra to gamma umami grass silage reduces dry matter digestibility.

Organic Matter Digestibility

Referring to the analysis of variance, the effect of the corn stalk and red Calliandra on organic matter digestibility had a highly significant effect ($p<0.01$). The Duncan multiple range test was used to examine the differences between treatments. From the test results, it was found that P2 had a mean score of 59.72, followed by P1 with a mean score of 59.07.This indicates that there is no significant difference between P1 and P2, but there is a significant difference compared to P3, which had an average yield of 56.74, and P4, with an average yield of 50.55. When compared to the study by Parwata et al. (2025), who tested the of elephant grass silage with various types of legumes (gamal leaves, calliandra leaves, Indigofera zollingeriana), the results were lower, with DMO values ranging from 58.78 to 70.00. However, these were lower than those reported in the study by Mudhita et al. (2024), which tested elephant grass silage with red calliandra and Lactiplantibacillus plantarum, which yielded an average ranging from 43.39 to 53.65.

The digestibility of organic matter is related to that of dry matter; that is, organic matter, which consists of major nutrients such as carbohydrates, crude fat, and protein, constitutes the major component of dry matter content Parwata et al. (2025). According to Dilaga et al. (2022), the results The digestibility of organic matter is directly proportional to the digestibility of dry matter, because the composition of dry matter is dominated by organic matter, which makes the results obtained almost consistent with dry matter digestibility, increasing in P1 and P2, then decreasing in P2, P3, and P4. The Crude protein content is one of the factors in increasing the digestibility of the material Crude protein has a positive effect on the digestibility of organic matter because rumen microbes can easily degrade crude protein Makmur et al. (2022).

Meanwhile, the decrease from P2 to P3 and P4 was caused by the increased tannin content tannins. According to Tanuwiria & Hidayat (2019), tannins are antinutritional compounds that can help protect proteins from microbial degradation in the rumen due to their ability to form complex compounds that reduce protein degradation. Microorganism growth can be inhibited by the presence of tannins, which can alter the rumen microbial population and affect the ability of rumen microbes to degrade nutrients in feed Bain et al. (2025); furthermore, excess tannins can bind to post-rumen enzymes, causing reduced hydrolytic activity and a decrease in digestibility.

CONCLUSIONS

The combination of corn stover and red calliandra proved to have an improvement in the digestibility of silage in vitro. Although this combination did not significantly alter the total dry matter and organic matter content, the proportion of raw materials determines the efficiency of nutrient degradation. A mixture of 80% corn stover and 20% Calliandra calothyrsus (P2) proved to be optimal, yielding dry matter digestibility of 54.03% and organic matter digestibility of 59.72%. While this combination did not significantly alter total dry matter or organic matter content compared to other treatments, it maximized nutrient degradation efficiency. The decline in digestibility at inclusion levels above 20% highlights the importance of balancing protein supplementation against the anti-nutritional effects of tannins.. To test direct effectiveness, Future in vivo studies should measure live weight gain, feed conversion efficiency, and methane emissions in cattle fed silage containing 80% corn stover and 20% Calliandra calothyrsus to validate these in vitro findings under practical farming conditions.

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