

The Effect of Corn Stover and Pineapple Waste Ratio in Silage on pH, Microbial Population, and Methane Gas in Rumen (In Vitro)

Susi Novi Amalia¹, Ujang Hidayat Tanuwiria², Iin Susilawati²

¹Student at the Faculty of Animal Husbandry, Department of Animal Nutrition and Feed Technology, Universitas Padjadjaran

²Faculty of Animal Husbandry, Department of Animal Nutrition and Feed Technology, Universitas Padjadjaran, Sumedang, Indonesia

*Corresponding Author

DOI: <https://dx.doi.org/10.51244/IJRSI.2025.12120141>

Received: 29 December 2025; Accepted: 06 January 2026; Published: 17 January 2026

ABSTRACT

This study aimed to evaluate the effects of different ratios of corn stover and pineapple waste silage on rumen pH, bacterial population, protozoa population, and methane production under in vitro conditions. The experiment was conducted using a Completely Randomized Design (CRD) with four treatments and five replications. The treatments consisted of T1 (90% corn stover + 10% pineapple waste), T2 (80% corn stover + 20% pineapple waste), T3 (70% corn stover + 30% pineapple waste), and T4 (60% corn stover + 40% pineapple waste). Data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test. The results showed that varying the ratio of corn stover and pineapple waste silage had no significant effect on rumen pH (6.77–6.78), bacterial population ($3.03\text{--}3.31 \times 10^9$ CFU/mL), or methane production (5.57–6.45 mM) ($P > 0.05$). In contrast, the protozoa population was significantly affected by the treatments ($4.21\text{--}4.70 \times 10^5$ cells/mL; $P \leq 0.05$), showing a decreasing trend by adding levels of pineapple waste. All measured parameters remained within normal physiological ranges, indicating stable rumen fermentation conditions. Although differences in methane production and bacterial population among treatments were not statistically significant, treatment T4 tended to exhibit a higher bacterial population and lower methane production. These findings indicate that combinations of corn stover and pineapple waste silage up to a 60:40 ratio are feasible for maintaining balanced rumen fermentation, with potential implications for improving fermentation efficiency and mitigating methane production. Further in vivo studies are required to confirm these results under practical feeding conditions.

Keywords: Corn Stover Silage, Pineapple Waste, Microbial Population, Rumen pH, Methane Gas

INTRODUCTION

Beef cattle are a strategic livestock commodity that plays a crucial role in meeting the animal protein requirements of the Indonesian population. West Java Province is the second largest beef producer in Indonesia after East Java, with production reaching 85,241.70 tons, equivalent to 17.80% of national beef production in 2024 (BPS West Java, 2024). Sustainable beef cattle production is strongly influenced by the availability of adequate feed, both in quantity and nutritional quality, as feed represents the largest production cost and is the primary factor determining livestock performance.

The utilization of agro industrial by products as alternative feed resources can help address feed shortages, reduce production costs, and minimize competition with human food resources. One such readily available feed source is corn stover, defined as the whole corn plant harvested at 60 to 80 days of age (Satiyarti et al., 2023). Corn stover is widely used as ruminant feed because it grows rapidly and adapts well to tropical environments (Tuturoong et al., 2020). According to Mustika & Hartutik (2021), corn stover contains 23.55% crude fiber, 7.80% crude protein, 2.4% ether extract, 7.43% ash, and 31.20% dry matter. Its relatively high dry matter content contributes to silage stability by reducing the risk of spoilage during fermentation. Corn stover is also

characterized by high fiber fractions, with Neutral Detergent Fiber values of 68.78% and Acid Detergent Fiber values of 42.36% (Tuturoong et al., 2020). In addition, corn stover is rich in lignocellulosic components, consisting of 28.38% cellulose and 19.16% hemicellulose (Angulo Padilla et al., 2018), which directly influence feed fermentability and digestibility in the rumen. Furthermore, the relatively high content of water soluble carbohydrates in corn stover supports the ensilage process by providing substrates for lactic acid bacteria, promoting lactic acid production and rapid pH reduction (Gao et al., 2019).

In addition to corn stover, pineapple (*Ananas comosus*) waste has considerable potential as a ruminant feed resource. Pineapple is a major tropical fruit commodity in Indonesia, with high production levels, particularly in West Java, where Subang Regency contributes more than 92.00% of provincial pineapple production (Andri, 2020). Approximately 47.00% of pineapple processing by products remain underutilized and may cause environmental pollution if not properly managed (Chrysostomus et al., 2020; Wahyuni et al., 2016). Pineapple waste contains carbohydrates, fiber, and various bioactive compounds that make it suitable for ruminant feeding. Its nutritional composition includes 14.22% dry matter, 81.90% organic matter, 8.10% ash, 3.50% crude protein, 19.69% crude fiber, 3.49% ether extract, and 57.27% Neutral Detergent Fiber, with an energy value of approximately 4,481.00 kcal (Septia et al., 2025). The relatively high carbohydrate content, approximately 11.39%, also provides a favorable substrate for microbial growth during fermentation (Cornelia & Kristyanti, 2021).

Beyond its nutritional value, pineapple waste contains several bioactive compounds, including flavonoids, saponins, and the proteolytic enzyme bromelain, which can influence rumen fermentation. Flavonoids have been reported to improve nitrogen utilization efficiency and suppress methane production by modulating rumen microbial populations (Liu et al., 2023). Saponins are known to reduce rumen protozoa populations by disrupting cell membranes, thereby altering fermentation pathways and potentially decreasing methane formation (Patra & Saxena, 2009). Bromelain enhances protein degradation into amino acids and ammonia, which can be more efficiently utilized by rumen microorganisms, supporting microbial protein synthesis and improving fermentation efficiency (Wurlina et al., 2020). However, pineapple waste also contains antinutritional compounds such as tannins and oxalic acid, which may reduce nutrient availability if not properly managed (Khajali & Rafiei, 2024; Reiza et al., 2019). Therefore, appropriate processing technologies are required to enhance its nutritional value while minimizing the negative effects of these compounds.

Silage fermentation is an effective preservation method to improve forage quality and extend the shelf life of high moisture materials such as corn stover and pineapple waste (Wati et al., 2018). Under anaerobic conditions, lactic acid bacteria convert soluble sugars into lactic acid, resulting in a rapid decline in pH and inhibition of spoilage microorganisms (Yuliyati et al., 2018). High quality silage is expected to support stable rumen fermentation, particularly by maintaining rumen pH within an optimal range. Rumen pH plays a critical role in regulating microbial activity, especially that of cellulolytic bacteria, which function optimally at a pH of 6.00 to 7.00 (Wajizah et al., 2015). A decline in rumen pH can inhibit fiber degradation, reduce fermentation efficiency, and disrupt the balance of rumen microbial populations.

Rumen fermentation is essential for the production of volatile fatty acids, which serve as the primary energy source for ruminant animals. However, this process also results in methane production, which represents a major limitation. Methane is a potent greenhouse gas contributing to climate change and also reflects an energy loss from the feed, estimated to range from 2.00% to 12.00% of gross energy intake. Methane production is strongly influenced by rumen microbial activity, particularly that of fiber degrading bacteria and protozoa, as well as rumen pH and dietary composition (Martin et al., 2010). Therefore, an appropriate combination of corn stover and pineapple waste is required to optimize silage quality, support efficient rumen fermentation, maintain microbial balance, and reduce methane emissions.

MATERIALS AND METHODS

Silage Production

The research was conducted at the Ruminant Animal Nutrition and Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Universitas Padjadjaran, from July to December 2025. The materials used for silage

preparation included corn stover obtained from Ben Buana Sejahtera Farm, located in Jatinangor District, Sumedang Regency, West Java Province. The corn stover was harvested at 90 days of age. Pineapple waste was obtained from a peeled pineapple supplier in Sarireja Village, Jalanjagak District, Subang Regency, West Java Province.

The moisture content of both corn stover and pineapple waste was reduced by wilting for approximately 24 hours. After wilting, the materials were chopped into pieces measuring approximately 2 to 4 cm, weighed, and mixed according to the designated treatment ratios. Molasses diluted with water at a 1:1 ratio was added as a fermentation additive. All ingredients were thoroughly mixed until homogeneous, then placed into plastic-lined jars, compacted to remove trapped air, and tightly sealed to create anaerobic conditions.

The silage fermentation process was carried out for 22 days at room temperature, following Paloa et al. (2025), who reported that silage fermentation generally occurs within 21 to 35 days. After the fermentation period, the silage was harvested and dried using a sun drying method until sufficiently dry. The dried silage was subsequently ground using a hammer mill for further analysis.

In Vitro

The fermented silage samples were evaluated for fermentability using the in vitro gas production technique described by Theodorou et al. (1994). A 0.5 g sample of each silage treatment was placed into a fermentation vial. Fresh rumen fluid collected from beef cattle was added, followed by McDougall's solution (McDougall, 1947) as an artificial saliva buffer with a pH of 6.9, at a ratio of 10 mL rumen fluid to 40 mL McDougall's solution. Carbon dioxide gas was flushed into the vial for approximately 15 seconds to ensure anaerobic conditions, after which the vial was sealed with a rubber stopper.

Rumen fluid was obtained from the Ciroyom Slaughterhouse Technical Implementation Unit (UPTD RPH), located at St. Arjuna No. 45, Husen Sastranegara, Cicendo District, Bandung City, West Java Province. After sealing, the vials containing the sample and reduction solution were shaken manually for approximately 30 seconds until homogeneous. The vials were then placed in a water bath set at a temperature of 38 to 40°C and incubated for 24 hours, with gentle shaking performed every 3 hours.

Observed Variables

Rumen pH

Rumen fluid pH was measured using a digital pH meter after 24 hours of incubation. First, the pH meter was standardized using a buffer solution at pH 7 for ±10 minutes, then with a buffer solution at pH 4 for ±10 minutes. The cathode was dipped into the solution until the pH meter reading reached a constant value. The pH meter reading was recorded as the rumen fluid pH (Hernaman et al., 2015).

Microbial Population

Fermented rumen fluid from each vial was transferred into a 50 mL Falcon tube for microbial analysis. Approximately 1 mL of the rumen fluid supernatant was collected for protozoa enumeration using a light microscope (Zeiss Primo Star, type no. 5, Jena, Germany). Protozoa counting was conducted by mixing 1 mL of fermented rumen fluid with 6 mL of 3.7% formalin and observing the sample twice under the microscope (Tanuwiria et al., 2025). Protozoa population was calculated using the following formula:

$$\text{Total Protozoa Population} = \text{Average protozoa} \times \frac{1000}{100} \times 7$$

Total bacterial population was determined using a Thoma counting chamber with a depth of 0.02 mm (BlauBrand, Wertheim, Germany). A volume of 20 µL of fermented rumen fluid was mixed with 6,980 µL of Hayem's solution. Approximately 10 µL of the mixture was loaded into each chamber, and observations were performed in five chambers (Tanuwiria et al., 2025). Bacterial population was calculated using the following formula:

$$\text{Total Bacterial Population} = \frac{\left(\frac{\text{Average bacterial}}{5}\right) \times 1000}{0,004}$$

Methane Gas

Gas production was measured at 2 hour intervals during the 24 hours incubation period using a 10 mL syringe fitted with a 0.1 mm diameter needle, which was inserted through the rubber stopper of the sealed vial. The produced gas was collected into a vacuum sealed 100 mL vial. Total gas production over 24 hours was calculated by summing the gas volumes collected at each interval. Approximately 5 mL of the accumulated gas was transferred into a 5 mL Vacutainer tube for methane concentration analysis. Methane concentration was determined using gas chromatography, with the injector temperature set at 90°C and the column temperature at 75°C.

Data Analysis and Method

This study employed an experimental method using a Completely Randomized Design (CRD) consisting of four treatments with five replications each. The treatments were as follows:

T1 = Silage with a ratio of 90% Corn Stover and 10% Pineapple Waste

T2 = Silage with a ratio of 80% Corn Stover and 20% Pineapple Waste

T3 = Silage with a ratio of 70% Corn Stover and 30% Pineapple Waste

T4 = Silage with a ratio of 60% Corn Stover and 40% Pineapple Waste

A total of 20 experimental units were used for the analysis of rumen pH, microbial populations, and methane production, with one additional unit serving as a blank. Data were analyzed using analysis of variance (ANOVA), and significant differences among treatments were further evaluated using Duncan’s multiple range test.

RESULT AND DISCUSSION

This study evaluated the effects of different ratios of corn stover and pineapple waste silage on rumen pH, microbial populations, and methane production under in vitro conditions. The results are presented in Table 1.

Table 1. The Effect of Corn Stover and Pineapple Waste Ratio in Silage on Rumen pH, Bacterial Populations, Protozoa Populations and Methane Gas (In Vitro)

Parameter	Treatment			
	T1	T2	T3	T4
Rumen pH	6.77±0.03	6.77±0.02	6.78±0.02	6.77±0.02
Bacterial Populations (10 ⁹ CFU/mL)	3.18±0.23	3.20±0.21	3.03±0.09	3.31±0.38
Protozoa Populations (10 ⁵ cells/mL)	4.70±0.10 ^c	4.21±0.14 ^a	4.28±0.09 ^a	4.52±0.16 ^b
Methane Gas (mM)	5.76±0.47	6.45±0.43	5.68±0.40	5.57±0.89

Data are presented as mean ± SD. Based on ANOVA, only the protozoa population was significantly affected by the treatments (P ≤ 0.05), whereas pH, bacterial population, and methane production showed no significant differences among treatments (P > 0.05). Different superscript letters indicate significant differences exclusively

within the protozoa row. T1 = 90% Corn Stover + 10% Pineapple Waste; T2 = 80% Corn Stover + 20% Pineapple Waste; T3 = 70% Corn Stover + 30% Pineapple Waste; T4 = 60% Corn Stover + 40% Pineapple Waste.

Rumen pH

Rumen fluid pH is an important indicator of the fermentation process occurring in the rumen. Analysis of variance showed that the treatments had no significant effect on rumen pH ($P > 0.05$). Rumen pH values across all treatments ranged narrowly from 6.77 to 6.78, indicating relatively uniform fermentation conditions. This suggests that differences in silage composition among treatments were not sufficient to alter rumen acidity.

The observed pH values were within the normal physiological range for optimal rumen fermentation. Bayne & Edmondson (2020) reported that the ideal rumen pH for efficient microbial fermentation ranges from 5.50 to 7.00. Rumen pH values were relatively similar among treatments, with pH levels of T1 (6.77), T2 (6.77), T3 (6.78), and T4 (6.77). These minor differences were biologically negligible and did not reflect meaningful changes in rumen fermentation dynamics.

A stable rumen pH reflects a balanced rumen ecosystem that supports the growth and activity of rumen microorganisms, including bacteria and protozoa. Hoy et al. (2023) reported that such conditions are essential for optimal feed fermentation, fiber degradation, and microbial protein synthesis. Similarly, Usman (2013) stated that maintaining rumen pH within the normal range is critical for cellulolytic bacteria, which are sensitive to acidic conditions and play a major role in fiber digestion.

Bacterial Population

Analysis of variance indicated that the treatments had no significant effect on rumen bacterial populations ($P > 0.05$). Bacterial populations across all treatments ranged from 3.03 to 3.31×10^9 CFU/mL, suggesting that rumen fermentation conditions were relatively uniform and stable. This indicates that combinations of corn stover and pineapple waste silage at different ratios provided sufficient fermentable substrates to support bacterial growth.

The bacterial population observed in this study remained within the optimal range to support nutrient degradation and volatile fatty acid (VFA) production. McDonald et al. (2010) reported that total bacterial populations associated with active rumen fermentation typically range from 10^9 to 10^{10} CFU/mL of rumen fluid.

Bacterial populations were relatively comparable among treatments, with values of T1 (3.18×10^9 CFU/mL), T2 (3.20×10^9 CFU/mL), T3 (3.03×10^9 CFU/mL), and T4 (3.31×10^9 CFU/mL). The higher proportion of pineapple waste in T4 tended to support greater bacterial abundance, likely due to the availability of readily fermentable carbohydrates that stimulate bacterial activity. These findings indicate that rumen conditions in T4 were particularly favorable for microbial growth and fermentation efficiency.

Similar trends have been reported in previous studies. Suksathit et al. (2011) found that *in vitro* fermentation of pineapple waste silage resulted in bacterial populations ranging from 9.90 to 12.07×10^9 CFU/mL, while Zhang et al. (2022) reported that corn stover supported bacterial populations of up to 10^9 CFU/mL. The bacterial populations observed in this study were within comparable ranges, indicating that the combination of corn stover and pineapple waste did not impair rumen microbial performance but instead maintained favorable fermentation conditions.

Protozoa Population

In contrast to rumen pH and bacterial population, analysis of variance showed that the treatments significantly affected protozoa populations ($P \leq 0.05$). Protozoa populations ranged from 4.21 to 4.70×10^5 cells/mL across all treatments and remained within the normal physiological range for rumen fluid. This finding is consistent with Soetanto (2019), who reported that protozoa populations in the rumen typically range from 10^5 to 10^6 cells/mL.

The highest protozoa population was observed in treatment T1 (4.70×10^5 cells/mL), followed by T4 (4.52×10^5 cells/mL), T3 (4.28×10^5 cells/mL), with the lowest population recorded in T2 (4.21×10^5 cells/mL). Overall,

the results indicate a decreasing trend in protozoa population with increasing proportions of pineapple waste in the silage. Although all treatments maintained protozoa populations within a range supportive of rumen fermentation, the significant differences observed suggest that dietary composition played an important role in modulating protozoa abundance.

Comparable findings have been reported in previous studies. Widiawati (2009) reported protozoa populations ranging from 0.37 to 2.86×10^6 cells/mL in rumen fluid when pineapple peel waste was included in the diet, while Zhang et al. (2022) observed protozoa populations of approximately 10^6 cells/mL in corn stover based diets. These studies support the present results, indicating that combinations of corn stover and pineapple waste silage are able to maintain rumen microbial balance while influencing protozoa dynamics.

The observed reduction in protozoa population with increasing pineapple waste inclusion may be attributed to the presence of bioactive compounds, particularly saponins and flavonoids, in pineapple waste. Saponins are known to exhibit antiprotozoal activity by forming complexes with sterols in protozoal cell membranes, leading to increased membrane permeability and cell lysis, thereby reducing protozoa populations in the rumen. Flavonoids have also been reported to modulate rumen microbial ecosystems by selectively inhibiting protozoa and altering microbial interactions, which can indirectly suppress protozoa growth. These mechanisms explain why treatments containing higher proportions of pineapple waste tended to exhibit lower protozoa populations.

In addition to bioactive compounds, differences in the ratio of corn stover to pineapple waste influenced the availability and type of carbohydrate substrates in the rumen. Treatment T1, which was dominated by corn stover, provided a higher proportion of structural carbohydrates and starch, supporting the highest protozoa population. Protozoa utilize carbohydrates as energy sources in addition to preying on rumen bacteria, as reported by Wandra et al. (2020). Conversely, increasing levels of pineapple waste in treatments T2 to T4 supplied more readily fermentable carbohydrates along with bioactive compounds, creating conditions less favorable for protozoa proliferation.

The reduction in protozoa populations observed in treatments with higher pineapple waste inclusion may have important implications for rumen fermentation efficiency and methane mitigation. Protozoa are known to form syntrophic associations with methanogenic archaea through hydrogen transfer (Patel, 2018). Therefore, a decrease in protozoa populations may reduce hydrogen availability for methanogenesis, contributing to lower methane production, even though differences in methane levels were not statistically significant in this study. This suggests that the inclusion of pineapple waste in corn stover based silage has the potential to modulate rumen microbial interactions in a direction favorable for improved fermentation efficiency and reduced methane emissions.

Methane Gas

Analysis of variance showed that the treatments had no significant effect on methane production ($P > 0.05$). Methane concentrations ranged from 5.57 to 6.45 mM across treatments. Methane production showed a narrow range across treatments, with concentrations of T1 (5.76 mM), T2 (6.45 mM), T3 (5.68 mM), and T4 (5.57 mM). Methane production trend decreasing by adding pineapple waste. These values were relatively low and uniform, indicating that differences in silage composition did not result in substantial increases in methane production during *in vitro* rumen fermentation.

Methane formation in the rumen occurs through methanogenesis, a process in which methanogenic archaea utilize hydrogen (H_2) and carbon dioxide (CO_2) to produce CH_4 (Martin et al., 2010). The hydrogenotrophic pathway ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) is the primary mechanism, although the acetylatic pathway may also occur under specific conditions (Martin et al., 2010). The relatively low methane production observed in this study suggests limited availability of hydrogen substrate for methanogens.

Methane production is closely associated with protozoa and rumen bacterial populations. Protozoa are known to form syntrophic associations with methanogens by producing hydrogen as a metabolic byproduct (Patel, 2018). In this study, treatments with relatively higher protozoa populations tended to exhibit slightly higher methane

production; however, this relationship was not linear and did not result in statistically significant differences, indicating that overall rumen microbial balance was maintained.

Compared with previous studies, methane production in this experiment was considerably lower. Ridla et al. (2025) reported methane production ranging from 18.40 to 21.03 mM from silage composed of pineapple and corn stover, which was nearly three times higher than the values observed in this study. This suggests that the ration formulation and ingredient ratios used here were more effective in suppressing methane formation during rumen fermentation.

The reduced CH₄ production may be attributed to the complementary carbohydrate characteristics of corn stover and pineapple waste, as well as the presence of bioactive compounds in pineapple waste. Plant secondary metabolites such as saponins and polyphenols are known to exhibit antiprotozoal activity and inhibit methanogenesis through substrate competition and direct suppression of methanogenic archaea (Yanuartono et al., 2019).

CONCLUSIONS

This study was conducted using an *in vitro* rumen fermentation system, which has inherent limitations. The *in vitro* approach does not fully represent the complexity of the rumen environment in live animals, particularly in terms of animal metabolism, feed intake regulation, absorption of fermentation end products, and long term microbial adaptation. In addition, the relatively short incubation period may not reflect the sustained effects of dietary treatments on rumen microbial dynamics and methane production. Therefore, further *in vivo* studies are recommended to validate the effects of corn stover and pineapple waste silage combinations on rumen fermentation characteristics and methane emissions under practical feeding conditions.

Varying the ratio of corn stover and pineapple waste silage up to 40% did not significantly affect rumen pH (6.77–6.78), bacterial population ($3.03\text{--}3.31 \times 10^9$ CFU/mL), or methane production (5.57–6.45 mM), with all values remaining within normal and stable ranges. In contrast, the protozoa population ($4.21\text{--}4.70 \times 10^5$ cells/mL) was the only parameter significantly affected by the treatments ($P \leq 0.05$), showing a decreasing trend as the proportion of pineapple waste increased, although still within the optimal range for rumen fermentation.

The relatively low methane production observed across treatments suggests the potential of combining corn stover and pineapple waste silage to support efficient rumen fermentation while contributing to methane mitigation. Overall, all tested silage ratios were feasible and capable of maintaining a balanced rumen microbial ecosystem. However, further *in vivo* investigations are necessary to confirm these findings and evaluate their applicability under animal production conditions.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided by Academic Leadership Grant (ALG) under Grant Number (3208/UN6.J/PT.00/2025). This support was instrumental in enabling the successful completion of this research project.

REFERENCES

1. Andri. (2020). Kang Jimat Bahas Kerjasama antara Subang dengan Okinawa dalam Program Sister City. Pemerintah Daerah Kabupaten Subang. <https://subang.go.id/berita/kang-jimat-bahas-kerjasama-antara-subang-dengan-okinawa-dalam-program-sister-city>
2. Angulo-Padilla, J., Lozano-De La Ossa, L., González-Delgado, Á., Sánchez-Tuirán, E., & Ojeda-Delgado, K. (2018). Potential for Degradation of Lignocellulosic Biomass via Alkaline Pretreatment using Corn Crop Residual Biomass. *Contemporary Engineering Sciences*, 11(14), 679–687. <https://doi.org/10.12988/ces.2018.8263>
3. Badan Pusat Statistik. (2024). Produksi Daging Sapi Menurut Provinsi (Ton). BPS Indonesia. <https://www.bps.go.id/indicator/24/480/1/produksi-daging-sapi-menurut-provinsi.html>

4. Bayne, J. E., & Edmondson, M. A. (2020). Diseases of the Gastrointestinal System. In *Sheep, Goat, and Cervid Medicine*, Elsevier, 63–96. <https://doi.org/10.1016/B978-0-323-62463-3.00014-1>
5. Chrysostomus, H., Koni, T., & Foenay, T. (2020). Pengaruh Berbagai Aditif terhadap Kandungan Serat Kasar dan Mineral Silase Kulit Pisang Kepok. *Jurnal Ilmu Peternakan dan Veteriner Tropis*, 10(2), 91–98. <https://doi.org/10.46549/jipvet.v10i2.100>
6. Cornelia, M., & Kristyanti, T. (2021). Utilization of Pineapple's (*Ananas comosus* L. Merr.) Peel Waste as Raw Material in Cider Making. *Proceedings of the International Conference on Food Science and Technology*, 258–263. <https://doi.org/10.5220/0010041402580263>
7. Gao, J., Wang, P., Zhou, C., Li, P., Tang, H., Zhang, J., & Chai, Y. (2019). Chemical Composition and In Vitro Digestibility of Corn Stover during Field Exposure and Fermentation Characteristics of Silage Prepared with Microbial Additives. *Asian-Australasian Journal of Animal Sciences*, 32(12), 1854–1863. <https://doi.org/10.5713/ajas.18.0886>
8. Hernaman, I., Budiman, A., Nurachman, S., & Hidrajat, K. (2015). Kajian In Vitro Substitusi Konsentrat dengan Penggunaan Limbah Perkebunan Singkong yang Disuplementasi Kobalt dan Seng dalam Ransum Domba. *Buletin Peternakan*, 39(2), 71–80. <https://doi.org/10.21059/buletinpeternak.v39i2.6710>
9. Hoy, C., Hartati, E., & Lestari, G. (2023). Pengaruh Silase Pakan Komplek Berbasis Sorgum *Clitoria ternatea* dengan Penambahan Berbagai Level Konsentrat Mengandung ZnSO₄ dan ZnCu Isoleusinat terhadap Fermentasi Rumen In Vitro. *Animal Agricultura*, 1(2), 79–89. <https://doi.org/10.59891/animacultura.v1i2.18>
10. Khajali, F., & Rafiei, F. (2024). A Review of Plant Anti-Nutritional Factors in Animal Health and Production: Classification, Biological Properties, and Passivation Strategies. *Journal of Agriculture and Food Research*, 18, 101290. <https://doi.org/10.1016/j.jafr.2024.101290>
11. Liu, J., Wang, Y., Liu, L., Ma, G., Zhang, Y., & Ren, J. (2023). Effect of Moringa Leaf Flavonoids on Production Performance, Immune Response, and Rumen Fermentation of Dairy Cows. *Veterinary Medicine and Science*, 9(2), 917–923. <https://doi.org/10.1002/vms3.993>
12. Martin, C., Morgavi, D. P., & Doreau, M. (2010). Methane Mitigation in Ruminants: From Microbe to the Farm Scale. *Animal*, 4(3), 351–365. <https://doi.org/10.1017/S1751731109990620>
13. McDougall, E. I. (1947). Studies on Ruminant Saliva: I. The Composition and Output of Sheep's Saliva. *Biochemical Journal*, 43(1), 99–109. <https://doi.org/10.1042/bj0430099>
14. McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A., & Wilkinson, R. G. (2010). *Animal Nutrition* (7th ed.). Pearson Education Limited.
15. Mustika, L. M., & Hartutik, H. (2021). Kualitas Silase Tebon Jagung (*Zea mays* L.) dengan Penambahan Berbagai Bahan Aditif Ditinjau dari Kandungan Nutrisi. *Jurnal Nutrisi Ternak Tropis*, 4(1), 55–59. <https://doi.org/10.21776/ub.jnt.2021.004.01.7>
16. Paloa, M., Waani, M., & Malalatang, S. (2025). Pengaruh Lama Ensilase dengan Penambahan Tepung Jagung terhadap Kualitas Fisik Rumput Gajah Dwarf (*Pennisetum purpureum* cv. Mott). *Zootec*, 45(2), 197–206.
17. Patel, S. (2018). Role of Rumen Protozoa: Metabolic and Fibrolytic Functions. *Advances in Biotechnology & Microbiology*, 10(4). <https://doi.org/10.19080/AIBM.2018.10.555793>
18. Patra, A. K., & Saxena, J. (2009). Effect and Mode of Action of Saponins on Microbial Populations and Fermentation in the Rumen. *Nutrition Research Reviews*, 22(2), 204–219. <https://doi.org/10.1017/S0954422409990163>
19. Reiza, I., Rijai, L., & Mahmudah, F. (2019). Skrining Fitokimia Ekstrak Etanol Kulit Nanas (*Ananas comosus* (L.) Merr.). *Proceedings of Mulawarman Pharmaceuticals Conferences*, 10, 104–108. <https://doi.org/10.25026/mpc.v10i1.371>
20. Ridla, M., Jayanegara, A., & Nahrowi, N. (2025). Evaluation of Silage Quality, Rumen Fermentation, Degradability, and Methane Emission of Total Mixed Rations from Agricultural By-Products: An In Vitro Study. *Journal of Animal and Feed Sciences*, 34(3), 442–450. <https://doi.org/10.22358/jafs/200863/2025>
21. Satiyarti, R., Yustisiana, S., & Sugiharta, I. (2023). Analisis Kualitas Silase Tanaman Jagung sebagai Pakan Ternak dengan Durasi Fermentasi Berbeda. *Organisms: Journal of Biosciences*, 3(2), 17–24. <https://doi.org/10.24042/organisms.v3i2.18043>
22. Septia, D., Muhtarudin, M., Tantalio, S., & Liman, L. (2025). Pengaruh Penambahan Kulit Nanas dengan Level Berbeda terhadap Kandungan Bahan Kering, Bahan Organik, dan Uji Organoleptik Silase Tebon

- Jagung. *Jurnal Riset dan Inovasi Peternakan (Journal of Research and Innovation of Animals)*, 9(3), 464–477. <https://doi.org/10.23960/jrip.2025.9.3.464-477>
23. Soetanto, H. (2019). *Pengantar Ilmu Nutrisi Ruminansia*. UB Press.
 24. Suksathit, S., Wachirapakorn, C., & Opatpatanakit, Y. (2011). Effects of Ensiled Pineapple Waste and Pangola Hay as Roughage Sources on Feed Intake, Nutrient Digestibility, and Ruminal Fermentation of Southern Thai Native Cattle. *Songklanakarin Journal of Science and Technology*, 33(3), 281–289.
 25. Tanuwiria, U., Zain, M., Syamsu, J., Yunilas, Y., Mushawwir, A., & Yanza, Y. (2025). Influence of Rumen Degradable Protein and Non-Fiber Carbohydrate Proportions on In Vitro Rumen Fermentation and Methane Emission. *Journal of Advanced Veterinary and Animal Research*, 12(3), 784–794. <https://doi.org/10.5455/javar.2025.1941>
 26. Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B., & France, J. (1994). A Simple Gas Production Method using a Pressure Transducer to Determine Fermentation Kinetics of Ruminant Feeds. *Animal Feed Science and Technology*, 48(3–4), 185–197. [https://doi.org/10.1016/0377-8401\(94\)90171-6](https://doi.org/10.1016/0377-8401(94)90171-6)
 27. Tuturoong, R. A. V., Malalantang, S. S., & Moningkey, S. A. E. (2020). Assessment of Nutritive Value of Corn Stover and King Grass in Complete Feed on Ongole Steer Calves Productivity. *Veterinary World*, 13(4), 801–806. <https://doi.org/10.14202/vetworld.2020.801-806>
 28. Usman, Y. (2013). Pemberian Pakan Serat Sisa Tanaman Pertanian terhadap Evolusi pH, N-NH₃, dan VFA Rumén Sapi. *Jurnal Agripet*, 13(2), 53–58. <https://doi.org/10.17969/agripet.v13i2.821>
 29. Wahyuni, S., Kadarusno, A., & Suwerda, B. (2016). Pemanfaatan *Saccharomyces cerevisiae* dan Limbah Buah Nanas untuk Pembuatan Bioetanol. *Sanitasi: Jurnal Kesehatan Lingkungan*, 7(4), 151–159. <https://doi.org/10.29238/sanitasi.v7i4.725>
 30. Wajizah, S., Usman, Y., & Mariana, D. E. (2015). Evaluation of Nutritive Value and In Vitro Digestibility of Oil Palm Fronds Fermented using *Aspergillus niger*. *Agripet*, 15(1), 13–19.
 31. Wandra, F., Pranowo, A., Hernaman, I., Tanuwiria, U., & Ayuningsih, B. (2020). Fermentabilitas Ransum yang Mengandung Ampas Bir dalam Cairan Rumén (In Vitro). *Jurnal Sain Peternakan Indonesia*, 15(2), 227–235. <https://doi.org/10.31186/jspi.id.15.2.227-235>
 32. Wati, W., Mashudi, M., & Irsyammawati, A. (2018). Kualitas Silase Rumput Odot (*Pennisetum purpureum* cv. Mott) dengan Penambahan *Lactobacillus plantarum* dan Molases. *Jurnal Nutrisi Ternak Tropis*, 1(1), 45–53. <https://doi.org/10.21776/ub.jnt.2018.001.01.6>
 33. Widiawati, Y. (2009). Pengaruh Substitusi Produk Samping Nenas (*Ananas comosus* (L.) Merr.) terhadap Ekosistem Rumén Domba. *Jurnal Ilmu Ternak dan Veteriner*, 14(4), 243–261.
 34. Wurlina, W., Hariadi, M., Mustofa, I., & Meles, D. (2020). Penggemukan Sapi Menggunakan Complete Feed dan Tape Jerami. *Jurnal Layanan Masyarakat*, 2(2), 63–68. <https://doi.org/10.20473/jlm.v2i2.2018.63-68>
 35. Yanuartono, Y., Nururrozi, A., Indarjulianto, S., & Purnamaningsih, H. (2019). Peran Protozoa pada Pencernaan Ruminansia dan Dampaknya terhadap Lingkungan. *Ternak Tropika: Journal of Tropical Animal Production*, 20(1), 16–28. <https://doi.org/10.21776/ub.jtapro.2019.020.01.3>
 36. Yuliyati, Y., Rachman, S., & Noviyanti, A. (2018). Pembuatan Silase Rumput Gajah untuk Pakan Ternak. *Jurnal Pengabdian kepada Masyarakat*, 2(7), 1–6.
 37. Zhang, M., Wang, R., Wu, T., Yang, Y., He, Z., Ma, Z., Tan, Z., Lin, B., & Wang, M. (2022). Comparison of Corn Stover Silages Harvested at Different Maturity Stages on Digestibility and Rumen Fermentation. *Animals*, 12(10), 1248. <https://doi.org/10.3390/ani12101248>