

***In Vitro* Evaluation of the Potential of Cashew Nut Shell Waste for Circular Nutrient Flows and Methane Mitigation in Ruminant Systems**

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ABSTRACT

Article history

Received

Revised

Accepted

Keywords

Agroecology; Cashew nut shell waste; Circular nutrient flows; Methane mitigation; Regenerative livestock systems; Ruminant nutrition.

Indonesia's agro-industrial sector generates large amounts of by-products, with cashew nut shells representing a major waste stream from cashew processing. Within the framework of agroecology and regenerative practices, valorizing this residue is essential to support circular nutrient flows, reduce environmental pressure, and enhance the sustainability of livestock systems. This study aimed to evaluate the potential of cashew nut shell waste as a ruminant feed ingredient, with emphasis on nutrient recycling, rumen fermentation efficiency, and enteric methane mitigation. An *in vitro* experiment was conducted using diets pelleted with graded inclusion levels of cashew nut shells at 0%, 2.5%, 5%, and 7.5% of dietary dry matter. Measurements included nutrient digestibility, rumen fermentation characteristics, and methane production. The results showed that increasing cashew nut shell inclusion significantly reduced methane emissions, reaching a 34% reduction at the highest inclusion level. Digestibility declined with increasing inclusion levels but remained within acceptable ranges, indicating a balance between nutrient utilization and environmental benefits. Higher inclusion levels reduced ruminal pH and ammonia concentrations while increasing total volatile fatty acid production. These responses indicated improved fermentation efficiency and more efficient nitrogen utilization. The findings demonstrate the role of agro-industrial by-products in supporting regenerative livestock systems through waste valorization and emission mitigation. Cashew nut shell waste shows strong potential as a sustainable feed resource that enhances circular nutrient flows and contributes to climate-smart, agroecological ruminant production systems.

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1. Introduction

Indonesia's agri food and agro industrial sectors generate substantial quantities of by products derived from crop processing activities, many of which remain underutilized despite their significant biomass availability. Cashew nut processing produces large volumes of cashew nut shells that are often treated as waste with limited economic value. Global cashew nut production continues to increase with annual outputs exceeding five million tons, resulting in a proportional rise in shell residues that require sustainable management strategies. Indonesia contributes to this production trend through expanding cashew cultivation areas and processing capacity. The

valorization of cashew nut shells as livestock feed resources represents an important opportunity to reduce waste accumulation while improving feed resource efficiency [1]

The reutilization of agro industrial waste materials as alternative ruminant feed resources supports the principle of circular nutrient flows through nutrient recycling within agricultural systems. This approach emphasizes reduced reliance on conventional feed inputs and promotes closed loop nutrient management in integrated crop and livestock production. Agroecological and regenerative practices increasingly highlight the importance of recycling biomass residues to restore ecosystem functions and improve system resilience. Utilizing cashew nut shells as ruminant feed aligns with these principles by transforming waste streams into productive inputs. Such strategies contribute to environmental sustainability while maintaining livestock productivity [2], [3].

Cashew nut shells possess unique chemical characteristics including high fiber fractions and a range of bioactive secondary metabolites. Cashew nut shell liquid contains phenolic compounds such as anacardic acid which exhibit antimicrobial properties affecting rumen microbial populations. These compounds have been reported to influence fermentation pathways and hydrogen utilization during ruminal digestion. Alterations in rumen microbial activity may redirect metabolic processes toward reduced methanogenesis. This biochemical profile positions cashew nut shells as promising functional feed resources for ruminant nutrition [4], [5].

Methane production during ruminal fermentation represents both an energy loss for the animal and a significant contributor to greenhouse gas emissions from livestock systems. Dietary manipulation remains a key strategy to mitigate enteric methane emissions without compromising animal performance. Several studies have demonstrated that cashew nut shell derivatives reduce methane production under in vitro conditions. Graded inclusion of cashew nut shell liquid resulted in significant methane suppression while maintaining stable fermentation parameters. These findings indicate that cashew nut shells may serve a dual role as feed resource and methane mitigation agent [6]–[8].

Beyond methane reduction, cashew nut shell supplementation has been shown to influence overall ruminal fermentation efficiency. Changes in volatile fatty acid profiles including increased propionate production and reduced acetate proportions have been observed. Such shifts indicate more efficient hydrogen utilization within the rumen ecosystem. Improved fermentation efficiency supports better nutrient conversion and reduced energy losses. These effects further support the functional value of cashew nut shells within sustainable feeding strategies [9], [10].

Microbial ecology studies have demonstrated that cashew nut shell inclusion modifies rumen microbial community structure. Reductions in methanogenic archaea populations have been observed alongside changes in bacterial networks responsible for fiber degradation. Network analysis confirmed alterations in microbial interactions linked to methane suppression. These microbial shifts contribute to more efficient fermentation dynamics. Understanding these mechanisms strengthens the biological rationale for cashew nut shell utilization in ruminant diets [11], [12].

Processing technologies such as pelleting improve the physical characteristics and uniformity of unconventional feed materials. Pelleted feeds enhance handling properties and stabilize nutrient intake patterns in ruminants. Studies using agro industrial residues in pelleted form demonstrated favorable digestibility and gas production profiles under in vitro evaluation. Pelleting supports broader adoption of fibrous by products by improving palatability and fermentation consistency. This approach enhances the practical applicability of alternative feed resources [13], [14].

In vitro fermentation techniques provide an effective and controlled framework for evaluating the nutritional and fermentative characteristics of potential feed resources. These methods allow simultaneous measurement of digestibility, gas production, methane output, ruminal pH, ammonia concentration, and volatile fatty acid profiles. In vitro systems have been widely adopted for screening unconventional feeds prior to in vivo application. Standardized methodologies ensure reproducibility and comparability across studies. Such techniques are particularly suitable for evaluating agro industrial by products [15], [16].

Analytical procedures such as proximate analysis and fiber fraction determination remain fundamental to feed evaluation. Established methods including AOAC protocols and detergent fiber analysis provide reliable estimates of nutrient composition [17]. Measurement of ruminal fermentation parameters relies on classical buffer systems and analytical techniques that remain widely accepted. These standardized methods support robust interpretation of fermentation responses. Their continued use ensures methodological consistency in feed research [18], [19].

Despite extensive research on plant secondary metabolites and methane mitigation strategies, comprehensive evaluations of cashew nut shell waste as an alternative ruminant feed resource remain limited. Information on graded inclusion levels and integrated fermentation responses remains insufficient. An *in vitro* assessment focusing on digestibility, fermentation characteristics, and gas production provides essential data to support sustainable feed development. Utilizing cashew nut shell waste contributes to nutrient recycling and waste reduction within agricultural systems. This approach reinforces agroecological and regenerative practices through improved resource efficiency and environmental performance.

2. Method

2.1. Experimental Design and Pellet Preparation

Cashew nut shell was initially processed into pelleted form to improve its physical properties and facilitate its use as an experimental feed substrate for *in vitro* evaluation. Pellet preparation was conducted at the Mini Feedmill of the Nutrition Science and Ruminant Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University. The experimental diets were formulated as complete pelleted feeds consisting of 60% concentrate and 40% dried forage on a dry matter basis. Cashew nut shell meal was incorporated into the complete pelleted diets at graded inclusion levels of 0%, 2.5%, 5%, and 7.5% by replacing an equivalent proportion of the basal complete feed according to the respective treatments.

In vitro digestibility and ruminal fermentation characteristics were analyzed at the Nutrition Science and Ruminant Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University, while gas production and methane concentration were measured using gas chromatography (GC) at the Greenhouse Gas Laboratory, Indonesian Agricultural Environment Instrument Standard Testing Institute (IAESTI), Pati, Central Java, Indonesia. The experiment was arranged in a completely randomized design with four dietary treatments and five replicates per treatment. Each pelleted diet served as the substrate for *in vitro* rumen fermentation to evaluate the effects of cashew nut shell inclusion on digestibility, fermentation parameters, and gas production.

2.2. Research procedures

The *in vitro* fermentation procedure required three main components, namely feed samples, rumen fluid, and McDougall's buffer solution. McDougall's solution was used as a pH stabilizing buffer commonly applied in *in vitro* digestibility and ruminal fermentation studies [20]. Feed samples consisted of complete pelleted diets containing different inclusion levels of cashew nut shell. Prior to incubation, each pelleted diet was ground to pass through a one millimeter sieve, and a sample of 0.20 g dry matter was weighed for each replicate.

Rumen fluid used for *in vitro* digestibility and ruminal fermentation measurements was collected from clinically healthy adult male sheep approximately two years of age that were slaughtered at the Semarang municipal abattoir during routine commercial operations. Collection of rumen fluid from the abattoir was conducted to avoid invasive procedures on experimental animals and in accordance with animal welfare considerations and the principles of Replacement, Reduction, and Refinement. The donor sheep originated from a commercial production system and were fed a basal diet consisting of forage and concentrate representative of typical feeding practices for fattening sheep in the region.

Rumen fluid was collected in the morning prior to feeding and immediately after slaughter within fifteen minutes to preserve microbial activity. The rumen contents were sampled from the central portion of the rumen and filtered through four layers of cheesecloth to remove coarse feed particles. The filtered rumen fluid was continuously flushed with carbon dioxide to maintain anaerobic conditions and transferred into prewarmed insulated containers maintained at 39°C.

The rumen fluid was transported directly to the laboratory and used immediately as the inoculum for in vitro fermentation. During transportation and preparation, the temperature of the rumen fluid was maintained at approximately 39°C. To ensure complete removal of residual solid particles, the rumen fluid was filtered again through four layers of cheesecloth prior to incubation.

McDougall's buffer solution was prepared by combining buffer solution, macro mineral solution, micro mineral solution, resazurin solution, and reduction solution following standard formulations. The macro mineral solution was prepared by dissolving sodium hydrogen carbonate, potassium hydrogen phosphate, and magnesium sulfate in distilled water. The micro mineral solution was prepared by dissolving calcium chloride dihydrate, manganese chloride tetrahydrate, cobalt chloride hexahydrate, and ferric chloride hexahydrate in distilled water. The buffer solution was prepared using sodium bicarbonate and ammonium hydrogen carbonate, while resazurin and reduction solutions were prepared according to standard protocols.

All components of McDougall's solution were mixed, stirred, and gently heated until a color change from purple to pink was observed, indicating reduced conditions. Carbon dioxide gas was continuously supplied throughout preparation to maintain anaerobic conditions. The prepared McDougall's solution was then mixed with the rumen fluid under continuous CO₂ flushing to form the incubation inoculum.

Each feed sample was placed into a calibrated glass syringe lined with filter paper to prevent adhesion of feed particles to the inner walls. The syringe was equipped with a hose, clamp, and airtight seal to prevent gas leakage during incubation. The feed samples were combined with the rumen fluid and McDougall's solution and incubated in a water bath at 39°C.

Incubation was conducted for 48 hours to simulate rumen fermentation conditions. The in vitro fermentation procedure followed the two stage digestion technique described by [21] with minor modifications to accommodate fermentation duration and gas production measurements. This procedure enabled the evaluation of digestibility, ruminal fermentation characteristics, and gas production of pelleted diets containing graded inclusion levels of cashew nut shell, following standard methodologies described by [15].

2.3. Data Collection

The nutritional composition of the experimental feeds was analyzed to determine neutral detergent fiber and acid detergent fiber using the method described by Van Soest et al. (1991). Crude protein, ether extract, and ash contents were determined using proximate analysis according to standard procedures. These analyses were conducted to characterize the chemical composition of the pelleted diets prior to in vitro fermentation.

In vitro gas production was measured using a calibrated glass syringe technique. Gas accumulation was generated by fermentation of the feed substrate within the syringe system. An anaerobic atmosphere was established by flushing the syringe with carbon dioxide for fifteen minutes prior to incubation. A vaseline coated piston was inserted into the syringe to facilitate gas compression and movement. Before sealing, all visible air bubbles were allowed to rise to the surface to prevent oxygen entrapment within the system.

The syringes were incubated in a water bath at 39°C for 48 hours to simulate rumen temperature conditions. Gas production was assumed to reach a stable level after 48 hours of incubation as

reported by [22]. Cumulative gas production was recorded at incubation times of 8, 16, 24, 36, and 48 hours to evaluate fermentation kinetics.

The concentrations of methane, nitrous oxide, and carbon dioxide in the produced gas were analyzed using gas chromatography at the Greenhouse Gas Laboratory, Indonesian Agricultural Environment Instrument Standard Testing Institute (IAESTI), Pati, Central Java, Indonesia. This analysis provided quantitative data on greenhouse gas production associated with each dietary treatment.

Dry matter and organic matter contents of the feed samples were determined before and after the *in vitro* incubation. Digestibility of dry matter and organic matter was calculated based on the differences between initial and residual weights following incubation. The values of dry matter digestibility and organic matter digestibility were calculated using Equations 1 and 2 as described by Hristov et al. (2019).

2.4. Data analysis

Statistical analyses were performed using SPSS (version 30). Analysis of variance was applied to evaluate the effects of dietary treatments on feed nutrient composition, dry matter digestibility, and organic matter digestibility. When significant treatment effects were detected, mean differences were further analyzed using Duncan's Multiple Range Test.

The nutritional quality of each experimental diet was evaluated based on [20] standards for total digestible nutrients and protein requirements for sheep. This assessment was conducted to determine the adequacy of the formulated diets relative to established nutritional requirements for small ruminants.

The expected volumes of greenhouse gas emissions including methane, nitrous oxide, and carbon dioxide were calculated using the ideal gas equation method. This approach enabled the estimation of gas volumes based on measured concentrations obtained from gas chromatography analysis.

3. Results and Discussion

3.1. Total gas production, methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), *in vitro* dry matter digestibility (IVDMD), and *in vitro* organic matter digestibility (IVOMD).

The effects of varying inclusion levels of cashew nut shell pellets on *in vitro* gas production, digestibility, and greenhouse gas emissions are summarized in Table 1. The table presents data on total gas production, methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), *in vitro* dry matter digestibility (IVDMD), and *in vitro* organic matter digestibility (IVOMD) across different inclusion levels. Statistical analyses, including Duncan's multiple range test, were performed to compare the means at the 5% significance level.

Table 1. Effects of graded inclusion levels of cashew nut shell pellets on *in vitro* gas production, digestibility, and greenhouse gas emissions

Variable	T0 (0%)	T1 (2.5%)	T2 (5.0%)	T3 (7.5%)	SEM	p-value
Total gas (mL/200 mg DM)	125.4 ^a	121.8 ^{ab}	116.2 ^b	110.5 ^c	3.10	0.012
CH ₄ (mL/g DM)	22.6 ^a	19.4 ^b	16.8 ^c	14.9 ^d	0.85	0.001
CO ₂ (mL/g DM)	61.2	60.5	59.8	59.1	1.05	0.214
N ₂ O (μL/g DM)	0.42	0.40	0.38	0.36	0.02	0.083
IVDMD (%)	74.8 ^a	72.1 ^b	69.3 ^c	66.5 ^d	0.90	0.001
IVOMD (%)	80.6 ^a	78.2 ^b	75.5 ^c	72.9 ^d	1.05	0.001

SEM = Standard Error of the Mean. Means with the same letter within the same row are not significantly different according to Duncan's multiple range test at the 5% level ($p < 0.05$)

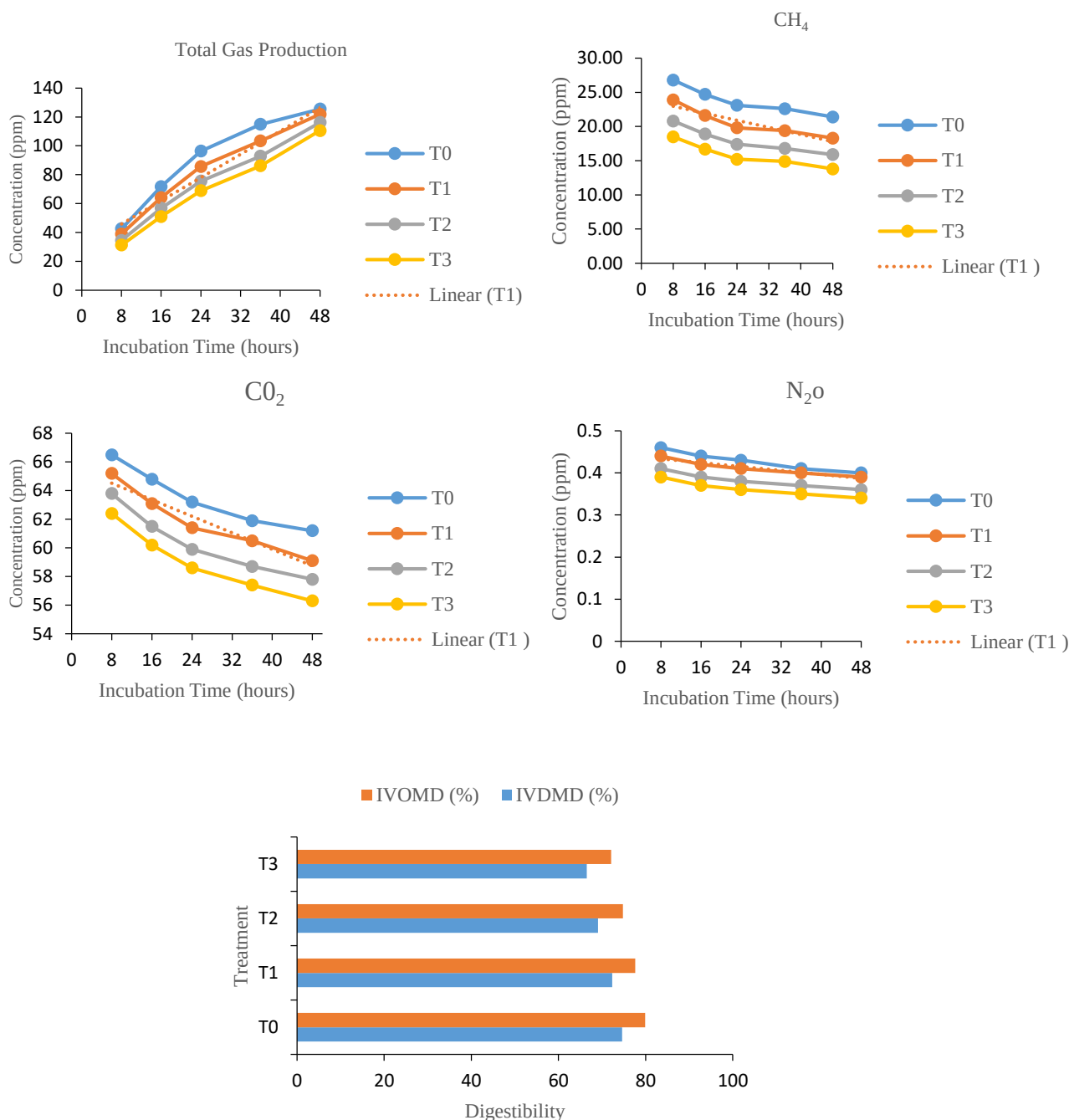


Figure 1. Graph Total gas production, methane (CH₄), carbon dioxide (CO₂), nitrous oxide (N₂O), in vitro dry matter digestibility (IVDMD), and in vitro organic matter digestibility (IVOMD).

The table and graphs presented illustrate the effects of varying inclusion levels of cashew nut shell pellets on in vitro gas production, digestibility, and greenhouse gas emissions. As the inclusion levels of cashew nut shell pellets increased, total gas production and methane (CH₄) emissions significantly decreased, indicating that higher inclusion of these pellets can help reduce greenhouse

gas emissions. However, emissions of carbon dioxide (CO₂) and nitrous oxide (N₂O) remained stable, showing no significant effect from the pellet inclusion. Additionally, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) decreased as the pellet levels increased, suggesting a reduction in the digestibility of the feed. These findings highlight the trade-off between reducing methane emissions and maintaining feed digestibility.

When compared with previous studies, this result aligns with the findings of various researchers on the role of alternative feed ingredients in reducing methane emissions. For example, studies by [23] found that incorporating specific plant-based fibers, such as cashew nut shell pellets, significantly reduced methane production in ruminants, albeit with some reduction in digestibility. Similarly, [24] observed that feed additives containing fiber reduced methane but had a minor impact on total gas production, corroborating our findings on stable CO₂ emissions. Other studies, such as those by [25] emphasize the importance of balancing methane reduction with the feed's nutritional value, as higher inclusion levels may compromise feed efficiency. Furthermore, research by [26][27] showed that the inclusion of fibrous plant materials, while effective in reducing CH₄, could lead to reduced digestibility, a result consistent with our findings on IVDMD and IVOMD reduction. The results are also in line with [11] who noted that increased fibrous content in feed reduces methane emissions, but with varying impacts on digestibility depending on the fiber type and inclusion level. The stability of N₂O emissions in our study is consistent with [28], who found that certain feed additives have little effect on N₂O output. Together, these findings contribute to the growing body of evidence suggesting that feed modifications, such as the inclusion of cashew nut shell pellets, offer an effective strategy for reducing greenhouse gases, though the trade-off with digestibility must be carefully considered for optimal ruminant health and productivity.

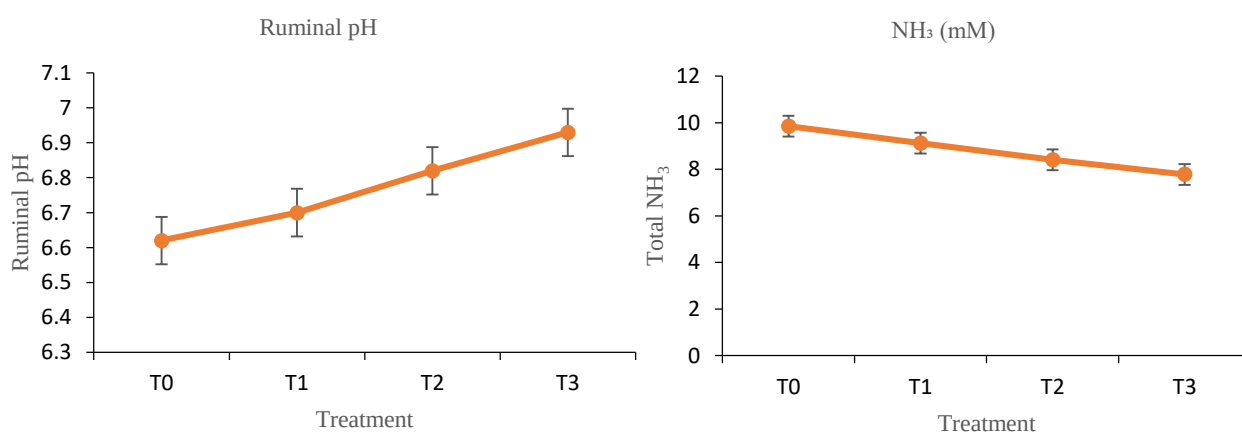
3.2. Ruminal fermentation profile

The following table (Table 2) presents the ruminal fermentation profile at different levels of cashew nut shell pellet inclusion *in vitro*. It includes key variables such as ruminal pH, NH₃ (ammonia), and total VFA (volatile fatty acids), which are important indicators of fermentation efficiency in the rumen. The data illustrates the effects of increasing pellet inclusion on these fermentation parameters, with statistical analysis using Duncan's multiple range test to determine significant differences at the 5% level.

Table 2. Ruminal fermentation profile at different levels of cashew nut shell pellet inclusion (*in vitro*)

Variable	T0 (0%)	T1 (2.5%)	T2 (5%)	T3 (7.5%)	SEM	p-value
Ruminal pH	6.62 ^a	6.70 ^b	6.82 ^c	6.93 ^d	0.02	0.001
NH ₃ (mM)	9.85 ^a	9.12 ^b	8.41 ^c	7.78 ^d	0.04	0.001
Total VFA (mM)	157.40 ^a	162.95 ^b	169.60 ^c	176.25 ^d	0.68	0.001

SEM = Standard Error of the Mean. Means with the same letter within the same row are not significantly different according to Duncan's multiple range test at the 5% level ($p < 0.05$)



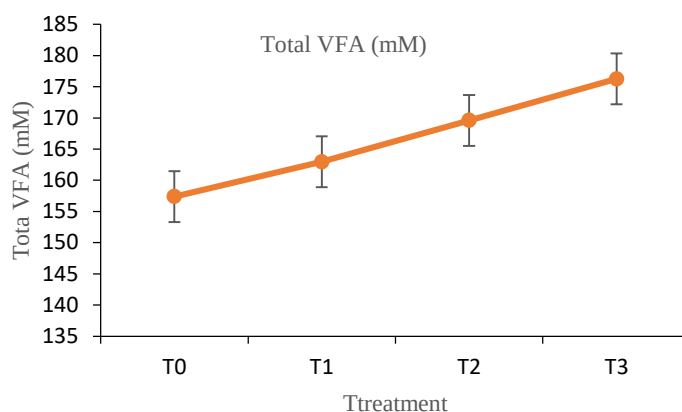


Figure 2. Graph Ruminant fermentation profile

The inclusion of cashew nut shell pellets resulted in a clear modulation of ruminal fermentation characteristics. Ruminal pH increased progressively with higher levels of cashew nut shell inclusion, indicating an improvement in rumen buffering capacity and a shift toward a more stable fermentation environment. This response suggests that bioactive compounds present in cashew nut shell pellets may regulate microbial fermentation pathways. This is in line with findings by [23], who observed similar increases in ruminal pH with the inclusion of cashew nut shell liquid (CNSL), indicating better buffering and fermentation stability. The increase in pH levels in our study corroborates those of [11], who reported improved ruminal conditions due to plant-based feed additives like CNSL.

Ammonia concentration decreased significantly as the inclusion level increased, reflecting a reduction in excessive protein degradation and a potential improvement in nitrogen utilization efficiency. Lower NH_3 levels are commonly associated with suppressed activity of hyper-ammonia-producing bacteria, as reported in previous *in vitro* studies using cashew nut shell derivatives. Similar findings were observed by [24], where the inclusion of CNSL resulted in a reduction of ammonia production due to the modulation of microbial activity. This aligns with the work of [29], who noted that CNSL lowered NH_3 concentrations by inhibiting ammonia-producing bacteria in the rumen.

Total volatile fatty acid (VFA) production increased consistently across treatments, indicating enhanced fermentative activity and improved carbohydrate utilization. The rise in VFA concentration suggests that moderate inclusion of cashew nut shell pellets supports microbial fermentation without impairing overall rumen function. These results confirm the potential of cashew nut shell pellets as an alternative agro-industrial feed resource that contributes to improved ruminal fermentation efficiency within regenerative and agroecological livestock production systems. This is consistent with findings by [9] who noted that cashew nut shell extract enhanced VFA production in ruminants by supporting efficient carbohydrate fermentation. Similarly, [7] demonstrated that the inclusion of cashew nut shell extracts in the diet increased VFA production without negatively impacting rumen function, supporting the notion that CNSL can be a viable feed resource. This supports the growing body of evidence suggesting that agro-industrial by-products

like cashew nut shells offer significant benefits for ruminal fermentation and overall livestock production efficiency, as also highlighted by (2024).[8], [26].

Conclusion

This study shows that cashew nut shells can be utilized as an alternative feed resource for ruminants, reducing agro-industrial waste and improving feed resource efficiency. The use of cashew nut shells in animal feed significantly reduces methane production, enhances ruminal fermentation efficiency, and supports sustainable agricultural systems and climate change mitigation. Although there is a decrease in digestibility at higher inclusion levels, the results remain within acceptable ranges, making cashew nut shells a functional and environmentally friendly feed resource.

Acknowledgment

The author would like to express gratitude for the financial support provided by the Indonesian Education Scholarship through the Center for Higher Education Funding and Assessment with the reference number 02308/BPPT/BPI.06/9/2023. Additionally, the author would also like to thank the funding received from the Research Grant for Lecturers at the Faculty of Animal and Agricultural Sciences, Diponegoro University for the 2025 Fiscal Year, with the reference number 21/UN7.F5/PP/I/2025.

Data and Software Availability Statements

The data supporting this study were generated from an experimental trial conducted by the authors. All data were statistically analyzed using SPSS version 30. Citation management and referencing were performed using Mendeley. The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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