The Effect of Corn Stover and Pineapple Peel Ratio in Silage on Fermentability, Total Gas Production, and Gas Kinetics in Rumen (In Vitro)

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ABSTRACT

Silage is one of the alternative feed sources produced through anaerobic fermentation, which can preserve nutrient quality and extend the storage life of feed materials, especially when fresh forage availability is limited. The utilization of agricultural by-products as silage materials also has the potential to improve the efficiency of sustainable ruminant feed production. This study aimed to evaluate the effect of different ratios of corn stover and pineapple peel silage on in vitro rumen fermentation. The experiment was conducted using a Completely Randomized Design (CRD) consisting of four treatments with five replications, namely T1 (90% corn stover + 10% pineapple peel), T2 (80% corn stover + 20% pineapple peel), T3 (70% corn stover + 30% pineapple peel), and T4 (60% corn stover + 40% pineapple peel).

The observed parameters included total volatile fatty acids (VFA) concentration, ammonia (NH_3) concentration, total gas production, and gas kinetics. The data were analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test. The results showed that different ratios of corn stover and pineapple peel silage significantly affected ($P < 0.05$) total volatile fatty acids (VFA) concentration, which ranged from 115.34 mM to 179.82 mM. Meanwhile, ammonia (NH_3) concentration ranged from 1.38 mM to 1.79 mM and was not significantly affected by the treatments ($P > 0.05$).

Total gas production ranged from 118.8 mL/g DM substrate to 132.9 mL/g DM substrate, while gas kinetics values ranged from 60.86 mL/g DM substrate to 67.51 mL/g DM substrate, and both parameters were not significantly affected by the treatments ($P > 0.05$). Overall, the ratio of 80% corn stover and 20% pineapple peel produced the most optimal and efficient rumen fermentation, indicating its potential as an alternative silage feed ingredient for sustainable ruminant nutrition.

Keywords: Silage, Volatile Fatty Acids, Ammonia, Gas Production, Gas Kinetics

INTRODUCTION

Ruminant productivity is strongly influenced by feed availability and quality, particularly forage as the primary component of ruminant rations. In Indonesia, the availability of fresh forage is highly dependent on seasonal conditions. Fluctuations in forage availability, especially during the dry season, often result in imbalances in feed supply, thereby leading to reduced nutrient intake, decreased feed utilization efficiency, and lower ruminant production performance. This condition constitutes one of the major limiting factors in improving ruminant productivity.

One of the strategies that can be implemented to address this issue is the application of feed preservation technology, such as silage production. Silage enables the continuous supply of ruminant feed throughout the year with relatively stable nutritional quality. The ensiling process, which occurs under anaerobic conditions, is capable of preserving the nutrient content of feed for an extended period, even up to two years when properly stored (McDonald et al., 2022).

Corn stover is the biomass of maize plants harvested at approximately 90 days of age. The biomass production of maize ranges from 20 to 30 tons per hectare per harvest, depending on the cultivation system, crop management practices, and the maize variety used (Neumann et al., 2024). Nutritionally, corn stover contains 33.21% crude fiber and approximately 2,350 kcal/kg of metabolizable energy, indicating its potential as an energy source for ruminants (Aling et al., 2020).

However, corn stover has limited storage stability if not properly processed due to its high moisture content, which reaches 80.26%. Under such conditions, silage production becomes one of the appropriate strategies to preserve the nutrient quality of corn stover. To optimize the ensiling process, the addition of other feed ingredients rich in soluble carbohydrates is required, considering that corn stover contains only 15.65% soluble carbohydrates (Despal et al., 2017).

One potential ingredient that can be used as a mixture with corn stover in silage production is pineapple peel. Pineapple peel contains a relatively high level of soluble carbohydrates, reaching 17.53%, which can accelerate the decline in pH during the ensiling process and create optimal conditions for the growth of lactic acid bacteria (Titisari et al., 2020). In addition, pineapple peel contains approximately 13.96% crude fiber and bioactive compounds such as tannins and saponins that play a role in supporting the rumen fermentation process. Although the protein content of pineapple peel is lower than that of corn stover, its soluble carbohydrate content can enhance fermentability and improve silage quality. Through the fermentation process, the crude fiber content of pineapple peel may also decrease, thereby improving its digestibility and utilization by rumen microbes.

Fermentability is an important parameter in evaluating the quality of ruminant feed, as it reflects the ability of rumen microbes to degrade feed substrates. The fermentation process produces volatile fatty acids (VFA), such as acetate, propionate, and butyrate, which serve as the primary energy sources for ruminants.

An optimal concentration of volatile fatty acids (VFA) plays a crucial role in supporting energy metabolism and maintaining the balance of rumen microbial populations (Rahayu et al., 2018). In addition, the ammonia (NH_3) concentration resulting from protein degradation serves as an indicator of nitrogen availability for rumen microbes. Excessive ammonia (NH_3) concentration indicates inefficient protein degradation and may reduce the nutritional value of the feed (Irmawati et al., 2023).

Rumen microbial fermentation activity can also be evaluated by measuring total gas production during in vitro fermentation. The gases produced mainly consist of carbon dioxide (CO_2) and methane (CH_4), which reflect the extent of organic matter degradation and the efficiency of nutrient utilization (Kusumaningrum et al., 2018). Furthermore, gas production kinetics analysis is required to understand the rate and pattern of fermentation, including the microbial adaptation phase, the maximum gas production rate, and the total gas production capacity (Ørskov & McDonald, 1979).

Different ratios of corn stover and pineapple peel in silage are expected to influence rumen fermentation characteristics, particularly fermentability, total gas production, and gas kinetics. However, scientific studies examining the effects of various ratios of corn stover and pineapple peel silage on in vitro rumen fermentation parameters remain limited. Therefore, this study aimed to evaluate the effects of different ratios of corn stover and pineapple peel silage on total volatile fatty acids (VFA) concentration, ammonia (NH_3) concentration, total

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gas production, and gas kinetics in vitro, in order to determine the most efficient combination as a high-quality and sustainable silage feed for ruminants in Indonesia.

MATERIALS AND METHODS

This study was conducted from June to December 2025 at the Ruminant Nutrition and Feed Chemistry Laboratory, Faculty of Animal Science, Universitas Padjadjaran, Jatinangor District, Sumedang Regency, West Java Province, Indonesia.

Silage Production

In this study, corn stover was obtained from Ben Buana Sejahtera Farm, located in Jatinangor District, Sumedang Regency, West Java, with a total of 45 kg used as silage raw material.

The material included the whole plant, namely stems, leaves, and cobs, harvested at 90 days of age to ensure optimal nutrient content, and the maize variety used was hybrid maize P40 Famili. Meanwhile, pineapple peel used as a silage mixture component was obtained from Sarireja Village, Jalancagak District, Subang Regency, West Java 41281, with a total usage of 15 kg.

Silage preparation began with wilting the corn stover for 2 hours and the pineapple by-product for 12 hours to reduce moisture content. Both materials were then chopped into 2–3 cm lengths. Corn stover and pineapple by-product were weighed according to the treatment ratios and mixed with molasses diluted with water (1:1) at 4% of the total fresh weight.

Following McDonald et al. (2022), the mixture was packed into vacuum-sealed bags, placed in tightly closed containers, and fermented for 21 days to maintain anaerobic conditions. The use of vacuum-sealed plastic bags and airtight containers was intended to maintain anaerobic conditions and prevent air infiltration during the fermentation process.

In Vitro

The fermented silage was dried for 2 days and then finely ground using a hammer mill. The in vitro procedure for volatile fatty acids (VFA) and ammonia (NH_3) analysis followed the method of Tilley & Terry (1963). Approximately 0.5 g of silage sample was weighed and placed into fermentation tubes, with a total of 20 units prepared. Each tube was added with 40 mL of artificial saliva solution and 10 mL of rumen fluid obtained from beef cattle. Carbon dioxide (CO_2) gas was then flushed into the tubes to establish anaerobic conditions, after which the tubes were sealed with ventilated rubber stoppers.

All tubes were incubated in a water bath at 38–40°C for 24 hours, with manual shaking every 3 hours. After incubation, three drops of saturated $HgCl_2$ solution were added to terminate rumen microbial activity. The mixture was subsequently transferred into centrifuge tubes and centrifuged at 4,000 rpm for 10 minutes to separate the residue from the supernatant. The resulting supernatant was then used for VFA and NH_3 concentration analysis.

Meanwhile, the in vitro procedure for measuring total gas production and gas kinetics followed the method of Theodorou et al. (1994). Labeled vial bottles were filled with approximately 0.5 g of silage sample according to treatment, with 20 units prepared, along with one bottle without sample serving as a blank containing only rumen fluid and artificial saliva solution to correct the actual total gas production.

In addition, 20 evacuated vials and one blank vial were prepared as gas collection containers during incubation. A mixture of 10 mL rumen fluid and 40 mL artificial saliva solution (total 50 mL) was introduced into each vial containing the sample while being flushed with CO_2 gas. The bottles were then sealed with aluminum caps and secured using a 20 mm crimper. All bottles were incubated in a water bath at 38–40°C for 24 hours, with shaking every 2 hours to maintain homogeneity throughout the fermentation process.

Observed Variables

Total Volatile Fatty Acids (VFA) Concentration

The total volatile fatty acids (VFA) concentration was determined using the steam distillation method (General Laboratory Procedure, 1996). Total VFA concentration was calculated using the following formula:

$$Total\ VFA\ (mM) = (a - b) \times N\ HCl \times 1000/5$$

Where:

a = Volume of blank titrant (5 mL NaOH)

b = Volume of sample titrant

Ammonia (NH_3) Concentration

Ammonia (NH_3) concentration was measured using the Conway microdiffusion method (Conway, 1948). NH_3 concentration was calculated using the following formula:

$$NH_3\ (mM) = V\ H_2SO_4 \times N\ H_2SO_4 \times 1.000$$

Where:

$V\ H_2SO_4$ = Volume of H_2SO_4 used for titration (mL)

$N\ H_2SO_4$ = Normality of H_2SO_4 (0.005 N)

Total Gas Production

Total gas production was measured based on data recorded every 2 hours for 24 hours during incubation, following Ørskov & McDonald (1979) with blank correction. The cumulative gas volumes were calculated using the formula:

$$TPG = (g1 + g2 + g3 + \ldots + ga) - Blank$$

Where:

TPG = Total gas production

ga = Gas volume at the nth measurement time

Gas Kinetics

Gas kinetics were estimated based on cumulative gas production data collected every 2 hours for 24 hours during incubation. The data were fitted to the exponential equation proposed by Ørskov & McDonald (1979), as follows:

$$P = a + b\ (1 - e^{-ct})$$

Where:

P = Cumulative gas production at time t (hours)

a = Gas production from the immediately soluble fraction

b = Gas production from the insoluble but fermentable fraction

c = Rate constant of gas production

The gas kinetics values presented in Table 1 represent the potential gas production (a + b).

Data Analysis and Method

The experiment was conducted using an experimental method under a Completely Randomized Design (CRD), consisting of four treatments with five replications each. A total of 20 experimental units were used for the analysis of volatile fatty acids (VFA) concentration and ammonia (NH_3) concentration. Meanwhile, 21 experimental units were used for total gas production and gas kinetics analysis, including one additional blank unit. The treatments were arranged as follows:

T1 = 90% corn stover + 10% pineapple peel

T2 = 80% corn stover + 20% pineapple peel

T3 = 70% corn stover + 30% pineapple peel

T4 = 60% corn stover + 40% pineapple peel

The collected data were analyzed using analysis of variance (ANOVA) to determine the effect of treatments on the observed parameters. When a significant effect was detected, Duncan's Multiple Range Test (DMRT) was performed to identify differences among treatment means.

RESULT AND DISCUSSION

The effect of different silage ratios on in vitro fermentation characteristics is presented in Table 1.

Table 1. Effect of Different Ratios of Corn Stover and Pineapple Peel in Silage on Total Volatile Fatty Acids (VFA) Concentration, Ammonia (NH_3) Concentration, Total Gas Production, and Gas Kinetics (In Vitro)

Parameter	Treatment			
	T1	T2	T3	T4
Total Volatile Fatty Acids (VFA) Concentration (mM)	115.34±5.77[a]	139.43±1.77[b]	160.31±9.12[c]	179.82±11.30[d]
Ammonia (NH_3) Concentration (mM)	1.79±0.45	1.60±0.55	1.57±0.54	1.38±0.54
Total Gas Production (mL/g DM substrate)	118.8±14.3	132.6±12.7	126.1±5.2	132.9±5.2
Gas Kinetics (mL/g DM substrate)	60.86±7.44	67.51±6.64	64.01±2.49	67.41±2.51

Data are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) showed that treatments significantly affected total volatile fatty acids (VFA) concentration ($F(3,16) = 62.63$; $P < 0.05$). In contrast, ammonia (NH_3) concentration ($F(3,16) = 0.52$; $P > 0.05$), total gas production ($F(3,16) = 2.00$; $P > 0.05$), and gas kinetics ($F(3,16) = 2.22$; $P > 0.05$) were not significantly affected by treatments. Different superscript letters within the same row indicate significant differences among treatments ($P \leq 0.05$). T1 = 90% corn stover + 10% pineapple peel; T2 = 80% corn stover + 20% pineapple peel; T3 = 70% corn stover + 30% pineapple peel; T4 = 60% corn stover + 40% pineapple peel

Total Volatile Fatty Acids (VFA) Concentration

Volatile fatty acids (VFA) are the primary end-products of rumen microbial fermentation and serve as the main energy source for ruminants. The major VFAs produced include acetate, propionate, and butyrate, which are formed through the degradation of structural and non-structural carbohydrates in the feed. Therefore, total VFA concentration is commonly used as an indicator of rumen fermentation intensity and efficiency (Rahayu et al., 2018).

The results presented in Table 1 show that total VFA concentration ranged from 115.34 to 179.82 mM. The lowest VFA concentration was observed in T1 (90% corn stover + 10% pineapple peel), while the highest value was recorded in T4 (60% corn stover + 40% pineapple peel). The increasing trend in VFA concentration indicates that the inclusion of pineapple peel improved feed fermentability by increasing the availability of readily fermentable carbohydrates as substrates for rumen microorganisms. Analysis of variance revealed that different ratios of corn stover and pineapple peel in silage significantly affected total VFA concentration ($P < 0.05$).

Physiologically, the optimal total VFA concentration for ruminants generally ranges between 70 and 150 mM, depending on diet composition and fermentation conditions, whereas concentrations exceeding 200 mM may increase the risk of ruminal acidosis (McDonald et al., 2022; Tanuwiria et al., 2023). Based on this range, VFA concentrations in T1 and T2 remained within the optimal limits, whereas T3 and T4 exceeded the upper optimal range, although still below the threshold associated with acidosis risk.

Among the treatments, T2 (80% corn stover + 20% pineapple peel) can be considered the most optimal, as it approached the upper limit of the optimal range without exceeding it. This condition indicates a balanced supply of readily fermentable carbohydrates and the ability of rumen microbes to efficiently convert them into energy. In contrast, the higher VFA production observed in T3 and T4 indicates increased fermentation intensity due to the greater soluble carbohydrate content derived from pineapple peel. However, VFA concentrations exceeding the optimal range may compromise rumen stability if not supported by adequate buffering capacity. Therefore, although T4 produced the highest total VFA concentration, it cannot be regarded as the most optimal treatment in terms of fermentation balance.

Ammonia (NH_3) Concentration

Ammonia (NH_3) is formed as the end-product of dietary protein degradation by rumen microorganisms and serves as the primary nitrogen source for microbial protein synthesis. The concentration of NH_3 in the rumen reflects the balance between the rate of protein degradation and microbial nitrogen utilization, and is therefore commonly used as an indicator of nitrogen use efficiency during rumen fermentation (Irmawati et al., 2023).

The results presented in Table 1 show that the average NH_3 concentration ranged from 1.38 to 1.79 mM. Sequentially, the mean NH_3 concentrations were 1.79 mM (T1), 1.60 mM (T2), 1.57 mM (T3), and 1.38 mM (T4). Analysis of variance indicated that the treatments did not significantly affect NH_3 concentration ($P > 0.05$). This finding suggests that variations in the ratio of corn stover and pineapple peel were insufficient to significantly alter protein degradation patterns or nitrogen utilization by rumen microorganisms.

The NH_3 concentrations observed in this study were below the optimal range reported to support maximal microbial protein synthesis, which is approximately 4–12 Mm (Hapsari et al., 2018). The relatively low NH_3 concentration is associated with the characteristics of the silage materials used. Corn stover is not a primary protein source, while pineapple peel mainly contributes readily fermentable carbohydrates (Triastuti et al., 2024). Consequently, the amount of degradable nitrogen available for conversion into NH_3 by rumen microbes was limited, resulting in relatively low NH_3 levels. This supports the statement of Rahayu et al. (2018) that ruminal

NH_3 concentration is influenced by several factors, including dietary protein content, feed solubility, nitrogen source, rumen pH, and feeding time.

Although no significant differences were observed, the numerical trend suggests the presence of an interaction between energy and nitrogen availability. NH_3 concentration tended to decrease as the proportion of pineapple peel increased, particularly in T4 (60% corn stover + 40% pineapple peel). This pattern indicates that increased availability of readily fermentable carbohydrates may have stimulated microbial activity, thereby enhancing nitrogen assimilation into microbial biomass and reducing NH_3 accumulation in the fermentation medium. This finding is consistent with Nurwildan et al. (2022), who reported that microbial NH_3 utilization is strongly influenced by the adequacy of fermentable energy, where sufficient energy supply promotes nitrogen incorporation into microbial cells and lowers free NH_3 concentration.

However, since all treatments produced NH_3 concentrations below the optimal range for maximal microbial synthesis, nitrogen can be considered the primary limiting factor in this fermentation system. This condition aligns with Rahayu et al. (2018), who stated that rumen fermentation based on low-protein feed ingredients generally results in insufficient NH_3 levels to support maximal microbial growth, even when energy availability is relatively high. Therefore, improving rumen fermentation efficiency in corn stover and pineapple peel-based silage may require supplementation with more readily available nitrogen sources to achieve a better balance between energy and nitrogen supply for rumen microorganisms.

Total Gas Production

Gas production reflects the metabolic activity of rumen microorganisms in degrading feed substrates through fermentation, which results in the formation of volatile fatty acids (VFA) and microbial biomass. The gas produced is a by-product of the fermentation of organic matter fractions by rumen microbes (Kusumaningrum et al., 2018). The magnitude of gas volume indicates the degree of feed digestibility and fermentability. Increased gas production generally reflects enhanced substrate degradation, whereas lower gas production may indicate a more efficient utilization of fermentation products for microbial biomass synthesis.

The results presented in Table 1 show that total gas production ranged from 118.8 to 132.9 mL/g DM substrate. Sequentially, the mean total gas production values were 118.8 (T1), 132.6 (T2), 126.1 (T3), and 132.9 mL/g DM substrate (T4). Analysis of variance revealed that different ratios of corn stover and pineapple peel in silage did not significantly affect total gas production ($P > 0.05$). The relatively lower gas production observed in T1 (90% corn stover + 10% pineapple peel) is associated with the dominance of corn stover, which contains a relatively high fiber fraction and lower levels of soluble carbohydrates, resulting in slower organic matter degradation by rumen microbes (Despal et al., 2017). Consequently, fermentation intensity was lower and gas production was more limited. Thus, the gas production value in T1 reflects restricted substrate fermentability rather than optimal fermentation conditions.

In contrast, T4 (60% corn stover + 40% pineapple peel) produced the highest gas volume (132.9 mL/g DM substrate). This increase is attributed to the higher proportion of pineapple peel, which contains substantial soluble carbohydrates (approximately 17.53%) that are readily utilized by rumen microbes (Titisari et al., 2020). This finding is consistent with Alfiansyah & Hartutik (2021), who reported that gas production during fermentation is strongly influenced by dietary carbohydrate content, with higher carbohydrate availability leading to greater gas formation.

However, higher total gas production does not necessarily indicate optimal feed utilization efficiency. Excessive gas production suggests that a portion of fermentation energy is lost in the form of gases, primarily CO_2 and CH_4, thereby reducing overall fermentation energy efficiency. Elevated gas production may also increase the risk of digestive disturbances such as bloat and contribute to greenhouse gas emissions. Conversely, moderate gas production may indicate that a greater proportion of fermented organic matter is directed toward microbial protein synthesis rather than being released as gas (Alfiansyah & Hartutik, 2021). Although no significant

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differences were observed among treatments, T2 (80% corn stover + 20% pineapple peel) exhibited a moderate and balanced gas production level, suggesting optimal fermentation activity without excessive energy loss in the form of gas. This pattern reflects a more efficient rumen fermentation process compared to the other treatments.

Gas Kinetics

Gas kinetics describe the rate of gas formation resulting from microbial fermentation of feed substrates during in vitro incubation. This parameter is closely related to the ability of rumen microbes to utilize readily fermentable feed fractions and is commonly used to evaluate fermentation dynamics, particularly during the early phase of incubation (Firsoni & Lisanti, 2017; Tatra & Husnaeni, 2024). Therefore, gas kinetics complement total gas production data by providing insight into fermentation processes in a dynamic manner rather than solely based on final gas volume.

The results in Table 1 indicate that gas kinetics values ranged from 60.86 to 67.51 mL/g DM substrate. Sequentially, the mean values were 60.86 (T1), 67.51 (T2), 64.01 (T3), and 67.41 mL/g DM substrate (T4). Analysis of variance showed that different silage ratios did not significantly affect gas kinetics ($P > 0.05$), indicating that variations in the proportion of corn stover and pineapple peel were insufficient to significantly influence fermentation rate.Nevertheless, a numerical trend was observed in which gas kinetics increased with a higher proportion of pineapple peel in the silage. This suggests that pineapple peel, as a source of readily fermentable carbohydrates, may accelerate microbial fermentation activity. The rapid availability of fermentable substrates stimulates microbial growth and enhances gas formation during incubation (Triastuti et al., 2024).

Overall, the response pattern was not strictly linear. T2 (80% corn stover + 20% pineapple peel) demonstrated the most balanced fermentation kinetics, characterized by a relatively high and stable rate of gas formation. Although T4 also showed a high kinetic value, this increase likely reflects intensified fermentation due to high levels of soluble substrates rather than superior fermentation efficiency compared to T2.

CONCLUSIONS

This study demonstrates that the silage ratio of 80% corn stover and 20% pineapple peel represents the most favorable composition under the conditions of this study among the treatments evaluated. This combination significantly increased total VFA concentration without adversely affecting NH_3 concentration, total gas production, or gas kinetics.

The significant effect observed on total VFA concentration indicates that different silage ratios influence carbohydrate fermentation intensity in the rumen. Increasing the proportion of pineapple peel at appropriate levels likely enhances the availability of soluble carbohydrates as primary substrates for rumen microbes, thereby increasing VFA production. Since VFA serve as the main energy source for ruminants, higher concentrations reflect a more active and efficient fermentation process.Meanwhile, the absence of significant effects on NH_3 concentration suggests that protein degradation and nitrogen utilization remained relatively stable across treatments. Similarly, the non-significant differences in total gas production and gas kinetics indicate that overall fermentation patterns remained within normal physiological conditions. Therefore, variation in silage ratio primarily optimized VFA production without causing imbalance in other fermentation parameters. However, considering that NH_3 concentrations were below the optimal range for rumen microbial growth, future application of this silage-based ration may require additional nitrogen supplementation to improve nitrogen availability and support more efficient microbial protein synthesis.

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