Effect of the Ratio Between Corn Forage and Red Calliandra on Ph, Lactic Acid, Ammonia, and Organic Matter Changes in Silage

Putri Ayu Winenggar[1], Iin Susilawati[2], Ujang Hidayat Tanuwiria[2]

[1]Student at the Faculty of Animal Husbandry, Department of Animal Nutrition and Feed Technology, Padjadjaran University

[2]Faculty of Animal Husbandry, Department of Animal Nutrition and Feed Technology, Padjadjaran University, Sumedang, Indonesia

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ABSTRACT

The objective of this study was to determine the effect of corn stover and red calliandra ratios on pH, lactic acid, ammonia, and organic matter (OM) change of silage. A Completely Randomized Design (CRD) was used with four treatments and five replications, consisting of P1 (90% corn stover + 10% red calliandra), P2 (80% corn stover + 20% red calliandra), P3 (70% corn stover + 30% red calliandra), P4 (60% corn stover + 40% red calliandra). All data were analyzed using analysis of varians (ANOVA) followed by Duncan's multiple range test. The observed variables included pH, lactic acid, ammonia, and changes in silage organic matter. The results showed that the ratios of corn stover and red calliandra had no significant effect on pH, lactic acid, ammonia or changes in silage organic matter ($p > 0,05$). The best treatment was obtained in P1 (90% corn stover + 10% red calliandra), which produced the lowest pH (4,07), the lowest ammonia level (4,68%), and the smallest organic matter loss (-0,35%), indicating slightly more efficient fermentation. It can be concluded that the combination of corn stover and red calliandra produces silage with relatively uniform chemical quality, while the ratio of 90% corn stover and 10% red calliandra tends to provide the most optimal fermentation outcome.

Keywords: Corn stover silage, Red calliandra, Fermentation quality, Ammonia concentration, Organic matter loss.

INTRODUCTION

The availability of reliable and nutritious forage is a key factor influencing the productivity of ruminant animals. Seasonal fluctuations, particularly during dry periods, often cause a reduction in forage quality and quantity. These conditions create challenges for farmers, making preservation methods such as ensiling an important option to maintain feed value over extended storage periods [1]. Ensiling depends on an anaerobic fermentation process in which lactic acid bacteria utilize water-soluble carbohydrates to form lactic acid, reduce pH, and limit the growth of undesirable microorganisms [2]. The efficiency of this process varies according to the characteristics of the material being ensiled, including dry matter content, carbohydrate supply, and the stability of anaerobic conditions throughout fermentation. Key chemical indicators such as pH, lactic acid, ammonia, and organic matter change provide a comprehensive evaluation of these fermentation processes and are widely used to assess overall silage quality.

Corn stover (*Zea mays*) is widely used as a silage material because it is available in large quantities and contains fermentable carbohydrates that support lactic acid bacteria activity [3]. Despite this advantage, its crude protein level is relatively low, making it less ideal as a standalone substrate. Red calliandra (*Calliandra calothyrsus*), on the other hand, is a leguminous plant with higher protein content and rumen-resistant protein fractions [4]. Its main limitation comes from its tannin content, which can suppress microbial activity and potentially slow fermentation. Combining these two materials may help balance their strengths and weaknesses, resulting in more stable fermentation.

Based on these considerations, evaluating silage formulated from corn stover and red calliandra is necessary. This study investigates how different ratios of the two forages affect silage pH, lactic acid content, ammonia concentration, and changes in organic matter during fermentation.

MATERIAL AND METHODS

This research was conducted at the Ruminant Nutrition and Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University, Indonesia for 1 months.

Tools and Materials

The tools and materials used in this study included 90-day-old corn stover, red calliandra, molasses, distilled water, 10-liter plastic jars, 40×60 cm plastic bags, an 8×6 m tarpaulin, a vacuum sealer, a machete, a Five Goats scale, a pH meter, a beaker glass, titration equipment, a drop pipette, Conway dishes, Erlenmeyer flasks, an electric oven, a muffle furnace, an analytical balance, aluminum cups, porcelain crucibles, a desiccator, and tongs.

Silage Production

The moisture content of the corn stover was reduced through wilting for approximately 2 hours, while red calliandra leaves were wilted for about 24 hours to achieve an appropriate dry matter level for the ensiling process. Both materials were then chopped using a machete into 2–4 cm pieces, weighed, and mixed according to the treatments. Molasses diluted with water at a 1:1 ratio was added at 4% of the total fresh weight for each treatment, and the mixture was stirred until homogeneous. The mixed materials were placed into plastic-lined jars and compacted to remove air. The jars were tightly sealed and fermented at room temperature for 22 days. After fermentation, the silage was opened and evaluated for quality by measuring pH, lactic acid concentration, ammonia (NH_3) content, and changes in organic matter as indicators of fermentation success.

Research Methods

The study was conducted using a Completely Randomized Design (CRD) with 4 treatments and 5 replicates, resulting in a total of 20 experimental units. The treatments carried out included:

P1 = 90% Corn stover + 10% red Calliandra

P2 = 80% Corn stover + 20% red Calliandra

P3 = 70% Corn stover + 30% red Calliandra

P4 = 60% Corn stover + 40% red Calliandra

Observed Variables:

Silage pH

Silage pH was measured to determine the acidity level as a primary indicator of fermentation quality. The pH was recorded directly inside the fermentation jars using a digital pH meter calibrated with pH 4.0 and pH 7.0 buffer solutions. Measuring pH under actual in-jar conditions provides an accurate representation of the fermentation environment and reflects the extent of lactic acid–driven fermentation and overall silage stability.

Lactic Acid

Lactic acid concentration was determined to evaluate the intensity of fermentation by lactic acid bacteria (LAB). The analysis followed an alkali titration method based on Cappuccino and Sherman (1991). A 10 g silage sample was homogenized with 10 mL distilled water, heated to remove dissolved CO_2, and subsequently cooled. A few drops of phenolphthalein indicator were added before titration with 0.1N NaOH until a stable pink endpoint was achieved. Final titration volume was used to calculate lactic acid concentration.

Ammonia (NH_3)

Ammonia content was analyzed as an indicator of protein deamination during fermentation. The Conway micro-diffusion technique (Conway, 1957) was employed. Silage extract (supernatant) and saturated Na_2CO_3 were placed in the outer compartments of a Conway dish, while boric acid solution containing mixed indicators was positioned in the central well. The sealed dish was incubated for 24 hours to allow volatilized NH_3 to be absorbed by the boric acid solution, which was subsequently titrated with standardized H_2SO_4 (e.g., 0.005 N) until a distinct endpoint color change occurred.

Measurement of Organic Matter (OM) Change

Organic matter (OM) change was assessed to quantify the loss of organic fraction during ensiling, representing the extent of substrate utilization by microorganisms. OM determination followed the ash method, in which samples were combusted at 550°C until constant weight. OM content was calculated as 100% minus ash content. The change in OM between pre-ensiling and post-fermentation samples was computed using the formula commonly applied in mixed-forage silage studies [4]:

$$Organic\ Matter\ Change\ (\%) = \frac{(OM\ before\ fermentation - OM\ after\ fermentation)}{OM\ before\ fermentation} \times 100$$

This parameter serves as an indicator of nutrient conservation efficiency throughout the fermentation process.

Statistical Analysis

Statistical analysis was tested with analysis of variance (ANOVA). If the results obtained were significantly different ($P<0.05$) between treatments, a further test was conducted using Duncan's multiple range test at a significance level of 5% to determine the differences between treatments. Data processing was analyzed using the SPSS program.

RESULT AND DISCUSSION

Table I Result Of Effect Of Corn Stover And Red Calliandra On Ph, Lactic Acid, Ammonia And Organic Matter Change

Parameter	Treatment			
	P1	P2	P3	P4
pH	4,07 ± 0,05	4,22 ± 0,09	4,26 ± 0,13	4,44 ± 0,08
Lactic Acid	1,55 ± 0,23	1,40 ± 0,15	1,81 ± 0,12	1,51 ± 0,26
Ammonia	4,68 ± 0,54	5,85 ± 0,60	6,17 ± 1,14	7,12 ± 1,48
Organic Matter Change	-0,35 ± 0,81	-1,31 ± 0,82	-1,17 ± 0,68	-0,74 ± 0,87

Data are presented as mean ± SD. All parameters showed no significant differences among treatments based on ANOVA ($p > 0.05$); therefore, superscript letters are not included.

Note: P1 = 90% Corn stover + 10% red calliandra; P2 = 80% Corn stover + 20% red calliandra; P3 = 70% Corn stover + 30% red calliandra; P4 = 60% Corn stover + 40% red calliandra.

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The overall results showed a relatively uniform pattern across all measured parameters, including pH, lactic acid, ammonia, and organic matter change. This uniformity indicates that the fermentation process proceeded in a similar manner across treatments, despite the differing proportions of red calliandra. The predominance of corn stover in the mixtures likely provided a consistent supply of fermentable carbohydrates that supported stable lactic acid bacteria activity at all inclusion levels. The similarity among treatments also suggests that the tannin content of red calliandra, at the proportions used in this study, was insufficient to alter the main microbial fermentation pathways. These findings offer a general overview of the fermentation dynamics and serve as a basis for the more detailed parameter-specific discussion presented in the following sections.

pH

The statistical analysis showed that varying the proportions of corn stover and red calliandra did not lead to significant changes in silage pH ($p = 0.474 > 0.05$). The observed pH values ranged from 4.07 to 4.44 (4.25 ± 0.03), indicating stable fermentation within the normal range for well-preserved silage, generally between 3.8 and 4.5. This stability suggests that including red calliandra up to 40% did not modify the acidification process. This result is consistent with [5], who noted that pH tends to remain steady when sufficient water-soluble carbohydrates are available to support lactic acid bacteria.

A similar trend has been reported in the review by [6], which states that materials with high carbohydrate content, such as corn, typically maintain relatively consistent pH values even when mixed with other forages, as long as anaerobic conditions are well maintained. Likewise, [7] observed that combining various forage sources with substrates rich in water-soluble carbohydrates generally does not produce major differences in final pH because lactic acid bacteria can still ferment the available substrate effectively. In the present study, the tannin content of red calliandra also appeared too low to suppress LAB activity, and therefore did not result in any notable increase in pH.

Overall, all treatments showed indications of proper fermentation. P1 recorded the lowest pH value (4.07), reflecting a slightly stronger acidification pattern, although the differences among treatments were small and remained within the acceptable range for good-quality silage.

Lactic Acid

The variation in corn stover and red calliandra ratios did not produce significant differences in the lactic acid concentration of the silage ($p = 0.730 > 0.05$). The average values were relatively close, ranging from 1.40 to 1.81% (1.57 ± 0.07%), which shows that adding up to 40% red calliandra did not substantially modify the activity of lactic acid bacteria during fermentation. Treatment P3 (70:30) had the highest lactic acid level, indicating a slightly better balance between the water-soluble carbohydrates supplied by corn stover and the nitrogen contribution from calliandra. This pattern supports the concept that lactic acid formation depends heavily on the supply of water-soluble carbohydrates, which serve as the primary substrate for lactic acid bacteria [7].

The lack of significant differences also suggests that the calliandra proportion used in this study was not high enough to shift the overall fermentation pattern, including any microbial inhibition potentially caused by tannins. Similar trends have been observed in alfalfa-based silages, where changing the level of legume inclusion or applying inoculants did not always alter lactic acid output when the availability of water-soluble carbohydrates and other fermentation conditions remained adequate [8].

All treatments produced lactic acid concentrations within the 1–3% interval, which is considered normal for high-quality silage [9]. This indicates that fermentation proceeded efficiently and without signs of butyric acid development, which is typically associated with off-odors. Even though the differences were not statistically significant, P3 showed a slight tendency toward more effective fermentation among the tested formulations.

Ammonia

The variance analysis showed that the different proportions of corn stover and red calliandra did not cause significant changes in silage ammonia concentration ($p = 0.818 > 0.05$). The observed values ranged from 4.68%

to 7.12% (5.96 ± 0.45%), indicating that protein breakdown during fermentation occurred at a low and fairly consistent level. Such values fall within the normal range for well-preserved silage, as ammonia levels below 10% of total nitrogen generally reflect limited proteolysis and a stable fermentation process [6].

The absence of treatment differences suggests that increasing red calliandra inclusion up to 40% was not enough to influence the extent of protein deamination. This outcome agrees with the findings of [5], who noted that ammonia accumulation remains modest when water-soluble carbohydrates are available in sufficient amounts to support the predominance of lactic acid bacteria. Although calliandra contains tannins that theoretically could reduce proteolysis, their concentration in this study did not appear strong enough to create measurable effects.

Among the treatments, P1 produced the lowest ammonia level (4.68%), while P4 showed the highest (7.12%). Even so, all values remained within acceptable limits and were statistically similar. The slightly lower value in P1 indicates a minor tendency toward more efficient fermentation, yet the overall differences were too small to be considered biologically important. These results demonstrate that the ratios of corn stover and red calliandra tested in this study did not alter ammonia formation, and that fermentation remained largely driven by lactic acid bacteria. This agrees with the review of [10], which emphasized that ammonia production is more strongly influenced by anaerobic stability and the availability of fermentable substrates than by differences in forage composition.

Organic Matter Changes

The variance analysis showed that different proportions of corn stover and red calliandra did not significantly affect changes in silage organic matter (OM) ($p = 0.310 > 0.05$). The OM values ranged from –0.35% to –1.31% (–0.89 ± 0.08), indicating that a portion of the organic fraction was used as a substrate during fermentation. The small magnitude of the decrease suggests that the fermentation process ran steadily and without notable energy loss. As noted in [6], a slight decline in OM generally reflects efficient fermentation because most of the energy remains preserved in organic acids and digestible nutrients.

The lack of treatment differences indicates that the inclusion of red calliandra up to 40% was not sufficient to alter the extent of OM reduction. The relatively high water-soluble carbohydrate (WSC) content in corn stover likely remained the main factor supporting fermentation, regardless of calliandra addition. This interpretation is consistent with [5], who reported that when WSC availability is adequate, OM losses tend to remain low because lactic acid bacteria can operate effectively.

The highest OM reduction occurred in P2 (–1.31%), whereas P1 experienced the smallest loss (–0.35%). Although the values did not differ statistically, the slightly lower reduction in P1 suggests a modest tendency toward more efficient fermentation. Overall, the mixtures of corn stover and red calliandra produced stable fermentation with minimal OM loss. This agrees with [11], who highlighted that successful ensiling is influenced more by sufficient fermentable substrates and proper anaerobic conditions than by differences in forage composition.

Although all parameters displayed relatively uniform patterns across treatments, this study has several limitations that should be considered when interpreting the results. The range of red calliandra inclusion used (10–40%) was relatively narrow, which may have limited the emergence of more pronounced biological differences among treatments. In addition, the fermentation period of 22 days may not have been sufficient to capture changes that typically appear during longer-term ensiling. Based on these limitations, future research is recommended to evaluate red calliandra levels above 40%, extend the fermentation duration, and explore more diverse ensiling conditions. Such approaches may provide a more comprehensive understanding of fermentation responses and nutrient stability in silages combining corn stover and red calliandra.

CONCLUSION

This study demonstrated that varying the ratios of corn stover and red calliandra up to 40% did not produce significant effects on silage pH, lactic acid concentration, ammonia levels, or changes in organic matter. All treatments yielded parameter values within the range of good-quality silage, reflecting stable fermentation dominated by lactic acid bacteria. Although not statistically different, the treatment with 90% corn stover and 10% red calliandra (P1) tended to produce the lowest pH (4,07), the lowest ammonia concentration (4,68%), and the smallest loss of organic matter (-0,35%), indicating slightly more efficient fermentation. Overall, all tested ratios of corn stover and red calliandra were suitable for producing silage with relatively uniform chemical quality and meeting the characteristics of well-conducted fermentation.

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